Hemocytometer Counting Alternative

Novel System Offers Cell Sizing, Count, and Assessment of Health

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Cell counts are routinely performed in life science, clinical, and industrial laboratories to monitor cell growth rates, to measure seeding densities, to establish counts for data normalization, and to determine initial counts for experimental protocols. Traditionally, these counts are performed manually using hemocytometers. However, in addition to being laborious, this approach suffers from large errors and variability.

Alternatives to hemocytometer-counting include high-end flow cytometers, Coulter-counting systems, and, more recently, imaging-based systems. The former systems are prohibitively expensive in both initial costs and the significant maintenance requirements. In contrast, imaging-based systems present researchers with the trade-off of realizing lower cost and ease-of-use at the expense of count precision and accuracy.

Orflo Technologies (www.orflo.com) recently introduced the Moxi Z mini-automated cell counter as a new alternative that bridges the gap in performance vs. cost and usability. Specifically, the Moxi Z utilizes the gold-standard Coulter Principle to provide three parameters of information regarding cell cultures: 1) precise cell sizing, 2) overall culture health, and 3) highly accurate cell counts.

Single-Cell Resolution —3D Cell Sizing and Health

At the core of the Moxi Z technology is a precise, volumetric (3D) electrical measurement of each cell or particle as it passes through an aperture (Coulter Principle). The resulting output is a high-resolution histogram that provides exact sizing information for each particle as well as a valuable perspective of the culture composition. The accuracy (r^2>0.999) and precision of the Moxi Z particle sizing was illustrated through comparison (*Figure 1A*) of Moxi Z measured diameters of precision calibrated beads with manufacturer-reported values (3.0 µm, 4.17 µm, 5.6 µm, 7.5 µm, 10.1 µm, 15.6 µm, and 25.0 µm diameters). Furthermore the high-resolution histogram (e.g., *Figure 1A* inset—five bead mixture) demonstrates the size discrimination capabilities of the instrument.

The quality of the sizing information provided by the Moxi Z strongly contrasts with the imprecision inherent in 2D image-based approaches that attempt to estimate cell size using image interpolation. Consequently, the Moxi Z uniquely enables size-based discrimination and analysis of cell sub-populations and a correspondingly more robust means of separating debris counts from cell counts.

This degree of information allows researchers to quantitatively monitor and study size-based changes to their cell populations.

The histogram sizing and shape also provides a means of monitoring culture health. One example of this is shown in *Figure 1B*, highlighting differences in histogram shapes for a healthy Jurkat population (*Figure 1B*, blue) versus a mix of healthy and heat-killed (60°C for 30 min, followed by overnight incubation at 37°C) Jurkat cells (*Figure 1B*, red). Increases in counts in the 4–8 µm range of the histogram reflect increased dead cell and debris counts.

The histogram change is quantified as an MPI value, that is a ratio of the core cell population to the total particle population. Changes in the MPI values and histogram shape can additionally uncover potential microbial contamination in cultures through their contributions to smaller particle counts as they colonize/aggregate.

In this regard, the MPI and monitoring of changes in histogram shapes can provide a level of quality control for the cultures by potentially signaling size changes associated with cellular mutations, cell cycle, variations in media composition, or other environmentally induced health changes.

Count Precision and Accuracy

Cell count information serves as the foundation for experimental protocols such

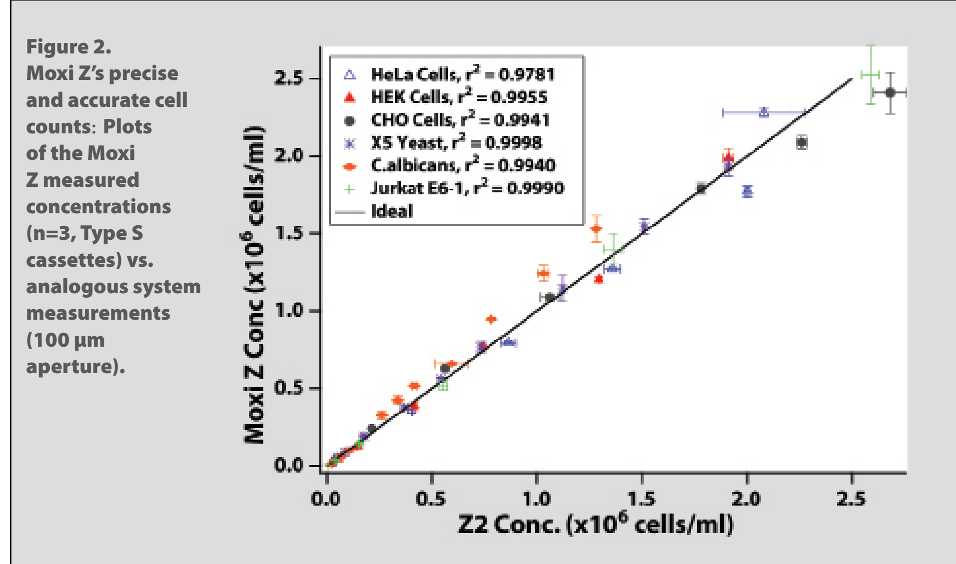

Figure 2. Moxi Z's precise and accurate cell counts: Plots of the Moxi Z measured concentrations (n=3, Type S cassettes) vs. analogous system measurements (100 µm aperture).

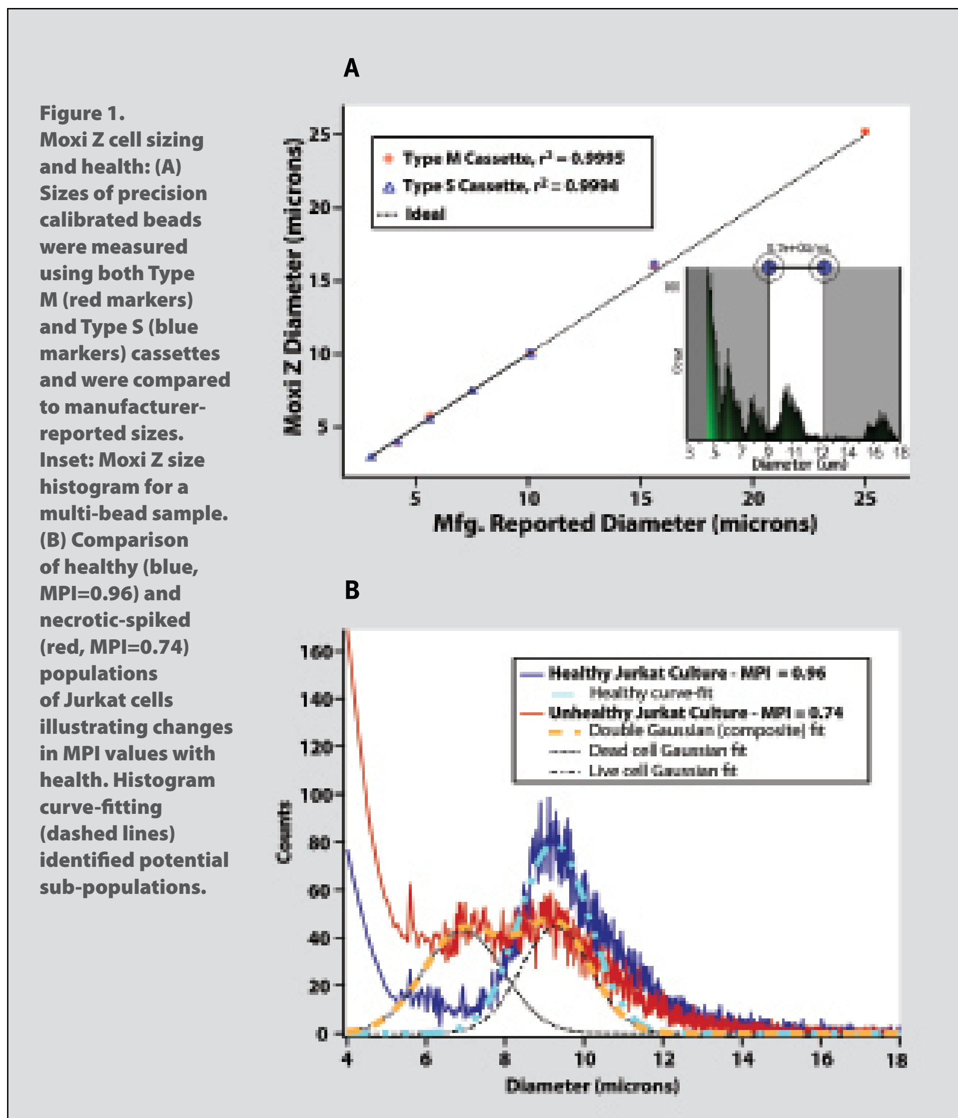

Figure 1. Moxi Z cell sizing and health: (A) Sizes of precision calibrated beads were measured using both Type M (red markers) and Type S (blue markers) cassettes and were compared to manufacturer-reported sizes. Inset: Moxi Z size histogram for a multi-bead sample. (B) Comparison of healthy (blue, MPI=0.96) and necrotic-spiked (red, MPI=0.74) populations of Jurkat cells illustrating changes in MPI values with health. Histogram curve-fitting (dashed lines) identified potential sub-populations.

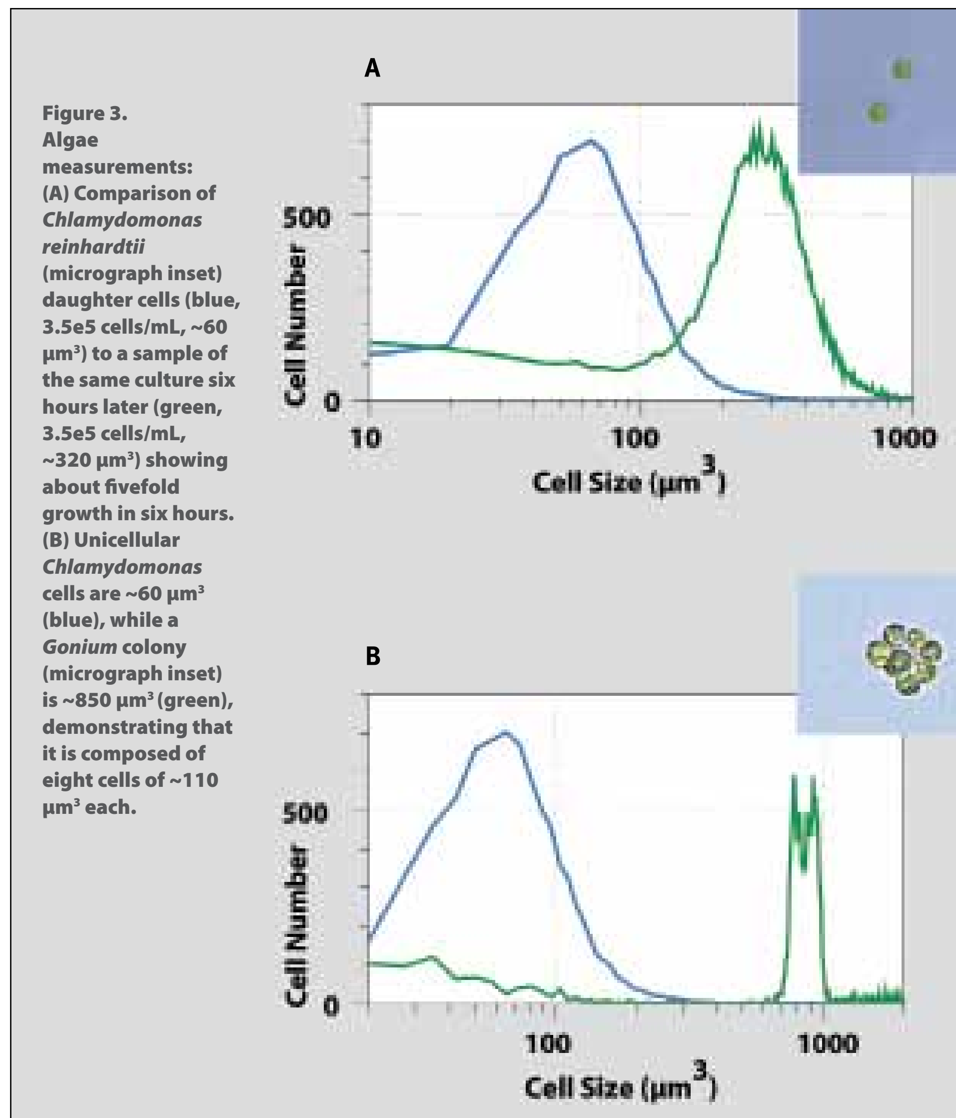

Figure 3. Algae measurements: (A) Comparison of *Chlamydomonas reinhardtii* (micrograph inset) daughter cells (blue, 3.5e5 cells/mL, ~60 µm³) to a sample of the same culture six hours later (green, 3.5e5 cells/mL, ~320 µm³) showing about fivefold growth in six hours. (B) Unicellular *Chlamydomonas* cells are ~60 µm³ (blue), while a *Gonium* colony (micrograph inset) is ~850 µm³ (green), demonstrating that it is composed of eight cells of ~110 µm³ each.

as in the determination of the quantities of (costly) reagents and the cell seeding densities necessary for downstream processing. Count information is also often applied to the normalization of results in data analysis, thereby imposing a strict requirement for both consistency and accuracy.

To gauge the Moxi Z counting performance, Moxi Z counts were compared to equivalent counts from a high-cost system that has long been used as a reference-standard in cell counting. Initial concentrations of 2e6–2.5e6 cells/mL for varying cell types were created through dilution in HBSS. Subsequent concentration levels were established through ratiometric serial dilutions and measured on both systems.

As shown in *Figure 2*, the Moxi Z achieves a comparable count accuracy ($r^2 > 0.994$) to that of the higher-cost system across a broad dynamic range (3e3–2.5e6 cells/mL). Furthermore, compared to manual and imaging-based approaches, the Moxi Z demonstrates a much higher level of repeatability with coefficients of variations of <5% vs. 30–40% for image-based systems (data not shown). As a result, the Moxi Z assures researchers a level of consistency and accuracy in their count data that is irrespective of the technician performing the counts.

Moxi Z Applied to Assessing Algal Growth

Measurement of algal growth parameters such as cell size, cell number, and growth rate is important for confirming optimal growth conditions and culture health. One of the best ways to simultaneously assess algal culture health is to take a series of cell size and number measurements during their growth cycle. The simplest method to do this is to use the Coulter Principle.

Other methods such as combining spectrophotometry (light scattering or absorbance assays) with manual hemocytometer counts are slow, cumbersome, and difficult to perform quickly, especially in the field. Until recently, simultaneously measuring cell size and number with the Coulter Principle required expensive stationary equipment. These systems were inaccessible for many laboratories and not amenable for field measurements.

Many algal species do not undergo binary fission, especially the model algal *Chlamydomonas reinhardtii*, which instead undergoes multiple fission, where cell growth and division are uncoupled. Moreover, being photosynthetic, many algal species, including *Chlamydomonas*, synchronize their cell cycles to the availability of light and divide in the dark. Consequently, when growing, *Chlamydomonas* cell numbers remains unchanged while cell size increases as much as 10-fold in a single day (*Figure 3A*).

Measurement of the growth of *Chlamydomonas* during light cycles is best performed with the Coulter method. The Moxi Z is being applied as an inexpensive portable instrument for measuring cell number and sizes in the laboratory at Kansas State University.

For example, in *Figure 3A*, a synchronous culture of *Chlamydomonas* was sampled just as growth had initiated and then six hours later of a total, 14 hour, growth cycle. While the number of cells in the synchronous sample has not changed (determined by the Moxi Z as 3.51e5 cells/mL, see inset), the volume of the cells has changed from ~50 μm^3 to ~320 μm^3, a fivefold increase in cell size in six hours.

In addition to being a valuable tool for measuring *Chlamydomonas* growth rates, the Moxi Z can also be used for determining physiological differences between species. For example the author's lab also uses *Chlamydomonas* and a closely related colonial multicellular alga, *Gonium pectorale*, as a model system for multicellular evolution.

Chlamydomonas is a unicellular strain, while *Gonium* species are colonies of *Chlamydomonas*-like cells that remain attached through cytoplasmic bridges (see micrograph insets). The lab uses a variety of *Gonium* strains in the course of research. Using the Moxi Z, it is straightforward to determine if a particular culture is in fact *Gonium*.

The Moxi Z is also ideal for characterizing *Gonium* colony size and cells per colony. For example, *Figure 3B* shows the corresponding size profiles on the Moxi Z (*Figure 3B* blue versus green). From these data, the *Gonium* strain is ~850 μm^3, suggesting it is composed of 8 cells of ~110 μm^3, a fact confirmed by microscopy.

Conclusion

The Moxi Z mini automated cell counter provides a valuable three-parameter cell assay (count, sizing, health) in just 8–15 seconds. Leveraging the single-cell resolution of the Coulter Principle, this information is provided with a degree of precision and accuracy rivaled only by higher-cost counting systems. However, the Moxi Z achieves this performance with enhanced usability, increased functionality, and a maintenance-free operation. GEN

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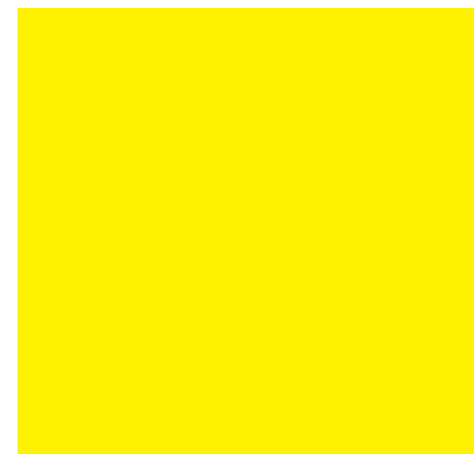

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