

Axion Cell Counter (Formerly Corning)

1. Materials:

- Trypan Blue (0.4%) (Gibco, 15250-061)
- Corning Counting Chamber for Corning Cell counter (Fisher Scientific, 07-202-554)
 - Disposable counting chambers are composed of two 10 μ L chambers
 - Depth (Metric) = 0.1mm

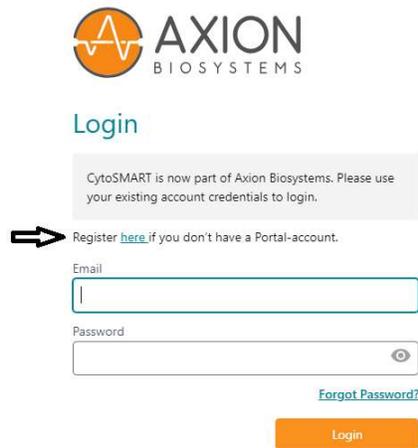


2. To create an account:

- Click on the CytoSMART Cell Counter Icon:



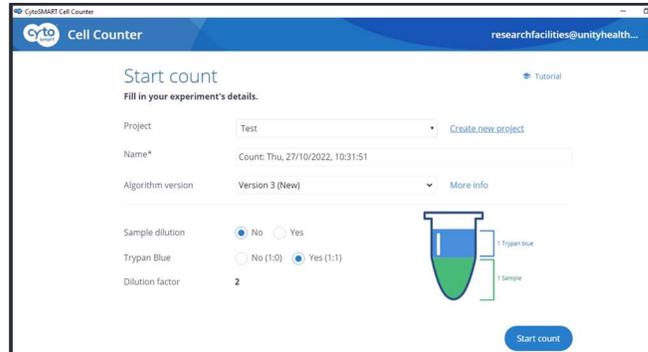
- Login: * for first time users create an account:



3. Start a Cell Count:

- To start a cell count, create a new project with your Name or select your project from the drop-down menu.
- Give the cell count a name.

- Recommendation- always include the date of the cell count in the file name to easily find the experiments at a later date.
- Select sample/Trypan blue dilutions



4. Sample Loading:

- Load your sample into the Counting chamber.
 - Recommendation- load 10 μL of the sample in the counting chamber with a 10 μL or 20 μL pipet.
 - Corning Cell Counter has a concentration range of 5×10^4 to 1×10^7 cells/mL. The system works within a cell size range between 4 to 70 μm . Out of range concentrations might result in false results.
 - If the cell solution is out of this concentration range, dilute the sample using culture medium and adjust the experiment dilution settings accordingly.
- Place the hemocytometer on the Cell Counter.



5. Focusing of the Cells:

- Use the focus knob to adjust the focus of the image.
- To focus, turn the knob clockwise or counter clockwise.
- For proper analysis, you should be seeing a contrast: living cells having bright centers and dark edges (see below).
- To achieve this, using the focus knob, set the focus just above the cell.
- Try multiple focus levels to determine which focus setting to use.

- You can try the auto-focus function. However, the AF (automatic focus) setting in the software is not always appropriate for all cell lines

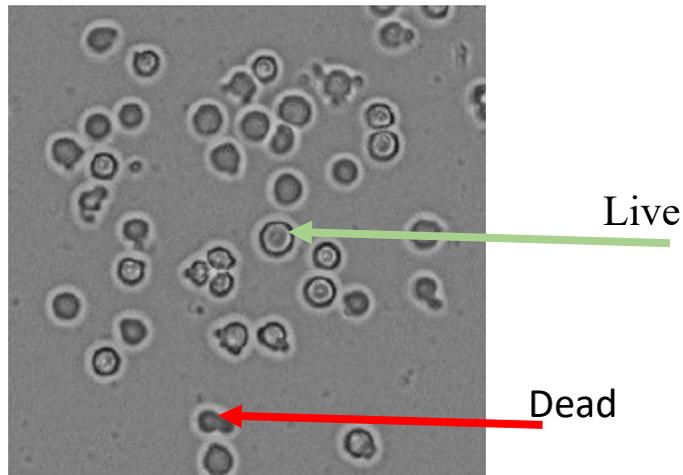


Tip: *Proper Focus settings are crucial for the accuracy of the counts**

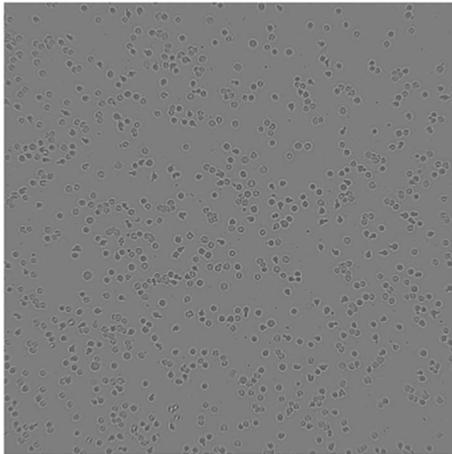
To optimize the focus for your cells perform a viability analysis. Prepare a live dead control (1:1).

How to prepare live/dead control: take an aliquot of cells, heat shock the cells at 65 degrees for 4 -5 min, immediately place on ice for 1 min. Following the treatment, the heat-killed cells (dead) should be combined 1:1 with live cells (untreated) (use 20 μ l of this mix, add 20 μ l of trypan blue, mix well, load 10 μ l into the counting chamber.

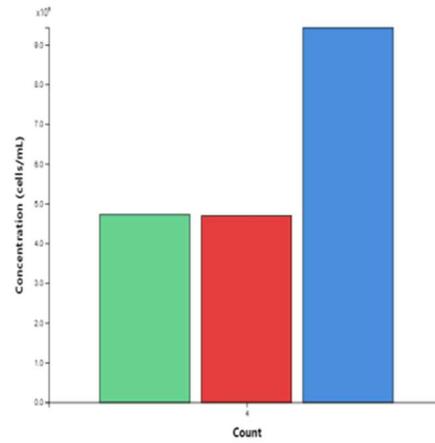
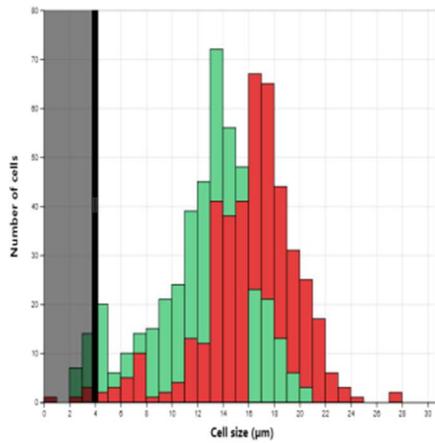
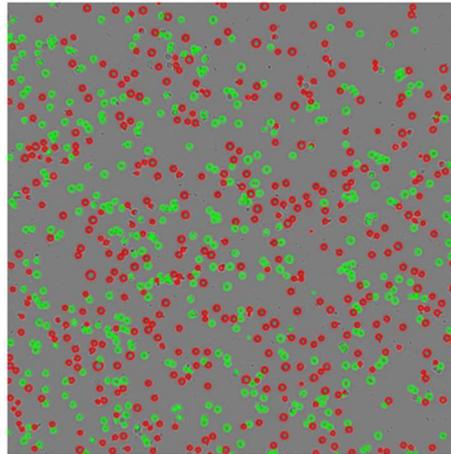
Live cells should have a sharper outline and ‘shiny’ middle, dead cells are opaque (and darker) throughout. The viability cell counts should be around 40-50%



Raw input image of count 4



Processed image of count 4

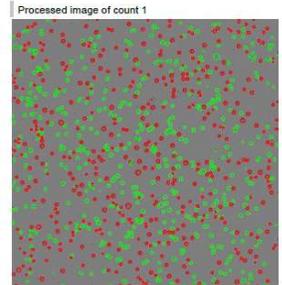


Count 4	
Gating	4 µm - 31 µm
Live cells	4.73e+6 cells/mL
Dead cells	4.70e+6 cells/mL
Total cells	9.43e+6 cells/mL
Viability	50.2 %
Live size	12.8 µm
Dead size	16.3 µm

6. Performing a Count:

- Make sure that the counting chamber is correctly placed.
- Check the image to see if the focus is correct.
- Select “Count” from the start screen

- Take images from 3-4 fields of view for most accurate counts (results from multiple images can be averaged in the software)
- The CytoSMART™ application will analyze the image and present the results.
- Note- Green circles represent living cells; red circles are dead cells. Data displayed-
 - number of (live and dead) cells
 - Total Cells and
 - Viability.
- Export the data as (i) pdf (ii) Excel or (iii) images.
- Transfer data via email.

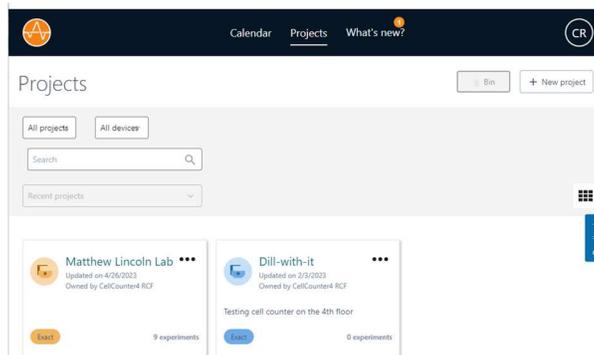


7. Connect to the Cloud:

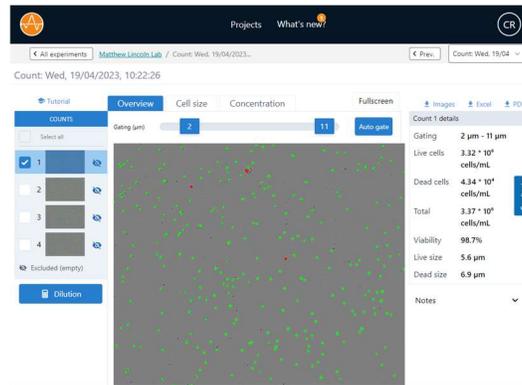
<https://cloud.cytosmart.com/>

- Log-in:

- Select your project:



- Reanalyze data, create pdf, excel files etc.



8. Videos:

Corning® Cell Counter Demo Video

https://www.youtube.com/watch?v=JWjckEHO6Wg&ab_channel=CorningLifeSciences

Counting Cells with the Corning® Cell Counter

https://www.youtube.com/watch?v=1rohagQn_10&ab_channel=CorningLifeSciences

Corning® Cell Counter Autofocus Functionality

https://www.youtube.com/watch?v=LyUwDk8IzEw&ab_channel=CorningLifeSciences

9. Troubleshooting

- No cells are visible-
 - Cell concentration of the sample is below the operating range- 5×10^4 cells/mL. To resolve this, pellet the solution by centrifuging and resuspend the cells in a smaller volume.
 - Bulk sample solution was left standing for some time and cells have gravitated towards the bottom of the vial. By pipetting the suspension up and down before loading the counting chamber, you should obtain a homogenous cell suspension
- Too many cells-
 - Cell concentrations are above 1×10^7 cells/mL. Dilute your cell suspension to obtain a lower concentration and count again.

- Cell clumps-
 - Cells can aggregate into clumps, leading to false results when analyzed. When cell clumps are visible, prepare a new sample. By gently resuspending the stock cell suspension, aggregates can be broken down.

- Bubbles-
 - While looking at the images in the CytoSMART™ application, you may see bubbles. They are recognizable as big black circles. Bubbles can lead to false results, as they influence the distribution of cells and the volume inside the counting chamber. The cause for bubbles is often due to incorrect pipetting.

- Debris-
 - When looking at the images from the CytoSMART application, you may see (cell) debris. Cleaned the counting chamber with 70% ethanol and Kim Wipe.

