

Advanced Cell Counting and Viability Assessment with the LUNA-III™ Automated Cell Counter

INTRODUCTION

Accurate cell counting is essential in biomedical research, impacting applications such as cell therapy, disease diagnosis, tissue regeneration, and bioassays. Traditional manual counting methods are time-consuming and prone to variability. Automated systems, like the LUNA-III™ Automated Cell Counter from Logos Biosystems, provide a solution with improved accuracy, reproducibility, and efficiency.

The LUNA-III™ Automated Cell Counter builds on the success of its predecessor, the LUNA-II™, with several key improvements that enhance its functionality and performance. These advancements include live cell detection for large or aggregated cells, new features such as internal storage and better autofocus, the ability to reanalyze data and Find cells feature, and outstanding linearity and low variability in cell concentration and viability measurements.

Introducing LUNA-III™ Automated Cell Counter

The LUNA-II™ was already a great device, known for its reliability and accuracy in cell counting. However, it had a limitation—declustering of dead cell cluster—that now have been addressed in the LUNA-III™ (Figure 1).

Traditional algorithms struggled with declustering dead cells stained with Trypan Blue (TB) due to minimal brightness differences at cell boundaries. LUNA-III™ resolves this with a machine learning algorithm that accurately distinguishes

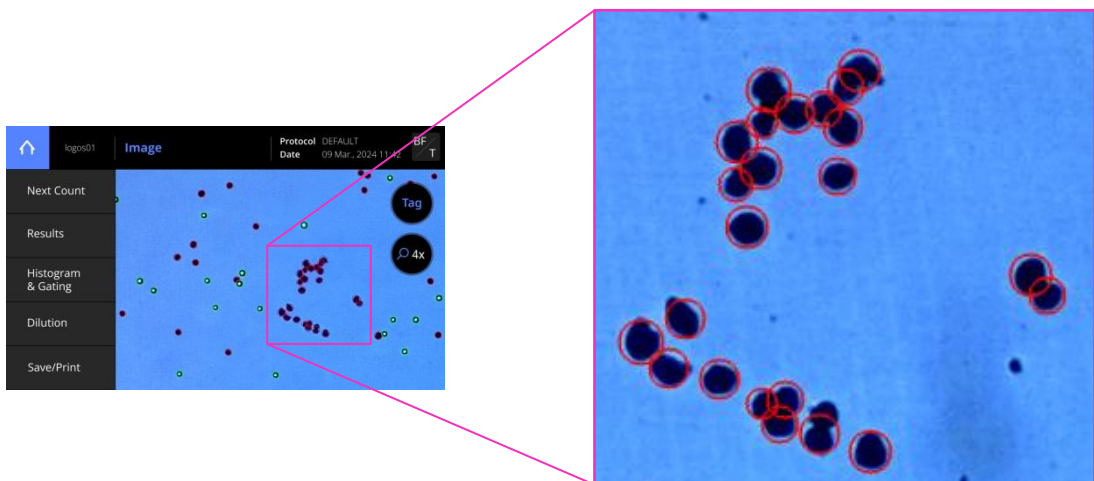
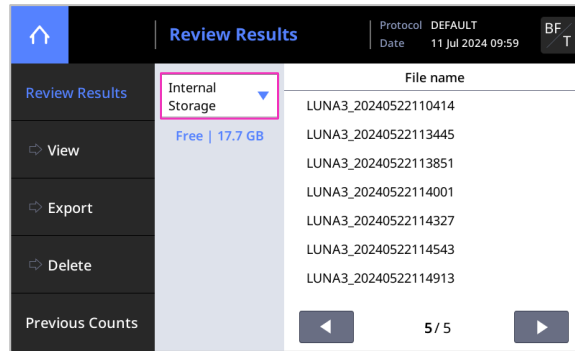


Figure 1. Green circles indicate live cells, and red circles indicate dead cells. The LUNA-III™ accurately identified dead cells, even in cases of cell aggregation and varying morphology, demonstrating the effectiveness of its new algorithm.

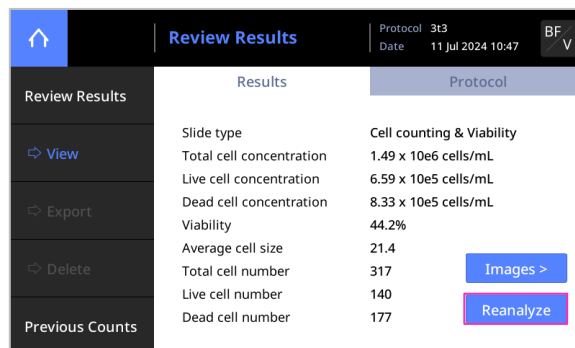
clustered cell shapes. Additionally, LUNA-III™ improves live cell detection by accurately identifying cells with irregular shapes or faint brightness to ensure more reliable results.

In addition to this crucial improvement, the LUNA-III™ boasts several new features designed to enhance user experience and workflow efficiency:

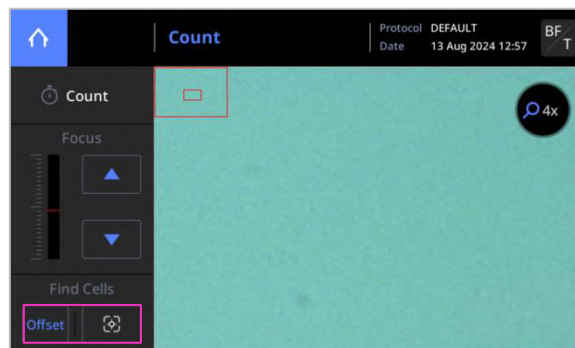
Internal Storage: Save and manage data directly on the device, streamlining your workflow and ensuring easy access to your results.



Reanalyze: Reanalyze previously captured images to ensure accuracy and consistency in your data analysis.



Find Cells: Utilize the 'Find Cells' feature to automatically adjust the focal plane and exposure level, ensuring thorough and precise analysis.



These advancements make the LUNA-III™ a superior tool for accurate and efficient cell counting and viability assessment, reinforcing its position as a reliable and innovative solution in the field of cell analysis.

MATERIALS AND METHODS

LUNA-III™ Automated Cell Counter

The default protocol of the LUNA-III™ was used for all measurements. Three LUNA-II™ devices were used to test intra-deviation after standard quality control procedures.

Cell Lines and Reagents

U937 (suspension) and 3T3 (adherent) cell lines were used. U937 cells were cultured in RPMI-1640 with 10 % FBS and 1 % penicillin/streptomycin. 3T3 cells were cultured in DMEM with 10 % FBS and 1 % penicillin/streptomycin. 0.4 % trypan blue (Cat No.T13001) was used for viability assessments.

Linearity of Cell Concentration and Viability

Cells were serially diluted starting from a maximum concentration of 1×10^7 cells/mL (3T3 cells started in maximum concentration 6×10^6 cells/mL). The cell concentration was measured using the LUNA FX7™ automated cell counter.

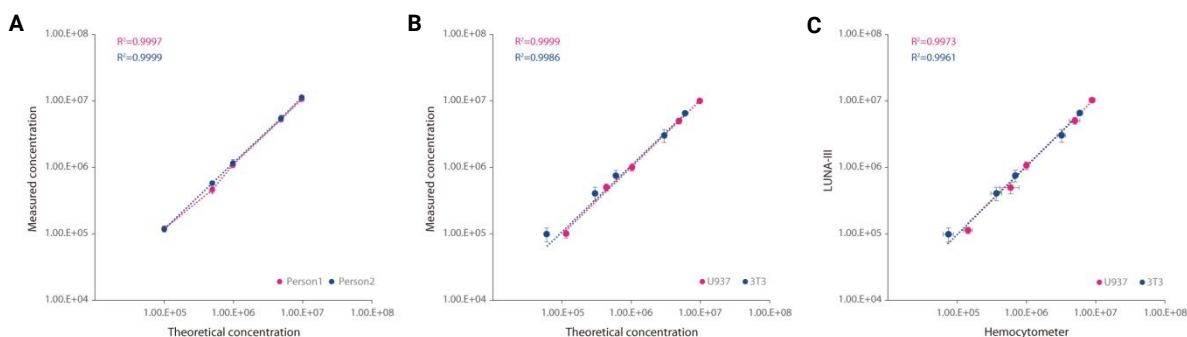
Live cells were obtained from exponentially growing cultures, while dead cells were prepared by heating cells at 100 °C for 30 min. Mixtures of live and dead cells at varying ratios were prepared to produce samples with different viabilities.

RESULTS

Linearity of Cell Concentration

To assess the cell concentration linearity of the LUNA-III™, we conducted tests from three different perspectives: discrepancies between different users (Figure 2A), discrepancies between two cell lines (Figure 2B), and comparison of LUNA-III™ cell counting results with those obtained using a hemocytometer (Figure 2C).

First, we measured linearity using two technicians with varying levels of experience, assessing serially diluted concentrations of U937 cells. Next, we evaluated linearity between U937 and 3T3 cells, which differ in size and characteristics, with a single technician assessing serially diluted samples. Finally, we compared the same samples using a hemocytometer. The LUNA-III™ consistently demonstrated linearity in all scenarios, with R^2 values of 0.9996 or higher across a broad concentration range. Images show the LUNA-III™ accurately detecting cells in these samples, further illustrating its precision and reliability (Figure 2D). This consistent performance highlights the LUNA-III™'s exceptional ability to accurately measure cell concentrations, showcasing its reliability and precision regardless of user experience.



D

Low concentration

High concentration

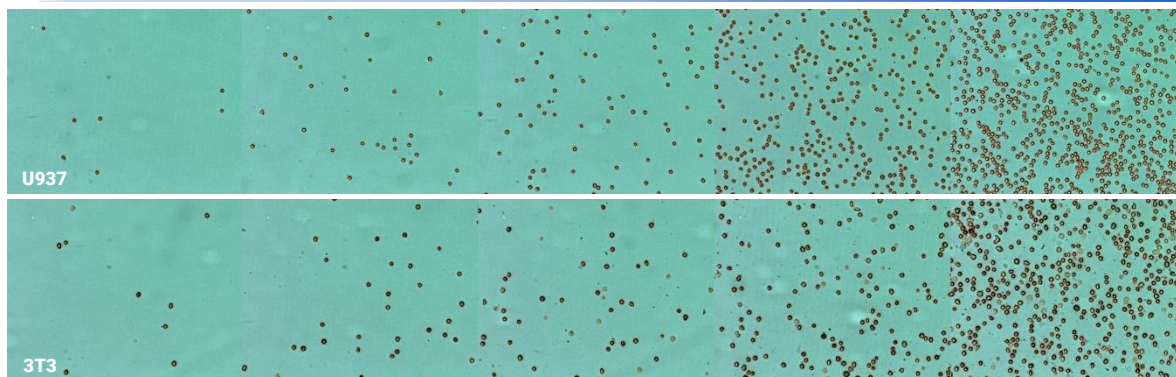


Figure 2. (A) U937 cells measured by two technicians with varying experience levels. The LUNA-III™ exhibited linearity with R^2 values of 0.9997 or higher. (B) Comparison of U937 and 3T3 cells measured by a single technician. The LUNA-III™ showed linearity with R^2 values of 0.9998 or higher. (C) LUNA-III™ versus hemocytometer measurements for U937 and 3T3 cells. The LUNA-III™ demonstrated linearity with R^2 values of 0.9996 or higher. (D) Images of U937 (top row) and 3T3 (bottom row) cells counted using the LUNA-III™. The images illustrate the accuracy and consistency of cell detection across a range of concentrations.

Linearity of Cell Viability

To evaluate the linearity of viability across different cell lines, we utilized the LUNA-III™. Live and dead U937 and 3T3 cells were mixed in varying proportions to create samples with a range of viabilities from 0 % to 100 %, which were then assessed by a single technician. The LUNA-III™ showed exceptional linearity in its viability measurements for both cell lines, achieving R^2 values of 0.9998 or higher across viability measurements (Figure 3A). The accompanying images show the LUNA-III™'s precise detection of live and dead cells, further highlighting its accuracy and reliability (Figure 3B). This consistent performance underscores the LUNA-III™'s extraordinary capability in measuring cell viability, demonstrating its robust reliability and precision across different cell types.

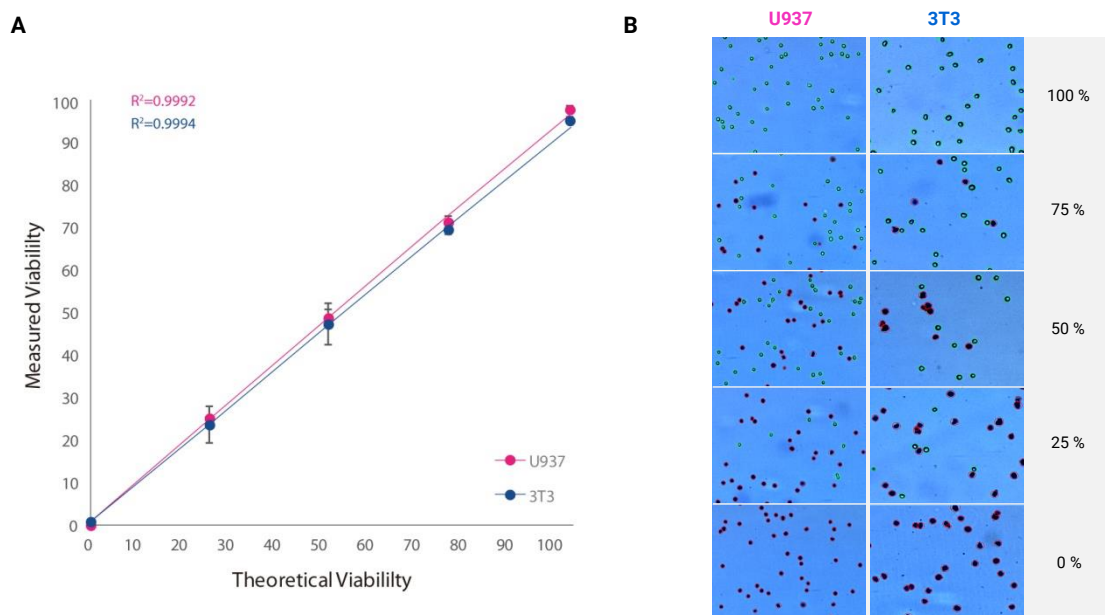


Figure 3. (A) Scatter plot showing the linearity of measured viability versus theoretical viability for U937 (blue) and 3T3 cells (red). (B) Images displaying accurately detected live (green circles) and dead (red circles) cells in U937 (top row) and 3T3 (bottom row) cell samples at various viability ratios.

CONCLUSION

The LUNA-III™ Automated Cell Counter represents a significant advancement in cell counting and viability assessment, building on the strengths of its predecessor, the LUNA-II™. By addressing key limitations such as cluster detection in dead cells and viability detection in irregularly shaped cells, the LUNA-III™ offers enhanced accuracy and reliability. Its new machine learning algorithms and features like internal storage, reanalysis capability, and the 'Find Cells' function make it a powerful tool for researchers. The system's outstanding linearity in both cell concentration and viability measurements confirms its reliability. Whether for routine cell counting or more complex viability assessments, the LUNA-III™ provides researchers with a reliable, efficient, and user-friendly platform to achieve precise results.

Ordering Information

Cat #	Product	Quantity
L60001	LUNA-III™ Automated Cell Counter	1 unit
L60002	LUNA-III™ Automated Cell Counter, Sustainable Package	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, 1,000 Slides	20 boxes
L12011	LUNA™ Reusable Slide	1 unit
L12012	LUNA™ Reusable Slides (2 pack)	2 units
L12014	LUNA™ Reusable Slide Coverslips	10 units
T13001	Trypan Blue Stain, 0.4%	2 x 1 mL
T13011	Trypan Blue Stain, 0.4%, Sterile-filtered	2 x 1 mL
L13002	Erythrosin B Stain	2 x 1 mL
B13001	LUNA™ Standard Beads	2 x 1 mL
P17001	LUNA™ Printer II	1 unit
U10005	USB Drive, 16 GB	1 unit
L72041	Cell Counter Validation Slide-BF II	1 unit
L64003	LUNA-III™ IQ/OQ Protocol	1 copy

