BECKMAN COULTER Presents

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MASTERING CELL COUNTING

ACCURATE CELL COUNTING IS CRITICAL TO ENSURE EXPERIMENTAL SUCCESS AND REPRODUCIBILITY FOR CELL-BASED ASSAYS AND CELL CULTURE APPLICATIONS.

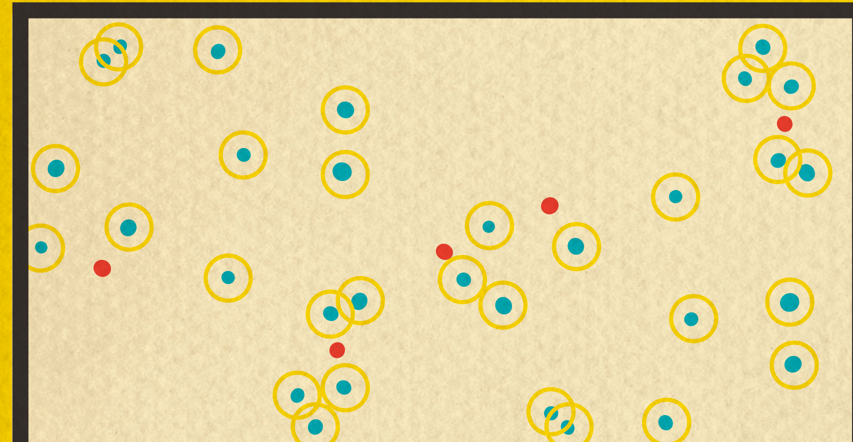
CALCULATING THE NUMBER OF CELLS IS REQUIRED FOR MONITORING CELL PROLIFERATION & VIABILITY, OPTIMIZING CELL CULTURE CONDITIONS, AND PREPARING CELL-BASED ASSAYS.

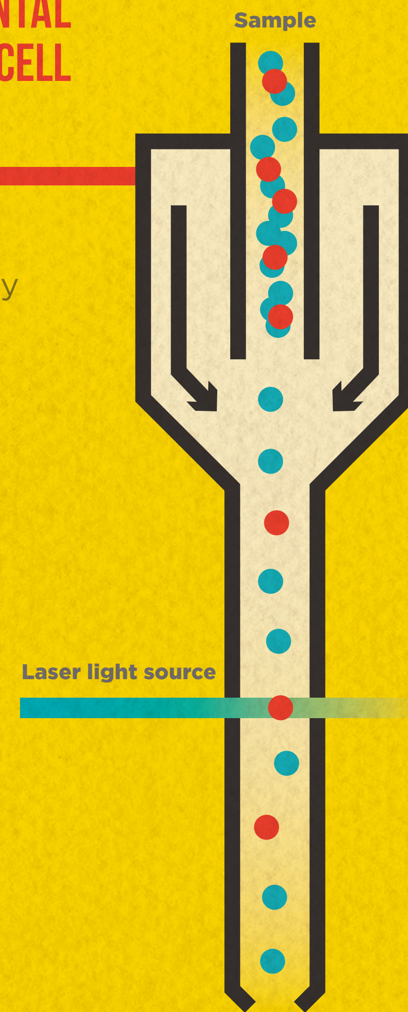
CELL COUNTING PROCEDURES NEED TO BE:

- Accurate • Reproducible
- Cell type independent, i.e., take into consideration cell property variables such as cell clumping, cell shape, and cell populations of varying size

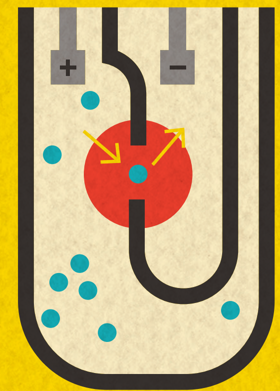
IMAGE-BASED COUNTERS

Image-based systems use bright field or fluorescent microscopy to capture an image of the cells. Some systems operate on a flow-based imaging methodology where cells are drawn into a capillary and the cells are imaged and counted as they pass a detector. Cell viability can be calculated using dye exclusion methods, such as Trypan Blue. Software analyzes the images based on system specific parameters such as cell diameter, brightness, and circularity to determine the number of cells and cell viability.





COULTER COUNTERS



Coulter counters measure changes in electrical resistance to determine the number, volume and size of the cells in the sample. Some Coulter counters offer the ability to distinguish live cells from dead cells and cell debris.

FLOW CYTOMETERS

Flow cytometers are not dedicated cell counters and report on relative values, such as the percent of cells in a given sample that have specific properties. The volume of sample counted needs to be determined to calculate the absolute cell count. To accomplish this, samples need to be spiked with fluorescent counting beads as a control.

HEMOCYTOMETER

DIMENSIONS	AREA	VOLUME AT 0.1 MM DEPTH
1 x 1 mm	1 mm^2	0.0001 mL
0.25 x 0.25 mm	0.0625 mm^2	0.00000625 mL
0.20 x 0.20 mm	0.04 mm^2	0.000004 mL
0.05 x 0.05 mm	0.0025 mm^2	0.00000025 mL

1. Apply the cell suspension so that the sample fills the chamber.
   Tip: Dilute the cell suspension if necessary. Cells should be uniformly distributed without clumping or overlap on the grid.
2. Using a microscope, count the number of cells in 4 squares.
   Tip: The lower the concentration of cells, the more squares should be counted to reduce statistical errors.
   Tip: Only count cells that are within the square or on the bottom or left lines; do not count cells that touch the top or right lines of the square.
3. Calculate the density of cells in the suspension.

CELL ANALYSIS

CELL BRIGHTNESS
Cells & Clutter Counted
Only Cells Counted

CELL SHARPNESS
Sharp & Fuzzy Cells Counted
Fuzzy Cells Not Counted

CELL SPOT AREA
2% Area - Viable
10% Area - Dead

DECLUSTER DEGREE
None
Low
Medium

MINIMUM CIRCULARITY
Value of 1 Fewer Cells Counted
Value of .05 More Cells Counted

SPOT BRIGHTNESS
Default Value 75 - Classified Viable
Default Value 90 - Classified Dead

AUTOMATED CELL COUNTING

VS.

MANUAL CELL COUNTING

$$\text{Cell density (cells/mL)} = \frac{\text{(Average number of cells counted per square) X (Dilution factor)}}{\text{Volume of square (mL)}}$$

Cell viability can be calculated by treating the cell suspension with cellular dyes, such as Trypan Blue. Live cells do not take up Trypan Blue, dead cells will stain blue.

$$\text{Cell viability (\%)} = \frac{\text{Total viable cells (unstained)}}{\text{Total cells (stained + unstained cells)}} \text{ X } 100$$

AUTOMATED CELL COUNTERS OFFER AN EFFICIENT AND RELIABLE WAY TO QUICKLY COUNT AND ANALYZE A CELL POPULATION.

MANUAL CELL COUNTING OFFERS AN ACCESSIBLE WAY TO DETERMINE THE CONCENTRATION OF CELLS IN A LIQUID SAMPLE, REQUIRING JUST A LIGHT MICROSCOPE AND HEMOCYTOMETER.

★PROs: FAST • ACCURATE • REPRODUCIBLE • ELIMINATES SUBJECTIVITY • ANALYSIS & GRAPHICAL DISPLAY • DATA STORAGE & EXPORT CAPABILITIES • SUPPORTS 21 CFR PART 11 ✖CONs: INITIAL COST FOR EQUIPMENT

★PROs: INEXPENSIVE • VERSATILE ✖CONs: TIME-CONSUMING • LABOR-INTENSIVE • VARIABLE RESULTS • LIMITED FEATURES • POOR REPRODUCIBILITY • SUBJECTIVE