

# Counting Peripheral Blood Mononuclear Cells Using the Corning® Cell Counter — A Brightfield Alternative to Manual Counting

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## Application Note

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### Introduction

#### About Peripheral Blood Mononuclear Cells (PBMCs)

Whole blood is collected from a donor and PBMCs are separated and saved while the remaining blood cells and components are returned to the body (apheresis)<sup>1</sup>. This process yields large amounts of PBMC which are suitable for further experimentation or study.

#### Counting of PBMCs

PBMCs are smaller in size than many mammalian cell types, and depending on the sample, may contain erythrocyte (red blood cell, RBC) contamination. It is recommended that RBCs are removed by centrifugal separation or lysis prior to counting with the Corning Cell Counter.

PBMCs are often counted using time-consuming manual methods such as a hemocytometer. While the hemocytometer has been considered the gold standard for cell counting since the 18<sup>th</sup> century<sup>2</sup>, results are frequently subjective, variable, and small differences in cell diameters cannot easily or reproducibly be discerned.

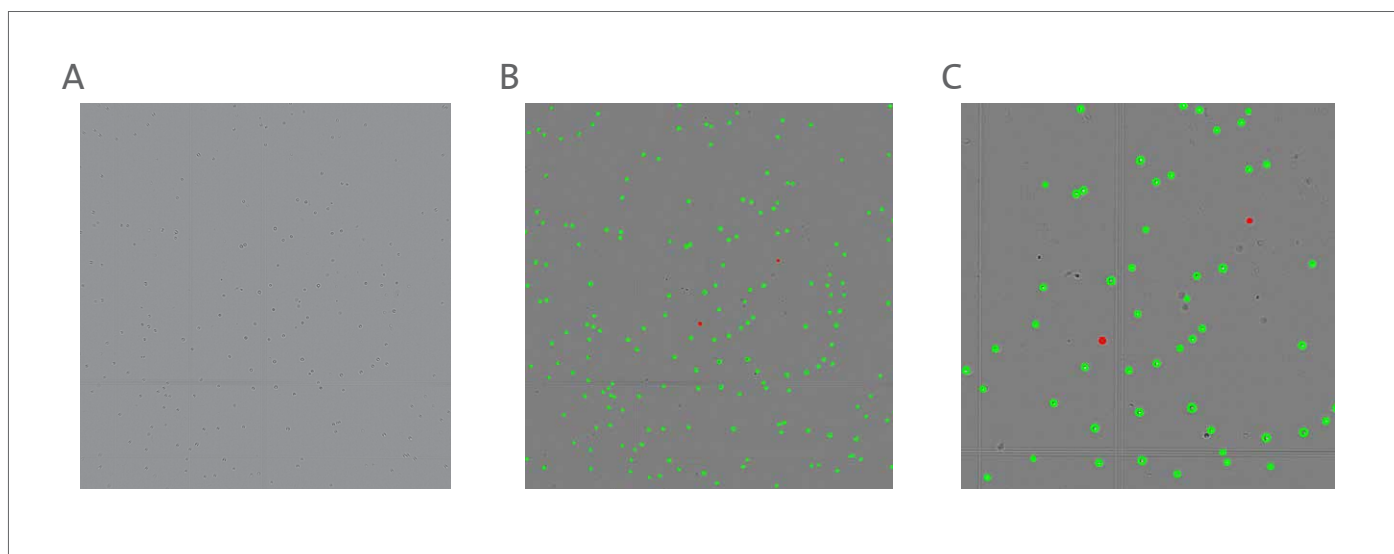
The Corning Cell Counter offers an alternative method to quickly and accurately count these cells. The counting algorithm was developed to identify and remove debris and account for dead cells, while the functionality of the application allows for the population to be gated to remove larger cells and debris from the final count.

### Materials and Methods

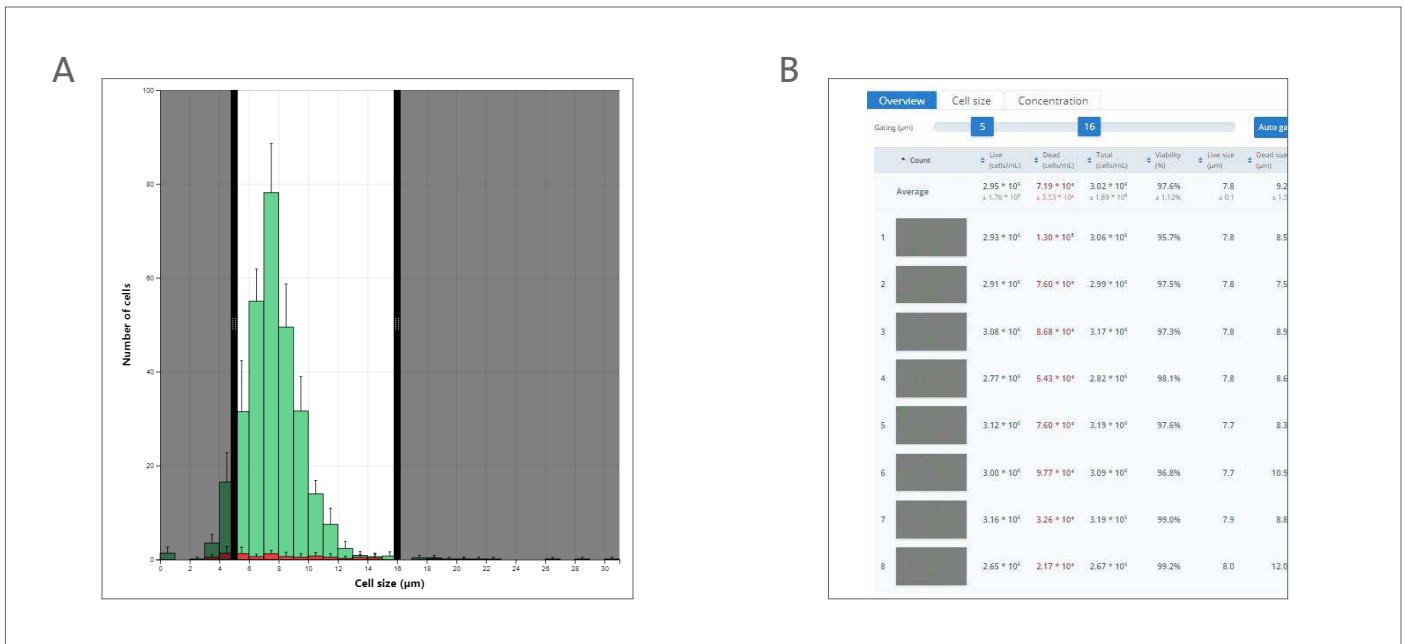
Cryopreserved PBMCs (without RBCs) from two donors (All Cells; Alameda, California) were counted on two different days using the Corning Cell Counter vs. manual counting using a hemocytometer.

Cells were counted on the Corning Cell Counter, then immediately manually counted, and viability was determined using a hemocytometer and the trypan blue exclusion method. Representative images of the area counted (1.96 mm<sup>2</sup>) are shown in Figure 1.

The Corning Cell Counter multicount feature was used (up to eight fields may be sampled as part of one count). In this experiment, eight different fields per count were sampled using the Corning Cell Counter (total area = 15.68 mm<sup>2</sup>), while the manual method counted five large squares of the hemocytometer (total area = 5 mm<sup>2</sup>).



**Figure 1.** Representative Corning Cell Counter images after focusing and after counting. (A) Properly focused image before count. (B) Results of count. Live cells (circled green); dead cells (circled red). Small cells and debris may be excluded from counting by the algorithm and by user-specified gating (example in Figure 2). (C) Magnified view of panel B.



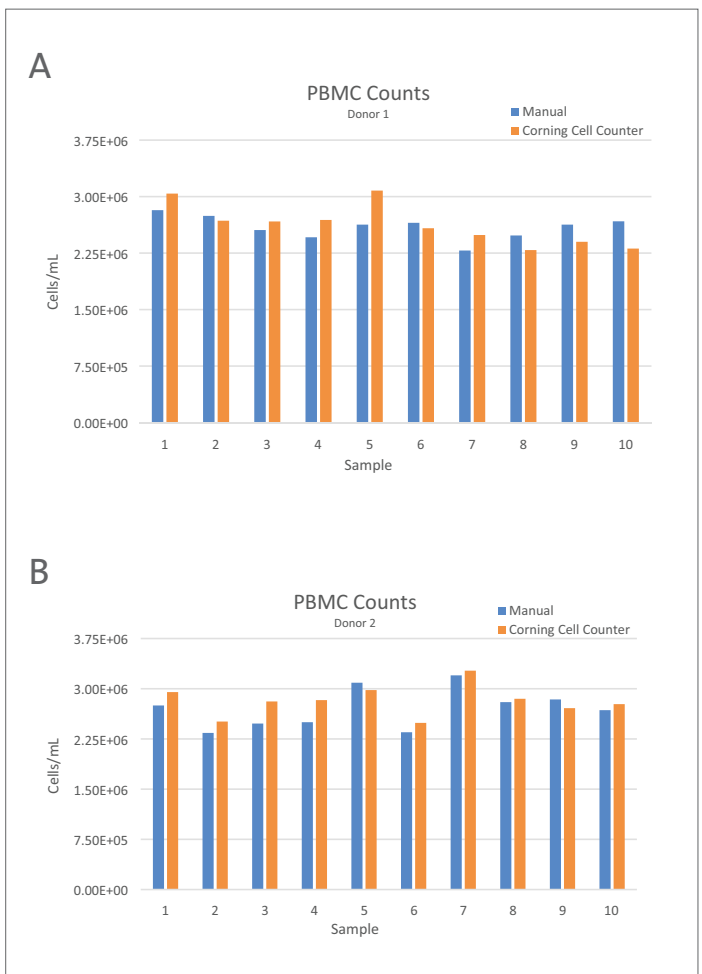
**Figure 2.** (A) Histogram showing gates set at 5 to 16 µm cell diameter. Cells outside this threshold were excluded from the count. Dead cells (red bars) and live cells (green bars) may be toggled to independently examine those subpopulations. Average ±SD of eight counts in one sample is shown; multicount functionality was used as described. (B) Expanded data available from use of multicount feature.

### Results and Discussion

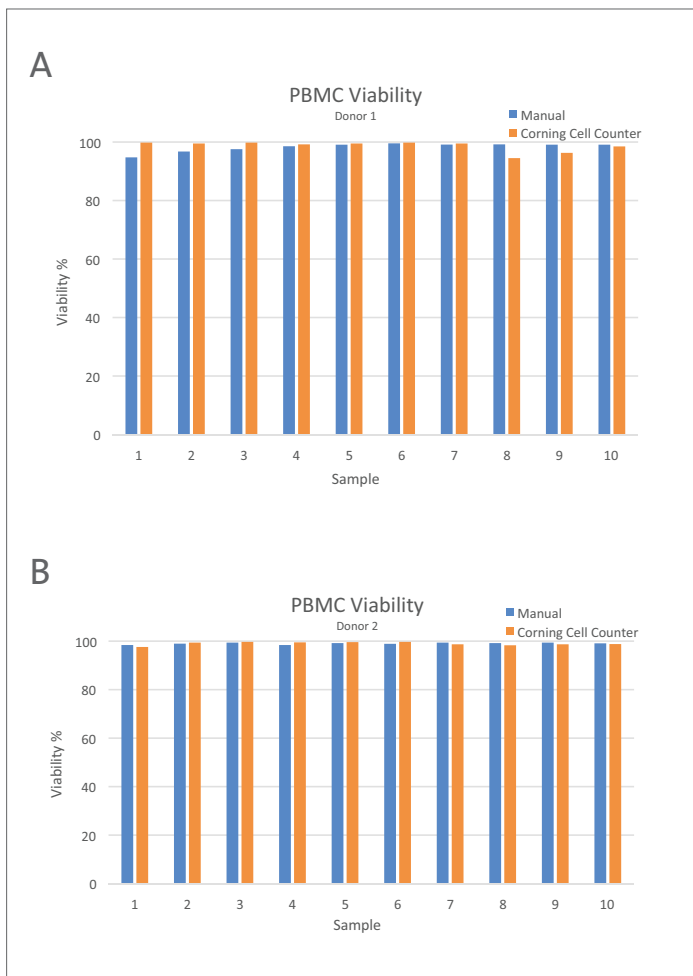
Using the *post hoc* histogram capability in the CytoSMART™ app or CytoSMART cloud, the gates were set at 5 to 16 µm (Figure 2). For the experimental data generated during this evaluation, the results of two donors over 10 independent samples each are shown in Figures 3 and 4.

When comparing the two counting methods using a Paired t-test, p values for both cell densities and percent viability were greater than 0.05, indicating there were no statistical differences between each data set. These results indicate the suitability of the Corning® Cell Counter for accurate counting of PMBCs by a trained operator.

It is important when counting PMBCs to avoid overcounting dead cells that are not cells. To be counted as a dead cell on the Corning Cell Counter, the cell must appear dark in all fields as in Figure 5. Additionally it is important that RBCs are not present in the sample to be counted as this may reduce the accuracy of the count.



**Figure 3.** Cell densities by count method (charts separated by donor). Paired t-test of combined donors p = 0.141.



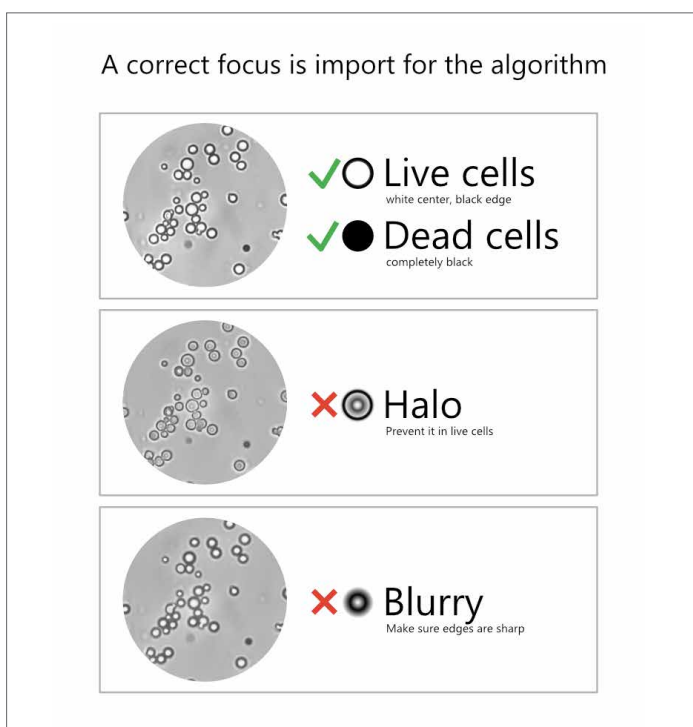
**Figure 4.** Cell viabilities by count method (charts separated by donor). Paired t-test of combined donors  $p = 0.718$ .

## Conclusions

- ▶ The Corning® Cell Counter may be used in PBMC counting where accurate cell counting is required, and is intended as a replacement method for manual counting with a hemocytometer.
- ▶ While the Corning Cell Counter is very easy to use, proper focusing techniques need to be observed to avoid subjectivity in counts from different users. With every count, users will gain experience and proficiency, leading to improved accuracy.
- ▶ It is possible to count cell suspensions of a higher density on the Corning Cell Counter than by manual means. Thus, the overall cell population will more accurately be represented, and the associated problem of ‘clicker fatigue’ is avoided altogether.
- ▶ Using the multicount feature is a quick and easy technique to count larger areas compared with manual counting.

## References

1. <https://www.allcells.com/tissue-products/leukopak/>
2. Lin-Gibson S, et al. Summary of the National Institute of Standards and Technology and US Food and Drug Administration cell counting workshop: Sharing practices in cell counting measurements *Cytotherapy*, 2018; 20:785-795.



**Figure 5.** Live vs. dead cell focusing images

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