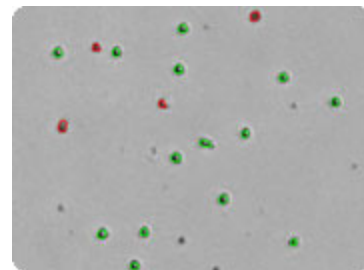
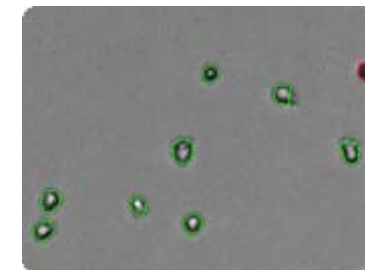
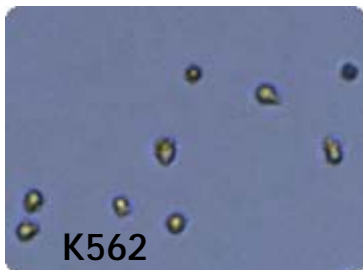
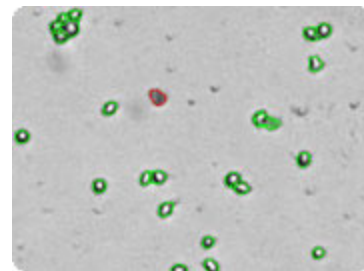
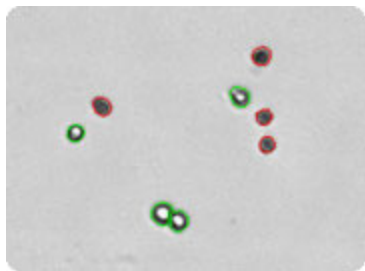
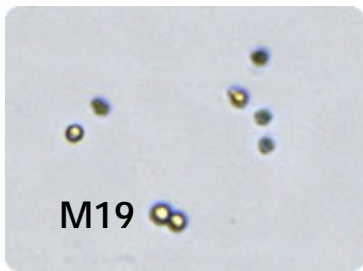
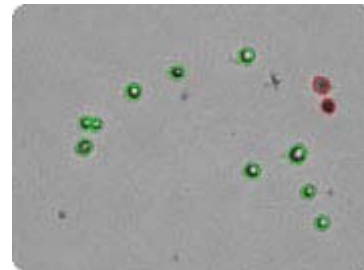
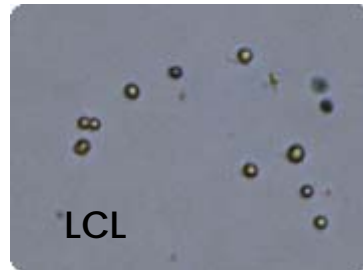
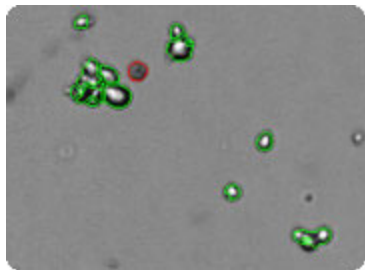
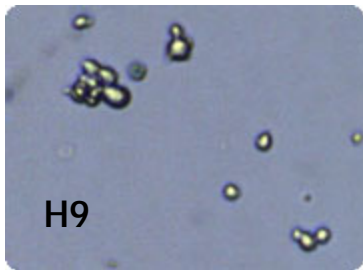


Cell Viability Measurement

- **Viability is a measure of the metabolic state of a cell population which is indicative of the potential for growth**
- **Most common method: membrane integrity**
 - Trypan blue exclusion: leaky membrane allows blue dye to get into cells
 - Good: capable for manual microscopy
 - Issues: operator dependent
- **Cellometer solution: use software algorithm**
 - Reduce operator dependency
 - Reduce calculation error
 - Speed up counting
 - Better management of data



Trypan Blue Viability for Cell Lines: Exclusion Assay



Cellometer has been used for more than 300 cell lines



Fluorescence Based Cell Viability Methods

- Membrane integrity
 - DNA binding dye with permeability dependency
 - AO (acridine Orange): permeable for live and dead cells
 - PI (propidium iodide), EB (Ethidium bromide), 7-AAD: not permeable for live cells
 - Others: SYTO® 13, SYTO 24®, SYTO® 14
- Enzymatic activity
 - Non fluorescent esterases diffuse into cells
 - Live cells with strong enzymatic activity produce fluorescent products
 - Live cells retain fluorescent products
 - Examples:
 - Calcein-AM to calcein
 - Fluorescein diacetate (FDA) to fluorescein
- Simultaneous detection of both live and dead cell populations for cell lines
 - Combination of the above with duo-fluorescence detection for each cell sample



References

- [1] S. A. Altman, L. Randers, and G. Rao, "Comparison of Trypan Blue-Dye Exclusion and Fluorometric Assays for Mammalian-Cell Viability Determinations," *Biotechnology Progress*, vol. 9, pp. 671-674, Nov-Dec 1993.
- [2] V. Boyd, O. M. Cholewa, and K. K. Papas, "Limitations in the Use of Fluorescein Diacetate/Propidium Iodide (FDA/PI) and Cell Permeable Nucleic Acid Stains for Viability Measurements of Isolated Islets of Langerhans," *Current Trends in Biotechnology and Pharmacy*, vol. 2, pp. 286-304, 2008.
- [3] J. M. Cocomartin, J. W. Oberink, T. A. M. Vanderveldendegroot, and E. C. Beuvery, "Viability Measurements of Hybridoma Cells in Suspension-Cultures," *Cytotechnology*, vol. 8, pp. 57-64, 1992.
- [4] J. A. Cook and J. B. Mitchell, "Viability Measurements in Mammalian-Cell Systems," *Analytical Biochemistry*, vol. 179, pp. 1-7, May 1989.
- [5] K. H. Jones and J. A. Senft, "An Improved Method to Determine Cell Viability by Simultaneous Staining with Fluorescein Diacetate Propidium Iodide," *Journal of Histochemistry & Cytochemistry*, vol. 33, pp. 77-79, 1985.



How Does It Work...

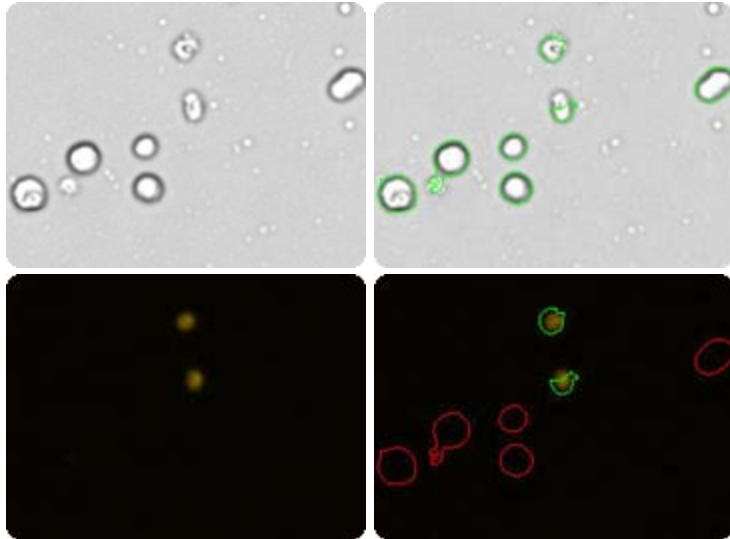


	Bright Field	Fluorescence	
Count	269	153	Show Size Distribution
Mean Size	13.6	13.5	Intensity Distribution
		(BR Size)	Size vs Intensity
Concentration	9.76×10^5	5.55×10^5	Sample Adjustment
$\frac{\text{F1 Count}}{\text{BR Total Count}} = 56.9\%$			Set Data File
Export			Save to Data File
Print			View Data File
		Done	

Output data generated instantly



Live Cell Concentration and PI Viability: Cell Lines



Data output:

- Total # of cells, FL+ cells
 - Cell concentration
- Live cell concentration
 - Viability
 - Cell images
 - Cell size histogram



Sample	Cell Concentration	Viability %
Jurkat	1.7 x 10 ⁶ /ml	91%
Mouse thymocyte	1.6 x 10 ⁶ /ml	81%
Mouse splenocyte	1.1 x 10 ⁶ /ml	85%
Mouse bone marrow	0.95 x 10 ⁶ /ml	81%

Other dyes: EB, 7-ADD

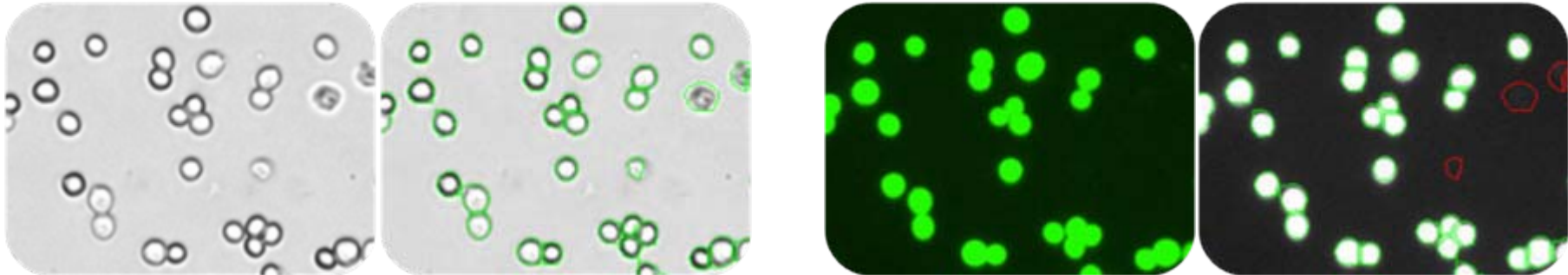


Count Live Cells and Measure Viability with Fluorescein Diacetate (FDA)

Assay principle

Fluorescein diacetate (FDA) (non- fluorescence) $\xrightarrow{\text{Esterases}}$ Fluorescein (Fluorescence)

Cellometer images and analysis



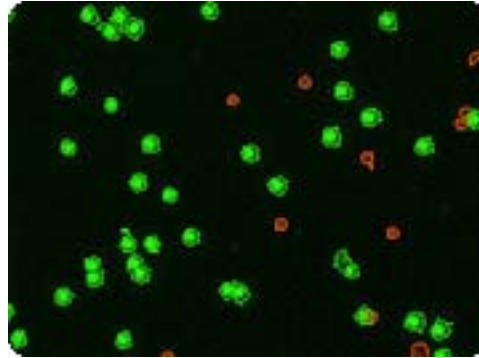
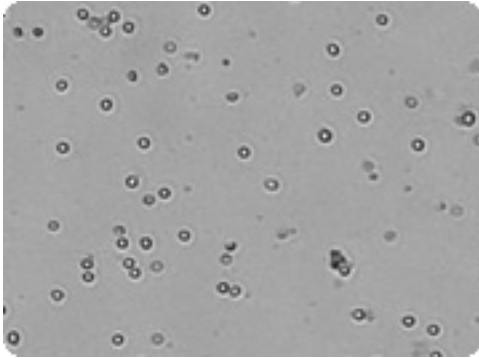
Cellometer cell sample analysis reports

- Total # of cells: Cell concentration measurement
- Count FL positive cells: live cell concentration measurement
- Calculate cell viability automatically
- Cell size measurement and population size histogram display
- Save all cell images

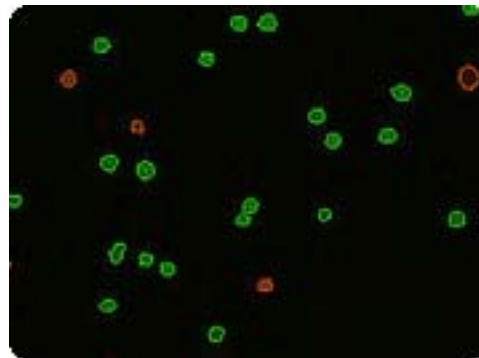
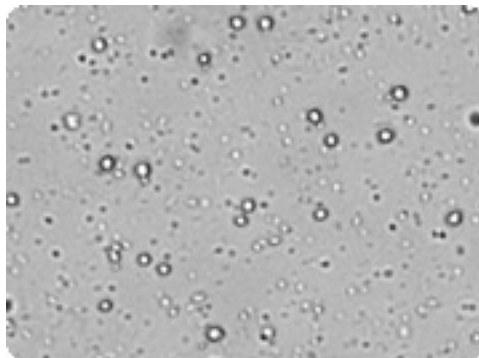


Live, Dead Cell Concentrations and Viability of Primary Cells Using Dual Staining Method

Spleenocyte: AOPI



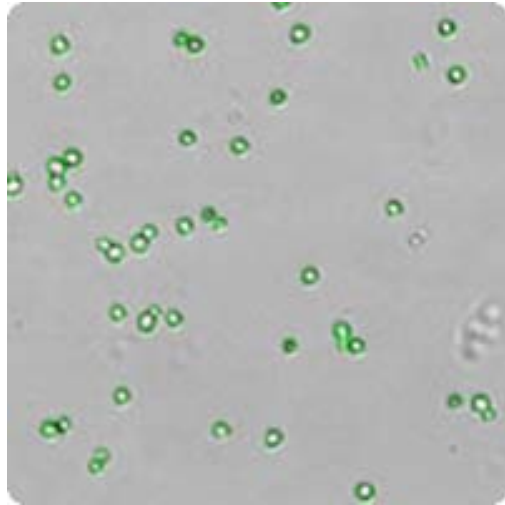
PBMC: AOEB



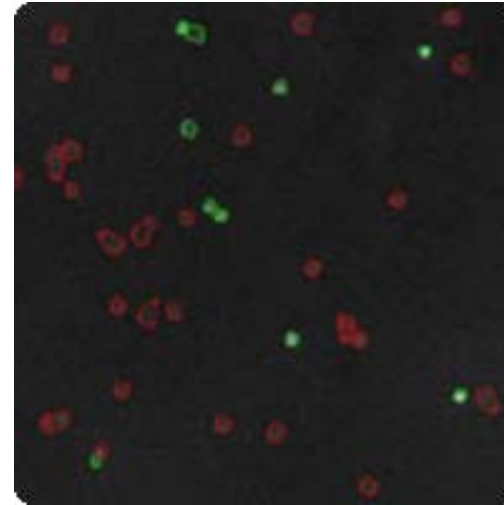
Fluorescent staining helps identify cells of interest for primary cell samples



Count and Determine Viability of Yeast Using Oxonol, Cellometer Vision 10x



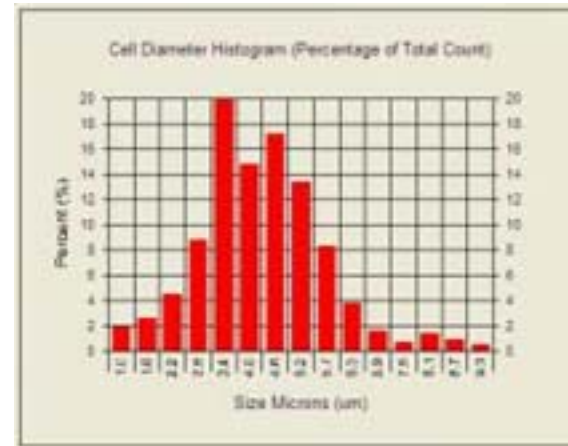
Green outline: all yeast cells



Green outline: dead cells, oxonol permeable
Red outline: live yeast cells



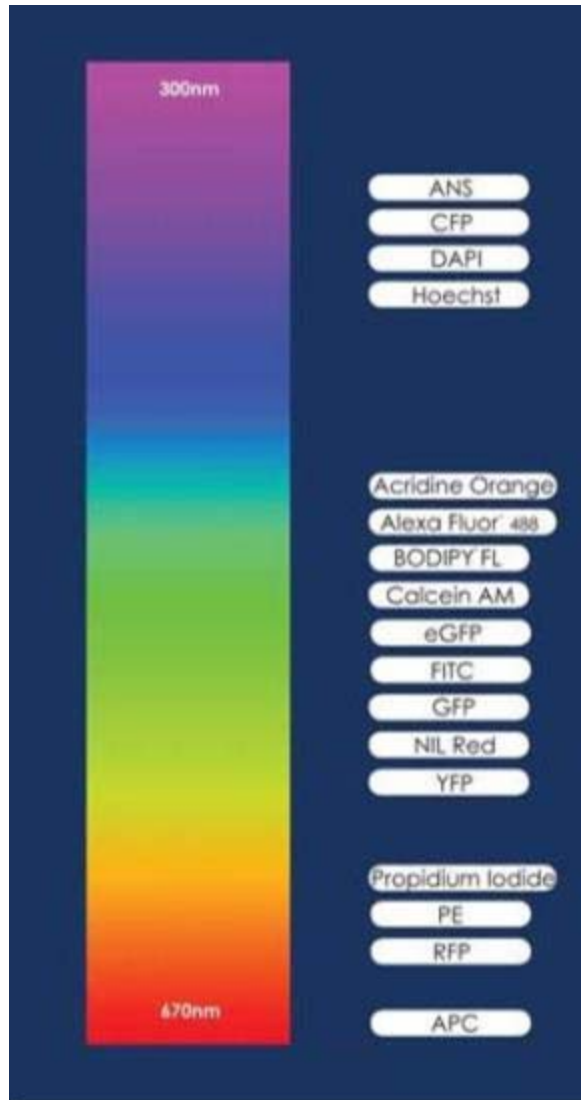
Automatically generate live cell concentration and viability



Automatically generate live cell size histogram



Multiple Optical Modules for Wide Range of Fluorophores



(Excitation / Emission: 475 nm/535 nm)

Example Fluorophores:
Acridine Orange (+DNA)
Alexa Fluor® 488
Calcein
Fluorescein (FITC)
SYTO® 9
SYTO® 13

(Excitation / Emission: 525 nm/595 nm)

Example Fluorophores:
Alexa Fluor® 546
Alexa Fluor® 555
Propidium Iodide
Ethidium Bromide
SYTOX® Orange

(Excitation / Emission: 375 nm/450 nm)

Example Fluorophores:
Alexa Fluor® 350
BFP (Blue Fluorescent Protein)
DAPI
Hoechst 33342 & 33258

(Excitation / Emission: 630 nm/695 nm)

Example Fluorophores:
Alexa Fluor® 647
Allophycocyanin (APC)
Cy5®

