Video Article

Determination of Mammalian Cell Counts, Cell Size and Cell Health Using the Moxi Z Mini Automated Cell Counter

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Abstract

Particle and cell counting is used for a variety of applications including routine cell culture, hematological analysis, and industrial controls[1-5]. A critical breakthrough in cell/particle counting technologies was the development of the Coulter technique by Wallace Coulter over 50 years ago. The technique involves the application of an electric field across a micron-sized aperture and hydrodynamically focusing single particles through the aperture. The resulting occlusion of the aperture by the particles yields a measurable change in electric impedance that can be directly and precisely correlated to cell size/volume. The recognition of the approach as the benchmark in cell/particle counting stems from the extraordinary precision and accuracy of its particle sizing and counts, particularly as compared to manual and imaging based technologies (accuracies on the order of 98% for Coulter counters versus 75-80% for manual and vision-based systems). This can be attributed to the fact that, unlike imaging-based approaches to cell counting, the Coulter Technique makes a true three-dimensional (3-D) measurement of cells/particles which dramatically reduces count interference from debris and clustering by calculating precise volumetric information about the cells/particles. Overall this provides a means for enumerating and sizing cells in a more accurate, less tedious, less time-consuming, and less subjective means than other counting techniques[6].

Despite the prominence of the Coulter technique in cell counting, its widespread use in routine biological studies has been prohibitive due to the cost and size of traditional instruments. Although a less expensive Coulter-based instrument has been produced, it has limitations as compared to its more expensive counterparts in the correction for "coincidence events" in which two or more cells pass through the aperture and are measured simultaneously. Another limitation with existing Coulter technologies is the lack of metrics on the overall health of cell samples. Consequently, additional techniques must often be used in conjunction with Coulter counting to assess cell viability. This extends experimental setup time and cost since the traditional methods of viability assessment require cell staining and/or use of expensive and cumbersome equipment such as a flow cytometer.

The Moxi Z mini automated cell counter, described here, is an ultra-small benchtop instrument that combines the accuracy of the Coulter Principle with a thin-film sensor technology to enable precise sizing and counting of particles ranging from 3-25 microns, depending on the cell counting cassette used. The M type cassette can be used to count particles from with average diameters of 4 - 25 microns (dynamic range 2 - 34 microns), and the Type S cassette can be used to count particles with and average diameter of 3 - 20 microns (dynamic range 2 - 26 microns). Since the system uses a volumetric measurement method, the 4-25 microns corresponds to a cell volume range of 34 - 8,180 fL and the 3 - 20 microns corresponds to a cell volume range of 14 - 4200 fL, which is relevant when non-spherical particles are being measured. To perform mammalian cell counts using the Moxi Z, the cells to be counted are first diluted with ORFLO or similar diluent. A cell counting cassette is inserted into the instrument, and the sample is loaded into the port of the cassette. Thousands of cells are pulled, single-file through a "Cell Sensing Zone" (CSZ) in the thin-film membrane over 8-15 seconds. Following the run, the instrument uses proprietary curve-fitting in conjunction with a proprietary software algorithm to provide coincidence event correction along with an assessment of overall culture health by determining the ratio of the number of cells in the population of interest to the total number of particles. The total particle counts include shrunken and broken down dead cells, as well as other debris and contaminants. The results are presented in histogram format with an automatic curve fit, with gates that can be adjusted manually as needed.

Ultimately, the Moxi Z enables counting with a precision and accuracy comparable to a Coulter Z2, the current gold standard, while providing additional culture health information. Furthermore it achieves these results in less time, with a smaller footprint, with significantly easier operation and maintenance, and at a fraction of the cost of comparable technologies.

Video Link

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Protocol

1. Preparation of Samples

1. Dilute the cell suspension with ORFLO diluent or an appropriate diluent so that the cell concentration is within the operating range of the instrument (3,000 to 500,000 cells/mL for Type M cassette, or 3,000 to 2,500,000 cells/mL for Type S cassette).
2. A dilution of 1:5 to 1:20 (Type M cassette) or no dilution to 1:5 dilution (Type S cassette) is recommended for most mammalian cell lines, but the appropriate dilution will depend on cell type and seeding density. The volume required for an accurate count is approximately 75 µL.

2. Running Samples

1. Turn the Moxi Z on by pressing the power button and the **Home** screen will be displayed.
2. Press the tray down and insert a cassette into the Moxi Z. The **Pipette 75µL Sample...** screen will be displayed.
3. Pipette a 75 µL sample into the fill port of the cassette (blue oval labeled 1 or 2, depending on which end of the cassette was inserted into the instrument). These are disposable cassettes, which can be used for 2 tests. Note: Place the pipette tip directly into the loading well at ~45° angle so that the tip is caught under the front lip of the well. When dispensing, it is important that the vacuum does not "get ahead" of the dispensing of the fluid as could cause pockets of air to enter the cassette. It is recommended to have a small bead (the size of entry well) of fluid form over the well during dispensing.
4. For counting most mammalian cells, touch the screen anywhere to start. For counting very small particles (4 to 8 µm average diameter for Type M and 3-8 µm average diameter for Type S), the Moxi Z can be run in **Small Particle Mode**. Note: the cassette type is indicated in the black bar at the top of the screen. In this mode, Moxi Z sets the diameter scale to 2 to 10 µm as the default and performs the count using optimized parameters for the detection of small cells. Press the **TOUCH HERE Small Particle Mode** button (black square) to initiate the test and run in this mode.
5. The Moxi Z will begin the test and the cell count results will be complete in approximately 8 seconds (type M cassette) or 15 seconds (Type S cassette).
6. The Curve Fitting and MVI calculations begin automatically and require only a few additional seconds. When a suitable fit is found, the results will then automatically be displayed on the screen.
7. To make **Gating** the default acquisition mode, press the **Curve Fit Count** button to toggle into Gating mode.
8. At this point the cassette can be removed from the unit.

3. Managing Data

1. If **AutoMode** scaling is off, the results are initially displayed for a curve fit count on a diameter scale of 2-34 µm (Type M cassette) or 2-26 µm (Type S cassette). If **Automode** scaling is on, then the Moxi Z will automatically scale the horizontal axis (x-axis) to the range that is closest to the width of the curve-fit population while ensuring all curve-fit data is displayed.
2. If **AutoMode** scaling is off, to manually display the results at a higher resolution, touch the 2-26 µm icon (Type M) or the 2-18 µm icon (Type S). Note: The cassette type is indicated in the blue box at the top left of the screen. This is recommended when counting smaller cells or particles to improve both the curve-fitting capabilities as well as the user's ability to visually discriminate nuances of the data.
3. Upon increasing the horizontal resolution, the scaling button will dynamically adjust to allow for cycling between the available x-axis scale ranges: 2-34, 2-26, 2-18, and 2-10 µm for the Type M cassette; or 2-26, 2-18, and 2-10 µm for the Type S cassette. This feature is only available immediately following a cell count.
4. Count information (cells/ml) for the curve-fit or gated region is displayed in a black box under the left side of the histogram. Mean cell size information is displayed in the black box under the right side of the histogram.
5. The MVI value is displayed a blue box at the top right of the histogram. If the test was run in small particle mode, the MVI does not apply and will be displayed as "MVI=SPM". Additionally if the concentration of cells is outside of the MVI operating range, a message of "MVI=Out of Range" will be displayed.
6. To save the current histogram, touch the **Done** icon. This saves the data with a name of "Test XXX" where XXX is the corresponding test number.
7. To manually gate the histogram, touch to toggle the **Curve Fit** count results button. This makes **Gating** the default acquisition mode. Then slide each blue gating marker to set the gates or tap the left and right arrows (these appear upon touching the blue gating marker) for fine gate adjustments Only the cells between the markers are counted. The histogram vertical scale is automatically scaled based on the curve-fit population amplitude. To adjust this vertical scale, swipe the screen up or down in the histogram region.
8. Touch the **Gated Count** results button to return the display to curve fit mode and make curve fit mode the default acquisition mode.
9. Then press the **Done** icon to save the results and return to the **Home** screen. To delete the results, press the **Delete** icon at any time to permanently delete the results of the test.

4. Analysis of Previously Acquired Data

1. To open a saved test, press the **Histogram** icon on the **Home** screen.
2. Icons for up to nine saved histograms will be displayed on the screen. Press the appropriate icon for the test of interest or press the **Page Up** or **Page Down** icons to view more test results.
3. Each histogram will be opened in either the **Curve Fit Count** mode or **Gated Count** mode, depending on how it was saved. In **Gated Count** mode, gating markers can be positioned as desired by sliding each blue gating marker independently. **Auto Gate** by touching on the desired peak. Touch to toggle between the **Gated Count** and **Curve Fit Count** modes.

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4. Press the **Zoom** and **Unzoom** icons to adjust the vertical scale of the histogram. The vertical scale can also be changed by vertically swiping a finger on the display up (to increase) or down (to decrease).
5. Press the **Delete** icon to permanently delete the results of the test.
6. Press the **Done** icon to close the test results and return to the **Home** screen. (Note: zoomed view will not be saved.)

5. Transferring Data to a PC

1. Data can be transferred to a PC using the Orflo **Moxichart** application or via USB transfer by plugging the Moxi Z USB cable to a PC or Mac. For Bluetooth transfer (MoxiChart), first determine the unit's Bluetooth ID by pressing the **Bluetooth** icon on the **Home** screen of the Moxi Z unit.
2. On the computer, open the **MoxiChart** application and click on **Bluetooth** icon in upper right corner. Then, choose the device which corresponds to the Bluetooth ID of the Moxi Z and select a destination folder for the uploaded files.
3. Moxichart will automatically transfer all the files to the specified directory via the Bluetooth connection.
4. Files transferred by Bluetooth (MoxiChart) or USB can be opened and analyzed directly with the MoxiChart application or, because they are formatted .csv files, they can be directly opened in Microsoft Excel or other data analysis programs for additionally/custom analysis.

6. Representative Results

To assess the precision and accuracy of the Moxi Z, counts of HEK-293 cells, HeLa cells, CHO-K1, Yeast, Jurkat E6-1 cells, PC12 cells, and precision-calibrated bead suspensions with concentrations ranging from 0-500,000 cells/mL (Type M cassette) and 0-2-2.5e^6 cells/ml (Type S cassette) were counted and compared using a Coulter Z2 and the Moxi Z. All mammalian cells were obtained from the American Type Culture Collection (ATCC) and cultured as previously described[7-11]. Briefly, cells were cultured in suspension in 75 mm^2 tissue culture flasks with core media of RPMI 1640 (Jurkat E6-1, PC12), MEM (HEK-293, HeLa) cells, or F12K(CHO-K1) cells. All media was supplemented with 10% fetal bovine serum and 100 U penicillin/100 µg Streptomycin. Flasks were maintained in an incubator at 37 °C with 5% CO_2. Cells were passaged every 3 days. Yeast cells (X5, *C. albicans*, Vin 13) were donated and used immediately as provided. Initial sample concentrations of ~500,000 cells/ml for the Type M cassette data and ~2-2.5e^6 cells/ml for the Type S cassette were established using the Coulter Z2. Subsequent theoretical concentration levels were prepared through serial dilutions with physiological buffers (Isoton II or Hank's Balanced Salt Solution). At each concentration, identical samples were measured by both systems and plotted against each other. As seen in **Figure 2**, there was a strong linear correlation (Type M r^2>0.9886, Type S r^2>0.9781) between the counts for all samples. Concentrations were also compared to expected/ theoretical cell concentrations with both the Moxi Z and the Z2 (**Figure 3**) and again demonstrated strong linear correlations (Type M r^2>0.9758, Type S r^2>0.9774).

To assess precision, HEK-293 and HeLa cell counts were performed using the Coulter Z2, Moxi Z, and a hemocytometer. The mean coefficient of variation (CV, n=19) of cell counts using the Moxi Z (Type M cassette), Coulter Z2 and a hemocytometer were calculated with CV's measured from 5 separate counts in the 200,000-300,000 cells/ml range. The small average CV (**Figure 4**) of the Moxi Z is comparable to that of the Z2 and is representative of the high level of precision only attainable with Coulter-based counting technologies.

Sizing precision of the Moxi Z instrument was evaluated next through the measurement of purchased, precision calibrated polystyrene beads. Averaged particle size measurements (n=5) from Moxi Z were plotted against manufacturer reported sizes. As can be seen in **Figure 5**, the Moxi Z measurements consistently matched the manufacturer values with a high linear correlation (r^2=0.9989).

Beyond size and count information, Moxi Z provides a reagent-free, general assessment of mammalian cell culture health with a reported Moxi Viability Index (MVI) value. This index is generated from a ratio of the number of cells in the population of interest with respect to the total particulate counts. To demonstrate the MVI measurement capabilities, MVI readings (Type M cassette) were compared to standard manual and flow cytometric live/dead measurement techniques. Populations of dead (<10% viable) HEK-293 and HeLa cells were created through overnight incubations of the live cells in a nutrient-free, sodium fluoride containing diluent (Isoton II, Beckman Coulter) at 37 °C. Controlled theoretical viability levels were prepared by ratiometrically mixing known concentrations of live and dead cells. Resulting solutions were analyzed for viability levels in duplicate with both hemocytometer counts and flow cytometer measurements. Hemocytometer measurements were made using a traditional 50/50 mixture of 0.1% Trypan Blue Solution and cells. Flow cytometry measurements were made using a Guava PCA cytometer (Merck) using the Viacount reagent and the manufacturer-specified protocol (Merck). As shown in **Figure 6**, the Pearson correlation coefficient values (r^2) "of the linear fit of the MVI data to analogous hemocytometer viability percentages for" HEK cells and HeLa cells were r^2=0.9937 and r^2=0.9728, respectively. These results demonstrate that this approach provides culture health information across a broad range of cell viabilities that compare favorably to the live/dead measurements obtained using a flow cytometer or hemocytometer.

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Tables and Figures (From Dittami *et al.*, 2011)[15]:

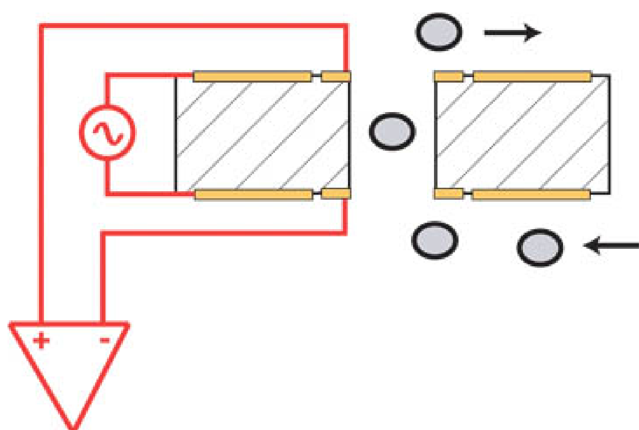

Figure 1. Thin-film Coulter Counting: Electrical current is passed through the Cell Sensing Zone of the thin-film cassette. As cells flow single file through the CSZ, the momentary increases in voltage are measured by the Moxi Z.

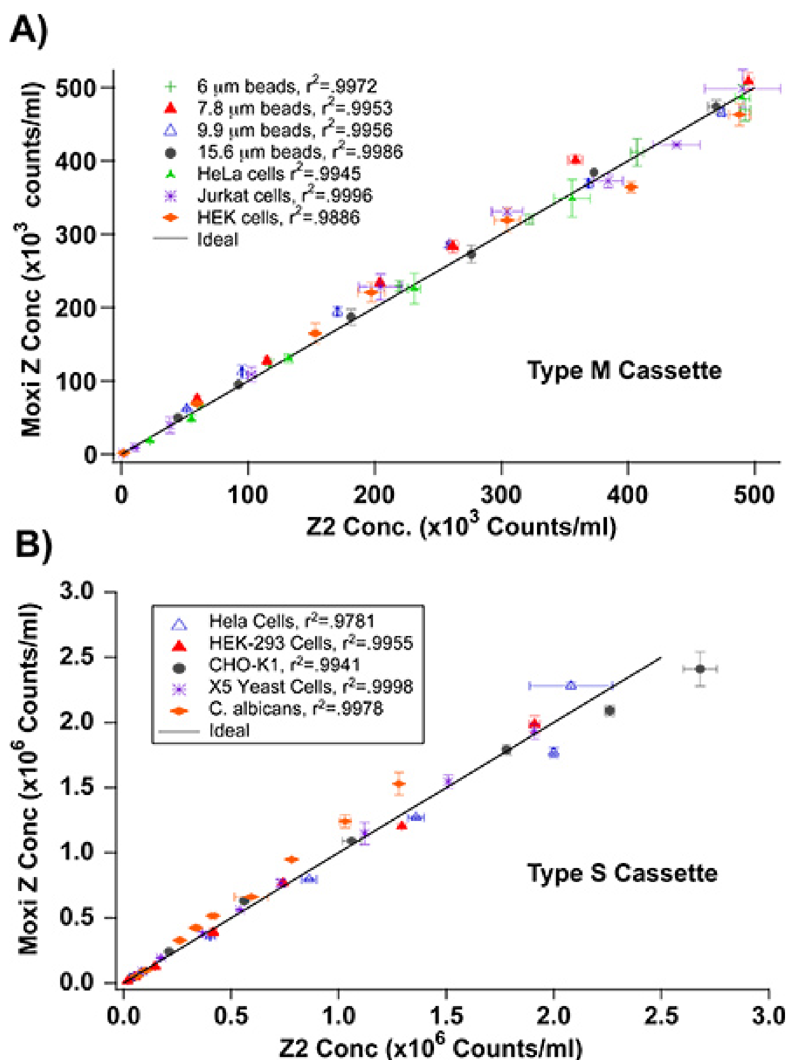

Figure 2. Moxi Z counts are comparable to Coulter Z2 counts. Identical counts of cell and bead suspensions were counted using both a Coulter Z2 and the Moxi Z. Averaged count values (n=3-4) of the Moxi Z counts were plotted with respect to the Coulter corresponding Z2 counts and r^2 values of the linear fit were determined. A) Type M cassette - concentration range of 0 - 500,000 cells/ml. B) Type S cassette - concentration range of 0 - 2-2.5e^6/ml.Error Bars indicate ± 1 standard deviation.

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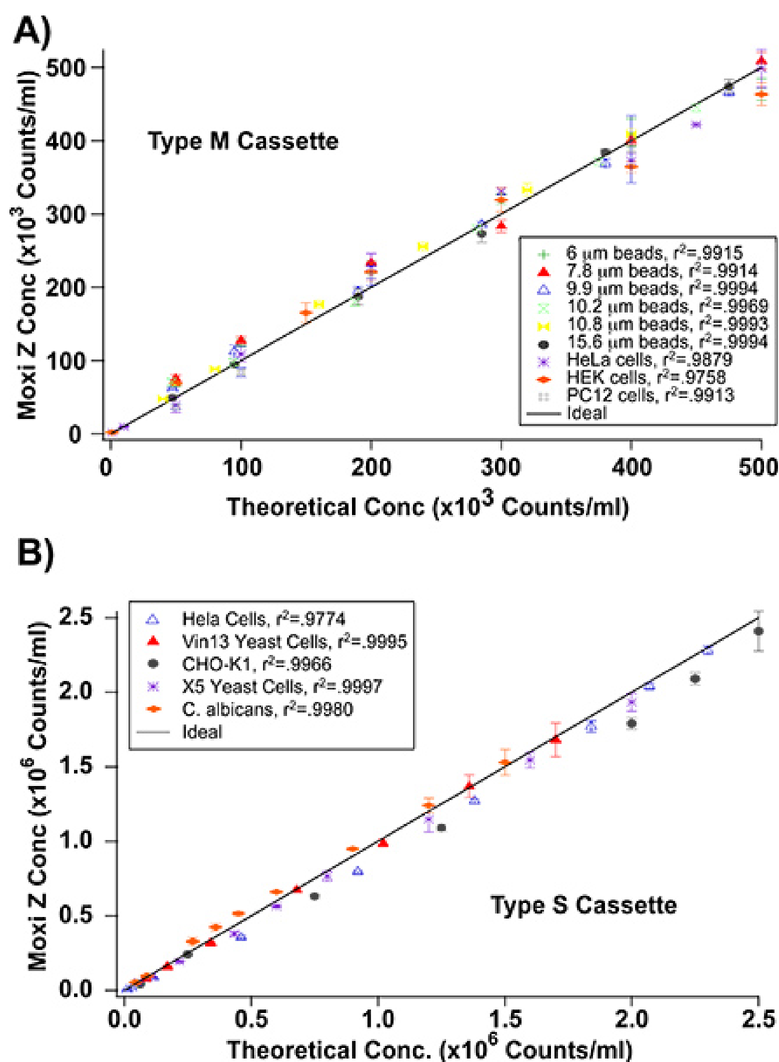

Figure 3. Moxi Z concentration measurements vs theoretical cell concentrations. A) Type M cassette -Theoretical concentration values were prepared from serial dilutions of an initial 500,000 cells/ml sample. **B) Type S cassette -**Theoretical concentration values were prepared from serial dilutions of an initial 2 - 2.5e^6 cells/ml sample. Error Bars indicate ± 1 standard deviation.

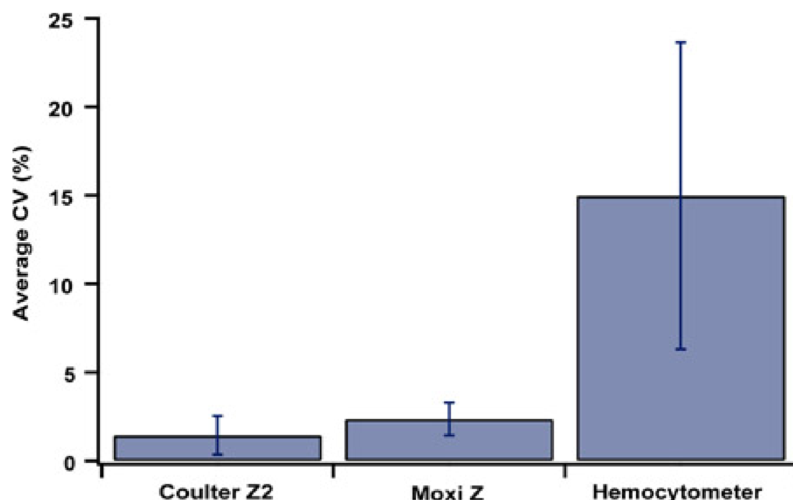

Figure 4. Coefficient of Variation for the Coulter Z2, Moxi Z (Type M cassette), and Hemocytometer. The mean coefficient of variation (CV, n=19) of HEK-293 and HeLa cell counts for each approach were calculated with CVs measured from 5 separate counts of HEK-293 and HeLa Cells in the 200,000- 300,000 cells/ml range. Error Bars indicate ± 1 standard deviation.

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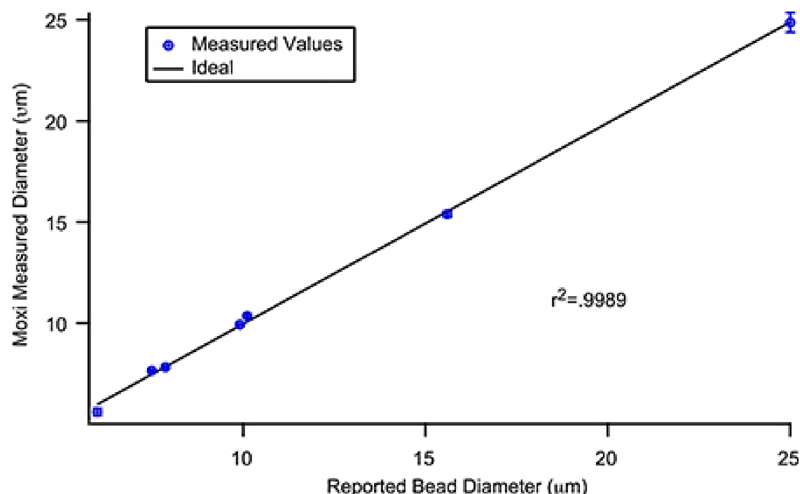

Figure 5. Precision of particle size measurements using Moxi Z- Moxi Z measured bead size (Type M cassette) vs. manufacturer reported bead size. Error Bars indicate ± 1 standard deviation.

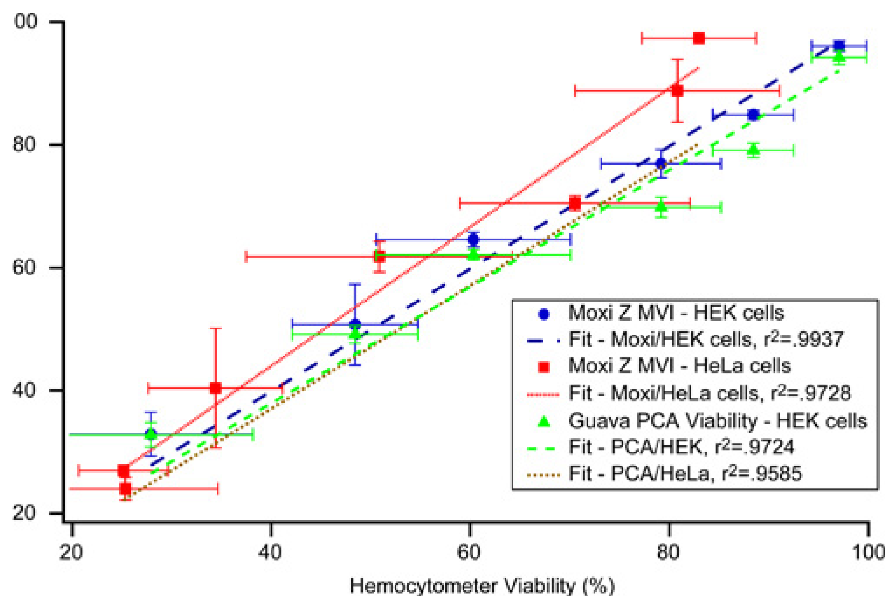

Figure 6. Culture Health Assessment using MVI - Comparison of MVI measurements (Type M cassette) and Guava PCA Viacount viability versus visual, trypan-blue stained viability counts using a hemocytometer.

Discussion

The underlying implementation the Coulter approach can be highly deterministic of the overall accuracy of the measurements. A critical area is the correction of the raw cell counts for "coincidence events" where two or more cells simultaneously pass through the aperture and are measured as a single electrical event. "Coincidence events" contribute to error which varies depending on a number of factors including cell size and degree of clustering (Davis et al 1967)[6]. As a result, the required coincidence correction for any given sample cannot be predicted, varying widely between cell/particle types and even between different cultures of identical cell types. Established theory, rooted in initial publications by Coulter, involves applying a logarithmic adjustment of the raw counts based on the number of events observed and an empirically-determined, particle-specific correction factor, z. While accurate counts can be properly achieved with this correction algorithm, it is limited in its practical applicability due to the large variations in z values between cell types and the corresponding difficulty in accurately predicting its value. Here, using the "true" cell count identified by the Moxi Z curve fitting in conjunction with the coincidence correction algorithm, the Moxi Z dynamically corrects for coincidence to achieve the true concentrations. When compared to results obtained using high-end Coulter Z2, the Moxi Z produced comparable counts across a concentration range of 0 - 500,000 cells/ml (Type M cassette) and 3,000 - 2-2.5e^{6} cells/ml (Type S cassette). Furthermore, the Moxi Z achieves this count accuracy with a similar low coefficient of variation in counts and a precision in particle sizing that is characteristic of the gold standard, Coulter-technique in cell counting.

Moreover, the data presented here indicate that the Moxi Z can provide valuable information regarding the overall health of cell cultures. The Moxi Z leverages the curve fitting approach with a proprietary software algorithm to determine this culture health assessment. Morphological changes associated with cell death, such as blebbing, breaking apart, and volumetric distortions (Kataoka and Tsuruo 1996, Liegler et al 1995, Sheridan et al 1981)[12-14], make it possible for the Moxi-Z to distinguish impedimetric differences between live populations and dead cell/debris populations. The MVI is generated by analyzing the culture/particle size distribution to identify the relative contributions of particle debris,

shrunken necrotic cells, and the curve-fitted cell population of interest. The MVI value thereby reflects a population index or ratiometric measure of the monodisperse population counts with respect to the overall particle population profile. This value is then algorithmically-adjusted based population statistics and empirical observations. Because the MVI looks at parameters other than traditional staining approaches, it is not expected to mirror these techniques but instead provides a valuable alternative view into the health of a cell culture, particularly with respect to the debris and microbial contaminants.

In summary, the Moxi-Z cell counter is an ultra small benchtop instrument that provides precise, reproducible assays of cell count, cell size, and cell health using the Coulter Principle and curve-fitting software algorithms combined with a patented thin-film sensor technology. With the Type M cassette it is capable of counting particles ranging from 4- 25 microns in diameter (dynamic range of 2 - 34 microns), in 8 seconds. With the Type S cassette it is capable of counting particles ranging from 3- 20 microns in diameter (dynamic range of 2 - 26 microns), in 15 seconds. Furthermore, the Moxi-Z performs health assessments without the use of reagents. It's much less labor intensive and subjective than manual counting. In comparison to standard Coulter counting, the Moxi is faster, significantly smaller, provides more information, requires considerably less maintenance, is easier to use, and is a fraction of the cost.

Disclosures

Gregory M. Dittami and H. E. Ayliffe are employees of Orflo Technologies, the company that designed, manufactures and sells the Moxi Z. Richard D. Rabbitt provides part-time, paid consulting expertise to Orflo Technologies.

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