Yeast Cell Counting using the LUNA-FX7™ Automated Cell Counter

Key features: Accurate yeast cell counts using the LUNA-FX7™ Automated Cell Counter

INTRODUCTION

Yeasts are used in a vast array of processes including basic research, brewing & distilling, and food production that require accurate cell counts and viabilities prior to and throughout the process.[1,2,3]
The small physical size, small genome size, morphology, and development have proven to be quite challenging for automated cell counters to overcome.[4,5] As a result, the tedious and error-prone method of manually counting yeast using a hemocytometer and vital stain remains the 'go to' method. Here we show how the LUNA-FX7™ can accurately and reliably count yeast cells.

MATERIALS

1	LUNA-FX7™ Automated Cell Counter	5	LUNA™ Cell Counting Slide [L12001] / PhotonSlide™ [L12005]
2	*EC1118*		
3	0.02 % Methylene Blue [L13003]	6	Cell Dilution Buffer II [F53002]
4	Acridine Orange [F23002] / Propidium Iodide [F2300]	7	Hemocytometer

METHODS

1) Yeast strain EC1118 was inoculated in YPD broth and incubated overnight at 30 ℃, 200 rpm
2) Yeast was diluted in 1XPBS at ratios of 1:3 to 1:5.
3) Diluted cultures were diluted into 2 equal samples. One sample was then heat killed (65 ℃ for 20 minutes) to generate 'Live and Dead' cells for viability testing.
4) Cells were further diluted using Logos Biosystems Yeast Cell Dilution Buffer II : 1:5 and 1:10 (cells: buffer)

Viability Testing

5) Live and dead cells were mixed as per the Table1

Table 1. *Mixture ratio of live cells and dead cells for viability test*

	0 %	25 %	50 %	75 %	100 %
Live Cells	0 µl	25 µl	50 µl	75 µl	100 µl
Dead Cells	100 µl	75 µl	50 µl	25 µl	0 µl

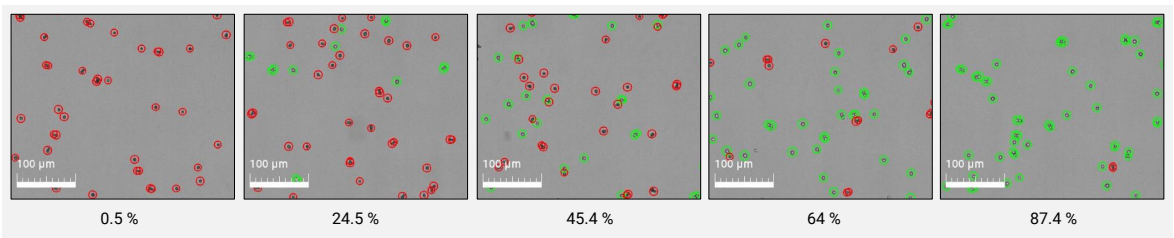
RESULTS

Linearity of viability of the LUNA-FX7™ Automated Cell Counter

To determine the linearity of viability, samples of various viability were prepared using live cells and dead cells and then counted using the LUNA-FX7™ Automated Cell Counter. With the LUNA-FX7™, viability can be checked in fluorescence mode and [CELL COUNTING & VIABILITY] of brightfield mode.

To examine viability in brightfield, yeast samples were mixed with 0.02 % methylene blue stain in the same volume, and 10ul was loaded on a LUNA™ Cell Counting Slide. Yeast samples were prepared as dead cells (0 %), 25 %, 50 %, 75 %, live cells (100 %). Referring to Figure 1A, five samples were stained with 0.02 % methylene blue stain, and images taken in the [CELL COUNTING & VIABILITY] of brightfield can be observed. Counting was repeated 3 times to calculate average viability, and the linearity of viability was confirmed accordingly. As shown in Figure 1B, viability measured by [CELL COUNTING & VIABILITY] of brightfield mode of LUNA-FX7™ was R^2=0.9964, which showed high agreement with the theoretical survival rate.

A



B

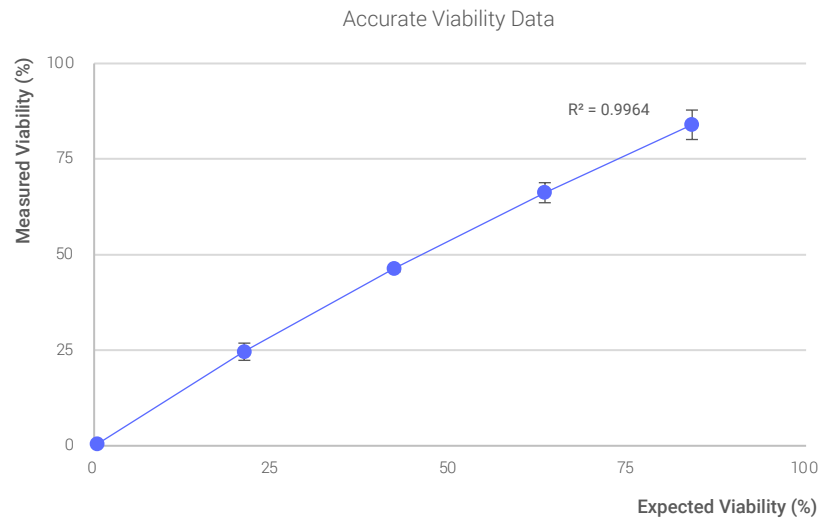


Figure 1. *Cell viability determined with [CELL COUNTING & VIABILITY] of brightfield mode of LUNA-FX7™ Automated Cell Counter. (A) It shows images of yeast cells stained with methylene blue and tagged. (B) The measured viability and the theoretically expected viability were confirmed with linear graph. (R^2=0.9964)*

In addition, to investigate viability in fluorescence, five yeast samples were stained with AO/PI, and 10ul was loaded onto a PhotonSlide™. [CELL LINES & PRIMARY CELLS] had been the only option in the fluorescence mode of LUNA-FX7™. Through the last software update, a new fluorescence mode was added [CELL LINES & PRIMARY CELLS, ADVANCED] to provide better results for cells with a lot of aggregation such as yeast. After AO/PI staining, images were acquired and observed as shown in Figure 2A. As seen in Figure 2B, five different viability samples were counted 3 times, and even in [ADVANCED] mode, the measured viability of yeast cells was R^2=0.9979, which had a significant correlation with the theoretical viability.

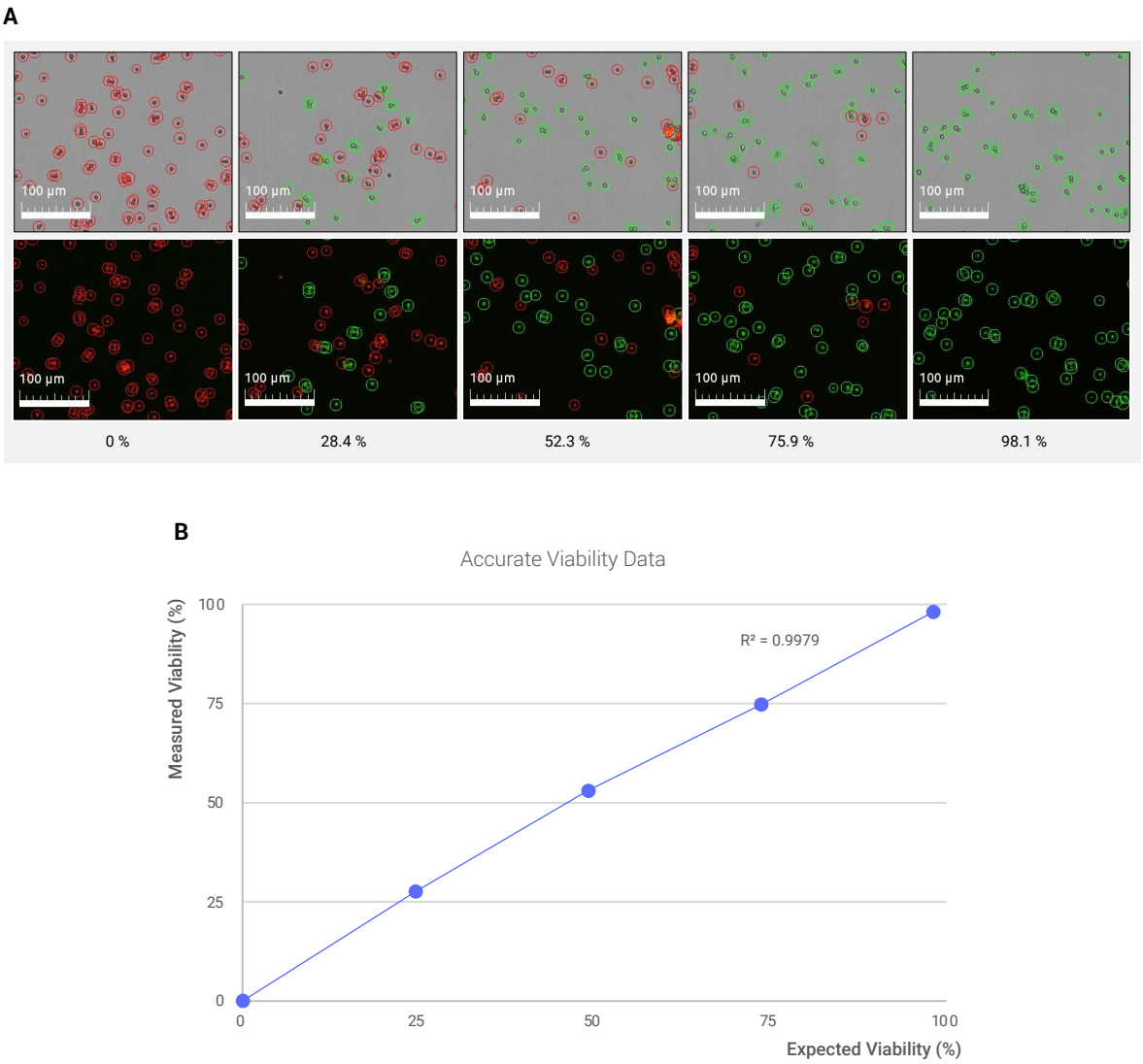


Figure 2. *Cell viability determined with [CELL LINES & PRIMARY CELLS, ADVANCED] of fluorescence mode of LUNA-FX7™ Automated Cell Counter. (A) It shows images of yeast cells stained with acridine orange/propidium iodide and tagged. (B) The measured viability and the theoretically expected viability were confirmed with linear graph. (R^2=0.9979)*

Linearity of concentration analysis of the LUNA-FX7™ Automated Cell Counter

To determine the linearity at various concentration in counting yeast using LUNA-FX7™ Automated Cell Counter, high concentration yeast was prepared, and the concentration was measured by serial dilution. For observation in

brightfield mode, it was stained with 0.02 % methylene blue, and counted in [TOTAL CELL COUNTING] and [CELL COUNTING & VIABILTIY] in brightfield mode, respectively. As can be seen in Figure 3, the R^2 value were measured to be 0.998 and 0.9991, respectively. This is similarly observed in the fluorescence mode, and when it was stained with AO/PI and captured in [ADVANCED] mode, as shown in figure 4, the R^2 value was 0.9753. This means that yeast counting using LUNA-FX7™ shows high linearity from high to low concentrations, and it means that more accurate measurement is possible with LUNA-FX7™.

Through these experiments and data from the previous application note [An analysis of optimal cell counting range of the LUNA-FX7™], we can think about the limit of quantification about yeast cells. The average size of yeast cells is 3~4 μm, and the size of a typical mammalian cell is 10μm. Therefore, compared to the range of 5.00E+4 to 1.50E+7 cells/ml for mammalian cells in the 2-ch slide, it can be adjusted to 5.00E+5 to 3.00E+7 cells/ml for yeast cells. Because the cell size differs by more than 2 times, it is impossible to count at a low concentration of 5.00E+4 cells/ml as in mammalian cells, but due to the size difference, it is possible to count cells with a high concentration that is twice as high as 1.50E+7 cells/ml.

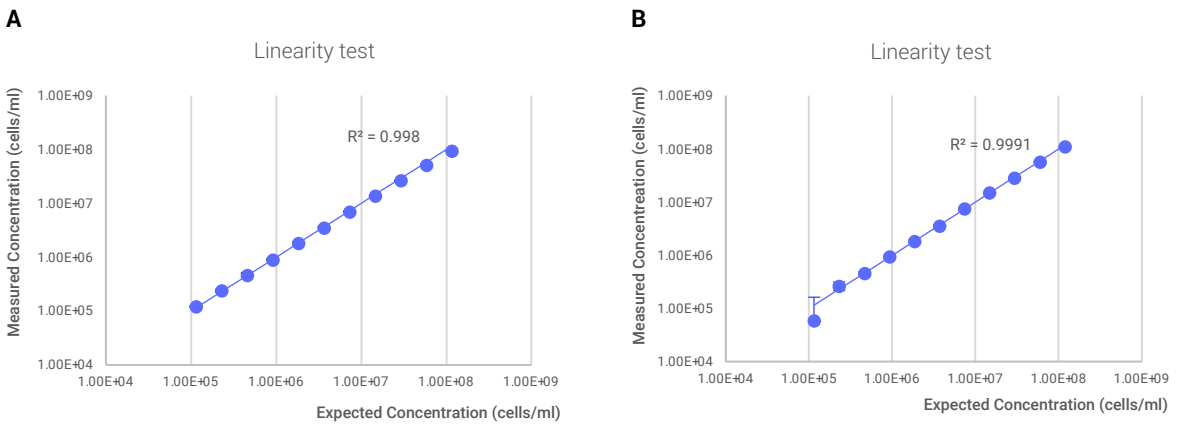


Figure 3. *Concentration of yeast sample measured in brightfield mode of LUNA-FX7™ Automated Cell Counter. (A) [TOTAL CELL COUNTING] (B) [CELL COUNTING & VIABILITY]*

Figure 4. *Concentration of yeast sample measured in [CELL LINES & PRIMARY CELLS, ADVANCED] of fluorescence mode of LUNA-FX7™ Automated Cell Counter.*

LUNA-FX7™ Automated Cell Counter vs. Hemocytometer

An experiment was conducted to compare the LUNA-FX7™ and hemocytometer, which have been traditionally used. As in the previous experiment, the sample was stained with 0.02 % methylene blue and AO/PI, and 10 µl each was loaded on a hemocytometer, LUNA™ Cell Counting Slide and PhotonSlide™. The images were taken in two modes of brightfield and fluorescence mode of LUNA-FX7™, and the concentrations were compared with those measured on a hemocytometer. As shown in Figure 5, no significant difference was observed between the values measured by the hemocytometer and the values measured by the LUNA-FX7™. When comparing the values in the hemocytometer and each mode, the P values are all over 0.05, and there is no statistically significant difference.

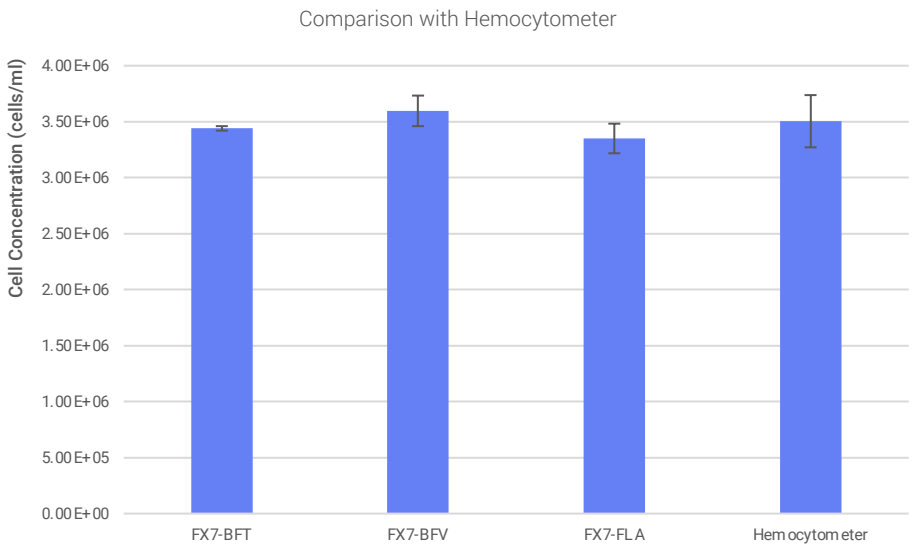


Figure 5. *Comparison of yeast counting results of hemocytometer and LUNA-FX7™ Automated Cell Counter.*

CONCLUSION

Here, we've shown that the LUNA-FX7™ can accurately and reliably count yeast cells. Further when compared to the traditional manual method, counting yeast with the LUNA-FX7™ is 20X faster, more reliable, and less prone to error.

REFERENCES

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