Corning® Cell Counter Autofocus Functionality for Mammalian Cells Counts

Application Note

Zhang Linyu, Wang Xuebin, and Chen Rui
Corning Incorporated, Life Sciences, Asia Technology Center
Shanghai, China

Introduction

The ability to assess cell viability in a cell population is a fundamental and critical procedure performed in molecular biology research labs.

The Corning Cell Counter is an automated cloud-based cell counter, which utilizes modern optics and intuitive image analysis tools. Its innovative design combines the best qualities of manual cell counting with those of automated image-based cell counting. The counting algorithm was developed to evolve to customer needs. By leveraging its cloud capabilities, enhancements can be implemented throughout the product lifecycle. Added features since launch include but are not limited to histogram graphs, live-gating, PDF project summary exports, enhanced viability detection within clusters, etc. With that, the Corning Cell Counter adds on an autofocus option; the report herein was used as a validating study to measure cell concentrations of mammalian cells accurately and precisely in 10 to 70 µm.

Materials and Methods

Three different cell lines (Vero, K562, and MSC) were used for comparing the accuracy of cell counts using the Corning Cell Counter (Corning 6749) with autofocus vs. manual focus. Vero cells were cultured in DMEM (Corning 10-013-CV) added with 10% FBS (Corning 35-081-CV). K562 were cultured in RPMI-1640 (Corning 10-040-CV) added with 10% FBS. MSCs were cultured in Corning MSC Xeno-free SFM (Corning 88-600-CV).

The adherent cells such as Vero and MSC were dissociated with TrypLE™ Express Enzyme (Thermo Fisher 12604039), then centrifuged at 1,000 rpm for 3 minutes. The pellet was resuspended in PBS to make single cell suspension. K562, a type of suspension cells, were harvested by centrifuging the culture medium, then the pellet was resuspended in PBS.

The cell suspension was mixed with trypan blue (Thermo Fisher T10282) at 1:1 ratio according to the manuals. A 10 µL of mixture was added to the Corning Counting Chamber (Corning 480200). Then the chamber was placed in the adapter plate that has been inserted on the stage of the Corning Cell Counter.

For manual focusing of the cells, the knob was turned clockwise or counterclockwise until optimal focus was met. For autofocusing of cells, the "AF" icon was selected to conduct the cloud-based autofocus of the cell suspension.

In this experiment, three samples of each type of cells were sampled using the Corning Cell Counter's autofocus and manual focus.

Results and Discussion

Counting of Vero cells

Representative images of the area counted (1.39 mm^2) are shown in Figure 1. Using the *post hoc* histogram capability in the Axion app or Axion cloud, the bottom gate was set at 6 µm (Figure 2). Three samples of Vero cells were sampled using the Corning Cell Counter using autofocusing and manual focusing, the results of live cell density and viability are shown in Figure 3. When comparing the counting results acquired by these two focusing methods, the data of live cell density and viability are similar between autofocus and manual focus.

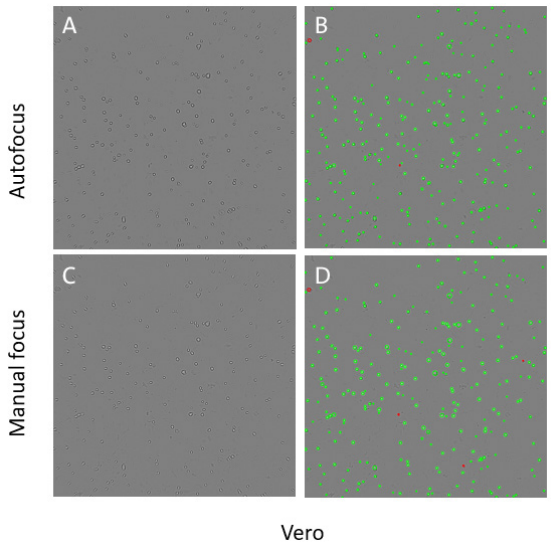


Figure 1. Representative Corning Cell Counter images after autofocus or manual focus and after counting. (A and C) Properly focused image before count. (B and D) Results of count. Live cells (circled green); dead cells (circled red). Small cells and debris may be excluded from counting by the algorithm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

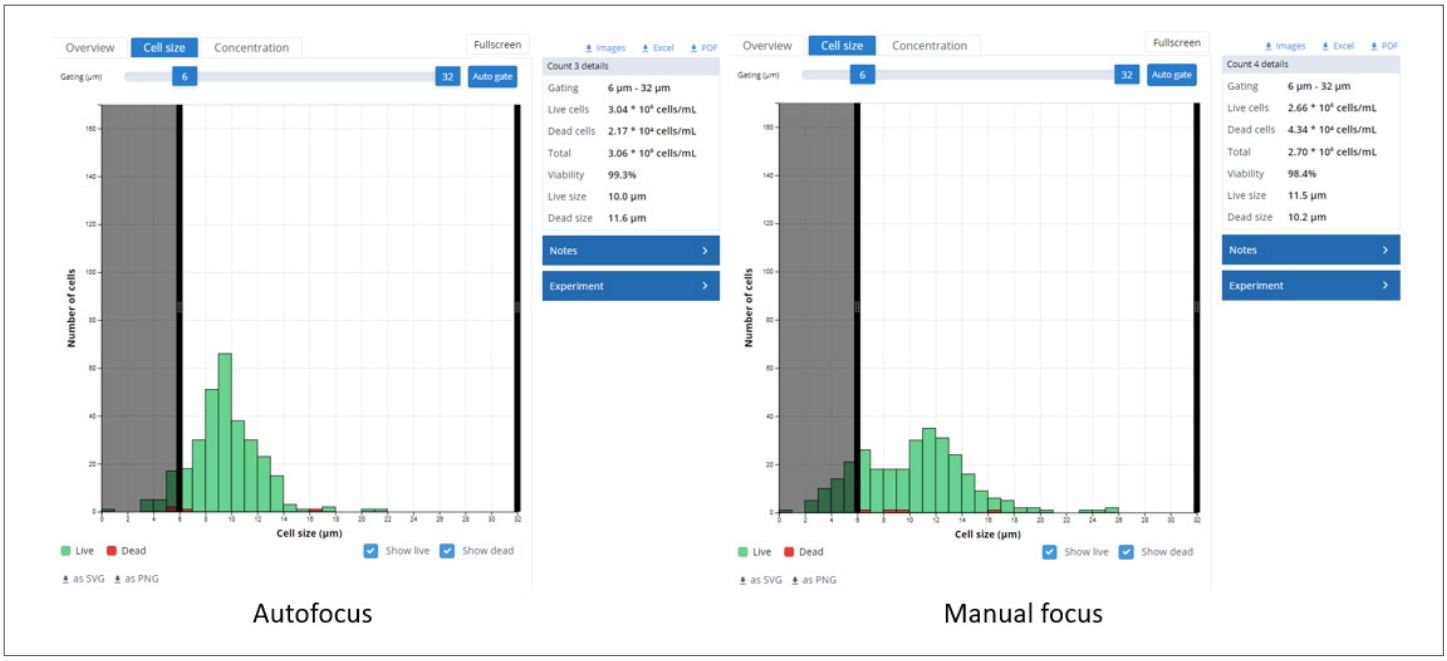
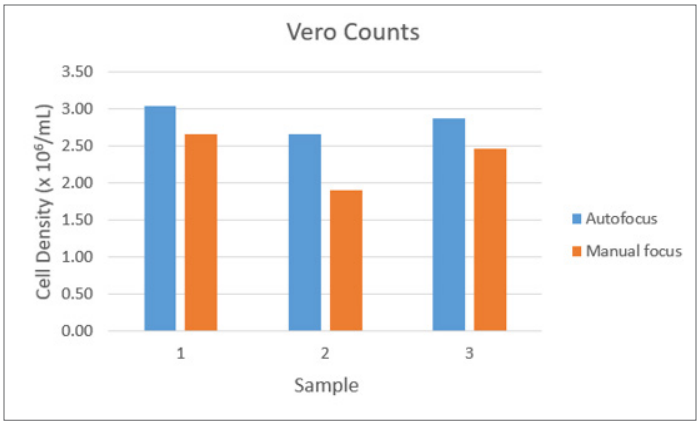


Figure 2. Histogram showing bottom gate set at 6 µm cell diameter. Cells below this threshold were excluded from the count. Dead cells (red bars) and live cells (green bars) can be toggled to independently examine each subpopulation.



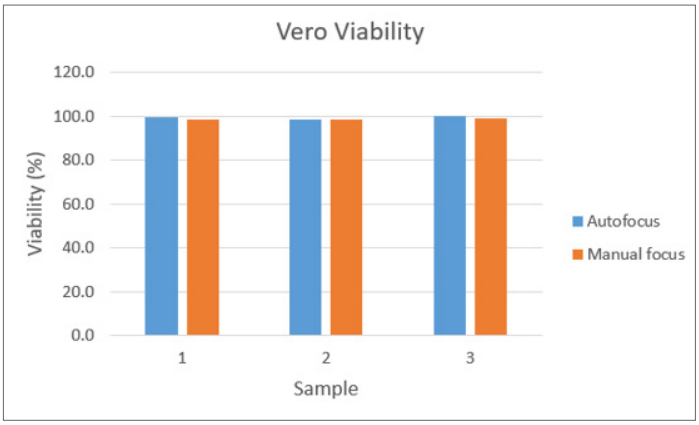


Figure 3. Cell densities and viabilities of Vero by autofocus and manual focus.

Counting of MSCs

Representative images of the area counted (1.39 mm^2) are shown in Figure 4. Using the *post hoc* histogram capability in the Axion app or Axion cloud, the bottom gate was set at 6 µm (Figure 5). Three samples of MSCs were sampled using the Corning® Cell Counter using autofocusing and manual focusing, the results of live cell density and viability are shown in Figure 6. When comparing the counting results acquired by these two focusing methods, the data of live cell density and viability are similar between autofocus and manual focus.

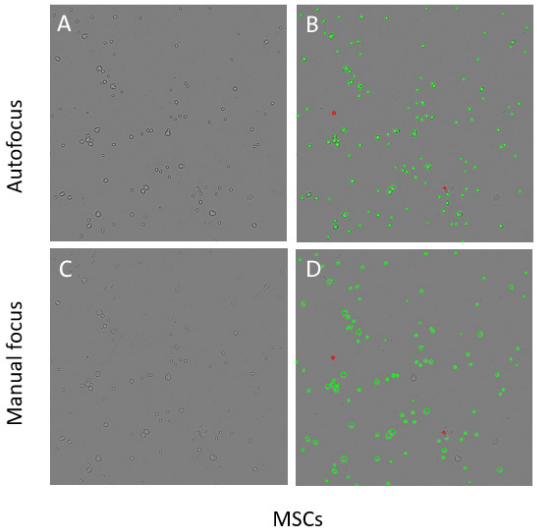
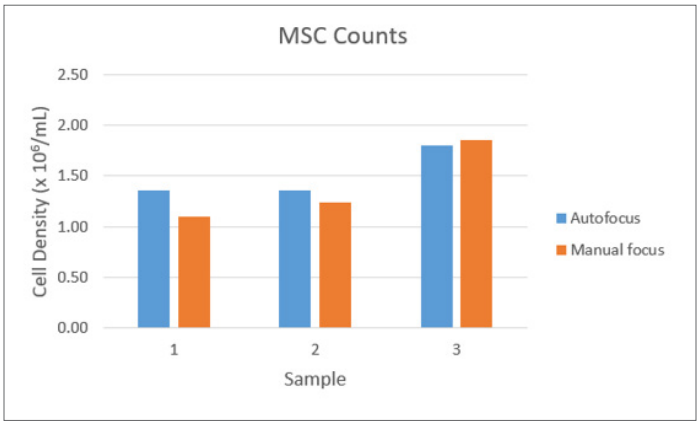


Figure 4. Representative Corning Cell Counter images after autofocus or manual focus and after counting. (A and C) Properly focused image before count. (B and D) Results of count. Live cells (circled green); dead cells (circled red). Small cells and debris may be excluded from counting by the algorithm and by user-specified gating (example in Figure 5).

Figure 5. Histogram showing bottom gate set at 6 µm cell diameter. Cells below this threshold were excluded from the count. Dead cells (red bars) and live cells (green bars) can be toggled to independently examine each subpopulation.



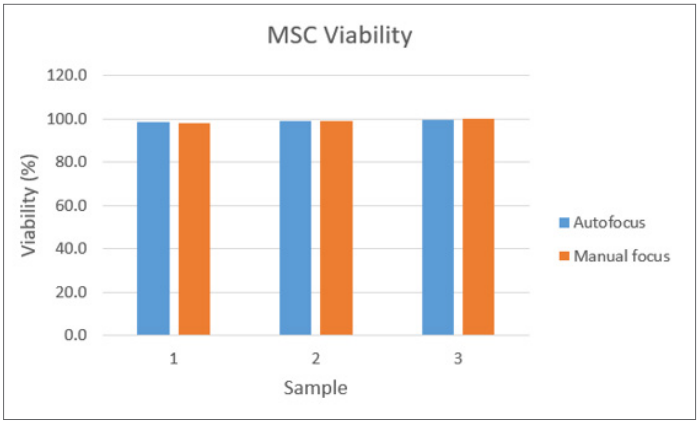


Figure 6. Cell densities and viabilities of MSCs by automatic and manual focus.

Counting of K562 cells

Representative images of the area counted (1.39 mm^2) are shown in Figure 7. Using the *post hoc* histogram capability in the Axion app or Axion cloud, the bottom gate was set at 5 µm (Figure 8). Three samples of K562 Cells were sampled using the Corning® Cell Counter using autofocusing and manual focusing, the results of live cell density and viability are shown in Figure 9. When comparing the counting results acquired by these two focusing methods, the data of live cell density and viability are similar between autofocus and manual focus.

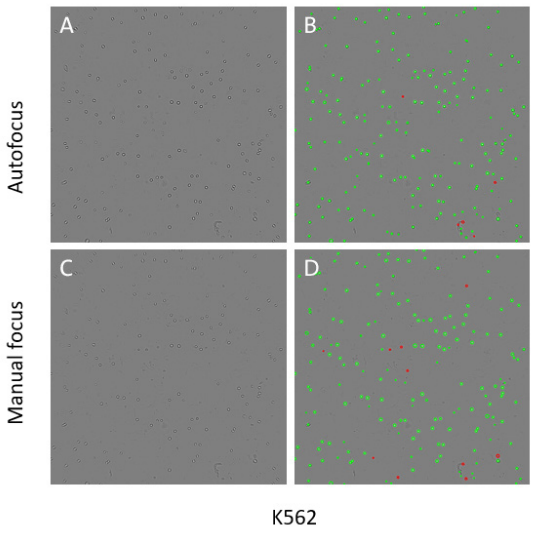


Figure 7. Representative Corning Cell Counter images after autofocus or manual focus and after counting. (A and C) Properly focused image before count. (B and D) Results of count. Live cells (circled green); dead cells (circled red). Small cells and debris may be excluded from counting by the algorithm and by user-specified gating (example in Figure 8).

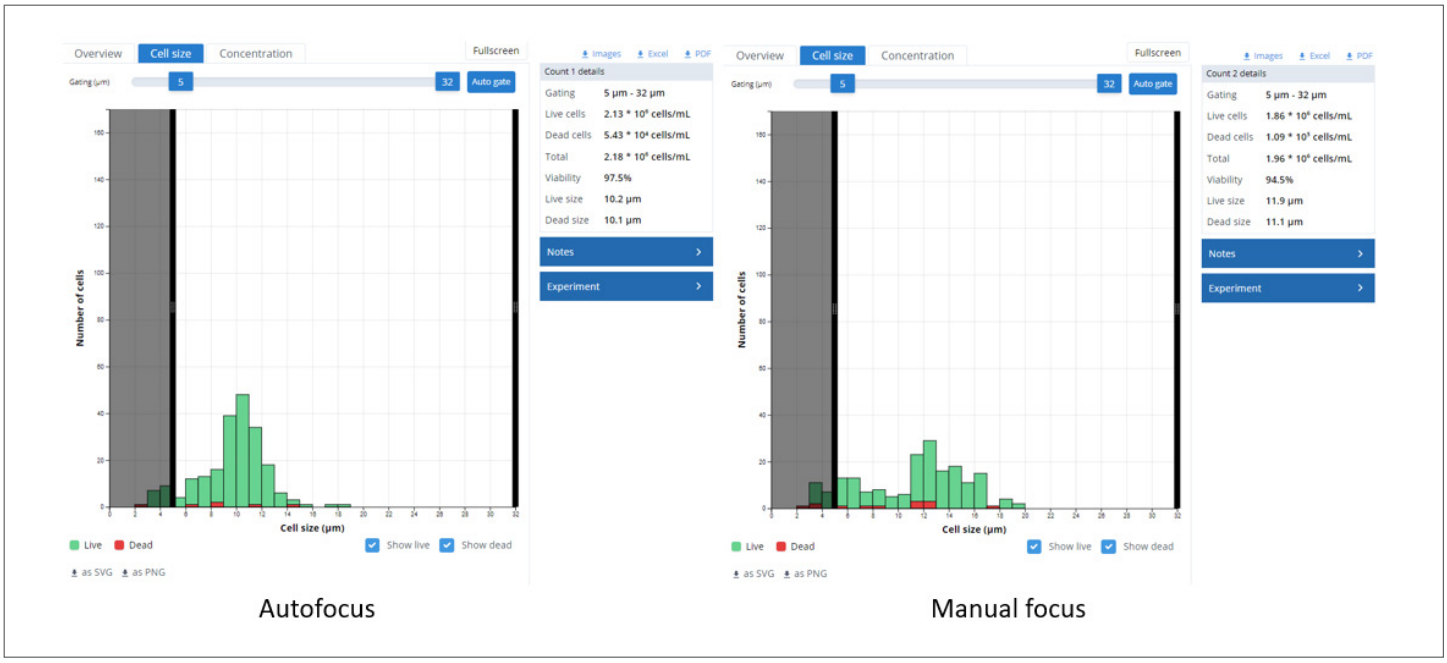
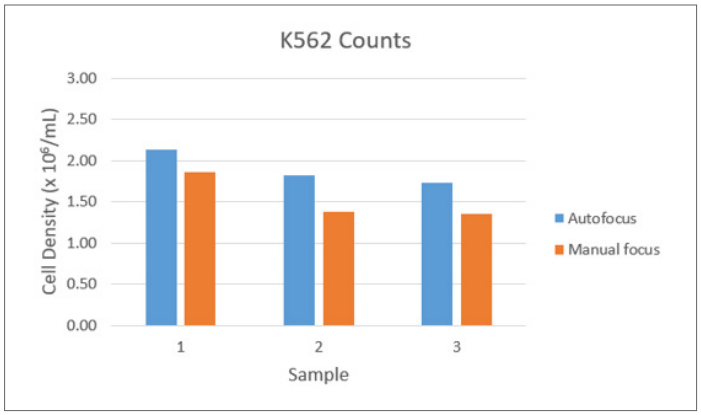


Figure 8. Histogram showing bottom gate set at 5 µm cell diameter. Cells below this threshold were excluded from the count. Dead cells (red bars) and live cells (green bars) can be toggled to independently examine each subpopulation.



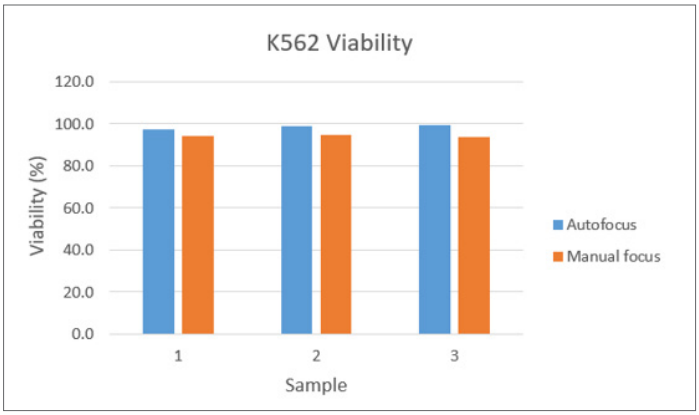


Figure 9. Cell densities and viabilities of K562 by autofocus and manual focus.

Conclusions

- The Corning® Cell Counter provides fast, accurate measurements of cell viability and concentration of mammalian cells.
- The results of cell density and viability are similar between autofocus and manual focus with a cell size ranging from 10 to 70 µm.
- The autofocus functionality is easy-to-use and can further save time and decrease user-to-user variability.

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