

Recommended procedure for comparison of cell concentration and viability using the Cellometer Auto 2000 automated cell viability counter and a manual hemacytometer.

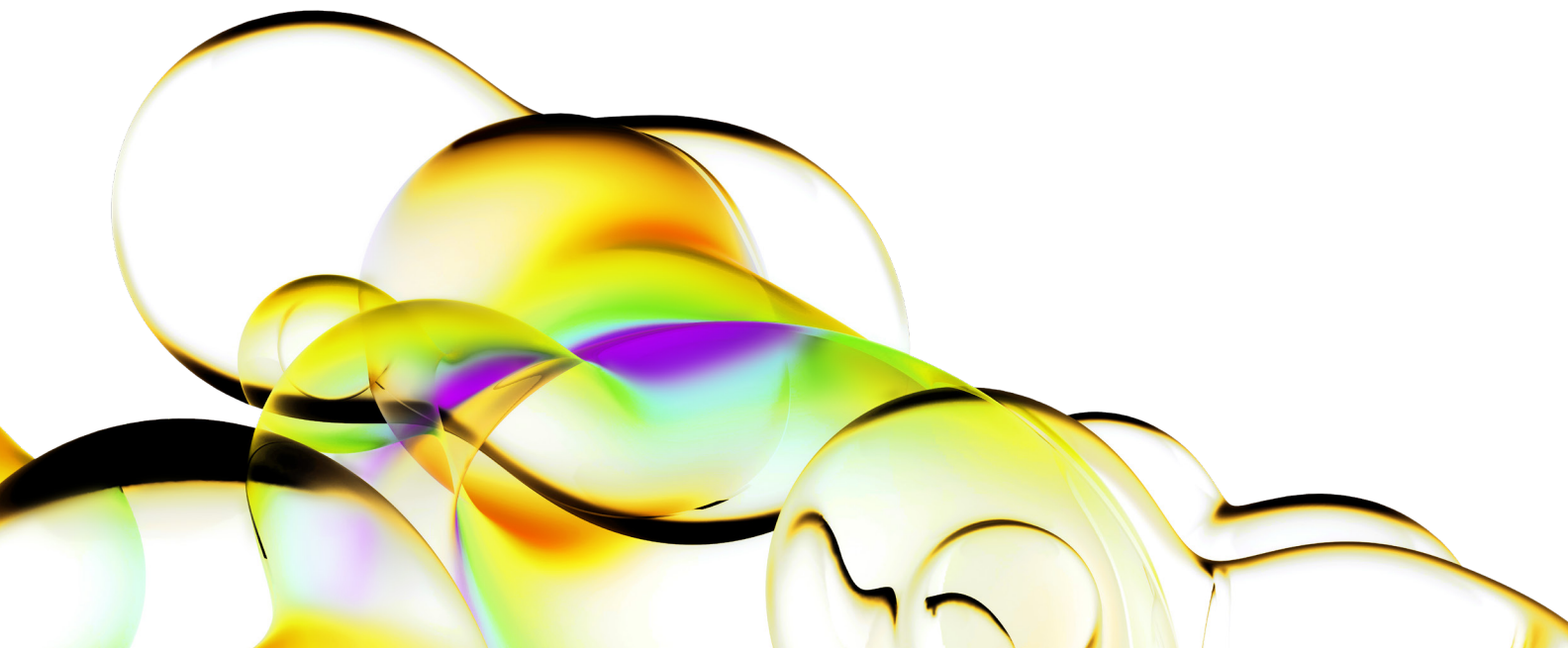
For researchers performing manual cell counting, a key factor involved in evaluation of an automated cell counter is comparison to their current method. Based on our experience supporting thousands of Cellometer® customers in the field, we have designed the following protocol to make comparison of the Cellometer Auto 2000 Cell Viability Counter to manual hemacytometer results as accurate as possible. *For the comparison to be valid, Cellometer Auto 2000 and hemacytometer counts must be done at the same time using aliquots from the same cell sample.*

Materials required:

- 200 μL cell sample (concentration of $\sim 1 \times 10^6$ cells/mL)
- 0.2% trypan blue staining solution
- 2 Cellometer Counting Chambers (CHT4)

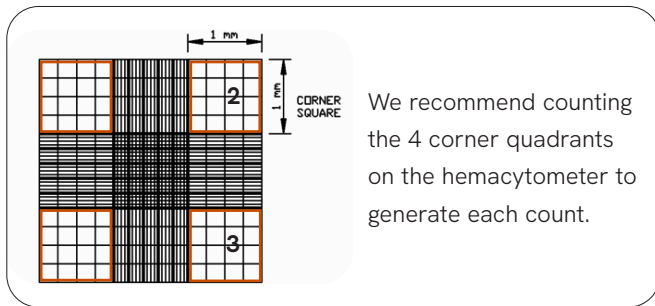
Cell preparation:

1. Invert stock cell solution 10x
2. Pipette gently up and down 10x
3. Transfer 50 μL of the stock cell solution into a sterile microcentrifuge tube for the "hemacytometer cell sample". Transfer 50 μL of the stock cell solution into a second sterile microcentrifuge tube for the "Cellometer Auto 2000 cell sample." These "cell samples" will be the only samples used to compare counts between the hemacytometer and Cellometer Auto 2000.



Cell concentration and viability using hemacytometer:

1. Pipette up and down gently 10x to break any potential cell clumps in the "hemacytometer cell sample"
2. Add 50 µL of Trypan blue staining solution to the "hemacytometer cell sample". The dilution factor is now 2. *The sample must be counted within 5 minutes.*
3. Load 10 µL onto the hemacytometer
4. Count all 4 quadrants and record the live and dead cell counts:



5. Use the equation below to calculate the cell concentration:

$$\frac{\text{Total cell count}}{4 \text{ Squares counted}} \times 10^4 \times \text{Dilution factor} = \text{Cell concentration (cells/mL)}$$

6. Use the equation below to calculate % viability:

$$\frac{\text{Live cell count}}{(\text{Live cell count} + \text{dead cell count})} \times 100\% = \% \text{ Viability}$$

7. Repeat procedure 3 additional times for a total of four counts.

8. Calculate the Standard Deviation for total cell concentration using the calculations below:

$$[(\text{Count 1} - \text{average})^2] + [(\text{Count 2} - \text{average})^2] + [(\text{Count 3} - \text{average})^2] + [(\text{Count 4} - \text{average})^2] = x$$

Divide the sum (x) by 4. Take the square root of this number. This is the standard deviation.

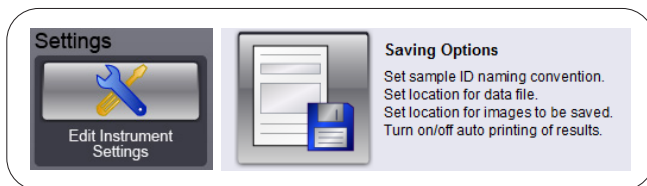
9. Calculate the Coefficient of Variation. CV should be less than 15%. If the CV is >15%, the experiment should be repeated with a fresh cell sample.

$$\frac{\text{Standard Deviation}}{\text{Average Cell Concentration}} \times 100\% = \% \text{ CV}$$

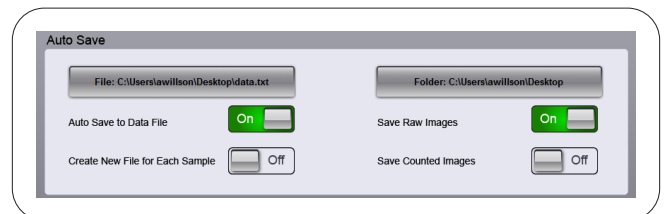
Sample #	Live Cell Count	Dead Cell Count	Total Cell Count	# squares counted	Total cell concentration	% Viability

Cellometer Auto 2000 set-up

1. Start Cellometer Auto 2000
2. Click on the Settings button, then the Saving Options button.



3. Click "Auto Save to Data File" and "Save Raw Images" to the "On" position. Click on the File and Folder buttons. In each case, select the USB drive and name the folder. Having saved images will help if any remote technical support is required and provides a permanent record of the raw data.

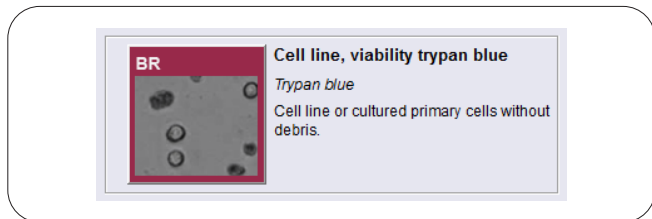


- Click "Done", then click "Done" again.

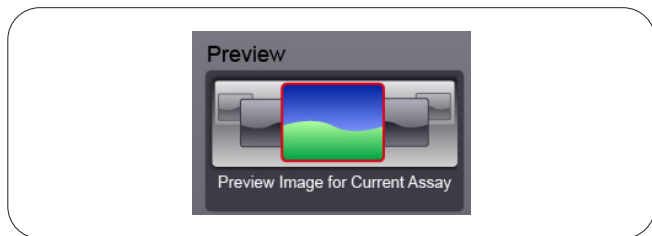


Cell concentration and viability using Cellometer Auto 2000

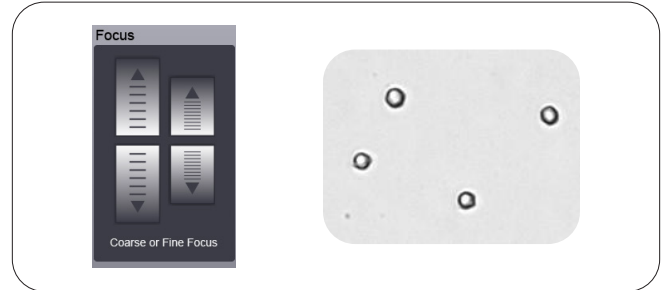
- Pipette up and down gently 10x to break any potential cell clumps in the "Cellometer Auto 2000 cell sample"
- Add 50 μ L of trypan blue staining solution to the "Cellometer Auto 2000 cell sample." The Dilution Factor is 2.
- Gently pipette up and down again 10x.
- Load 20 μ L of sample into the Cellometer Counting Chamber (CHT4)
- Insert counting chamber into instrument.
- Click on the "Cell line, viability trypan blue" assay. When prompted, enter your desired Sample ID and a dilution factor of 2.



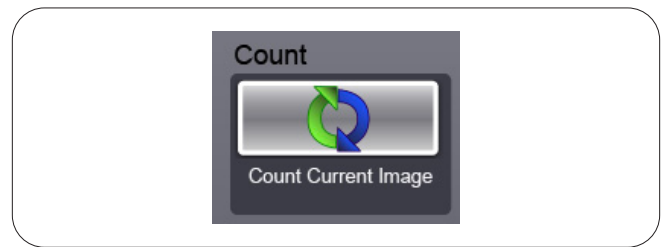
- Click on "Preview Image for Current Assay"



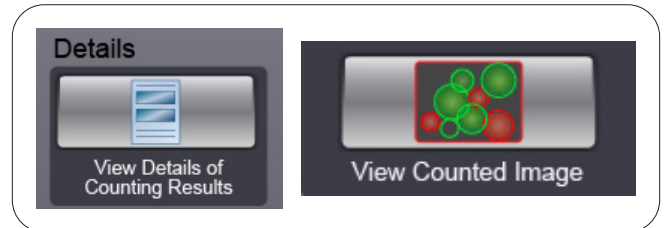
- Adjust the focus if necessary using the coarse and fine adjustments on the left-hand side of the screen. Cells in focus will have a bright center and crisp edge.



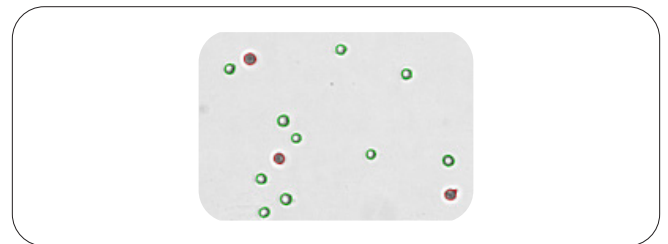
- Click the Count button at the bottom of the screen.



- Click on the "View Details" button at the bottom left of the screen, then click on the "View Counted Image" button on the left-hand side of the screen.



- Review the counted image. Live cells should be circled in green. Dead cells, stained dark with trypan blue, should be circled in red.



- Record cell concentration and viability data from Cellometer Auto 2000. Repeat 3 additional times (with fresh 20 μ L aliquots of stained sample) for a total of four values.

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- Remove the current slide from the Cellometer Auto 2000 and insert a new chamber containing the next 20 μL sample. Click on the "Next Sample" button at the bottom right of the screen. When prompted, enter the Sample ID, then click "Count".



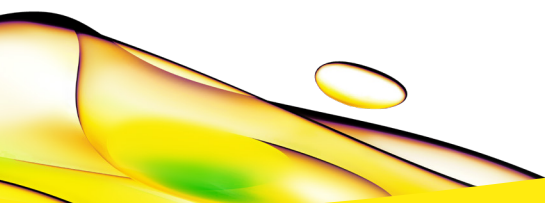
- Calculate the Standard Deviation and the Coefficient of Variation for the Total Cell Concentration.
CV should be less than 15%.

Sample #	Live Cell Conc	Total Cell Conc	% Viability

Analysis of results/conclusions

The average cell concentration obtained using the Cellometer Auto 2000 should vary less than 10% from the average cell concentration calculated using the manual hemacytometer. This data can be used as validation of the Cellometer Auto 2000 Cell Viability Counter as a replacement method for cell counting.

Most researchers find that the standard deviation is much lower and counting is much faster for the Cellometer Auto 2000 when compared to the manual hemacytometer, especially when dealing with cell clumps and irregular-shaped cells that may be difficult to count manually.



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