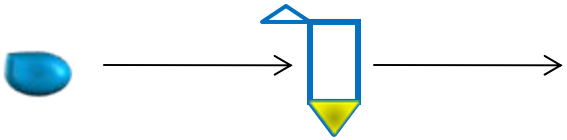
Primary Cells: Concentration and Viability

- Reliable method to count primary cells: nucleus staining
  - Just using bright field imaging mode is hard to get consistent, highly accurate data when sample variations are large
- Cellometer systems for analysis of primary cell samples:
  - Auto X4 or Vision
- PBMC sample variation study: 42 samples
- Body fluids: whole blood, cord blood, bone marrow, BAL…
- Digested tissue: spleenocyte, digested tumor suspensions

How Does It Work…

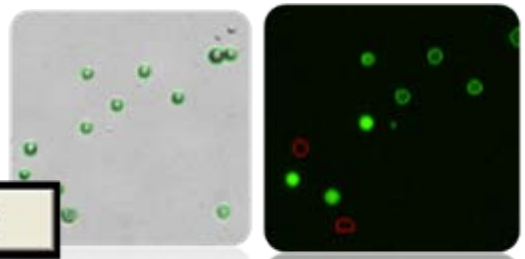


~20µL sample

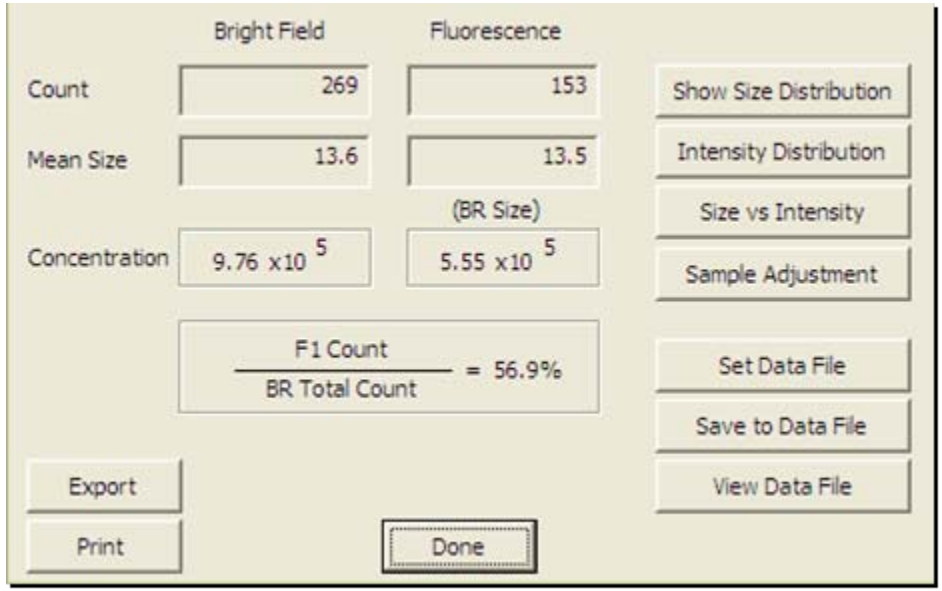
Add 20µL sample+20µL fluorescent dye

Pipette 20µL of sample into disposable counting chamber

Insert chamber in Cellometer

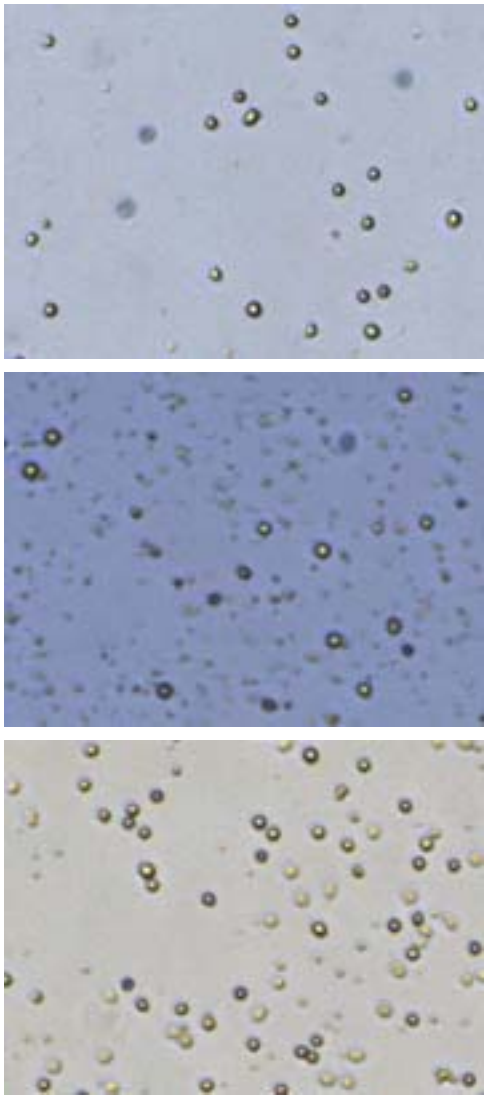


Bright field and fluorescent cell images acquired and analyzed



Output data generated instantly

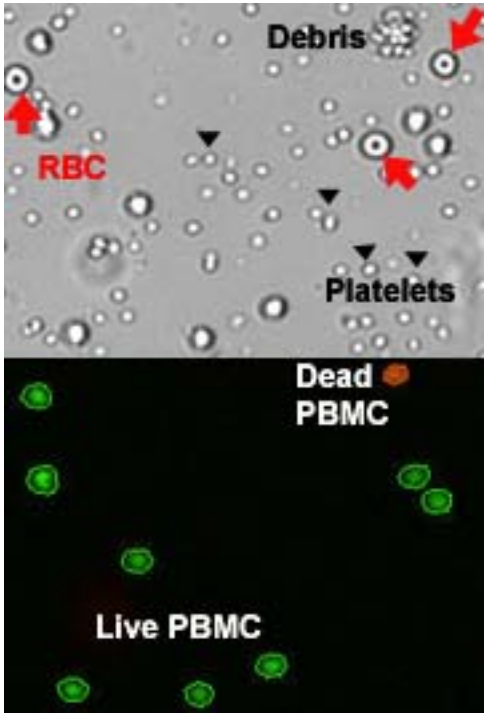
A Survey of PBMC Samples



42 samples

- Multiple labs
- Human, mouse, monkey
- Fresh and frozen
- 69% with high degree of contamination
- Varied from sample to sample from the same lab

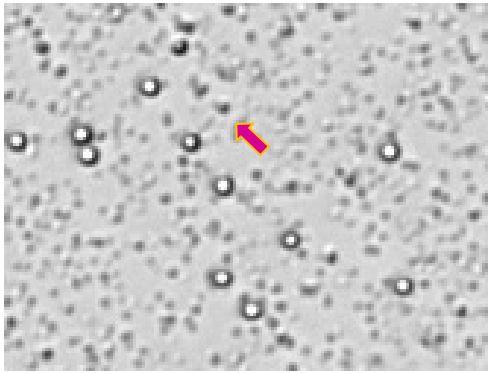
Understand PBMC contamination using Cellometer Vision 10 x



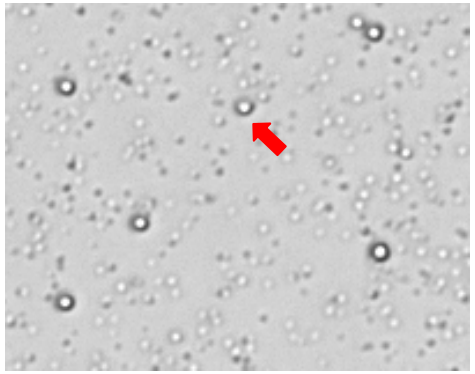
- **Platelets**
- **Un-lysed RBC**
- **Debris**

PBMC with Cellometer AutoX4 or Vision

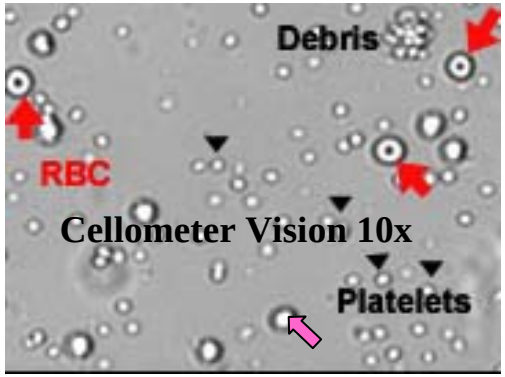
Contaminated with platelets

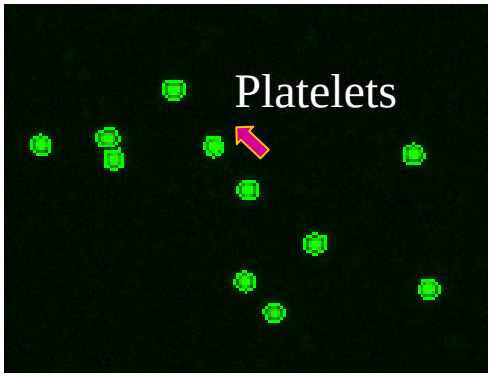


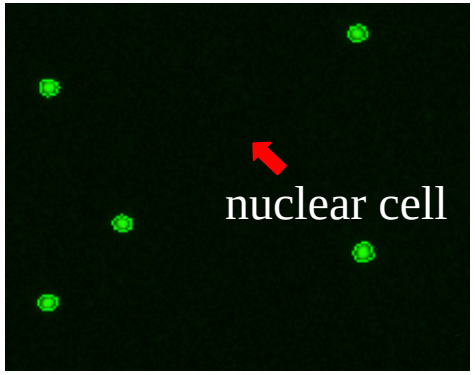
Contaminated with nuclear cell



Contaminated with RBC, platelet, debris



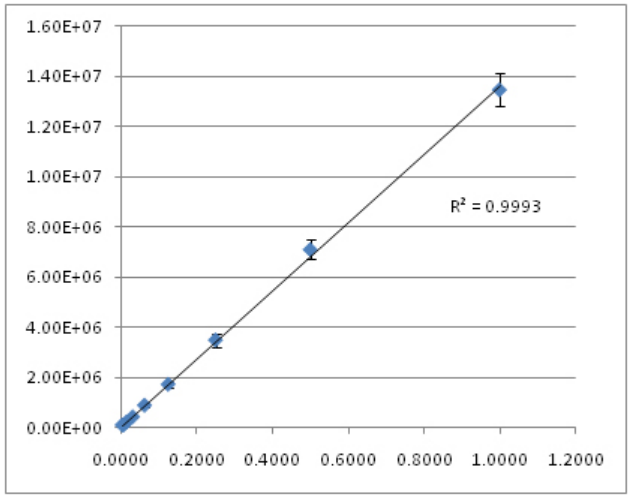




Strong fluorescent signal above any background for more accurate cell measurement

Counting Range and Consistency for Primary Cells

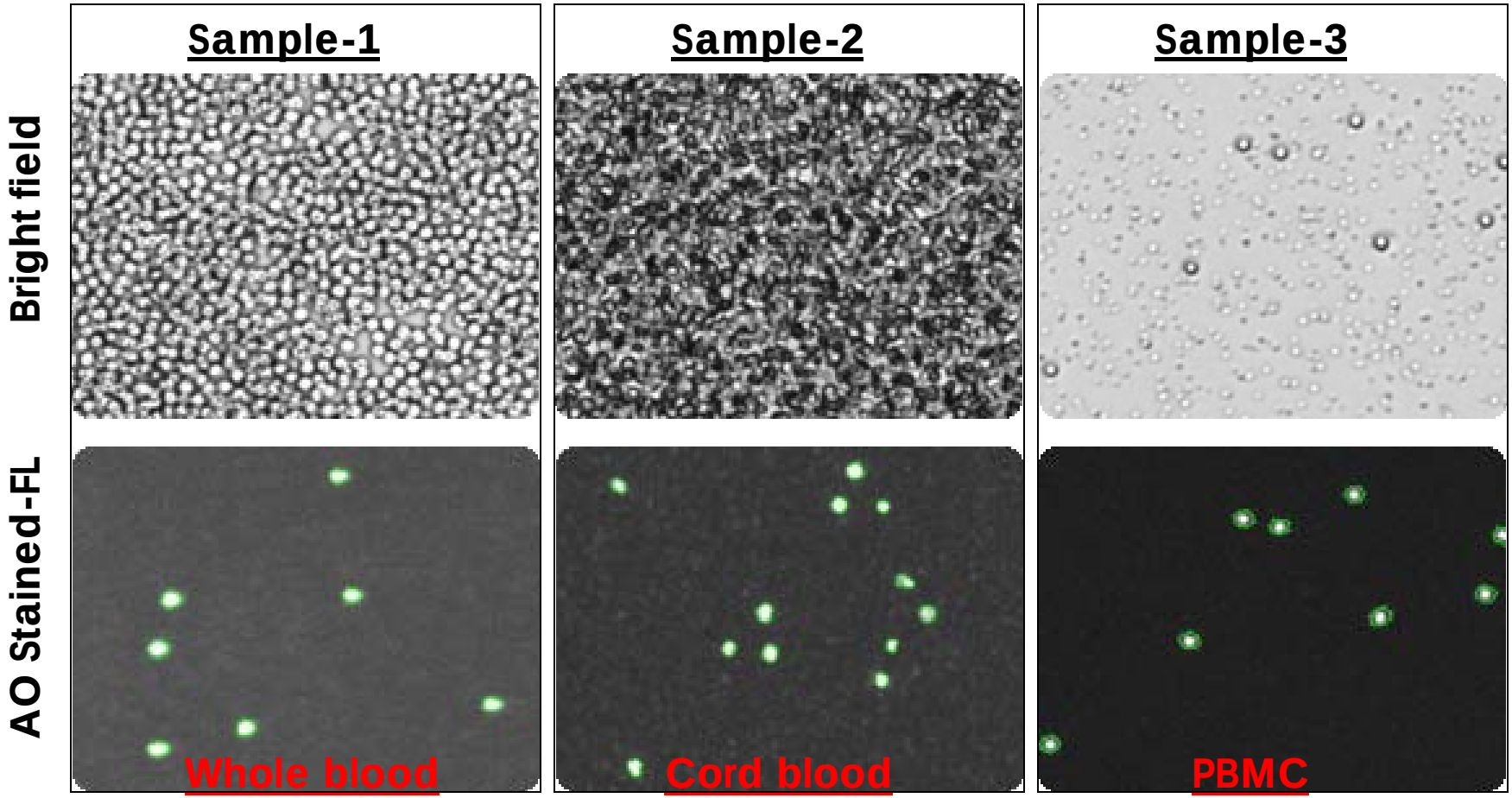
Measure live cell concentration using Cellometer Auto X4: PBMC dilution series



Frozen PBMC cell concentration, standard deviation and coefficient of variance

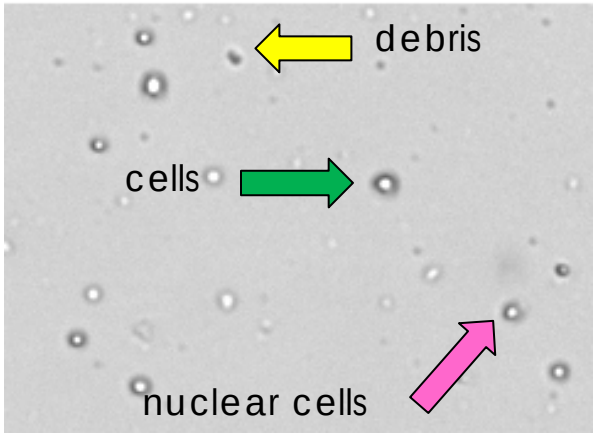
Cell sample	N	Concentration mean	Standard deviation	CV %	Cell Stain
1	15	6.84 x 106	4.20 x 105	6.1%	AO
2	17	6.14 x 106	4.43 x 105	7.2%	AOPI

Primary Cell Samples:
Using DNA Staining Dye to Identify & Count

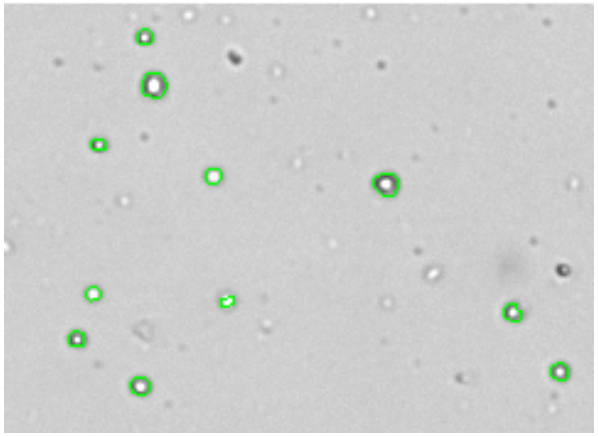


Counting Acridine Orange Stained Cells from Bronchoalveolar Lavage Fluid

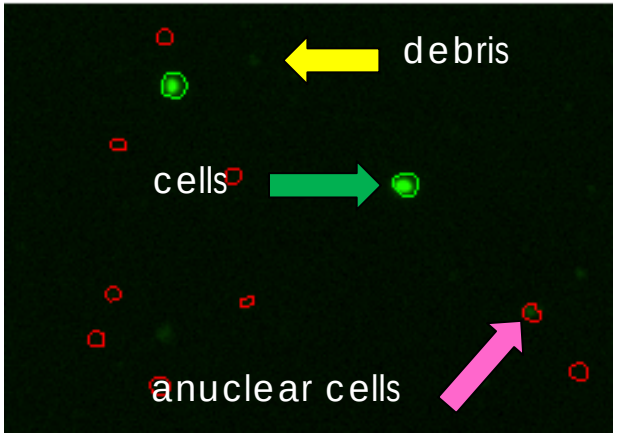
Bright field image



Processed bright field image



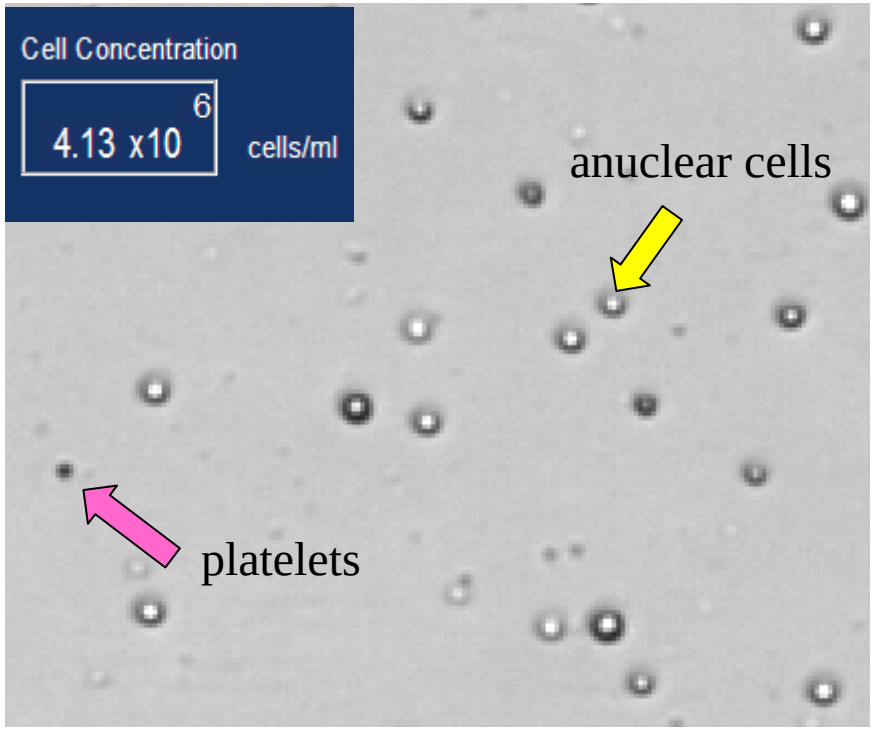
Processed Fluorescence image

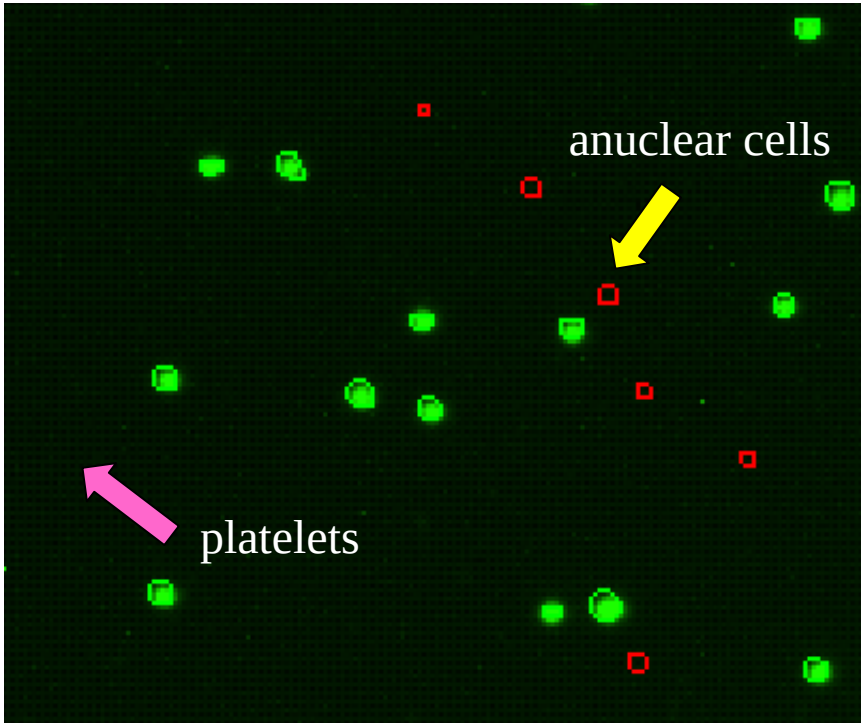


Only use bright field counts all cell like particles

More accurate count of nucleated cells

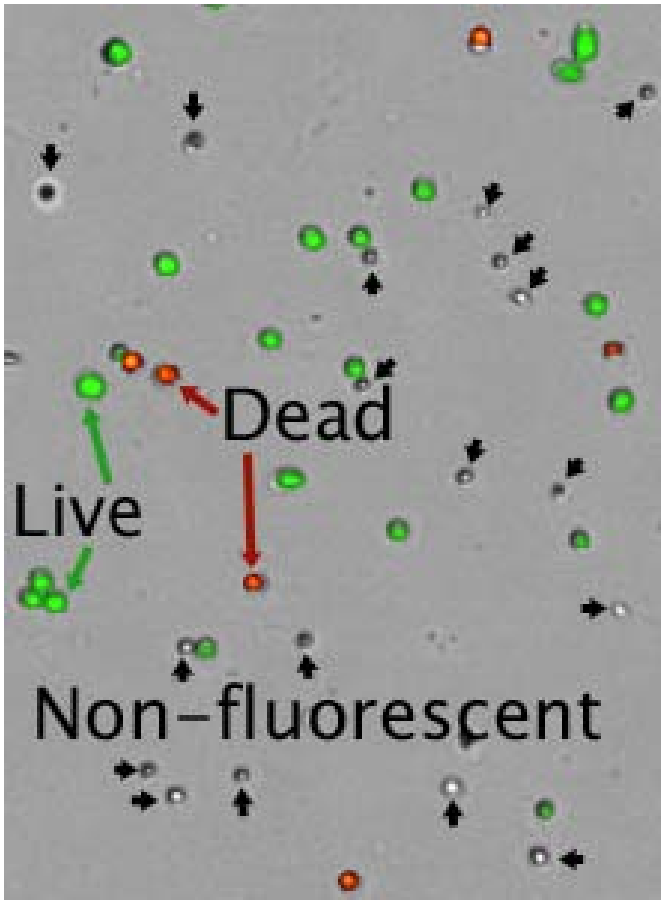
Count Acridine Orange Stained Spleenocytes





More accurate count of spleenocytes by exclude un-lysed anuclear cells

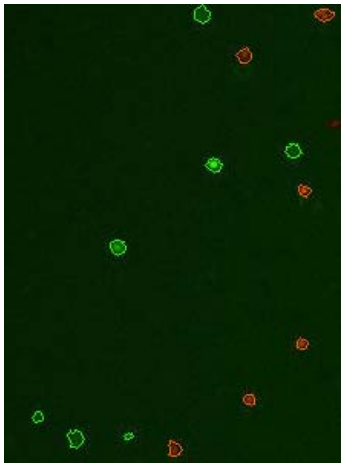
Digested Mammary Gland Tumor, Single Cell Suspension
Live Cell Concentration and Viability



- Incubate with CalceinAM

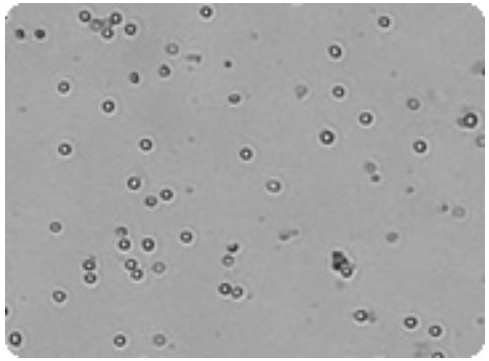
- Green indicate live cells, orange is for the EB stained dead cells

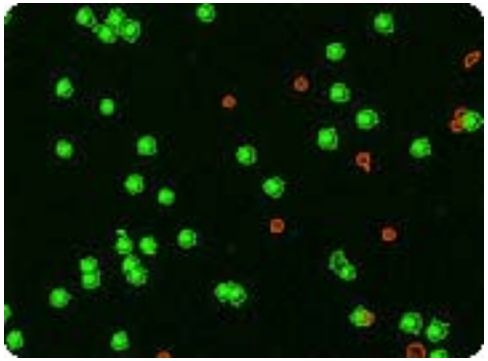
- There are some cells that are not fluorescent in either channel. They are also likely to be red blood cells



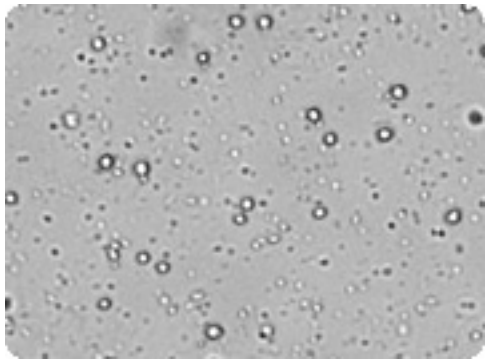
Primary Cell Sample
Live, Dead Cell Concentrations and Viability Using Dual Staining Method

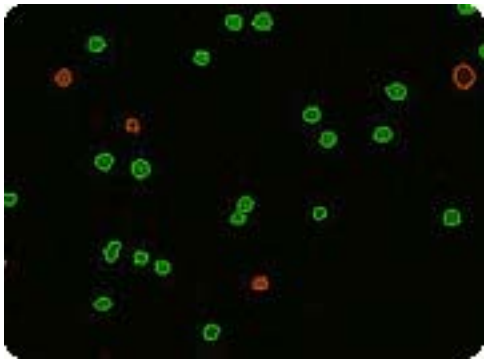
Spleenocyte: AOPI





PBMC: AOEB





Sample name	Cell count	Live cell concentration	Viability
PBMC #1	4588	1.29E+07	85.30%
	788	2.21E+06	
PBMC #2	6864	1.93E+07	68.20%
	3196	8.98E+06	
PBMC #3	1796	5.05E+06	73.80%
	639	1.79E+06	
PBMC #4	1090	3.07E+06	85.30%
	188	5.31E+05	
PBMC #5	1080	3.04E+06	92.80%
	84	2.37E+05	

Conclusions

- Primary cell sample contains cell like debris which is very hard to exclude using bright field only counting method
- Cell sample varies with different degree of contamination
- Fluorescent staining helps identify cells of interest
- Cellometer Auto X4 or Vision provide the simplest and accurate cell concentration for primary cells