Overview of Module 5

1. What is one reason we need to count cells?

   counting cells allows us to seed accurately, which ensures experimental reproducibility and that our method of disease diagnosis is reliable

2. What is the primary, manual method of cell counting used in research?

   hemocytometer grid countingl

3. Why do we use hemocytometers?

   they're basically everything a researcher wants (fast, cheap, reusable, small, easy to use (and thereby easy to teach people how to use)

4. In flow cytometry, what does forward scattered light help measure? What does side scattered light help measure?

   FSC helps measure cell size (as it correlates to cell volume

   SSC helps measure cell type (as it correlates to internal complexity

5. In FACS, how are different cell types sorted?

   Fluorescence-tagged antibodies are added to the mixture and bind to their target cells. The fluorescent cells are then assigned a charge (positive or negative) that is picked up by the photodetector and used to sort the cells

6. Why is it important that a Coulter Counter contains electrolytic solution?

   The electrolytic solution makes it so that cell movement changes electrical resistance (the factor measured by the coulter counter)

7. Why do we perform Viability Assays?

   To quantify and visualize cells

8. What is the function of mitochondrial reductase in an MTT Assay?

   Reduces yellow MTT to purple Formazan, which absorbs maximally at 570 nm

9. What is the function of luciferase in Cell Titer Glo?

   Oxidizes Luciferin to Oxyluciferin, releasing light in the process

10. What is the function of esterase in Live-Dead Assay?

    Convert non-polar Calcein-AM (which can move freely across the cell membrane) into polar Calcein (which is trapped inside of the living cell) to mark live cells

Cell Counting Techniques Vocabulary

1. Match the key term to its role in cell counting or viability assays:

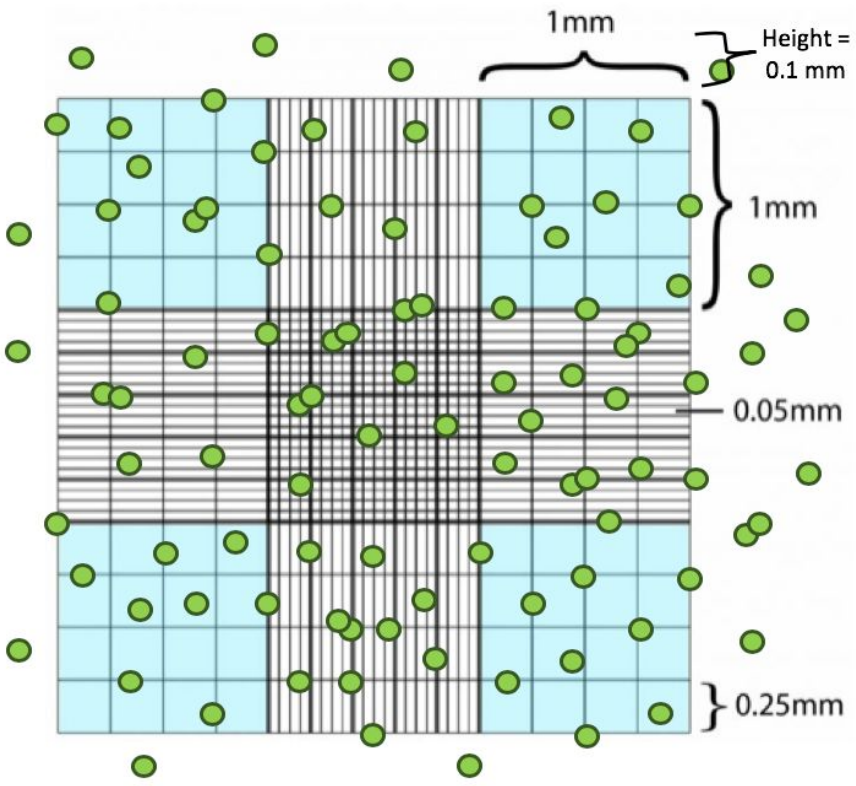
A. Calcein	___G___	Assay that uses bioluminescence to measure ATP
B. MTT Assay	___H___	One-step process that estimates metabolic activity through Formazan
C. Flow Cytometry	___J___	Two chamber cell counting system that measures changes in resistance
D. DMSO	___C___	Measures cells by detecting scattered light
E. ImageJ	___B___	The first modern cell viability assay
F. Hemocytometer	___E___	Common image analysis software for cell counting
G. Cell Titer Glo	___A___	Marks live cells in Live/Dead Assay
H. MTS Assay	___I___	Marks dead cells in Live/Dead Assay
I. Ethidium Homodimer	___F___	Manual cell counting method that uses counting grids
J. Coulter Counter	___D___	Reagent that creates pores in the cell membrane

Using a Hemocytometer

1. To ensure consistency in your hemocytometer count, you include cells on the __top__ edge and _left__ edge of your quadrant in your cell count, but don't include cells on the _bottom_ edge and _right_ edge of your quadrant in your cell count.

2. To obtain the average number of cells per counting square, you count the cells in each counting square and divide by __4__.

3. To obtain the cell concentration in # cells / mL, you take the average number of cells per counting square, divide by the volume of each counting square (_0.1_ mm^3) and convert mm^3 to mL.
4. Practice obtaining a hemocytometer count!



Quadrant Counts

Square 1: _____7_____ cells

Square 2: _____5_____ cells

Square 3: _____8_____ cells

Square 4: _____8_____ cells

Average: ___7___ cells/square

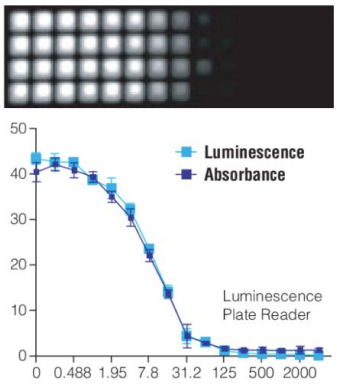
Cell Concentration:

$$\frac{cells}{mL} = (\ \_7\_\ \ \frac{cells}{square})*(\frac{counting\ square}{0.1\ mm^3})*(\frac{1\ mm^3}{10^{-3}cm^3})*(\frac{1\ cm^3}{1\ mL}) = \_7 \cdot 10^4\_\ \frac{cells}{mL}$$

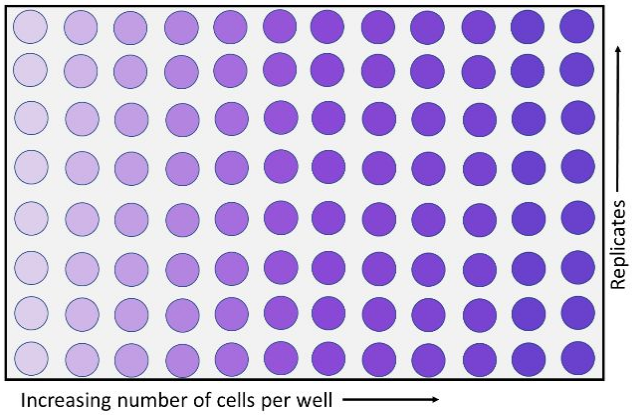
Examining Cell Viability

5. Match the cell viability assay to the image below:

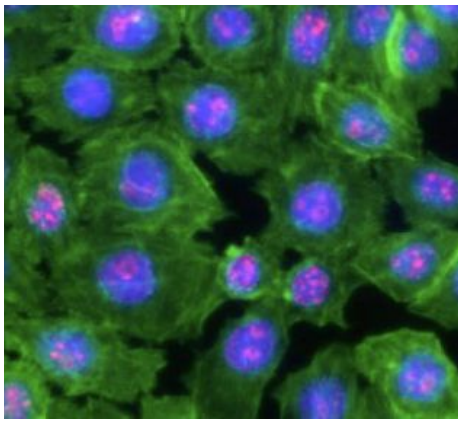
MTT/MTS, Live/Dead, Immunostaining, Cell Titer Glo



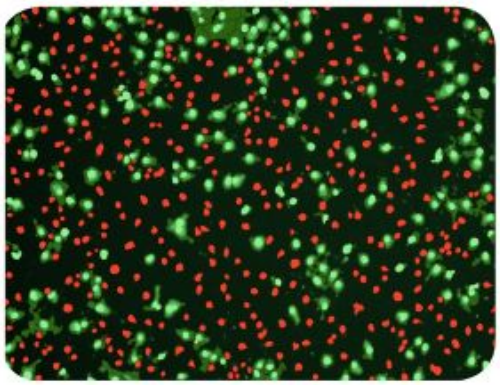
(A)



(B)



(C)



(D)

(a) Picture A shows the results of a ___Cell Titer Glo _____ assay.

(b) Picture B shows the results of a ___MTT/MTS____ assay.

(c) Picture C shows the microscopy images from a __Immunostaining____ assay.

(d) Picture D shows the microscopy images from a ___Live/Dead___ assay.