logos biosystems

application note

The Accuracy and Low Variability of the LUNA-II ™ Automated Cell Counter

INTRODUCTION

With a growing emphasis on both inter- and intra-lab reproducibility in biomedical research, the seemingly simple act of obtaining accurate cell counts is now more important than ever. In addition to negatively affecting reproducibility, inaccurate cell counts have downstream consequences for determining the potency and/or efficacy of cell therapy treatments, disease diagnoses, rate of growth for regenerated tissues, and various bioassays [1]. Traditionally, the gold standard for ascertaining viable cell numbers has involved placing cells stained with a vital dye such as trypan blue in a hemocytometer and manually counting them under a microscope [2-5]. Manually counting cells has a low cost per count and provides versatility [2], but the procedure is time consuming and labor intensive. Additionally, the results obtained from manual counts may suffer from cross-contamination through the reusable hemocytometer and significant variations may arise from different hemocytometer volumes, pipetting inconsistencies, and inter-user variation [2,3,5]. Recent advances in automated cell counting systems such as the LUNA-II™ from Logos Biosystems make it possible to obtain more consistent, accurate, and reproducible results at a reasonable cost per count [2-4].

The LUNA-II™: the First Automated Cell Counter Equipped with a Liquid Lens

The LUNA-II™ Automated Cell Counter is an advanced cell counting system with a state-of-the-art liquid lens technology that allows for autofocusing. The LUNA-II™ rapidly performs highly accurate cell counts by employing statistical observations of the intensity characteristics in live and dead cells. Liquid lenses have several advantages over other traditional optical lenses: reduced size, large range of optical variation, long lifetime, shock resistance, speed, and low power consumption [6,7].

Due to the liquid lens and advanced counting algorithm, the LUNA-II™ also provides considerably lower levels of variability in cell concentration and viability determinations. Technical variability in cell counting may occur when repeated measurements of the same sample are 1) made with a single device (intra) or 2) made with different devices (inter). The advanced engineering and high quality control exhibited in the LUNA-II™ results in a low level of device-to-device variability, ensuring both intra- and inter-lab consistency and reproducibility.

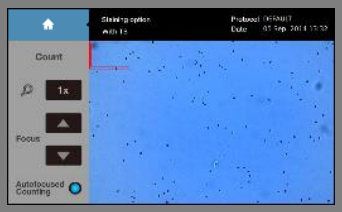
Here we demonstrate how the LUNA-II™ will help you obtain more accurate and consistent cell counts in various experimental settings.

BASIC PROCEDURE

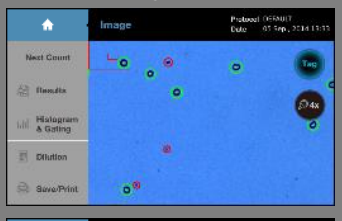
Select count

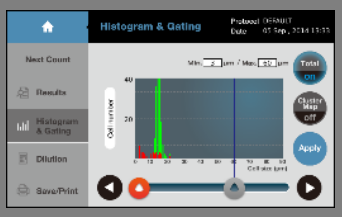
Load your sample

Press [Count]



Review your results





MATERIALS AND METHODS

LUNA-II™ Automated Cell Counter

The DEFAULT protocol of the LUNA-II™ was used for all counts. Three LUNA-II™ devices were used to test intra-deviation after standard quality control procedures. To determine device-to-device variability, seven LUNA-II™ devices were tested.

Cell lines and reagents

A human promyelocytic leukemia cell line, HL-60, was maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 100 units/ml penicillin/streptomycin. HeLa (a human cervical adenocarcinoma cell line) was maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS and 100 units/ml penicillin/streptomycin. 0.4% trypan blue stain (Cat No. T13101) and LUNA™ Standard Beads (Cat No. B13101) were used.

Measurement of cell viability

Live cells were prepared from exponentially growing cells. Dead cells were prepared by incubating an appropriate number of cells at 70℃ for 30 min. A series of cell suspensions with different viabilities was prepared by mixing live and dead cells. Theoretical viabilities were calculated from the measured viability of live and dead cells, respectively. The measured viabilities were determined by trypan blue staining.

Results

Linearity of concentration analysis of the LUNA-II™

To determine the linearity of the LUNA-II™, the concentrations of serially diluted LUNA™ Standard Bead solutions were measured by technicians with different levels of experience. As shown in Figure 1, the LUNA-II™ exhibited a high degree of linearity as shown by R^2 values ($R^2 \geq 0.9999$) across a wide linear operating range, regardless of experience level. These data indicate that the LUNA-II™ measures the concentration of particles with high accuracy.

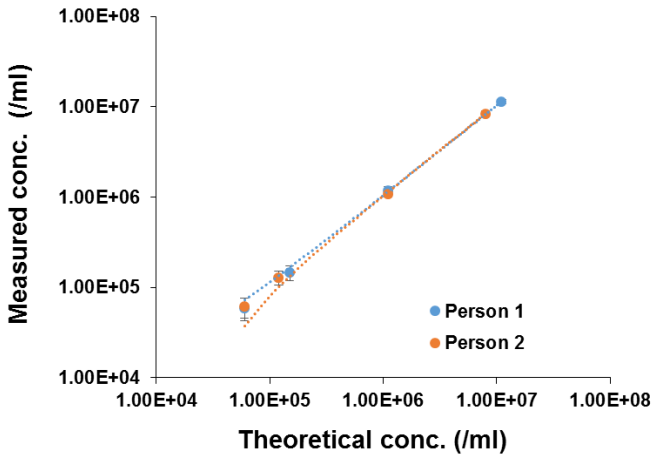
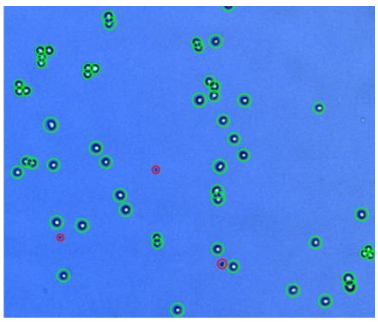


Fig 1. Concentration of LUNA™ Standard Beads determined with a LUNA-II™. Data represented as mean ± SD from a representative experiment performed in decuplicate.

Cell Images Captured with the LUNA-II™



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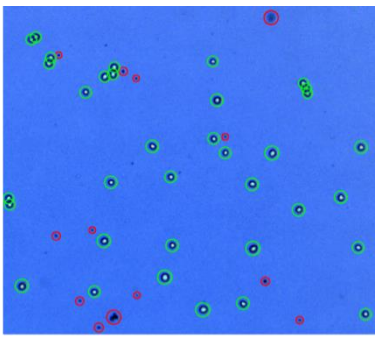

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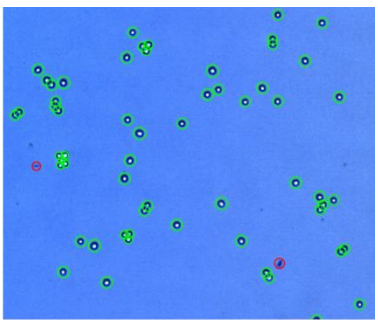

HCT116

The linearity of cell counting of the LUNA-II™ was further determined with live cultured cell lines. Two different cells lines were used in the study: 1) HL-60 (suspension) and 2) HeLa (adherent) cells. Prior to counting, exponentially growing cells were serially diluted and subsequently mixed with an equal volume of 0.4% trypan blue stain. Again, a high degree of linearity ($R^2 > 0.997$) was obtained, irrespective of cell type (Figure 2).

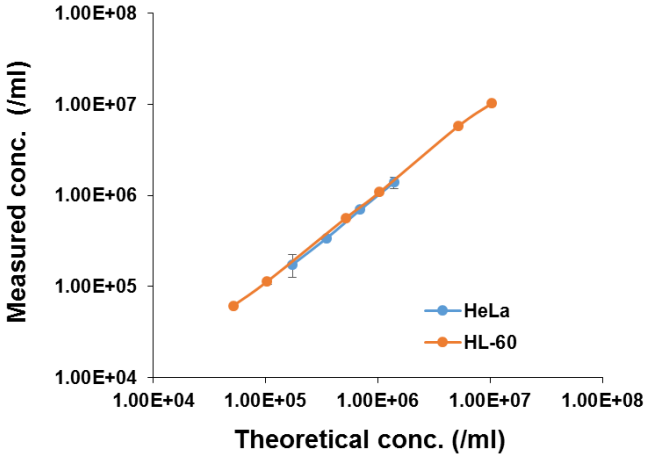

Fig 2. Determination of cell concentration with the LUNA-II™. Data represented as mean ± SD from a representative experiment performed in triplicate

Linearity of cell viability of the LUNA-II™

The linearity of cell viability measured with the LUNA-II™ was also determined with HeLa and HL-60 cells. A range of cell viability solutions were prepared as described in the Materials and Methods section. As shown in Figure 3, the viabilities measured with the LUNA-II™ closely matched the theoretical viabilities with $R^2 > 0.997$.

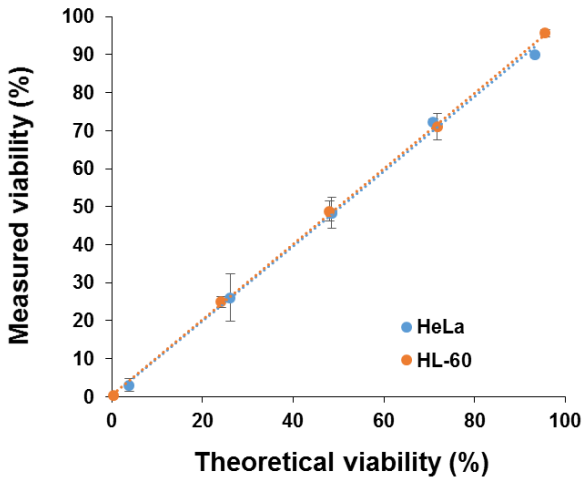

Fig 3. Cell viability determined with the LUNA-II™. Data represented as mean ± SD from a representative experiment performed in triplicate (for HeLa) or heptaplicate.

Intra- and inter-deviation of the LUNA-II™

Cell counting reproducibility is important for subsequent applications of cultured cells. To demonstrate the ability of the LUNA-II™ to deliver reproducible and consistent results, two different experiments were conducted. First, intra-deviation was determined by the repeated counting of LUNA™ Standard Beads: the beads were loaded into a LUNA™ Cell Counting Slide and counted seven times per device with three different devices. Second, inter-deviation was measured by counting the beads across seven different LUNA-II™ devices.

As shown in Table 1, repeated counting of the beads showed similar results for total cell concentration, average cell size, and the total cell number. In fact, devices 2 and 3 demonstrated great correlation. The minor differences from device 1 were mainly caused by the Brownian movement of beads and/or evaporation. During the counting process, evaporation of the cell solution may cause minor changes in particle distribution in the field of view.

Device 1

Repeat	Total cells (cells/ml)	Avg. cell size (µm)	# Total cells
1	4.48E+05	10.5	97
2	4.53E+05	10.4	98
3	4.53E+05	10.5	98
4	4.53E+05	10.5	98
5	4.53E+05	10.5	98
6	4.48E+05	10.5	97
7	4.53E+05	10.6	98
Mean	**4.52E+05**	**10.5**	**98**
SD	**2.44E+03**	**0.1**	**0.5**
CV	**0.54%**	**0.55%**	**0.50%**

Device 2

Repeat	Total cells (cells/ml)	Avg. cell size (µm)	# Total cells
1	5.45E+05	10.1	119
2	5.45E+05	10.1	119
3	5.45E+05	10.1	119
4	5.45E+05	10.1	119
5	5.45E+05	10.1	119
6	5.45E+05	10.1	119
7	5.45E+05	10.1	119
Mean	**5.45E+05**	**10.1**	**119**
SD	**0.00E+00**	**0.0**	**0.0**
CV	**0.00%**	**0.00%**	**0.00%**

Device 3

Repeat	Total cells (cells/ml)	Avg. cell size (µm)	# Total cells
1	5.80E+05	11.0	128
2	5.80E+05	11.0	128
3	5.80E+05	11.0	128
4	5.80E+05	10.9	128
5	5.80E+05	10.9	128
6	5.80E+05	11.0	128
7	5.80E+05	11.0	128
Mean	**5.80E+05**	**11.0**	**128**
SD	**0.00E+00**	**0.0**	**0.0**
CV	**0.00%**	**0.44%**	**0.00%**

Table 1. Intra-deviation of the LUNA-II™. Three different preparations of LUNA-II™ Standard Beads were counted by three LUNA-II™ devices, respectively. The counting of each preparation was repeated seven times.

Table 2 demonstrates inter-deviation across seven different LUNA-II™ devices. These data suggest that device-to-device variability of the LUNA-II™ is significantly low as coefficient of variance (CV) values are less than 5% in all of the measurements.

Standard beads, prep 1

Device	Total cells (cells/ml)	Avg. cell size (µm)	# Total cells
1	5.37E+05	10.1	118
2	5.21E+05	10.4	113
3	5.82E+05	10.6	128
4	5.52E+05	10.1	121
5	5.59E+05	11.0	121
6	5.63E+05	10.2	123
7	5.40E+05	10.7	119
Mean	**5.51E+05**	**10.4**	**120**
SD	**1.99E+04**	**0.3**	**4.6**
CV	**3.62%**	**3.26%**	**3.83%**

Standard beads, prep 2

Device	Total cells (cells/ml)	Avg. cell size (µm)	# Total cells
1	6.92E+05	9.8	152
2	6.68E+05	10.1	145
3	6.27E+05	10.3	138
4	6.62E+05	10.2	145
5	6.51E+05	10.1	141
6	6.55E+05	10.3	143
7	6.62E+05	10.2	146
Mean	**6.60E+05**	**10.1**	**144**
SD	**1.95E+04**	**0.2**	**4.4**
CV	**2.96%**	**1.78%**	**3.04%**

Standard beads, prep 3

Device	Total cells (cells/ml)	Avg. cell size (µm)	# Total cells
1	6.15E+05	10.5	135
2	6.08E+05	10.2	132
3	5.63E+05	10.5	124
4	6.02E+05	10.4	132
5	6.19E+05	10.3	134
6	6.23E+05	10.7	136
7	6.21E+05	10.6	137
Mean	**6.07E+05**	**10.5**	**133**
SD	**2.09E+04**	**0.2**	**4.3**
CV	**3.44%**	**1.64%**	**3.26%**

Table 2. Inter-deviation of the LUNA-II™. A preparation of LUNA-II™ Standard Beads was counted by seven LUNA-II™ devices. The counting was repeated two more times with different preparations of beads.

CONCLUSIONS

The LUNA-II™ Automated Cell Counter provides:

- extremely accurate measurements of cell concentration and viability,
- a high degree of linearity in cell concentration and viability in a wide working range, and
- a high degree of reproducibility and low technical variability.

References

1. Sarkar S. ISCT Webinar: Measurement assurance for cell enumeration. ISCT North American Legal and Regulatory Affairs (LRA) Committee. 2015. https://isct-beacon.app.box.com/isctwebinar29042015. Accessed 9 June 2015.
2. Johnston G. Automated handled instrument improves counting precision across multiple cell lines. BioTechniques. 2010;48:325-7.
3. Cadena-Herrera D, Lala JEE-D, Ramírez-Ibañez ND, López-Morales CA, Pérez NO, Flores-Ortiz LF, Medina-Rivero E. Validation of three viable-cell counting methods: manual, semi-automated, and automated. Biotech Rep. 2015;7:9-16.
4. Tholudur A, Giron L, Alam K, Thomas T, Garr E, Weatherly G, Kulowiec K, Quick M, Shepard S. Comparing automated and manual cell counts for cell culture applications. BioProcess Int. 2006;4:28-34.
5. Strober W. Trypan blue exclusion test of cell viability. Curr Protoc Immunol. 1997;A3.B.1-A3.B.2.
6. Lin H-C, Chen M-S, Lin Y-H. A review of electrically tunable focusing liquid crystal lenses. Trans Electr Electron Mater. 2011;12:234-240.
7. Berge B. Liquid lens technology: principle of electrowetting based lenses and application to imaging. Proceed IEEE Int Conf Micro Electro Mech Syst 2005;227-230.

• Specifications

LUNA-II™ Automated Cell Counter Specifications

Instrument Type	Benchtop cell counter
Dimensions (W x D x H)	16 x 18 x 28 cm (6.3 x 7.0 x 11.0 in)
Weight	1.6 kg (3.5 lb)
Cell Concentration Range	5 x 10^4 – 1 x 10^7 cells/mL
Cell Diameter Range	3 – 60 µm (optimal range: 8-30 µm)
Cell Viability Range	0 – 100%
Image Resolution	5 MP
Image Type	TIFF
Documentation	PDF
Processing Time*	10** sec (without autofocusing) or 15** sec (with autofocusing) at 1 x 10^6 cell/mL

**Processing time may vary according to cell type and concentration).*
***This is the minimum processing time for each focusing option at the specified concentration of HeLa or HL-60 cells.*

LUNA™ Cell Counting Slide Specifications

Material	Polystyrene
Dimensions (W x D x H)	25 x 75 x 2.4 cm
Chamber Depth	100 µm
Chamber Volume	10 µL

• Ordering Information

Cat #	Product	Size
L40001	LUNA-II™ Automated Cell Counter (with built-in printer)	1 unit
L40002	LUNA-II™ Automated Cell Counter (without printer)	1 unit
L12001	LUNA™ Cell Counting Slides, 50 Slides	1 box
L12002	LUNA™ Cell Counting Slides, 500 Slides	10 boxes
L12003	LUNA™ Cell Counting Slides, 1000 Slides	20 boxes
T13001	Trypan Blue Stain, 0.4%	2 x 1 mL
L13002	LUNA™ Biosafe Stain	2 x 1 mL
B13101	LUNA™ Standard Beads	2 x 1 mL
P12002	LUNA-II™ Printer Paper - thermal, 700 prints	P12002

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