A p p l i c a t i o n   N o t e

Cell size determination, cell health and quality, bioresearch, biomanufacturing, precise size gaiting before and after counting, accurate and reliable size measurement

Incomparable cell size measurement with the LUNA-FX7™

INTRODUCTION

Cell size may matter in bioresearch. Notably, the intricate correlations between cell size and phenotypic traits such as stemness features, proliferative ability, and metabolic status are becoming better understood. In more applied workflows such as biomanufacturing, cell size may be a critical indicator for cell health in assessing batch culture or source material in cell therapy[1]. Thus, reliable cell size determinations in automated cell counting have become valuable output data. The LUNA-FX7™, Logos Biosystems' newest and most powerful automated cell counter, provides cell size data. In addition to the standard cell size gating features common to all LUNA™ Family, the LUNA-FX7™ counting output includes size determinations in conjunction with each cell viability status. Moreover, the LUNA-FX7™ has multiple means to analyze and categorize cell populations based on size during and after counting.

Here, we demonstrate the accuracy and reliability of the LUNA-FX7™ in measuring cell size and its precise size gating ability. For evaluating the accuracy in determining cell size, six different NIST Traceable Particle Size Standards and two cell lines, AsPC-1 (Pancreatic carcinoma) and DLD-1 (Colon carcinoma), were counted in the Brightfield Total Cell Counting mode.

LUNA-FX7™ Automated Cell Counter

APPLICATION

Accurate measurement of various particle sizes

The six different NIST Traceable Particle Size Standards (10 - 60 μm) were counted in the Brightfield Total Cell Counting mode of the LUNA-FX7™. The accuracy in determining particle size was 96.7% or more. Conversely, the cell counter manufactured by a competitor was inaccurate on the particles 10 μm or smaller and 50 μm and larger (Figure 1).
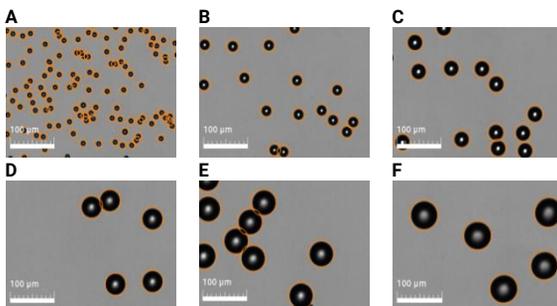
	NIST	LUNA-FX7™		Competitor	
	Size (μm)	Size (μm)	Accuracy (%)*	Size (μm)	Accuracy (%)*
(A) NT27N	9.98	9.60	97.2	12.27	77.1
(B) NT30N	20.85	20.40	97.8	21.25	98.1
(C) NT32N	30.04	30.40	98.5	31.04	96.7
(D) NT33N	43.33	41.90	96.7	42.56	98.2
(E) NT34N	50.00	49.90	98.8	38.48	77.0
(F) #64200	57.43	57.80	98.1	41.00	71.4

* Accuracy (%) = (1 - | $\frac{measured\ size - NIST\ provided\ size}{NIST\ provided\ size}$ |) x 100

Figure 1. *Size determination of NIST Traceable Particle Size Standards. (A-F) The images show well-tagged particles, indicating the precise measurement of particle diameter size. (G) The table shows measured particle sizes and the size measuring accuracy of the LUNA-FX7™ and a competitor's counter.*

Counting of target size particles in mixed condition

To evaluate the accuracy of size measurement in the LUNA-FX7™, we created a mixture of four NIST Traceable Particle Size Standards (~10 µm, 20 µm, 40 µm, and 60 µm) in different proportions. Initially, we counted the mixed particles with the minimum cell size value of 3 µm and the maximum cell size value of 60 µm, modified from the default protocol. After counting, the histogram of cell concentration over cell size was generated, and the NIST Standards were correctly categorized (Figure 2). The selected size gating was performed and resulted in the perfect tags for the target sizes and high accuracy in size measurement of over 96.2% (Figure 3). Also, size gating can be easily applicable in the LUNA-FX7™; by the protocol preset before counting, through the histogram adjusting right after counting, or reanalysis of stored count data utilizing a protocol with different size parameters.


A

B

Total cells

C

NIST Traceable Particle Size Standards (µm)	
a	9.98
b	20.85
c	43.33
d	57.43
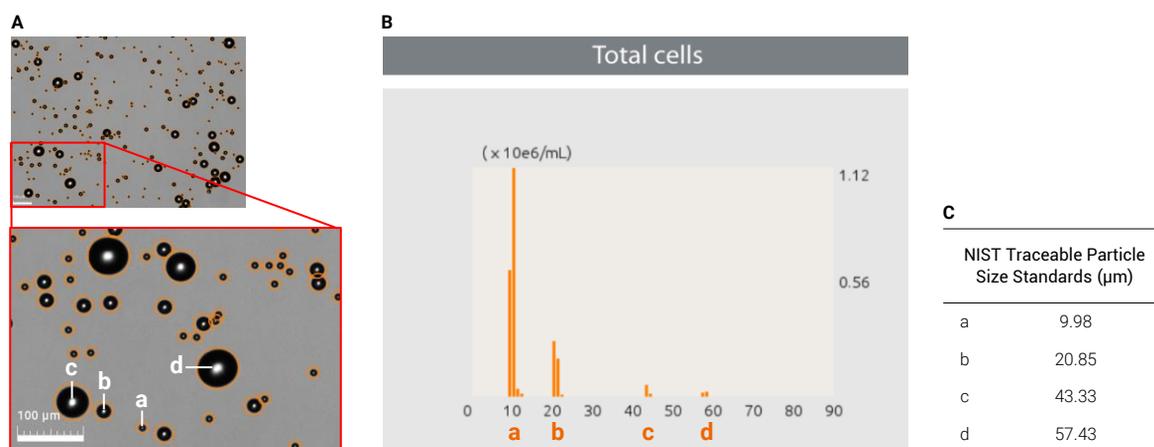
Figure 2. *Precise size determination. (A) The image shows well-tagged particles in various sizes. (B-C) The histogram indicates the exact distribution of the NIST particles by size.*


NIST Beads	9.98 µm	20.85 µm	43.33 µm	57.43 µm
Tagged Image				
Histogram				
Gating size	8 µm - 15 µm	15 µm - 25 µm	35 µm - 45 µm	55 µm - 65 µm
Average size	9.70 µm	20.40 µm	41.70 µm	58.40 µm
Size accuracy	97.2%	97.8%	96.2%	98.3%
Total count	3.03E+06 cells/ml	3.98E+05 cells/ml	5.05E+04 cells/ml	2.53E+04 cells/ml
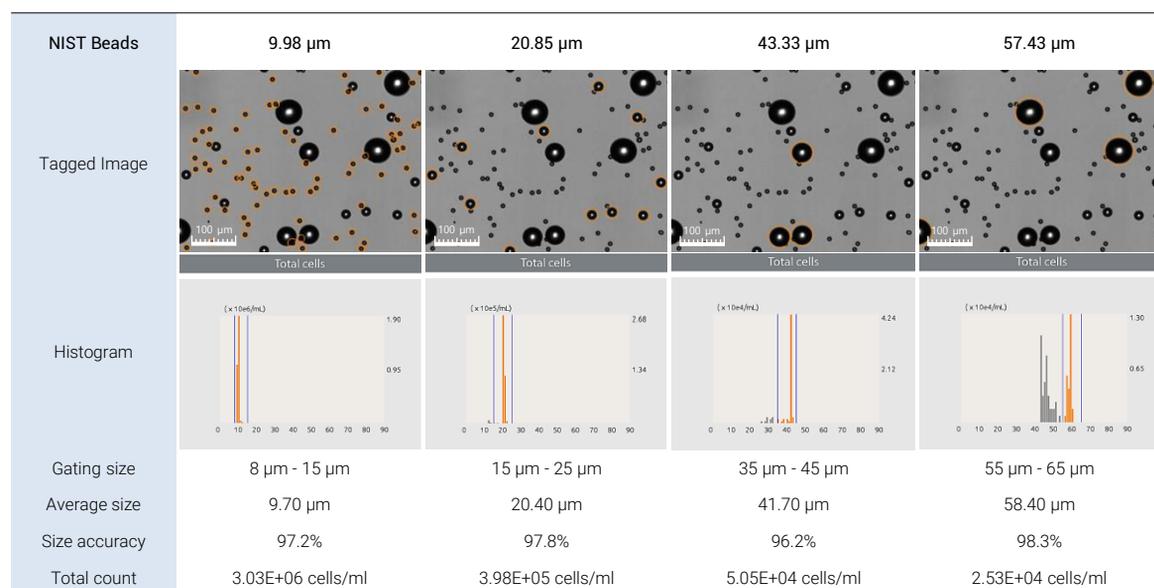
Figure 3. *Specific size gating in the LUNA-FX7™. The tagged images and the histograms show that the target size particles, indicated in orange, are intensively, selectively, and exclusively detected by gating.*

The counting and size gating application to cell lines

We then counted two cell lines with different size ranges: AsPC-1 (Pancreatic carcinoma) and DLD-1 (Colon carcinoma). Healthy AsPC-1 cells exhibit a range of 8 - 40 µm, while healthy DLD-1 cells are expected to have a narrower size range of 10 - 20 µm. The LUNA-FX7™ accurately captures the entire size range of both populations (Figure 4).
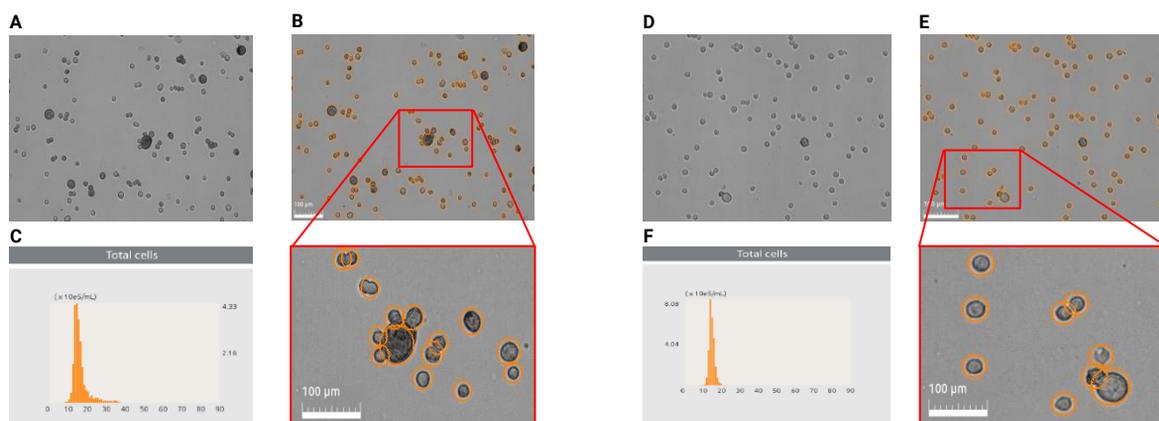

Figure 4. *Cell counts and size determinations of AsPC-1 and DLD-1 cells. AsPC-1 (A-C) and DLD-1 (D-F) cell lines were applied to confirm the counting performance in various sizes mixed.*

CONCLUSION

The LUNA-FX7™ has multiple means to measure, differentiate, and determine the size of cell populations. With the options of pre-defined size gating during counting and after counting, the LUNA-FX7™ provides the flexibility to assess the health and quality of cell populations. Moreover, the reanalysis feature of the LUNA-FX7™ allows more extensive evaluations of precious preserved counts for downstream analysis.

REFERENCES

[1]Björklund, M. (2019). Cell size homeostasis: Metabolic control of growth and cell division. Biochim Biophys Acta Mol Cell Res, 1866(3), 409-417. doi:10.1016/j.bbamcr.2018.10.002

Find out more at https://logosbio.com/automated-cell-counters/fluorescence/luna-fx7