An analysis of optimal cell counting range of the LUNA-FX7™

INTRODUCTION

Most automated cell counters have a reliable detection range of 1.00E+05 to ~1.00E+07 cells/mL[1]. Sometimes, though, cell cultures may require cells to be concentrated or diluted to obtain accurate counts. The LUNA-FX7™ Automated Cell Counter provides several slide options that can be used for various augmentations of applications in cell counting. Here, we demonstrated the highest performance of the LUNA-FX7™ in wide concentration ranges with HL60 cells and beads. The LUNA-FX7™ ensured the reliable cell counting in the dynamic range of 1.00E+04 cells/mL to 2.50E+07 cells/mL, and even broaden with smaller beads up to 5.03E+07 cells/mL. To determine the accurate counting range in the LUNA-FX7™ slide options, we analyzed counting results obtained from 1, 2, and 8-channel slides.

LUNA-FX7™ Automated Cell Counter

MATERIALS AND METHODS

For the LOQ (Limit of Quantification) measurement of the LUNA-FX7™, HL60 cells, the LUNA-FX™ Calibration Beads Kit (Cat# F73101) and the LUNA™ Fluorescence Calibration Beads (Cat# F23102) were prepared[2]. The diameter of the LUNA-FX™ Calibration Beads and the LUNA™ Fluorescence Calibration Beads is 12 µm and 7 µm, respectively.

HL60 cells of 2.50E+07 cells/mL and the serial dilutions were prepared to be counted in the Brightfield and Fluorescence Cell Counting modes of the LUNA-FX7™. Prior to counting, the cells were stained with the 0.4% Trypan Blue Stain (Cat# T13001) and loaded in the 2-channel LUNA™ Cell Counting Slides (Cat# L12001) for brightfield counting. For fluorescent counting, the Acridine Orange/Propidium Iodide Stain (Cat# F23001) and the 2-channel PhotonSlide™ (Cat# L12005) were used. The conventional hemocytometer count was performed to cross-check the count results of the LUNA-FX7™.

For determining the capacity of LOQ ranges of the LUNA-FX7™ Automated Cell Counter, the typical cell size comparable beads (12 µm) were applied in both the Brightfield and the Fluorescence Cell Counting modes using three types of slides, 1-channel, 2-channel, and 8-channel slides*[1]. The default protocols were adopted for all the counts, and the LOQ ranges were statistically evaluated based on the differences of measured vs. expected counts, combined with co-efficiency of replications. To further verify the higher concentrations, the beads of 7 µm were counted on the Fluorescence Cell Counting mode.

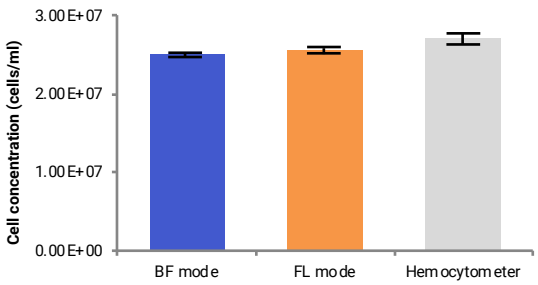
*[1] *1-channel: LUNA™ 1-Channel Slides (Cat# L72011), 2-channel: LUNA™ Cell Counting Slides (Cat# L12001) for brightfield counting, PhotonSlide™ (Cat# L12005) for fluorescence counting, 8-channel: LUNA™ 8-Channel Slides (Cat# L72001)*

RESULTS

Comparable HL60 cell counting with the LUNA-FX7™ vs. a hemocytometer

The 2.50E+07 cells/mL of HL60 cells were enumerated in LUNA-FX7™, while the same sample was also manually counted with a hemocytometer. Compared to the hemocytometer calculation, the counts with the LUNA-FX7™ exhibited a difference of 5.53% and 5.03%, respectively, in the Brightfield Cell Counting mode and the Fluorescence Cell Counting mode (Figure 1). Indeed, the tighter deviations of standard errors indicate that the LUNA-FX7™ is more reproducible in counting than the hemocytometer (Figure 1). We also intuitively scrutinized the counting ability of the LUNA-FX7™ in serially diluted HL60 cells and the enlarged inlet images reveal that each cell was remarkably well tagged, even in the almost occupied field of view with the high concentration of 2.50E+07 cells/mL (Figure 2).

A



B

		Average	St. Error	Δ (%)*
LUNA-FX7™	BF total mode (TB)	2.51E+07	4.26E+05	5.53
	FL mode (AO/PI)	2.52E+07	5.03E+05	5.03
Hemocytometer		2.65E+07	9.49E+05	0.00

$$* \Delta\ (\%) = \frac{|FX7\ value - Hemocytometer|}{Hemocytometer} \times 100\ (\%)$$

Figure 1. *The comparable counts of HL60 cells: the LUNA-FX7™ vs. a hemocytometer. The comparable cell concentrations (A) and the corresponding table (B) show comparable cell count values of the LUNA-FX7™ and a hemocytometer. The results in both counting modes of the LUNA-FX7™ are not significantly different from that of the standard manual count with a hemocytometer.*

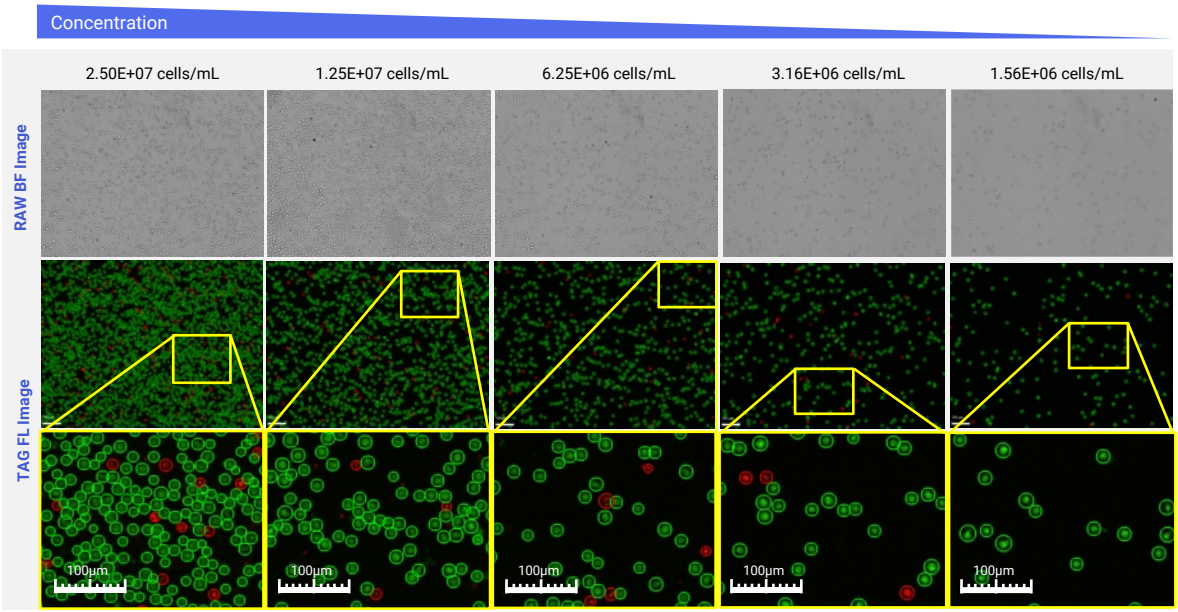
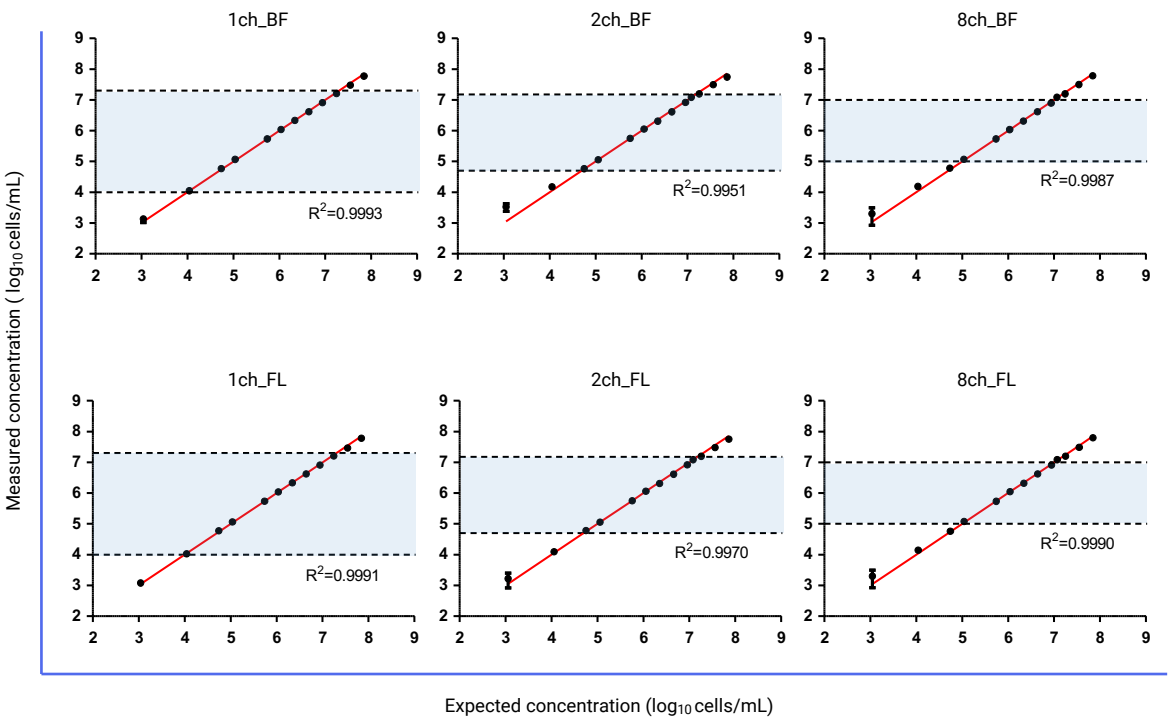


Figure 2. *The images of HL60 cell counting in serial dilutions. The brightfield raw images and corresponding fluorescent-tagged images express the counting status from the concentration of 1.56E+06 cells/mL to 2.50E+07 cells/mL.*

Limit of quantification of the slide options

When the bead concentration range was tested with 1.00E+03 to 1.00E+08 cells/mL, the R^2 values of the plots in three slide options and the two counting modes were all greater than 0.99. The dynamic ranges of cell concentrations, indicated as the blue boxes with dashed lines in Figure 3B, were established where the differences between the expected and actual measured values are 10% or less. Among three slide options, the 2-channel slide option is comfortable and familiar to use for cell counting, just like using a hemocytometer. The 8-channel slide option has the advantage of counting eight samples at once in a high-throughput manner and being compatible with multi-channel pipettes. Besides, the 3-channel slides are available to measure the same volume as the 2-channel slides, and the LOQ range may be equated with the 2-channel option. Notably, the 1-channel slides, measuring larger volume up to 5.1 µL and total 47 fields, can be most effectively used in the extreme cases of the low or high concentration of cells in 1.00E+04 cells/mL to 2.00E+07 cells/mL without extra labor of concentrating or diluting the cell samples. Furthermore, smaller beads can be placed with decent declustering in a higher number; thus, the 7 µm of dual fluorescent beads were employed and resulted in an even broader comprehensive range of concentrations (Figure 4).

A



B

	1-channel slide	2-channel slide	8-channel slide
Number of fields	47 fields	12 fields	5 fields
LOQ (< 10% of Δ)	1.00E+04 - 2.50E+07	5.00E+04 - 1.50E+07	1.00E+05 - 1.00E+07

Figure 3. The LOQ ranges of slide options. *The plots correspond to counting limits of 1-ch, 2-ch, and 8-ch slides in the LUNA-FX7™, and the blue boxes with dashed lines indicate the suggested ranges of LOQ for the slide option and the mode applied (A). The suggested ranges of LOQ of each slide type are defined with numbers in the table (B). Specifically, the 1-ch slide shows the most comprehensive range among the three slide options.*

Table 1. *Limit of quantification of slide options on brightfield and fluorescence counting.*

		Brightfield counting		Fluorescent counting	
		Lowest conc.	Highest conc.	Lowest conc.	Highest conc.
1-ch slide	Expected conc. (cells/mL)	1.00.E+04	2.00.E+07	1.00.E+04	2.00.E+07
	Measured conc. (cells/mL)	1.02.E+04	1.83.E+07	9.64.E+04	1.84.E+07
	St. Dev.	9.17.E+03	4.61.E+05	7.71.E+02	4.34.E+05
	CV (%)	9.00%	2.52%	8.00%	2.36%
	Δ (%)	1.83%	8.57%	3.64%	8.00%
2-ch slide	Expected conc. (cells/mL)	5.00.E+04	1.50.E+07	5.00.E+04	1.50.E+07
	Measured conc. (cells/mL)	5.110E+04	1.50.E+07	5.28.E+04	1.51.E+07
	St. Dev.	4.90.E+03	5.03.E+05	3.76.E+03	5.02.E+05
	CV (%)	9.59%	3.35%	7.11%	3.32%
	Δ (%)	2.12%	0.11%	5.64%	0.83%
8-ch slide	Expected conc. (cells/mL)	1.00.E+05	1.00.E+07	1.00.E+05	1.50.E+07
	Measured conc. (cells/mL)	1.09.E+05	1.04.E+07	1.09.E+05	1.55.E+07
	St. Dev.	1.02.E+04	5.01.E+04	5.86.E+03	7.29.E+04
	CV (%)	9.31%	0.48%	5.37%	0.47%
	Δ (%)	9.26%	4.31%	9.09%	3.38%

$$* \Delta = \frac{|Expected\ conc. - Measured\ conc.|}{Expected\ conc.} \times 100\ \%$$

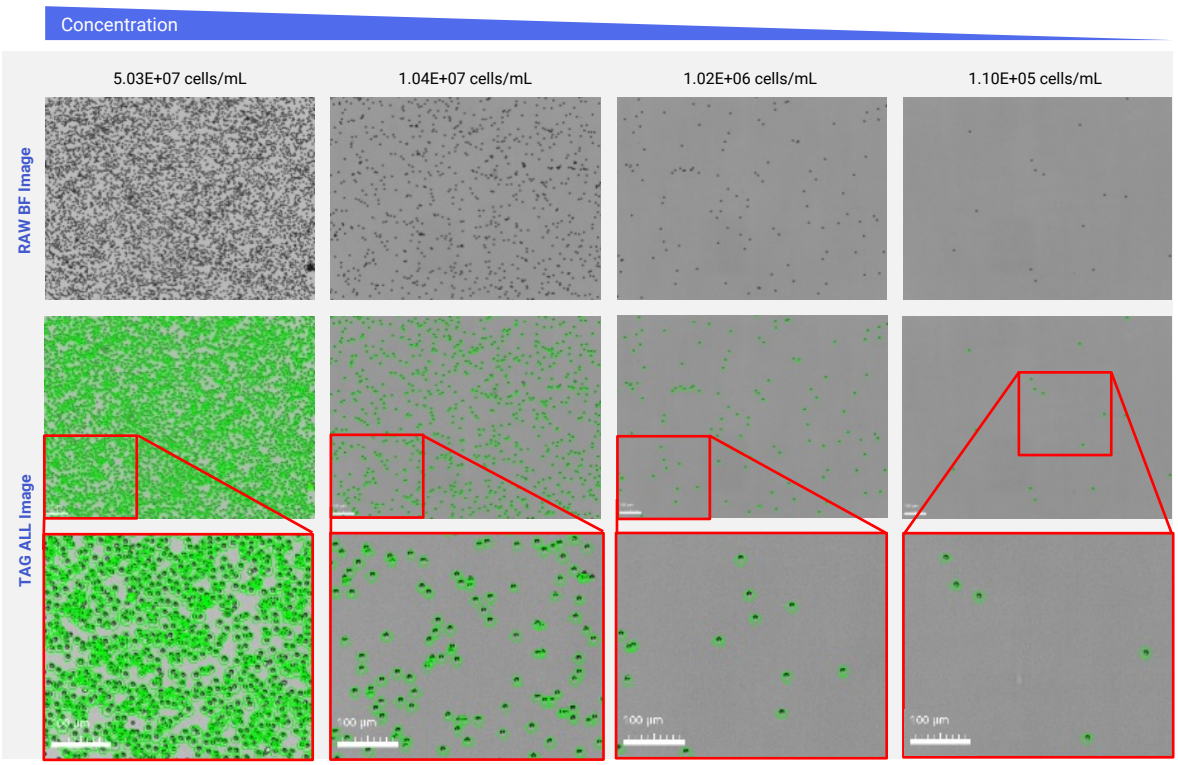


Figure 4. ***Limit of quantification of small-sized bead counts.*** *In the smaller bead counting (7 µm), the raw images and the corresponding tagged images in the Fluorescence Cell Counting mode display more comprehensive ranges to the highest concentration (5.03E+07 cells/mL).*

CONCLUSION

The LUNA-FX7™ Automated Cell Counter has well performed the counting in a wide range of concentrations on both the Brightfield and the Fluorescence Cell Counting modes. Expressly, evaluating the LOQ ranges of the typical cell size has confirmed the utmost concentrations from 1.00E+04 to 2.50E+07 cells/mL (Figure 3B). The detection ability on a wide concentration range provides more convenient calculation results for the user by directly counting cells without diluting or concentrating in most cell cultures. Furthermore, we successfully provided the counting limits up to 5.03E+07 cells/mL with 7 µm beads. There are possible alterations in the maximum range of LOQ, which may be affected by cell sizes and other conditions, such as the tendency of the cell clustering or occupancy of counting fields.

REFERENCES

[1] Seo D, Oh S, Lee M, Hwang Y, Seo S. A Field-Portable Cell Analyzer without a Microscope and Reagents. Sensors (Basel). 2017 Dec 29;18(1):85. doi: 10.3390/s18010085. PMID: 29286336; PMCID: PMC5795886.

[2] Buoro S, Seghezzi M, Manenti B, Mecca T, Candiago E, Vidali M, Ottomano C, Castelli CC, Ferrari R, Zappalà G, Lippi G. Reliability of automated synovial fluid cell counting with Mindray BC-6800 body fluid mode. Int J Lab Hematol. 2017 Jun;39(3):337-346. doi: 10.1111/ijlh.12632. Epub 2017 Mar 6. PMID: 28263031.