ORFLO | Personal Cell Analysis

TECH NOTE | 11/2011

Comparison of Moxi Z Cell Counter with an Imaging Cell Counter and a High-End Coulter System

Introduction

Cell counts are routinely performed in life science, clinical, and industrial laboratories to ensure proper cell growth rates, to measure passage/seeding densities, as well as to establish initial counts for experimental protocols. Traditionally, these counts are performed manually by researchers using a hemocytometer, mechanical counter, and a microscope. In addition to being extremely laborious, this approach suffers from large errors and variability in the resulting count information due to subjective interpretation of cells and debris, loading errors, improper counting technique, difficulty in tracking high concentration counts, challenges in counting of (3-D) clustered cells, and poor statistical robustness of low cell concentration counts.

Alternatives to this unreliable and painstaking approach of counting include high-end flow cytometers, Coulter-counting systems, and most recently imaging-based systems. The former systems are prohibitively expensive and can require significant training for proper use. Imaging based systems present researchers with the tradeoff of realizing lower cost, enhanced convenience, and ease of use at the expense of the count precision and accuracy provided by their higher end counterparts. Recently, the Orflo Moxi Z cell counter has been introduced as a new alternative that bridges this gap in performance vs. cost and usability. Specifically, the Moxi Z delivers cell count and sizing information that mirrors the performance of the higher-end systems while simultaneously offering the significantly improved ease of use, functionality, speed, lower cost, and maintenance-free operation that has characterized the newer imaging systems. This application note examines and compares the features and performance of the Moxi Z cell counter as compared to a high-end Coulter system[t] and a leading imaging system[*].

Fig. 1 – Photograph of the three systems evaluated in this application note. Juxtaposition of the systems highlights the relative size of each and the small footprint of the Moxi Z. From back to front: Beckman Coulter Z2, BioRad TC10, Orflo Moxi Z.

Precision and Accuracy

The foremost criterion in evaluating a counting system's performance is the quality of the count information that is generated. This information often serves as the foundation for experimental protocols such as in the determination of the quantities of (costly) reagents and the cell seeding densities necessary for downstream processing. Count information also is often applied to the normalization of results in data analysis and presentation, thereby imposing a strict requirement for both consistency and accuracy.

To achieve accuracy and precision, the Moxi Z cell counter has implemented the same Coulter Technique of cell counting that is used in the higher-end Coulter systems. At the core of this technique is a precise, volumetric (3-D) electrical measurement of cells as they pass through an aperture. In contrast, imaging systems take an image (2-D) of a cell sample and apply software algorithms to extract cell profiles and corresponding counts. This interpretive approach is subject to errors in focusing, debris contamination and overall processing limitations, all of which are reflected in the quality of the corresponding count results. As would be expected, the image processing approach provides extremely coarse information regarding sample size profiles, particularly as compared the exact volumetric information reported by the Moxi Z. An example highlighting the discrepancy in size quality is shown in Fig. 2 for a mixed sample of five types of precision calibrated beads (mean diameters of 4.1 µm, 6 µm, 7.9 µm, 10.1 µm, and 15.6 µm) displayed on the Moxi Z and imaging system (note: the Coulter system was not displayed as there was not a sufficient amount of beads to generate the 5 mL of sample required for a test).

Differences in the performance of an imaging system vs. the Moxi Z were evaluated through serial dilution experiments of CHO-K1 and Jurkat E6-1 cells and comparisons to corresponding counts from the gold-standard Coulter system. As the resulting data shows (Fig. 3), the Moxi Z yields improved linearity of the counts (Moxi Z r^2=.9958 vs. imaging system r^2=.9317 with CHO-K1 cells and Moxi Z r^2=.9972 vs. imaging system r^2=.9939 for Jurkat E6-1 cells) vs the imaging system. More notably, the imaging system routinely and significantly underestimates the true counts of the sample and also exhibits substantial count-to-count variations (large error bars) for identical samples. Fig 3b quantifies this error (as a percentage of the Coulter system results) and presents

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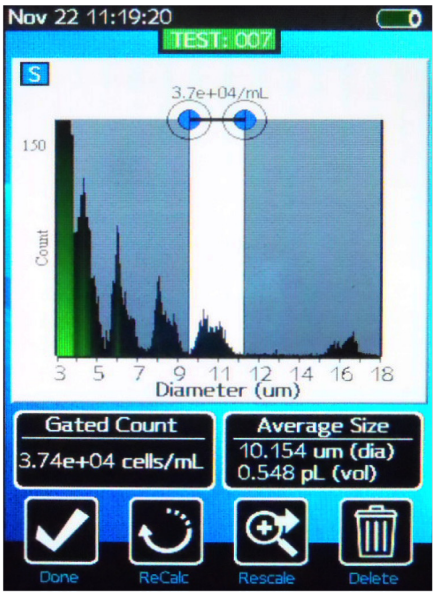

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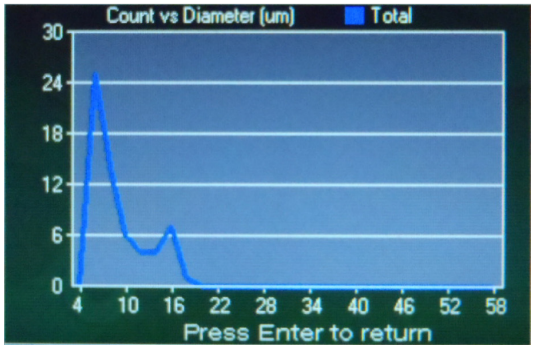


Fig. 2 – Comparison of histogram displays on the a) Moxi Z versus the b) Imaging system for a mixed sample of five types of precision calibrated beads (mean diameters of 4.1 µm, 6 µm, 7.9 µm, 10.1 µm, and 15.6 µm). Moxi Z dynamic gating enables precise counting and sizing of particle sub-populations (10.1 µm bead gated count and size shown here). The imaging system presents only a total count (on a different screen).

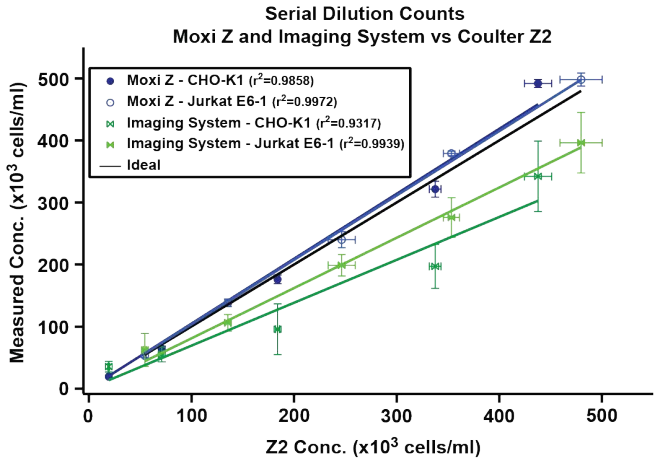
the overall variability (coefficient of variation, CV %) of the counts for each system. As the underlying implementation of the imaging system is similar to (and therefore subject to many of the same limitations of) the hemocytometer technology, it expectedly exhibits a similar error value (31%) and CV (~21%) range. In stark contrast, the Moxi Z achieved similar performance to the reference standard with an error of just 5% and a CV of only 4%.

Cassette Architecture

Automated cell counters have brought dramatic improvements in eliminating system maintenance. A key breakthrough in this regard is the use of disposables for test processing. This format, used by both imaging systems and the Moxi Z, minimizes the potential for sample contact with the system and contrasts with the high-end Coulter systems in which samples are in direct contact with the system aperture and internal fluidics. This sample exposure adds a substantial degree of complexity to the operation and maintenance of the system. At a minimum, considerable care has to be taken to prevent frequent blockage of the fluidic path. Beyond this, significant cost and effort is

Fig. 3 – a) Serial Dilution Counts were performed with the Moxi Z, imaging system and Coulter system. As the Coulter Z2 is the established standard in counting technologies, both the Moxi Z counts (Blue circles) and imaging system counts (green triangles) were plot with respect to this system (black line ideal count). Error bars are representative of ± one standard deviation of the mean. b) Bar graph representation of the count errors (% with respect to Coulter Z2 system, blue bars) and coefficients of variation (CV's %, red bars) for the different systems.

A)



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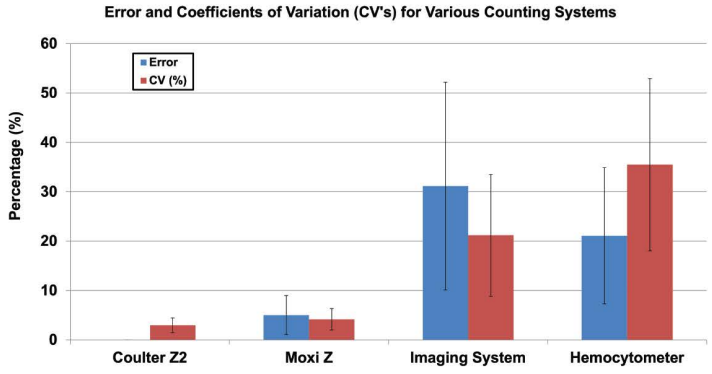


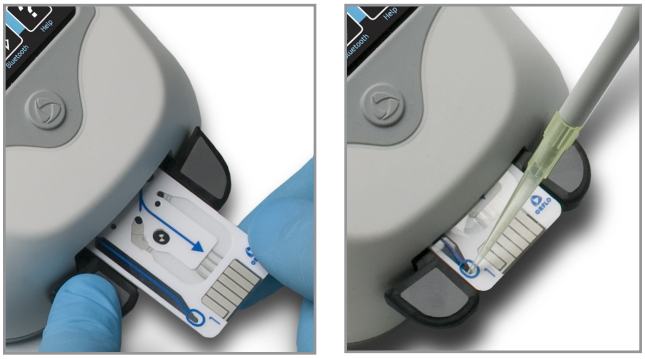


Fig. 4 – Loading procedure of the Moxi Z. The cassette-based architecture and exterior sample loading eliminate the potential for instrument contamination.

required in the routine disinfecting of system components. No such maintenance is required by the Moxi Z. In addition, the Moxi Z sample loading method (Fig. 4) offers an added degree of protection from instrument contamination (compared to imaging systems) by pulling the sample into the detection chamber from the exterior (as opposed to insertion of the sample-loaded end of the disposable into the system).

Finally, while the imaging systems use simple clear plastic slides, the Moxi Z cassettes are sophisticated microfluidics sensors that include pre-filters for dissociation of cell aggregates and clog prevention as well as electrical circuitry for cell detection. This circuitry is also used to make the electronic volumetric measurement of each particle for precise particle sizing. Furthermore, because of the cassette-based circuitry, new capabilities of the system can be implemented via new cassette types. This allows all existing Moxi Z instruments to take advantage of evolving technology improvements.

Culture Health Assessment

Through the Moxi Population Index (MPI), the Moxi Z provides a rapid, general assessment of the on-going health of cell cultures without the need for reagents. The underlying principle of MPI is that there are morphological changes that occur with time as cells die including a shrinking of the cells and, ultimately, a breaking apart of the cells. This, in addition to potential microbial contamination, contribute to increased sample debris/particulate counts. All these changes generate cell/particle populations that can be size-differentiated from the healthy cell populations by Moxi Z. In this regard, the Moxi Z MPI provides a valuable, new, and different perspective from traditional live/dead viability staining regarding the health of a culture. Furthermore, because the MPI and detailed particle size histogram are automatically calculated with each curve-fit test, users can automatically track the on-going health of a culture. Alternatively, the imaging system requires mixing of the Trypan Blue stain with the cell sample for each health assessment test. The instrument then attempts to isolate the dead cells from the live cells colorimetrically, based upon dye exclusion from the cell membranes. However, because of the automated processing of the image lacks the subjective (user) interpretation of the traditional hemocytometer counts, the imaging system analysis is subject to unpredictable interference from non-specifically stained debris. Finally, the Coulter system does not provide any health assessment information.

Operation - Ease of Use and Functionality

In addition to the overall maintenance of the systems, the Moxi Z delivers considerably improved ease of use and overall functionality with respect the higher-end counting systems and even the newer imaging systems. To begin with, the Moxi Z implements a fully automated paradigm that requires no pre-

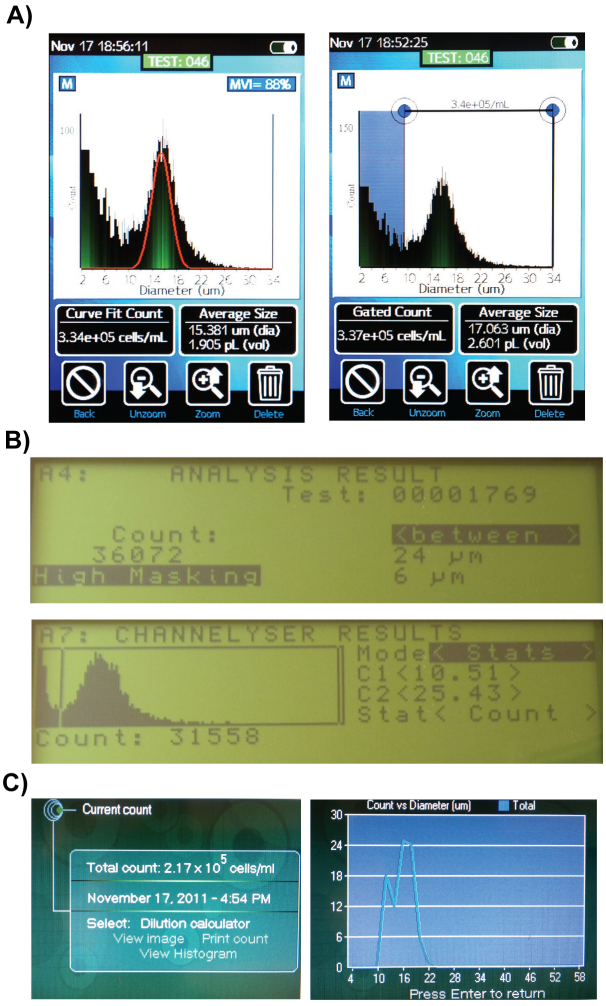


Fig. 5– Images of an identical HEK-293 cell sample processed on the a) Moxi Z, b) Coulter Z2 and c) TC10 cell counters. The Moxi Z interface has advantages over the other technologies with its color touchscreen display, high-resolution histogram, post-processing analysis and the quantity of information presented.

test configuration or focusing of the sample. Furthermore, the Moxi Z also generates a high resolution histogram of the data that, coupled with the color touchscreen display, enables interactive analysis of the results (Fig 5a). This analysis includes both user-adjustable gating (Fig 5a-right) for regional size analysis (i.e. multi-cell populations) as well as curve-fitting (Fig 5a-left) for more precise counts, more accurate cell size information, and reagent-less assessment of cell culture health (MPI). Regional analysis with gating enables the identification of counts and mean diameters within a mixed population of cells/particles (i.e. 10.1 µm bead count and size gating example in Fig. 2a). Unlike the Coulter system, this analysis can be performed after the test has been run as well as with saved tests. Furthermore, the Coulter system provides no information on the health of the sample. The imaging system lacks the post-processing capabilities and dynamic size analysis all together.

In addition to count and histogram data, the Moxi Z automatically displays average diameter and volume information for each test, and provides functionality to quickly rescale both the vertical (counts) and horizontal (diameter) axes. This compares to the Coulter system which requires the scaling (gating range) to be specified a priori and creates lower resolution histograms (Fig 5b) only after pressing the menu option after each test. The imaging system provides only simple count information initially (Fig 5c - left). A low-resolution, coarse size histogram (Fig 5c - right) can be accessed immediately after a test after navigating through the menu. However, it lacks critical mean cell size information and is not saved with the count results.

Data Transfer and Management

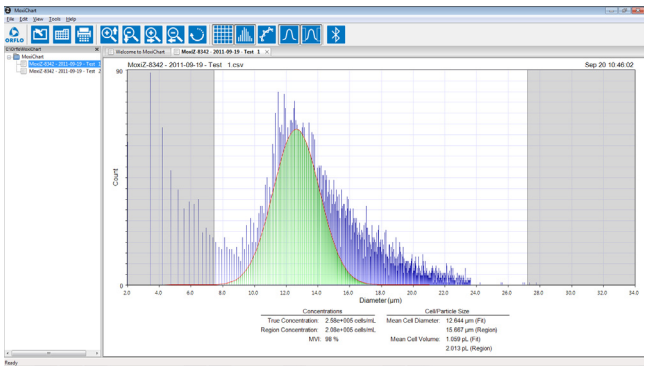
Up to 500 tests can be stored at a time on the Moxi Z unit. This compares to 100 tests for the imaging system and no data storage for the Coulter unit. Furthermore, in stark contrast to the simple count-only information stored by the imaging system, the Moxi Z stores the full histogram information and enables on-unit analysis by switching from curve-fit mode to gating mode, adjustable gating, and vertical scaling. ORFLO also provides a free PC and Macintosh compatible software package, MoxiChart (Fig 6a), that enables data transfer from the Moxi units via Bluetooth or USB, firmware updates for functionality improvements, data management, and data analysis capabilities. Additionally, the complete histogram information is transferred and stored in a comma separated value (.csv) file format for facilitated loading and subsequent processing by external data analysis programs such as Microsoft Excel or Wavemetrics IGOR Pro (Fig 6b). This is a significant enhancement over the functionality provided by the imaging system that merely provides a text file output (Fig 6c) with only count totals only and is transferrable only by using a USB flash drive.

Summary

The Moxi Z cell counter is a revolutionary cell counting system that combines the count precision and accuracy of higher end cell counters and flow cytometers with the ease of use, maintenance-free operation and lower cost of imaging systems. In addition, Moxi Z adds to the existing technologies with dramatically improved overall functionality including dynamic gating following a test, curve-fitting for more accurate counts, reagent-less cell health assessment and improved data management capabilities. As a result, the Moxi Z provides a level of performance and usability that is unparalleled in the industry.

Fig. 6– a) Orflo MoxiChart application enables data transfer from the Moxi Z unit, data management, analysis and image generation/printing. b) The .csv file output format has complete histogram information for each test enabling further analysis in data analysis programs. c) Imaging system output consists of a text file with count total information.

A)



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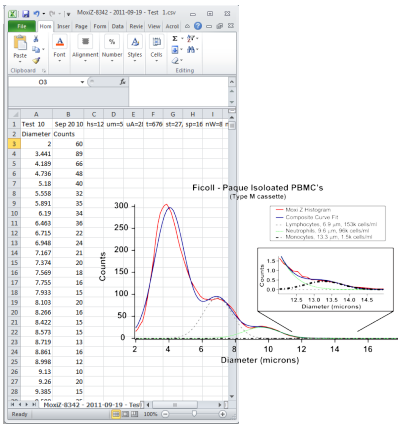

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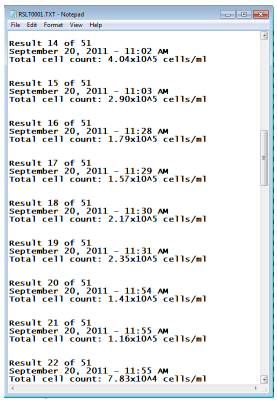

	Moxi Z	Imaging System	High-end Coulter counting system
Error (% vs Z2)	5%	31%	N/A
CV (%)	4%	21%	3%
Touch Screen	Yes	No	No
Test Speed	8 sec	30 sec	2.5 - 50 sec (50 - 100 µm apertures , .1 - .5 ml metered volumes)
Size	7.5" x 4.25" x 2.75" (1.4 lbs)	7.5" x 6" x 10" (2.2 lbs)	17.5" x 10.4" x 14" *main unit only (29.7 lbs)
Wireless operation (use in cell culture hoods)	Yes (4400 mAh Li Ion battery) and Bluetooth data transfer	No	No
Risk of Sample Contamination of Instrument	None	Some (cassette are loaded into the unit with the sample end first)	Yes (direct sample contact)
Maintenance	None	None	Routine cleaning
Size information	High resolution (1200 bin) Size Histogram based on 3D (volumetric) measurement, Mean Diameter AND Mean Volume for every test, Curve-fit for more accurate cell size or regional gated size infromation.	Low resolution histogram based on 2-D (image analysis) measurement, no mean diameter or volume	Low resolution histogram, mean diameter OR mean volume. Gated size information only
Culture Health Assessment	MPI	Live/Dead (Trypan –Blue)	None
PC/Mac Software	MoxiChart	None	None
Data transfer	Bluetooth or USB direct to PC/Mac	USB flash drive	None
Post – processing on unit	Dynamic gating and scaling after a run	None	None
Exported raw histogram	Yes (.csv format)	No	No