Application Note

Precise and Accurate Bead Counting Using the Scepter™ 2.0 Handheld Automated Cell Counter

Introduction

Micron-sized beads are used in a variety of biological applications, ranging from daily validation of flow cytometer performance to purification of fusion protein constructs from cell lysates. Depending on the nature of the assay, the beads may either possess magnetic properties or be labeled internally with a fluorescent dye. In bead-based multiplexed immunoassays, these particles are coated with unique recognition molecules, such as epitope-specific antibodies, permitting capture and precise quantification of desired analyte(s). Accurate determination of bead counts at the onset of each assay allows for standardization of bead concentrations across multiple samples and minimizes errors and variation in downstream results.

A number of methodologies are currently available for particle counting. While inexpensive, manual counting using a hemocytometer is laborious and error-prone due to user subjectivity. The range of commercially-available automated devices can be divided into two formats: vision-based platforms and flow-based systems. Most vision-based counters use the standard Trypan Blue exclusion assay to assess viability and employ a digital camera and image analysis software to determine particle size and concentration[1]. Flow-based devices measure particles in a stream using impedance-based detection. By precisely controlling flow, volumetric measurements can be obtained, thereby permitting estimation of sample bead concentrations[2]. For most researchers, the main barrier to using an automated vision-based or flow-based system is the price associated with large benchtop instruments[3].

Performance	40 µm aperture sensor	60 µm aperture sensor
Volume required	≥ 100 µL	≥ 100 µL
Particle diameter range	4-16 µm	6-36 µm
Particle concentration	5×10^4 – 1.5×10^6 particles/mL	1×10^4 – 5×10^5 particles/mL
Process time	<40 seconds	<30 seconds

Table 1. Scepter™ sensor specifications

With the Scepter™ cell counter, EMD Millipore has captured the ease of automated instrumentation and accuracy of impedance-based counting using the Coulter principle in an affordable, handheld format. The instrumentation has been collapsed into a device the size of a pipette, and uses a combination of analog and digital hardware for sensing, signal processing, data storage, and graphical display in the form of a histogram. The 40 µm- and 60 µm-aperture sensors placed at the tip of the instrument are engineered with a microfabricated, sensing zone that enables discrimination by bead size and bead volume at sub-micron and sub-picoliter resolution, respectively. Table 1 outlines the specifications for each sensor type.

While the Scepter™ cell counter was initially optimized for cell counting, here we report that the device is also well suited for precise counting for beads of numerous types commonly employed in a wide range of biological applications.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Bead Description	Sensor Aperture Size (µm)	Average Measured diam. (µm)	Bead Composition	Source	Catalogue No.
Latex Microparticles	40	5	Polystyrene	Fluka	79633
Innovatis Cedex® Control Beads	40	8	Polystyrene	Roche Applied Science	05650542001
Latex Microparticles	40	10	Polystyrene	Fluka	72986
MILLIPLEX® MAP Microspheres	40	6	Polystyrene	EMD Millipore	MXHIL-4
MILLIPLEX® MAP Magnetic Microspheres	40	6	Super-paramagnetic	EMD Millipore	HCYTNFA-MAG
Dynabeads® Cell Isolation	40	4	Super-paramagnetic	Invitrogen	11035
PureProteome™ Protein A Beads	40	9	Magnetic	EMD Millipore	LSKMAGA10

Table 2.
Bead types tested

Materials and Methods

Bead counting using the Scepter™ cell counter

Sample preparation:

Bead suspensions (see Table 2 for list of beads tested) were serially diluted in phosphate-buffered saline (PBS, EMD Millipore Catalogue No. BSS-1006-A). The concentration range tested, 50,000 - 1,500,000 beads/mL, corresponds to the upper and lower limits of detection for the 40 µm sensor. PBS contains an optimal salt concentration for sufficient conductivity required for optimal counting performance. For each test, we used the recommended sample volume of 100 µL in a 1.5 mL microcentrifuge tube. Other tubes may not be able to accommodate the width of the sensor, or provide sufficient sample depth for the instrument to function properly. Since beads settle quickly, we kept the bead suspension well mixed prior to testing to ensure reproducible counts.

Scepter™ Bead Counting:

Operation of the Scepter™ cell counter is similar to using a standard laboratory pipette. The Scepter™ cell counter is turned on by depressing and holding the toggle on the back of the instrument. Once on, the instrument will prompt the user to attach a sensor. The Scepter™ unit displays detailed on-screen instructions for each step of the counting process. Briefly, depress the plunger and submerge the tip into the solution. Next, release the plunger to draw 50 µL of bead suspension into the sensor. The Scepter™ cell counter detects each particle passing through the sensor's aperture, then calculates concentration and displays a histogram of bead diameter or volume on its screen.

Scepter™ Data Analysis:

The upper and lower limits of the histogram, called gates, are either set automatically based on the histogram profile, or can be set to the same gates used in the previous count. After the count is complete and the histogram is displayed on the instrument, the gates can be moved manually to fine-tune the analysis. Up to 72 histograms can be stored on the instrument itself. All test data files can be uploaded to a computer and further analyzed using Scepter™ Software Pro.

Bead counting by other methods

In certain cases, counts were also performed using the Z2 Coulter Counter® (Beckman Coulter) and a vision-based automated cell counter. Counts were performed according to manufacturer's instructions using the same starting suspension and serially diluted samples.

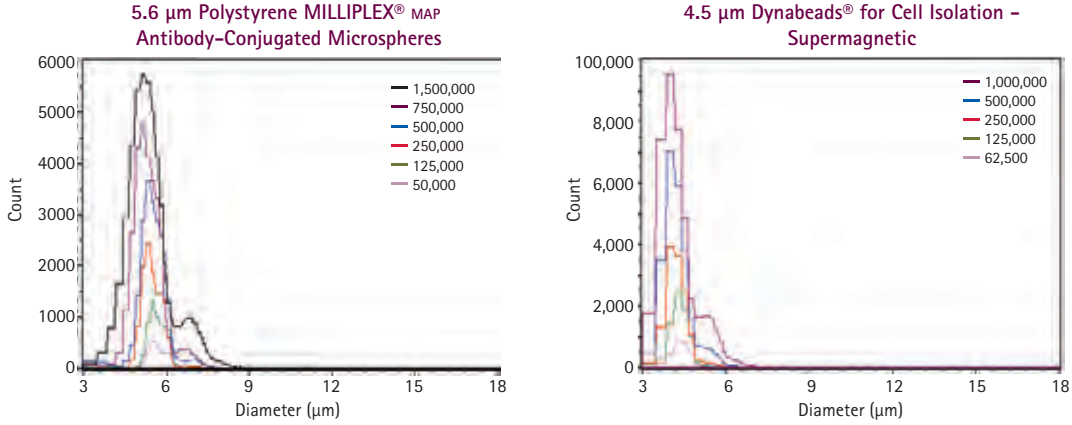

Figure 1.

Scepter™ histogram overlays showing serial dilution of two bead types. Scepter™ Software Pro displays imported size distribution histograms as either a single sample histogram or as overlaid histograms for multiple samples. Shown are overlaid histograms for serially diluted 5.6 µm MILLIPLEX® MAP microspheres and 4.5 µm Invitrogen Dynabeads®.

Results

Scepter™ precision counting

A variety of bead types ranging in size from 4-10 µm were counted using the Scepter™ counter fitted with 40 µm sensors. For each bead type, the original sample was diluted to an approximate concentration of 1.5 x 10^6 beads/ mL and counted using a Coulter Counter® fitted with a 50 µm aperture to determine the theoretical starting concentration. Next, a serial dilution series (2-fold) was prepared and assayed to determine theoretical concentrations at each dilution step. All seven bead types tested yielded interpretable histograms that could be gated and used to calculate sample bead size and concentration. Examples of these histograms are shown in Figure 1.

In order to validate the reproducibility of measured concentration values, we plotted the mean derived concentration values (4 replicates) versus their theoretical counterparts across the dilution range. The precision of Scepter™ cell counting was determined by calculating coefficients of variation at each data point, for each bead type individually (Figure 2 and Table 3). As shown, the overlap of data points and small error bars suggest that, regardless of bead type, concentration values were accurate, precise, and reliable up to 1.5 x 10^6 beads/mL. The highest variability, both within and between bead types, was found at 1.5 x 10^6 beads/mL which coincides with the upper limit of detection for the 40 µm sensor. Overall, the high degree of linearity (as shown by the R^2 values) indicates that Scepter™ counting is a reliable method for the bead types tested, across a wide linear operating range.

Comparative platform analysis

Comparing the %CV values for the Scepter™ cell counter, the Z2 Coulter Counter®, and the vision-based cell counter acquired during measurement of

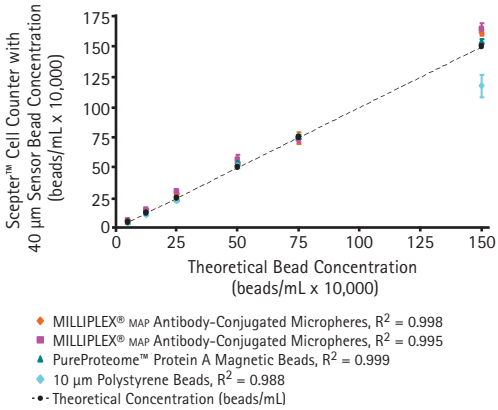

Figure 2.

The Scepter™ cell counter performs with high linearity ($R^2 \geq 0.99$) across multiple, diverse bead types, over a wide operating range. Shown here are bead concentration data for four representative samples out of seven bead types tested.

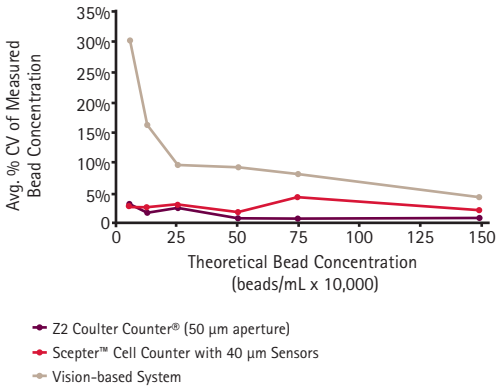

Figure 3.

The Scepter™ cell counter performs bead counting with smaller coefficients of variation than vision-based automated counting. Shown are the average %CVs (3 bead types: MILLIPLEX® MAP Antibody-Conjugated Microspheres, MILLIPLEX® MAP Antibody-Conjugated Magnetic Microspheres and PureProteome™ Protein A Magnetic Beads) with respect to bead concentration and counting method.

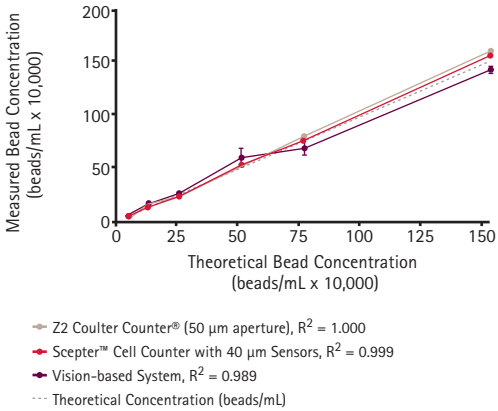

Figure 4.

The Scepter™ cell counter counts PureProteome™ Protein A magnetic beads with greater linearity and smaller standard deviation than vision-based automated counting. Beads were counted using the methods shown. Data points represent average of four replicates. Error bars represent standard deviation. These beads represent one out of 7 total bead types tested.

Theoretical concentration (beads/mL)	MILLIPLEX® MAP antibody-conjugated magnetic beads		PureProteome™ Protein A magnetic beads		10 µm polystyrene beads	
	Conc.	%CV	Conc.	%CV	Conc.	%CV
1,500,000	1,650,000	2.7	1,545,667	1.2	1,176,000	8.0
750,000	735,700	3.7	755,767	2.3	n/a	n/a
500,000	567,700	2.2	537,933	0.6	514,000	3.2
250,000	300,600	2.8	239,733	3.5	224,467	0.7
125,000	153,367	2.7	131,667	2.6	108,200	2.6
50,000	61,533	2.7	51,980	2.3	39,067	5.6

Table 3.
Precise counting of serial dilutions of 3 bead types using the Scepter™ cell counter.

the serial dilution samples revealed that the Scepter™ was only slightly less precise than the gold standard Coulter Counter® but significantly more precise than the vision-based automated counting system (Figure 3). Furthermore, the Scepter™ cell counter data displayed smaller standard deviations than the vision-based counting system data for all bead types and concentrations tested (Figure 4).

Size measurement

For the Scepter™ cell counter, the ability to accurately determine particle size is an inherent property of the sensor's aperture. For example, the 40 µm sensor is capable of sizing particles in the 4-16 µm range. To determine the accuracy in reporting bead size, we compared our results to the known bead diameter values (derived from the certificates of analysis) for 2 bead types. Calibrated latex microparticles (5 µm and 10 µm) were measured and results are shown in Figure 5. Accurate sizing was observed using both the Scepter™ cell counter and the Coulter Counter®.

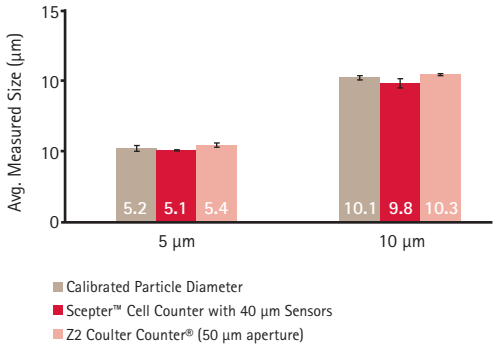

Figure 5.

The Scepter™ cell counter and the Coulter Counter® accurately measure bead diameter. The average diameters of serially diluted samples of calibrated latex microparticles (5 µm and 10 µm) were measured with the Scepter™ cell counter and Coulter Counter®. Measured diameters were averaged across all samples within the counting range (50000-1500000 beads/mL). Results demonstrate the accuracy of the particle size measurements.

Discussion

Comparing the performance of the Scepter™ cell counter to results from other counting methods, we conclude that this new handheld, automated cell counter delivers precise, fast, and reliable bead counts and bead size measurements over a wide operating concentration range. The superior functionality of Scepter™ counting is likely a result of the precision-engineered technology embedded into the sensor and the sophisticated counting instrumentation based upon the Coulter principle. This performance quality, combined with the Scepter™ cell counter's convenient, intuitive form, suggests that Scepter™ counting will be quickly integrated into the workflow of researchers wishing to alleviate the pain of rudimentary bead counting and improve reproducibility of bead-based assays, such as immunoprecipitation and multiplexed detection.

References

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2. Houwen, B. Fifty years of hematology innovation: the Coulter principle. Medical Laboratory Observer 2003 Nov.
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