

Accurate Size Measurement with the LUNA-III™ Automated Cell Counter

INTRODUCTION

Cell size is a crucial parameter in biomedical research and applications like biomanufacturing, where it indicates cell health and quality. In workflows such as biomanufacturing, cell size is essential for assessing batch culture or source material in cell therapy. This makes reliable cell size determination in automated cell counting valuable. The **LUNA-III™** offers standard cell size gating features common to all LUNA™ Family devices, known for their precise cell size measurements. It also provides methods to analyze and categorize cell populations based on size during and after counting. We demonstrate the accuracy and reliability of the **LUNA-FX7™** in measuring cell size and its precise size gating ability using six different NIST Traceable Particle Size Standards and the 3T3 cell line.



MATERIALS AND METHODS

Cell Lines and Reagents

3T3 cell lines were used for the experiments. 3T3 cells were cultured in DMEM with 10 % FBS and 1 %. For viability assessments, 0.4 % **trypan blue** (Cat No. T13101) was utilized. Additionally, six different NIST Traceable Particle Size Standards (9.98, 20.85, 30.04, 43.33, 50.00, and 57.43 μm) were employed to verify size measurement accuracy.

Accuracy with NIST Traceable Particle Size Standards

NIST Traceable Particle Size Standards provide an official, objective comparison to known measurements maintained by the National Institute of Standards and Technology. These standards were used to verify the accuracy of the **LUNA-III™** for size measurement. The **LUNA-III™** demonstrated an accuracy of 95.2 % or higher in determining particle size, confirming its precision for size measurement (Figure 1).

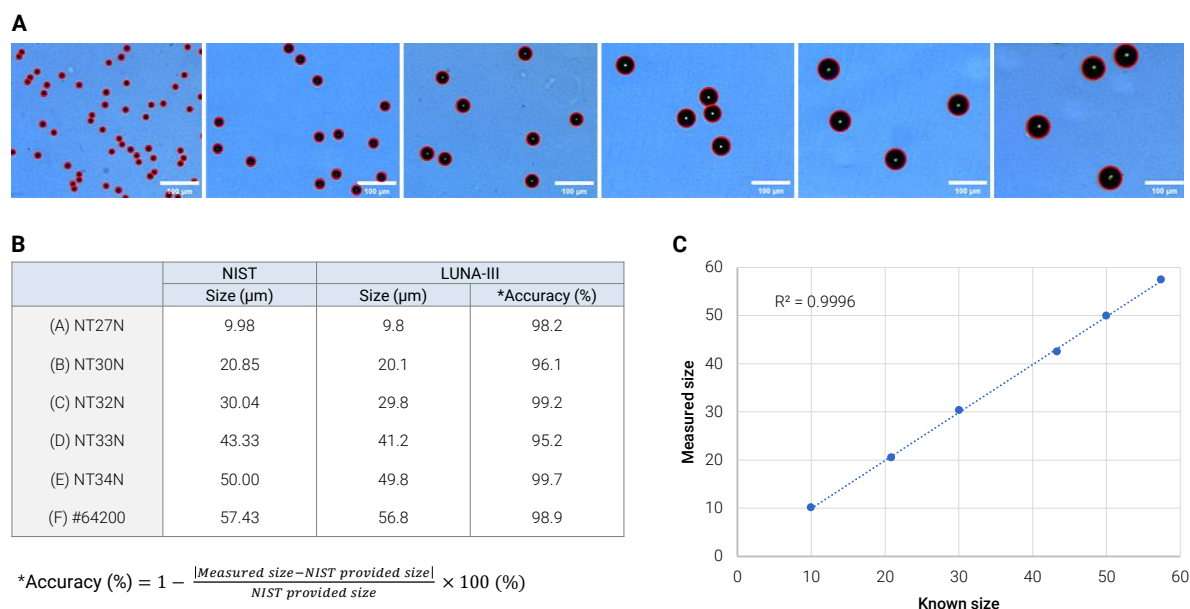


Figure 1. (A) Images of NIST Traceable Particle Size Standards (NT27N, NT30N, NT32N, NT33N, NT34N, #64200) counted using the LUNA-III™. The red circles indicate the detected particles.

(B)*Table showing the known sizes of the NIST standards compared to the sizes measured by the LUNA-III™ and the corresponding accuracy percentages.

(C) Scatter plot illustrating the linearity of the measured sizes versus known sizes of the NIST standards. The LUNA-III™ demonstrated an R^2 value of 0.9999, indicating excellent linearity and accuracy in size measurement.

Cell Lines Size Measurement

To evaluate the accuracy of size measurements in the LUNA-III™, we prepared a mixture of six NIST Traceable Particle Size Standards. We adjusted the counting protocol to include cell sizes from 1 µm to 90 µm. After counting, a histogram of cell concentration versus cell size was generated, accurately categorizing the NIST Standards. Different size beads were separately gated using flexible size gating based on the histogram, which can be adjusted immediately after counting. This size gating allowed for precise tagging of target sizes, achieving over 98 % accuracy in size measurement.

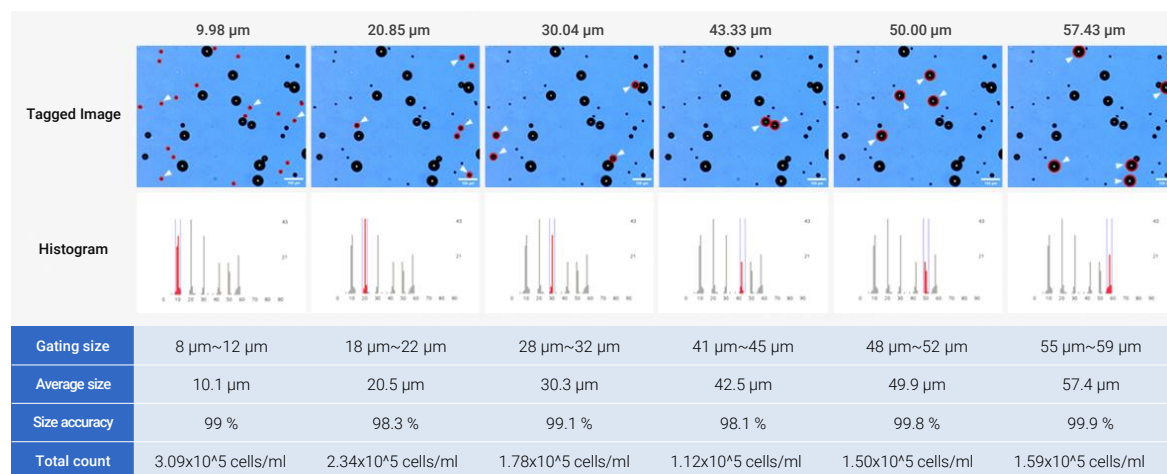


Figure 2. Images showing the detection and size gating of NIST Traceable Particle Size Standards using the LUNA-III™ across different size ranges: 8 µm ~ 12 µm, 18 µm ~ 22 µm, 28 µm ~ 32 µm, 41 µm ~ 45 µm, 48 µm ~ 52 µm, and 55 µm ~ 59 µm. Each image is paired with a corresponding histogram illustrating the distribution of particle sizes within the specified gating range.

Cell Lines Size Measurement

To evaluate the LUNA-III™'s ability to filter 3T3 cells (known size: about 18 μm) based on size, we applied specific size gating criteria across various ranges: 7 μm – 24 μm , 1 μm – 11 μm , 12 μm – 24 μm , and 16 μm – 20 μm (Figure 3). The images show the accurate detection and sizing of 3T3 cells within each gating range, with corresponding histograms illustrating the distribution of cell sizes. The LUNA-III™ effectively filters and categorizes 3T3 cells by size, ensuring reliable and precise measurements across different cell size ranges.

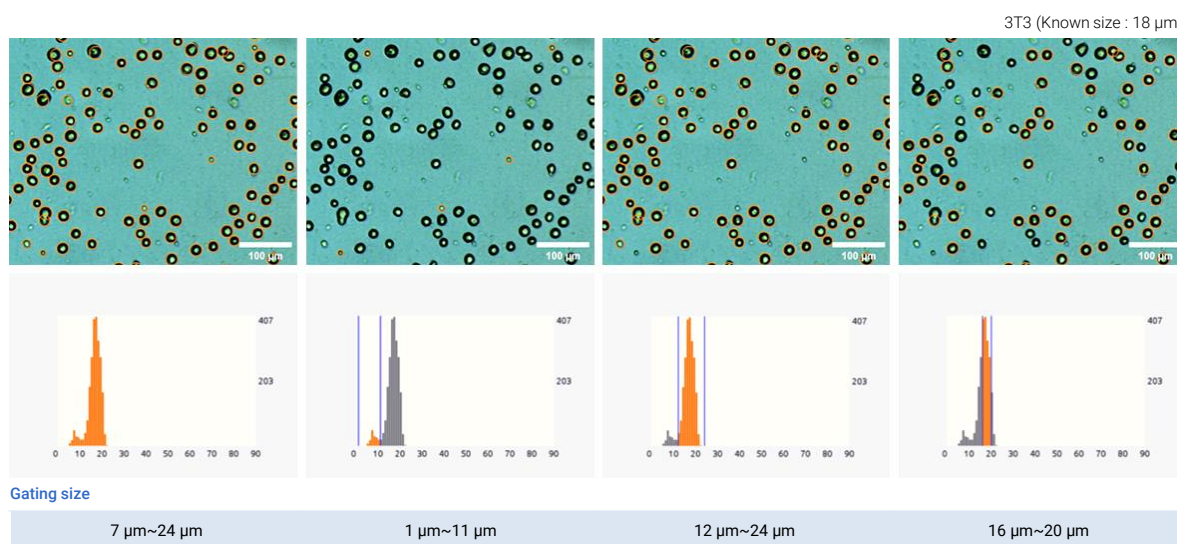


Figure 3. Images of 3T3 cells filtered and counted using the LUNA-III™, showing cells within the specified gating sizes: 7~24 μm , 1~11 μm , 12 ~24 μm , and 16 ~20 μm . The orange circles indicate detected cells. Corresponding histograms displaying the distribution of cell sizes within each gating range.

CONCLUSION

The LUNA-III™ Automated Cell Counter provides highly accurate and reliable cell size measurements. Using NIST Traceable Particle Size Standards, the LUNA-III™ demonstrated an accuracy of 95.2 % or higher. It effectively categorized mixed particle sizes and accurately gated 3T3 cells across various size ranges, achieving over 98 % accuracy.

The LUNA-III™ offers:

- Precise cell size measurement across various particle sizes and cell types.
- Reliable size gating for accurate categorization of cell populations.
- High reproducibility and consistency in measurements.

These capabilities make the LUNA-III™ a valuable tool for assessing cell size, health, and quality.