

Bethany Brown¹, Michelle Devoe¹, Jean Paul Habumugisha², Kathryn H. Roache-Johnson¹, Benjamin Spaulding¹, Heather Anne Wright¹

¹Fluid Imaging Technologies, ²University of Southern Maine

INTRODUCTION

The FlowCam is an imaging particle analysis instrument that can count and morphologically analyze microscopic particles in a fluid medium. The FlowCam Automated Liquid Handling (ALH) system integrates with the FlowCam 8000 series to provide for unmonitored and uninterrupted processing of up to 96 samples. This study uses ThermoFisher 10 µm and 20 µm COUNT-CAL™ beads to compare size and counting between two methods of sample introduction (manual pipetting and ALH dispensing) into a FlowCam 8100.

OBJECTIVE

To compare the percent recovery and size accuracy between manual sampling and ALH sampling into a FlowCam 8100.

MATERIALS & METHODS

The count (concentration) 10µm and 20 µm COUNT-CAL polystyrene beads (ThermoFisher Scientific) and size of 20 µm COUNT-CAL were measured and compared between Manual sampling and Robotic Automated Liquid Handler (ALH) sampling methods.

- Manual sampling: 50 µL, 100 µL, 150 µL and 200 µL aliquots of COUNT-CAL beads were pipetted into the FlowCam. Four replicates were performed for each volume.
- ALH sampling: 50 µL, 100 µL, 150 µL and 200 µL aliquots of COUNT-CAL beads were robotically pipetted from a 96-well plate and a dispensed into the FlowCam.
- Concentration was calculated using VisualSpreadsheet®.
- Percent Recovery was calculated using the following equation:
 $\% \text{ Recovery} = \left[\frac{\text{Concentration (particles/mL)}}{3000 \text{ p/mL}} \right] * 100$
- Thermo Fisher COUNT-CAL™ Lot# 44663 NIST Traceable Size (µm) = 20.06 +/- 0.12
- Thermo Fisher Concentration (p/mL) = 3000

REFERENCES

- 1) Automated liquid handler <<http://www.fluidimaging.com/products/automated-liquid-handling-system>>
- 2) Spaulding, B. W., Krueger, A. B., Carpenter, J. F., Utilization of a FlowCam® 8100 to Detect and Image Particle Concentration Difference in Prefilled Syringes
- 3) COUNT-CAL™ Count Precision Standards <<https://www.thermofisher.com/order/catalog/product/CC02-PK>>

RESULTS

COUNTING RESULTS

10 and 20 µm COUNT-CAL beads, averages of 4 replicates per aliquot volume

MANUAL SAMPLING

Avg. Count=3049 p/ml, Avg % Rec=102%, STD DEV=99.5, AVG % CV=6.2

Beads size	Manual Sampling			
	Volume (µl)	Mean count (particles/mL)	%Recovery	%CV Conc.
CC10	50	3266	109	10.3
CC10	100	3037	101	2.9
CC10	150	2961	99	7.3
CC10	200	2977	99	4.9
CC20	50	2959	99	5.2
CC20	100	3072	102	9.1
CC20	150	3071	102	6.0
CC20	200	3050	102	4.2

ALH SAMPLING

Avg. Count=3079 p/ml, Avg % Rec=103%, STD DEV=106.3, AVG % CV = 6.0

Beads size	ALH Sampling			
	Volume (µl)	Mean count (particles/mL)	%Recovery	%CV Conc.
CC10	50	3230	108	12.4
CC10	100	3033	101	7.1
CC10	150	2948	98	4.1
CC10	200	3060	102	4.7
CC20	50	3092	50	6.5
CC20	100	2936	27	3.3
CC20	150	3155	47	6.9
CC20	200	3180	39	2.9

SIZING RESULTS

20 µm COUNT-CAL beads, averages of 4 replicates per aliquot volume

MANUAL SAMPLING

Avg. Size (µm) = 20.18
 STD DEV= 0.10, % CV=0.51

Beads size	Manual Sampling			
	Volume (µl)	Mean Size (µm)	STD DEV	%CV
CC20	50	20.23	0.25	1.3
CC20	100	20.30	0.11	0.5
CC20	150	20.1	0.04	0.21
CC20	200	20.09	0.08	0.39

ALH SAMPLING

Avg. Size (µm) =20.34
 STD DEV=0.51, % CV = 2.5

Beads size	ALH Sampling			
	Volume (µl)	Mean Size (µm)	STD DEV	%CV
CC20	50	20.08	0.06	0.3
CC20	100	20.12	0.09	0.4
CC20	150	21.10	1.74	8.2
CC20	200	20.06	0.08	0.4

CONCLUSIONS

- The average particle count for the sample pipetted manually was 3049 particles/mL which was 102% of the expected 3000 particles/mL with 99.5 standard deviation, and average 6.2% CV.
- The average particle count for the sample pipetted with the ALH was 3079 particles/mL which was 103% of the expected 3000 particles/mL with 106.3 standard deviation., and average 6.0% CV.
- The two methods have similar counting performance with low %CV.
- The two methods have similar sizing performance with low %CV and standard deviation.
- The use of the ALH saves hands-on time because the analysis can be performed without a person monitoring.

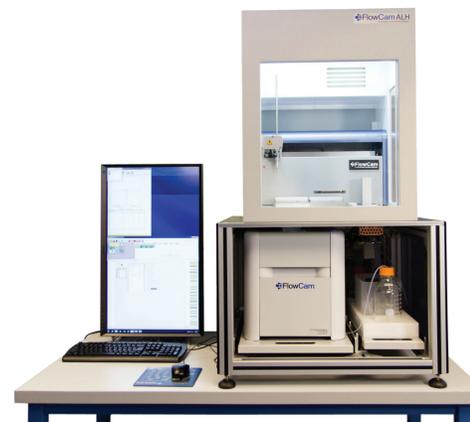


Figure 1: FlowCam 8100 and ALH System