Assay Name: HTS suspension cell count and viability using AO/PI

Assay ID: Celigo_02_0007

Description: Measure cell count and viability using Acridine Orange (AO) and Propidium Iodide (PI) on suspension cells to increase the efficiency of cell-based analysis for high-throughput screening

Stains: Acridine Orange (AO) and Propidium Iodide (PI)

Imaging channels: Green and Red

Image analysis algorithm: Celigo software Target 1 + 2

Methods:

1. Culture and collect Jurkat cells
2. Prepare the AO/PI working concentration by diluting AO/PI (Nexcelom, Cat# CS2-0106) by 10X in PBS
3. Pipette 25 µL of AO/PI and 5 µL of Jurkat cells into each well on a 96-well half area microplate

Plate 1: Serial dilution of cells.

Dilution	1	2	3	4	5	6	7	8	9	10	11	12
A	Neat	2X dilutions -->										0.0005
B	Neat	2X dilutions -->										0.0005
C	Neat	2X dilutions -->										0.0005
D	Neat	2X dilutions -->										0.0005
E	Neat	2X dilutions -->										0.0005
F	Neat	2X dilutions -->										0.0005
G	Neat	2X dilutions -->										0.0005
H	Neat	2X dilutions -->										0.0005

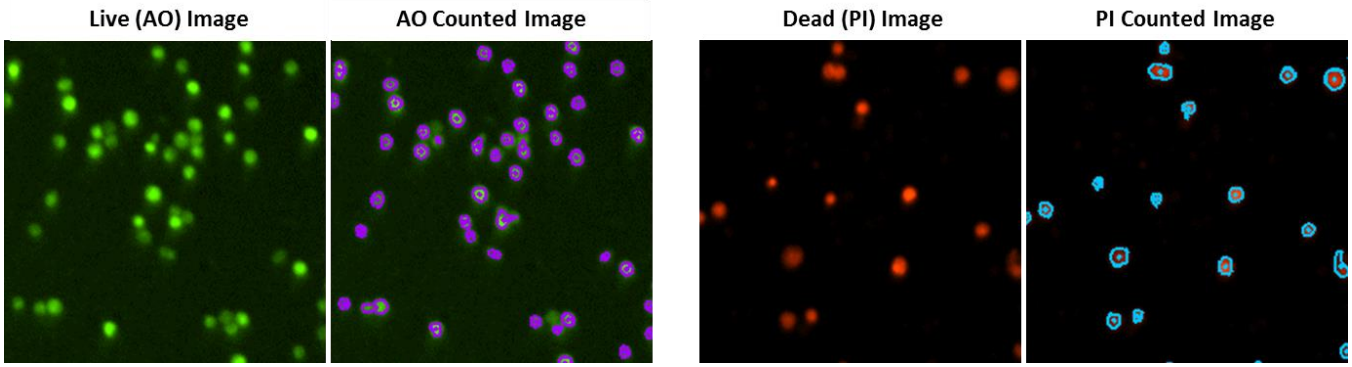
Plate 2: Five viability percentages

Viability	1	2	3	4	5
A	100%	75%	50%	25%	0%
B	100%	75%	50%	25%	0%
C	100%	75%	50%	25%	0%
D	100%	75%	50%	25%	0%
E	100%	75%	50%	25%	0%
F	100%	75%	50%	25%	0%
G	100%	75%	50%	25%	0%
H	100%	75%	50%	25%	0%

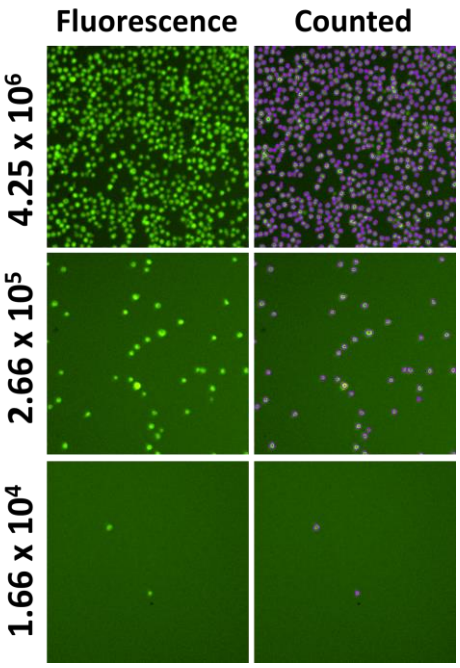
4. Use Celigo and capture images and analyze total AO and PI positive Jurkat cells in the wells
5. Use the equation below to calculate viability %
   a. $Viability\ \% = \frac{AO\ positive\ cells}{AO+PI\ positive\ cells} x\ 100$

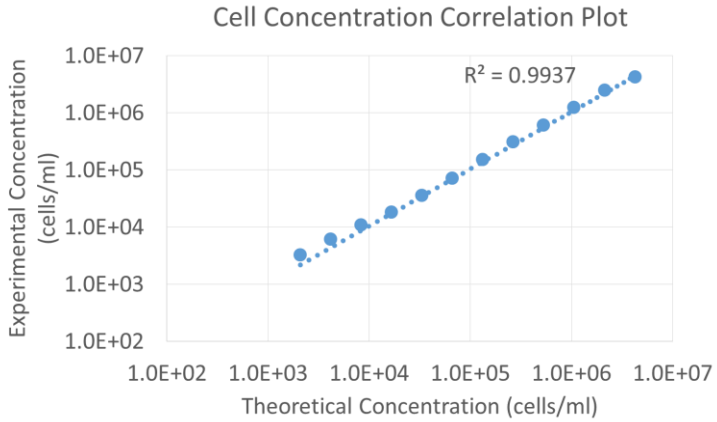
Results:

Counting AO and PI positive cells using Celigo image cytometer



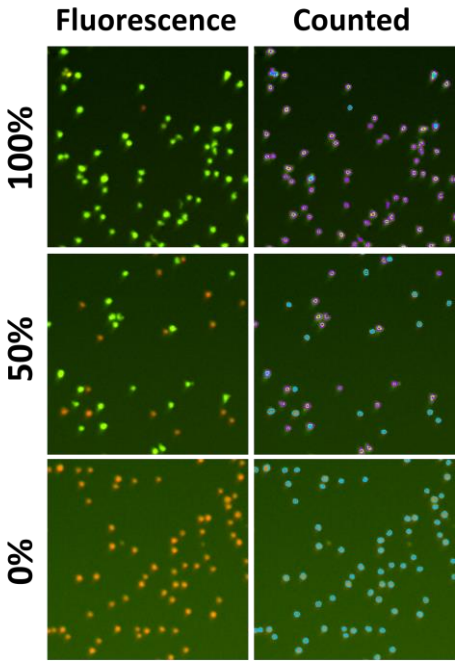
- Example AO/PI fluorescent images, and counted images at different Jurkat cell concentrations
- The fluorescent images showed decreasing number of AO positive cells as the dilution factor increased

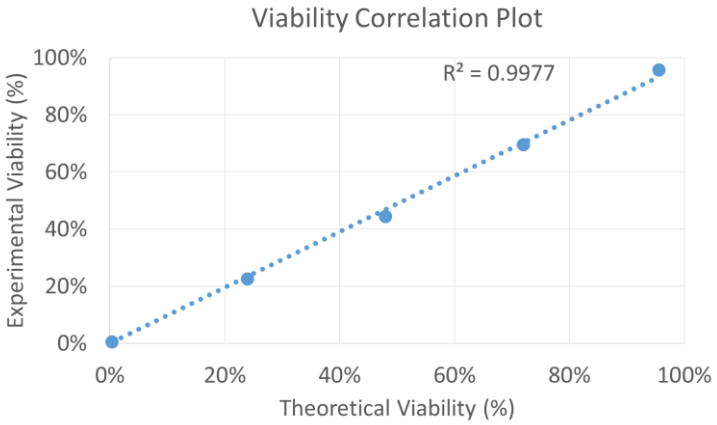




- The correlation plot is the actual counting accuracy against theoretical counts
- The R^2 value was highly linear (0.9937)
- Depending on the cell volume pipetted into the well, the concentration range can go from 1 cell to approximately 1 x 10^7 cells/ml

- The AO/PI fluorescent images showed increase in PI positive cells as the viability decreased





- The linearity plot is the measurement of cell viability
- The Celigo can measure accurate cell viability from 0 to 100%
- The R^2 value was highly linear (0.9977)

- The Celigo image cytometer was able to perform high-throughput cell count and viability measurement
  - The method was compared directly to Cellometer image cytometer and was highly comparable