# Application Note

Peripheral Blood Mononuclear Cells (PBMC), rapid evaluation, concentration and viability
AO/PI staining, dual fluorescence, 1, 2, 3, 8-channel slide options

# Rapid evaluation of PBMC counting and viability

## INTRODUCTION

Peripheral Blood Mononuclear Cells (PBMCs) are a vital source material used in myriad research applications, including single-cell sequencing to vaccine development and toxicological studies. Furthermore, PBMC derivatives such as T cells, B cells, NK cells, and stem cells are based on cell therapies, including CAR-T cell therapies and regenerative medicines. Therefore, accurately measuring numbers and viability of PBMCs after collection, isolation, or expansion are essential to making experimental or manufacturing decisions about downstream processes. Yet, directly obtaining leukocyte counts in whole blood using traditional counting techniques is complicated by the presence of mature, anucleated RBCs and platelets. Advantageously, the use of nucleic acid stains like Acridine Orange and Propidium Iodide (AO/PI) allow the nucleated leukocytes to be differentiated and accurately counted within a whole blood sample (Figure 1). Here, we exhibit the ability of the new dual fluorescent LUNA-FX7™ Automated Cell Counter to meet diverse cell counting needs by demonstrating its use in counting leukocytes in whole blood and the PBMCs isolated from whole blood samples.



*LUNA-FX7™ Automated Cell Counter*
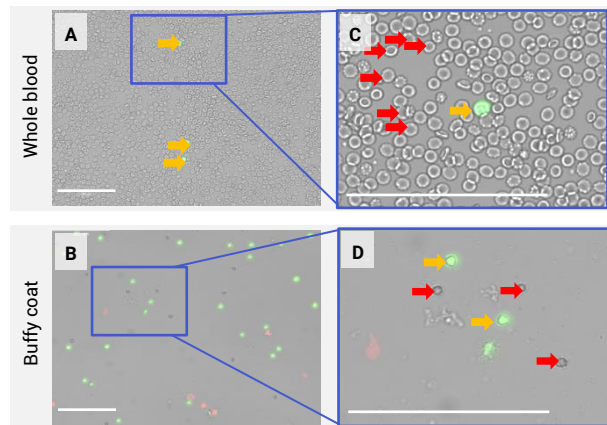


**Figure 1.** *Diluted whole blood and PBMC-enriched buffy coat stained using AO/PI fluorescent dye. The microscopic overlay images of the whole blood stained with AO/PI (A,C), and PBMCs from enriched buffy coat stained with AO/PI (B,D) were acquired using the CELENA® X High Content Imaging System with a 20X fluorite objective (www.logosbio.com). The yellow arrows indicate nucleated cells in AO/PI positive stained cells, while the red arrows indicate RBCs. The scale bar is 100 µm.*

## MATERIAL AND METHODS

One milliliter of the human peripheral blood sample was prepared, and the PBMC sample was obtained by standard density gradient centrifugation technique using Histopaque-1083 (Sigma, #10831)[1]. After final washing, the PBMCs enriched in the buffy coat were resuspended in 100 µl PBS or RPMI +10% FBS media. Cell counts were performed in

the LUNA-FX7™ with either the 2-channel PhotonSlide™ (Cat# L12005) or LUNA™ 8-Channel Slides (Cat# F72001) and used a modified default protocol in the Fluorescence Cell Counting mode (Table 1). Before loading the cells, cells were stained at the standard ratio of the AO/PI reagent (Cat# F23001); 18 µl of cells + 2 µl of AO/PI, and then 10 µl of the mix was loaded into a slide chamber.
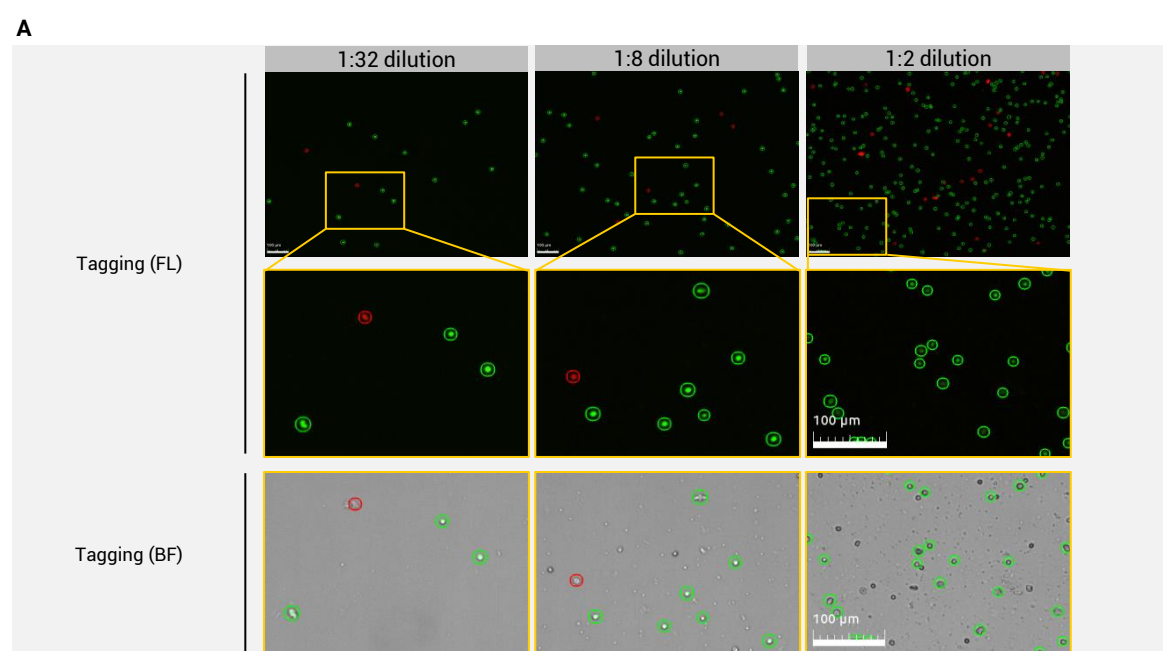
## RESULTS

### Evaluating the PBMC concentration and viability with the LUNA-FX7™

The separated PBMCs were analyzed by counting a series of 2-fold dilutions using both the 8-channel and 2-channel slides (Figure 2). The counts using both slide types showed linearity with an R-square value of 0.99 or above on the logarithmic scale of concentrations over the serial dilutions. Not surprisingly, with a greater volume of analysis, the 2-channel slide showed slightly better consistency than the 8-channel slide.

**Table 1.** *The optimized parameter settings for PBMC or leukocytes counting of the LUNA-FX7™ on Fluorescence Cell Counting mode*

| Counting mode | Fluorescence Cell Counting |
|---|---|
| GF exposure level | 5 |
| RF exposure level | 5 |
| Cell size calculation | FL* |
| Min. cell size | 4* |
| Max. cell size | 70 |
| GF threshold level | 5 |
| RF threshold level | 5 |
| Dilution factor | 1.11 |

*\* Changed from the DEFAULT values.*

**A**
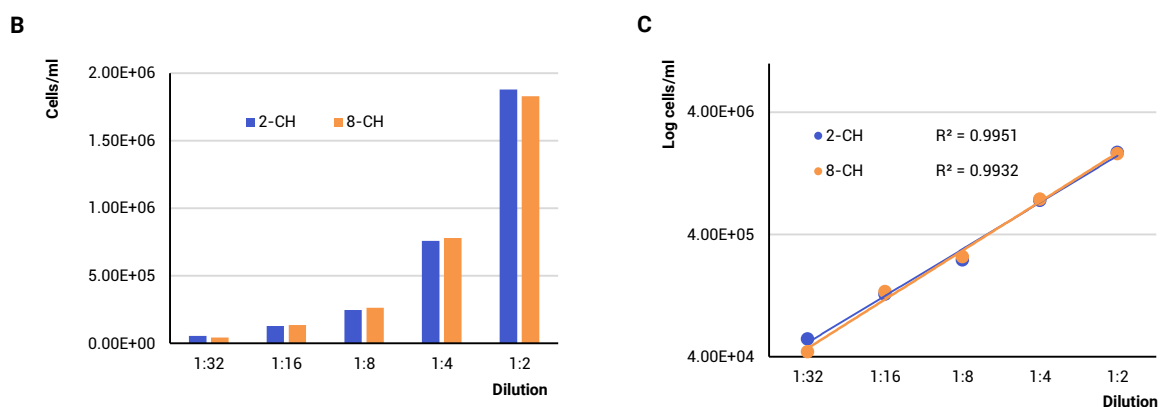
**Figure 2.** *The linearity of counting in serial dilutions of PBMCs. (A) Tagged (live or dead) fluorescent and brightfield overlay of several dilutions. (B) Bar graph showing the results of 5 serial dilutions. (C) The logarithmic scale of the counts over the dilutions using both 2-channel and 8-channel slides down to concentrations less than 4.00E+04 cells/ml. The scale bar represents 100 µm.*

### The easy counting of leukocytes in whole blood

The leukocytes in whole blood were visualized by fluorescence and also accurately enumerated with the LUNA-FX7™. Among countless mature RBCs, the fluorescence leukocytes were distinctively counted from diluted whole blood (Figure 3).

**B**



**C**
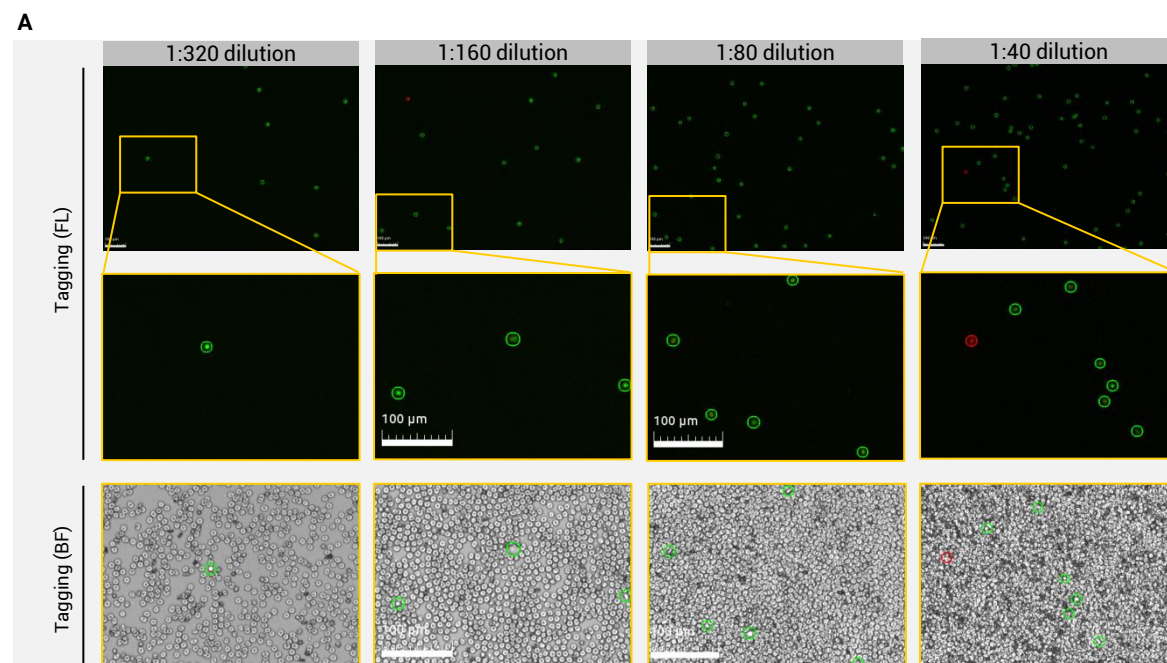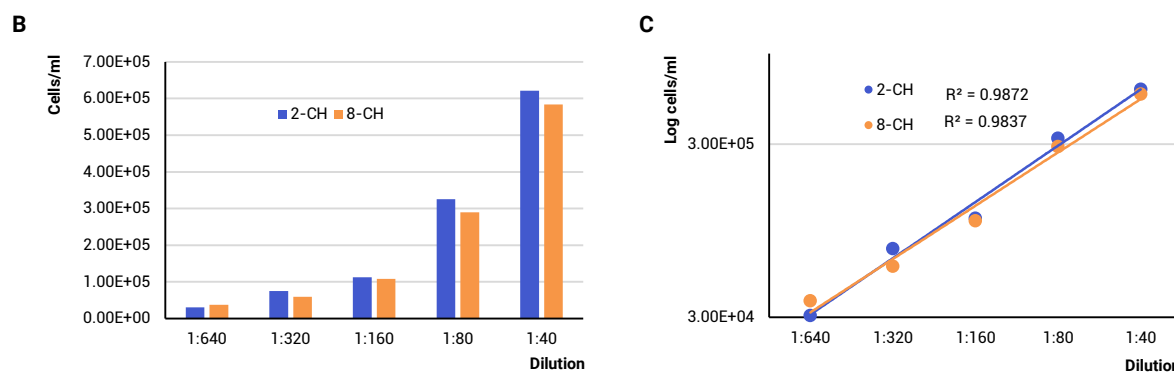


*Figure 3. The Leukocyte count of whole blood on Fluorescent Cell Counting mode in the LUNA-FX7™. (A) Tagged leukocyte images of fluorescent and brightfield overlay in serial dilutions of whole blood samples. (B) Bar graph displaying counting of 5 serial dilutions from 1:40 to 1:640 of whole blood cells. (C) The linearity of counts appears across concentration on both 2-channel and 8-channel slides. The scale bar represents 100 µm.*

## CONCLUSION

The enumeration of PBMCs was evaluated adequately on the LUNA-FX7™ Automatic Cell Counter with the 2-channel PhotonSlide™ and LUNA™ 8-Channel Slides. With more slide options for the LUNA-FX7™, LUNA™ 1-Channel Slides (Cat# L72011) may be applied for a more comprehensive concentration range by analyzing 47 image fields, and LUNA™ 3-Channel Slides (Cat# L72021) are beneficial for preset of triplicate analysis as counting options. Indeed, leukocyte counting is much more straightforward by loading the diluted blood after AO/PI fluorescence staining. So, you can use the LUNA-FX7™ to respect the archived records of PBMCs along with leukocyte counts without intensive labor.

## REFERENCES

[1] S Parasuraman, R Raveendran, and R Kesavan Blood sample collection in small laboratory animals J Pharmacol Pharmacother. 2010 Jul-Dec; 1(2): 87−93. doi: 10.4103/0976-500X.72350

Find out more at https://logosbio.com/automated-cell-counters/fluorescence/luna-fx7