

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 concentration across multiple samples, and minimizes errors and variation in downstream results.

A number of methodologies are currently available for particle counting. While manual counting using a hemocytometer is accessible and inexpensive, it is laborious and error-prone due to user subjectivity. Automated counting devices are commercially-available in 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 with image analysis software to determine particle size and concentration¹. Flow-based devices measure particles in a stream using

impedance-based detection. By precisely controlling the flow, volumetric measurements can be obtained, thereby permitting estimation of sample bead concentration². For most researchers, a critical barrier to using an automated vision-based or flow-based system is the price associated with large benchtop instruments³.

With the Scepter™ cell counter, MilliporeSigma has combined the ease of automated instrumentation with the accuracy of impedance-based counting using the Coulter principle in an affordable, handheld tool. 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. The 40 μm and 60 μm aperture sensors are engineered with a microfabricated sensing zone that enables discrimination by bead size and bead volume at submicron and subpicoliter resolution, respectively. **Table 1** outlines the specifications for each sensor type.

While the Scepter™ device was initially optimized for cell counting, herein we report that the device is also well suited for the precise counting of diverse types of beads commonly employed in other biological applications.

Scepter™ sensor specifications

Specification	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

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, Cat. No. BSS-1006-A). The concentration range tested (50,000 to 1,500,000 beads/mL), corresponds to the upper and lower limits of detection for the 40 µm sensor. PBS is at an optimal salt concentration for appropriate conductivity that is required for accurate 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 may not provide sufficient sample depth for the instrument to function properly. Since beads can settle quickly, we kept the bead suspension well mixed prior to testing ensuring reproducible counts.

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.

Bead types tested

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

Table 2

Scepter™ Software

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. All test data files can be uploaded to a computer and further analyzed using Scepter™ Software Pro.

Bead counting by other methods

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

Results

Precision counting with Scepter™ cell counter

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 1.5×10^6 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**.

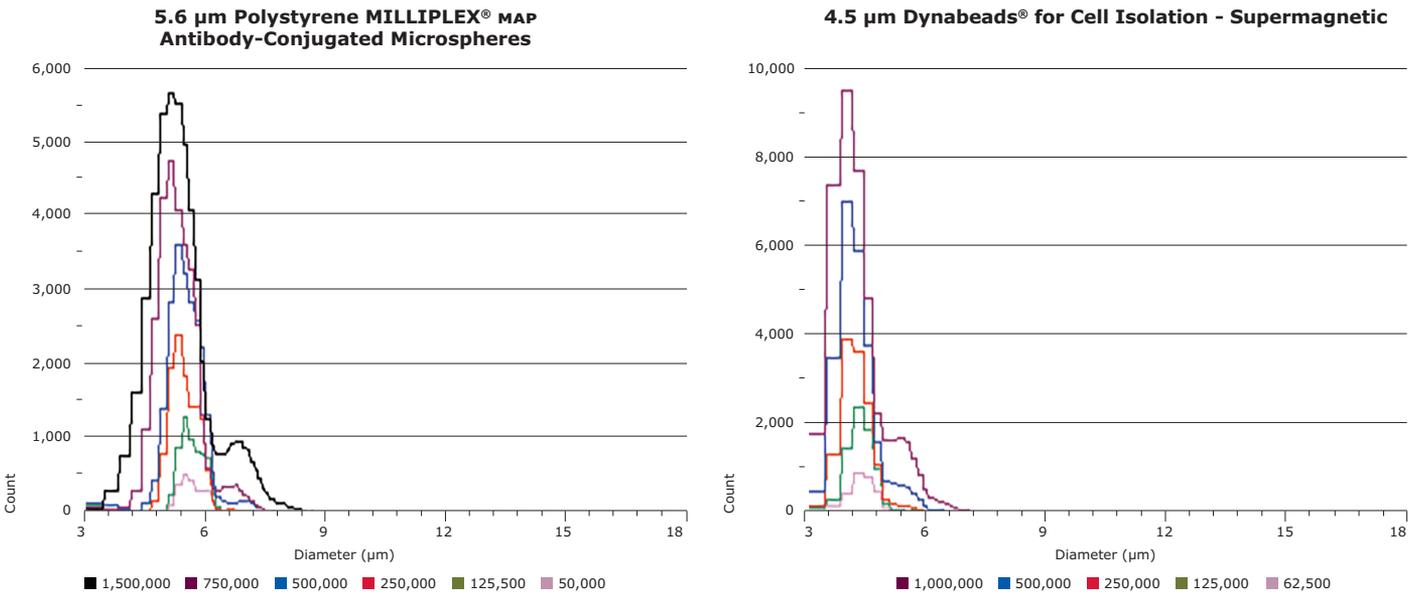


Figure 1

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 (continued)

To validate the reproducibility of measured concentration values, we plotted the mean derived concentration values ($n=4$) versus their theoretical values across the dilution range. The bead counting precision using the Scepter™ counter 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,

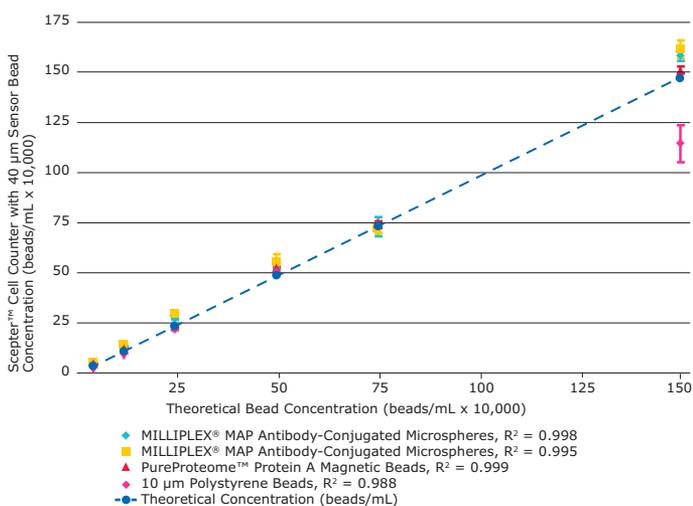


Figure 2

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.

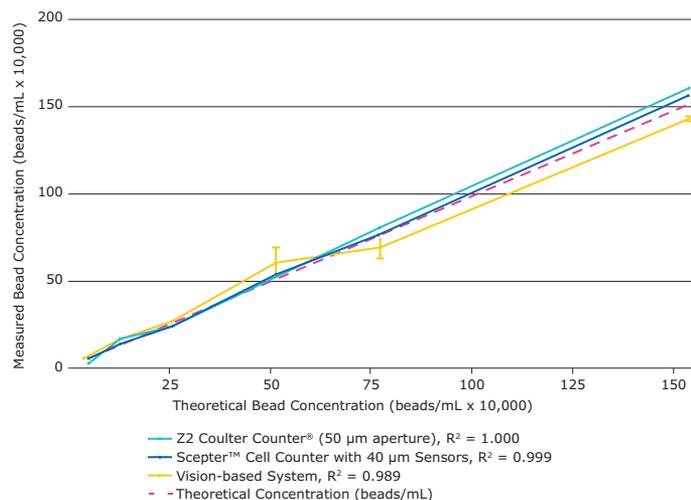


Figure 4

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.

and reliable up to a concentration of 1.5×10^6 beads/mL. The highest variability, both within and between bead types, was found at 1.5×10^6 beads/mL which coincides with the upper detection limit of detection for the 40 μm sensor. Overall, the high degree of linearity (as shown by R^2 values) indicates that counting with the Scepter™ cell counter is a reliable method for the bead types tested, across a wide linear operating range.

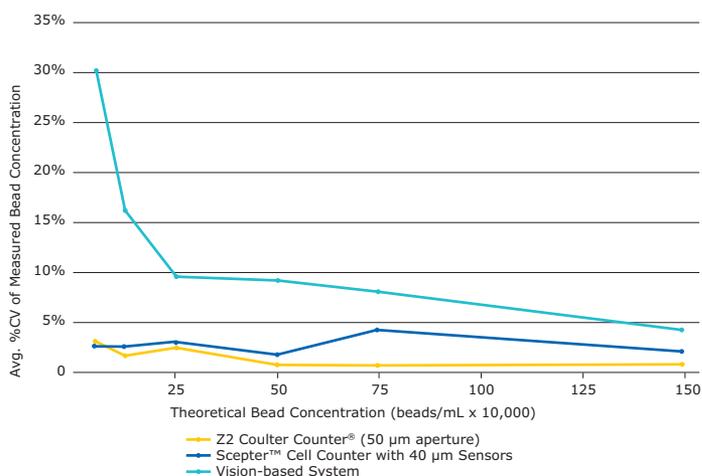


Figure 3

The Scepter™ cell counter performs bead counting with smaller coefficients of variation (%CV) than vision-based, automated counting. Shown are the average %CVs of three 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.

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

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

Comparative platform analysis

Comparing the coefficients of variation (%CV) values for the Scepter™ cell counter, the Z2 Coulter Counter®, and the vision-based cell counter acquired during measurement of the serial dilution samples revealed that the Scepter™ cell counter was only slightly less precise than the gold standard Z2 Coulter Counter® instrument 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 (obtained from the certificates of analysis) for two 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® instrument.

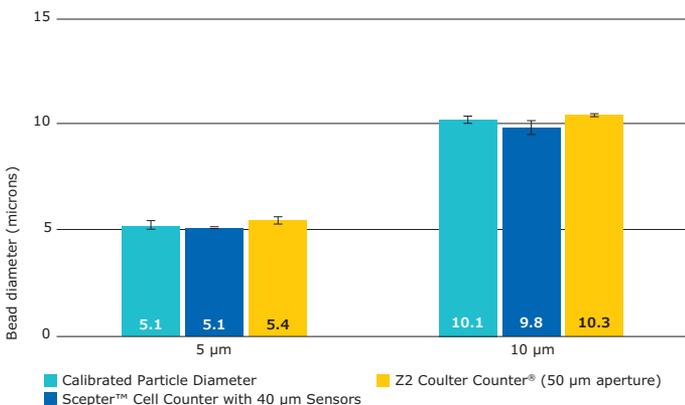


Figure 5

The Scepter™ cell counter and the Z2 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).

Discussion

Comparing the performance of the Scepter™ cell counter to results from other counting methods, we conclude that this handheld, automated cell counter delivers precise, fast, and reliable bead count and size measurements over a wide operating concentration range. The superior functionality of the Scepter™ cell counter is due to the sensor's precision-engineered technology and sophisticated, Coulter principle-based counting instrumentation. High performance quality combined with its convenient and intuitive form, suggest that the Scepter™ handheld counter will quickly be integrated into the standard workflow of researchers who need to enhance the efficiency of rudimentary bead counting and improve the reproducibility of bead-based assays, such as immunoprecipitation and multiplexed detection.

References

1. Tucker KG, Chalder S, al-Rubeai M, Thomas CR. Enzyme Microb Technol 1994 Jan. 16(1):29-35.
2. Houwen, B. Fifty years of hematology innovation: the Coulter principle. Medical Laboratory Observer 2003 Nov.
3. Barghshoon, S. Cell Counting Survey. MilliporeSigma 2009 Feb.

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