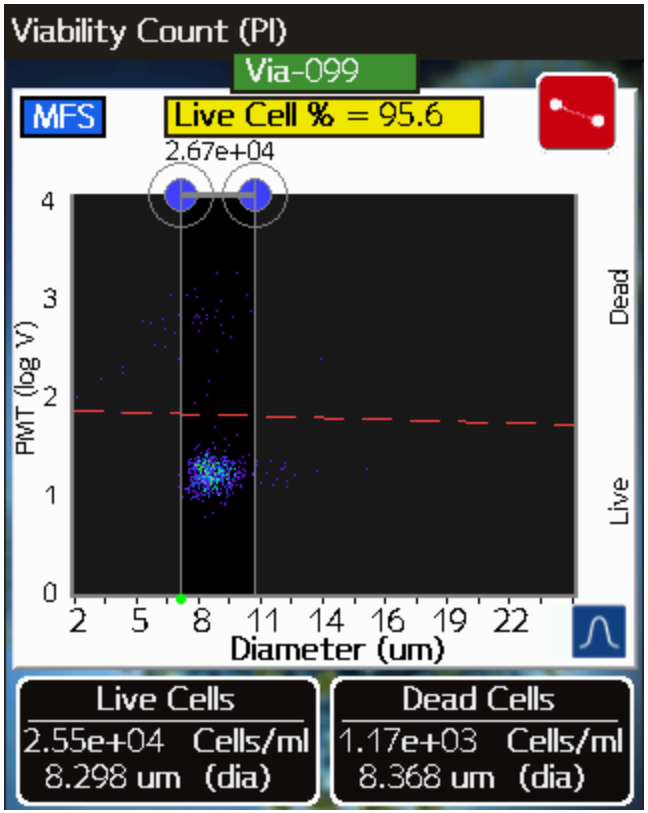
Distinguishing Activated and Resting CD4+ T-Cell Populations on the Moxi Flow

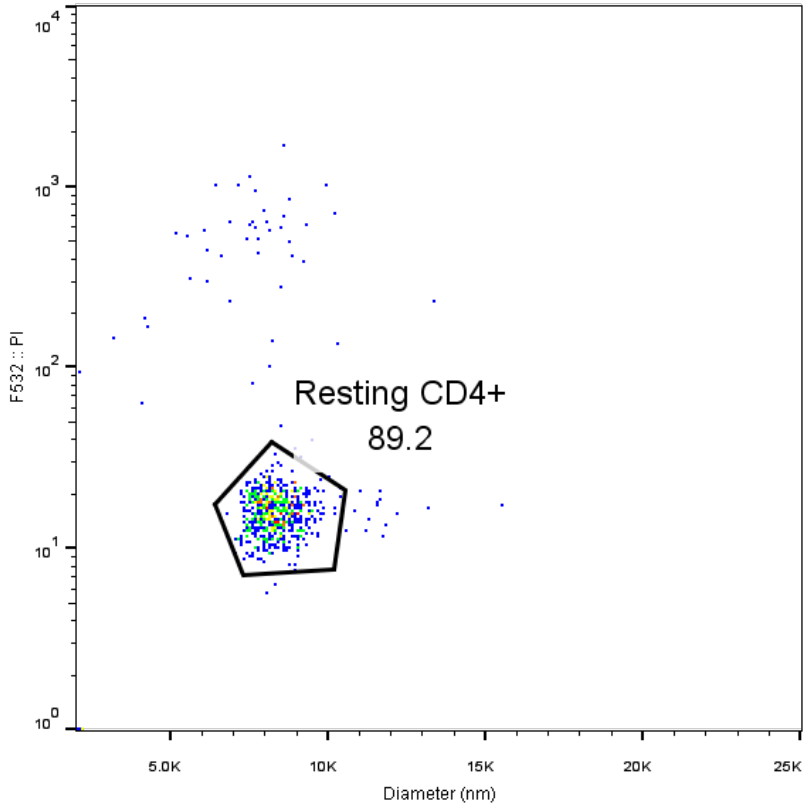
Dylan Koundakjian

Ryan Park

Ragon Institute of MGH, Harvard, & MIT

Resting CD4+



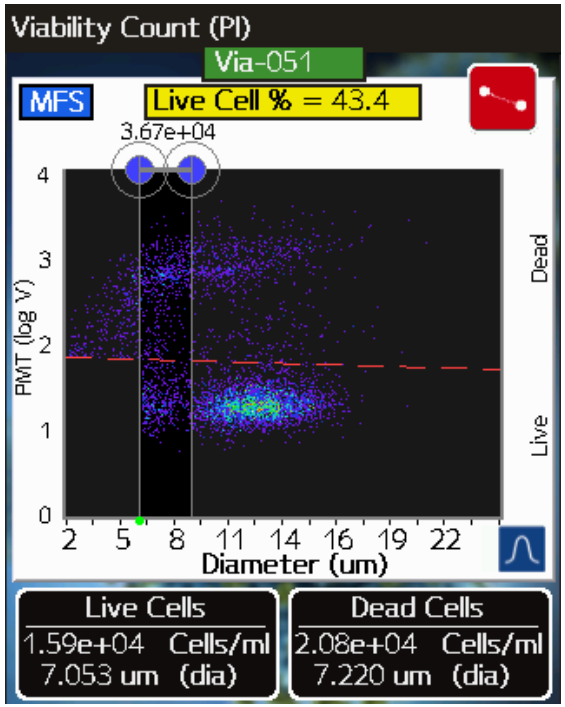


Diluted 1:10 in propidium iodide

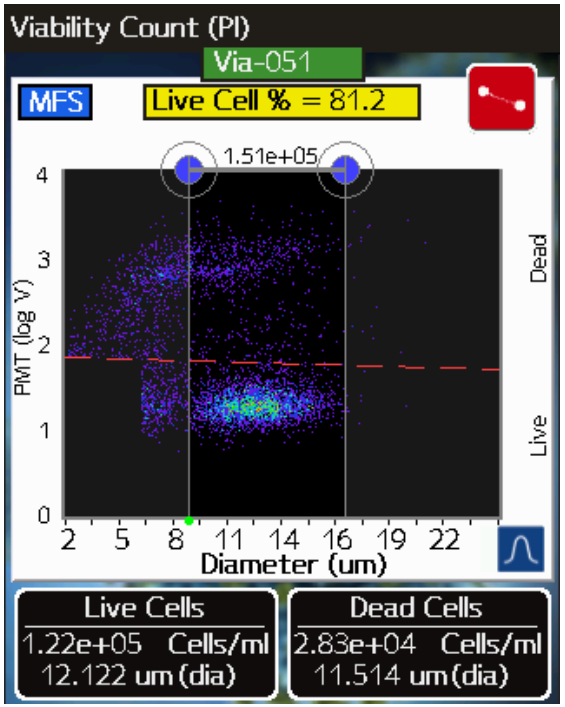
Activated CD4+ (Dynabeads)

Life Technologies

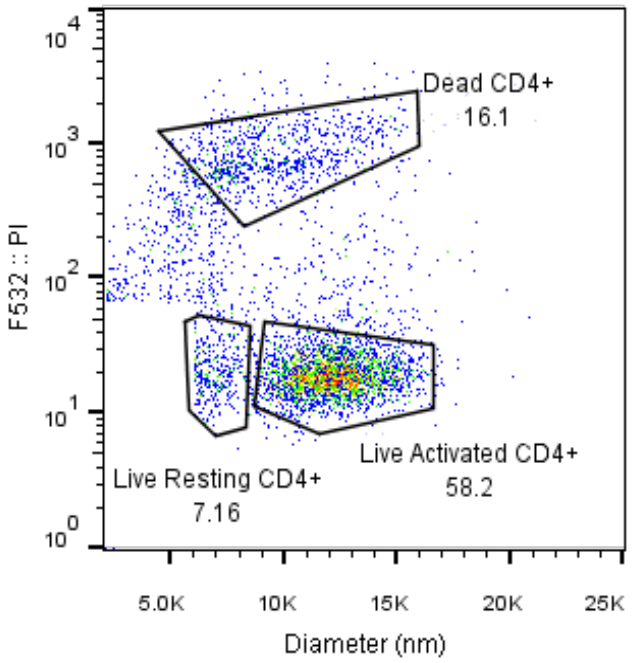
Resting Gate



Activated Gate



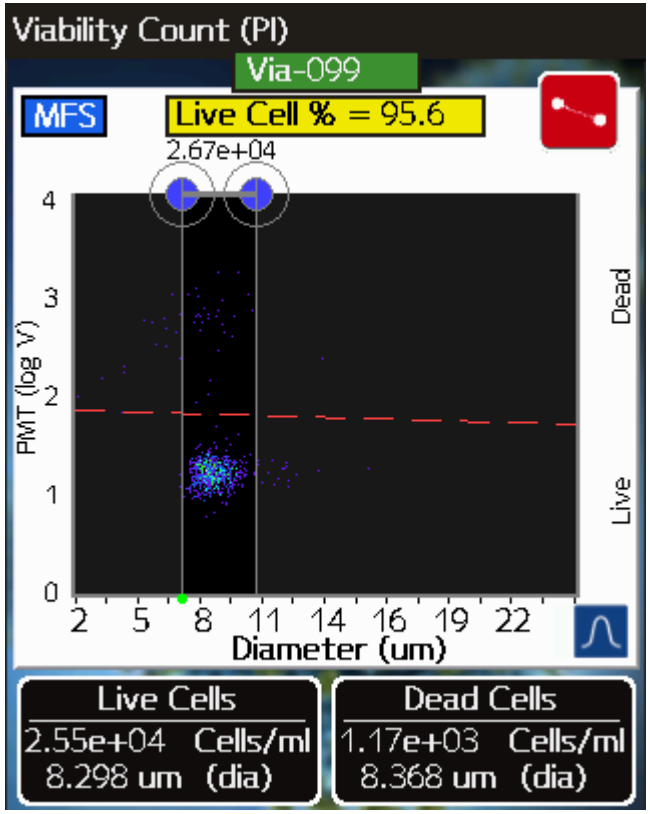
Both Gates



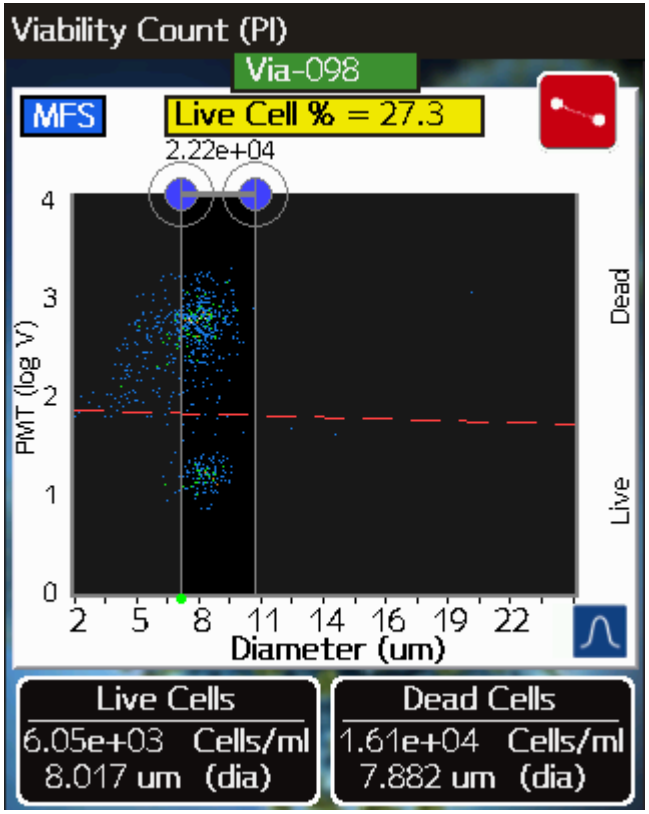
Resting and Activated populations distinguished by Coulter principle
(direct volumetric measurement)

Observing Cell Death with Antibiotics (Resting cells only)

Untreated Resting CD4+



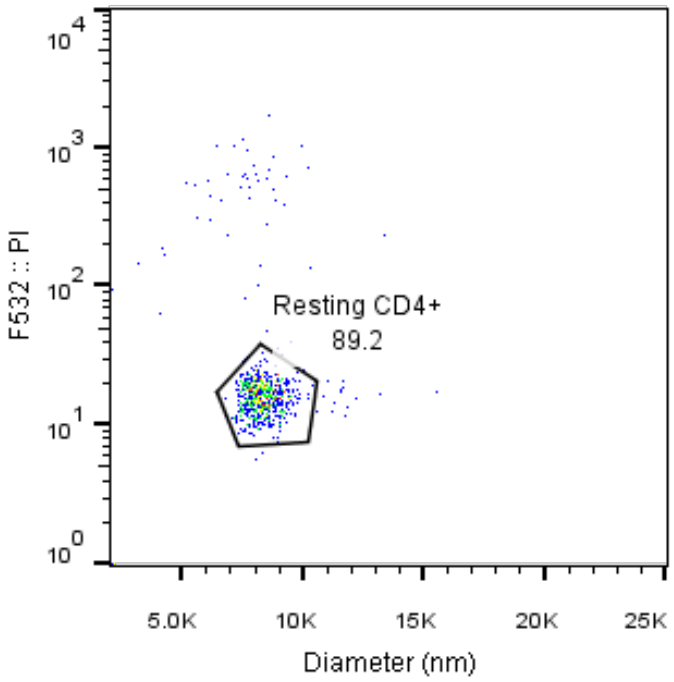
CD4+ with 2mg/mL Zeocin



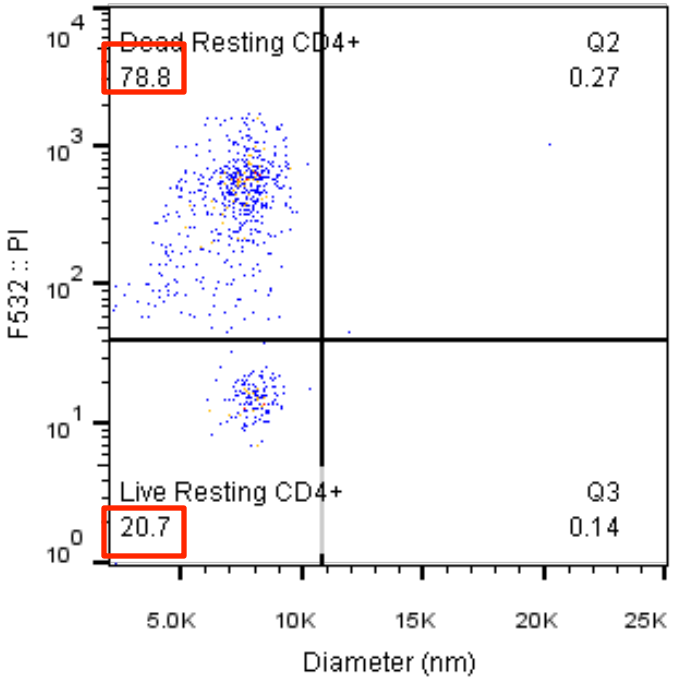
Note decreased live cell count and large dead cell population in Zeocin treated C

Observing Cell Death with Antibiotics, FCS View (Resting cells only)

Untreated Resting CD4+



CD4+ with 2mg/mL Zeocin



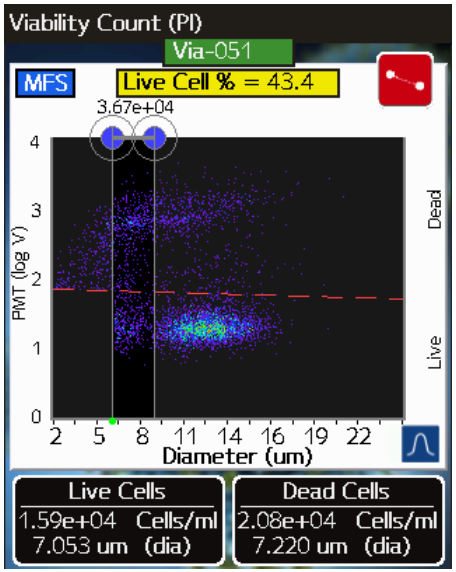
Note decreased live cell count and large dead cell population in Zeocin treated CD

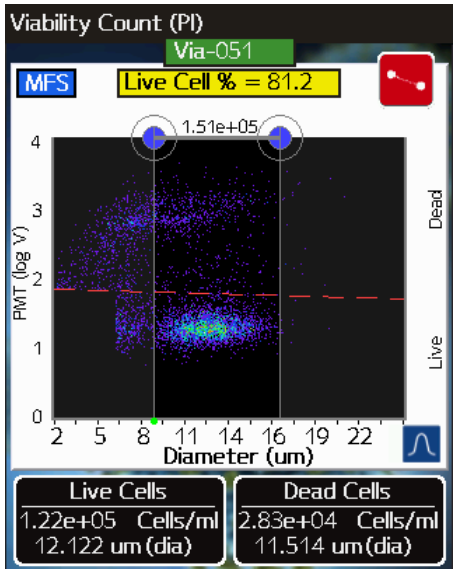
Observing Cell Death with Antibiotics (Resting and Activated populations)

Resting Gate

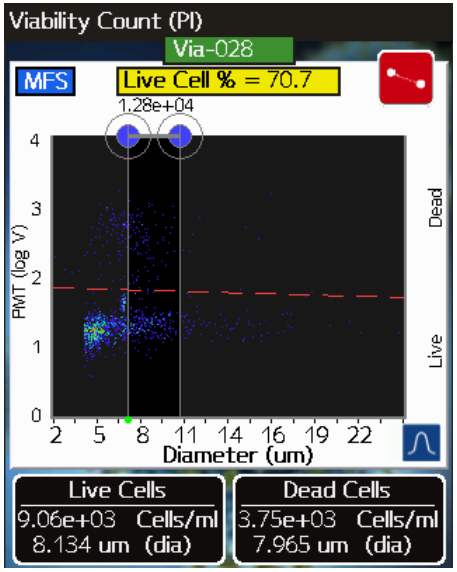
Activated Gate

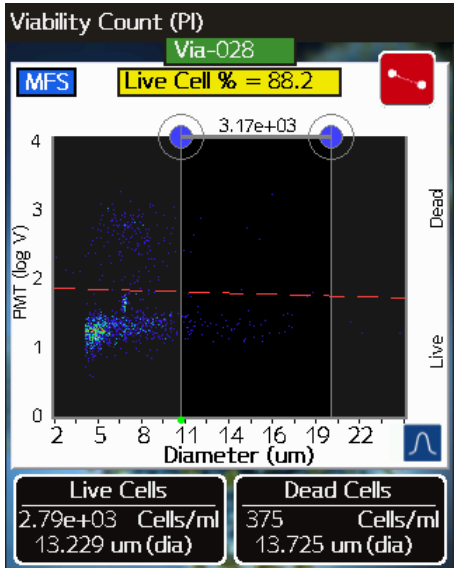
Untreated CD4+





CD4+ with 100ug/mL Zeocin

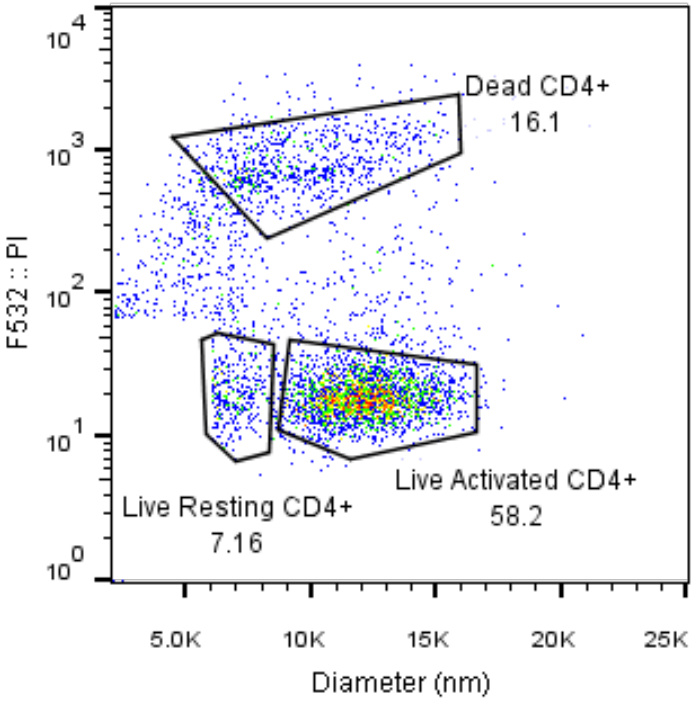




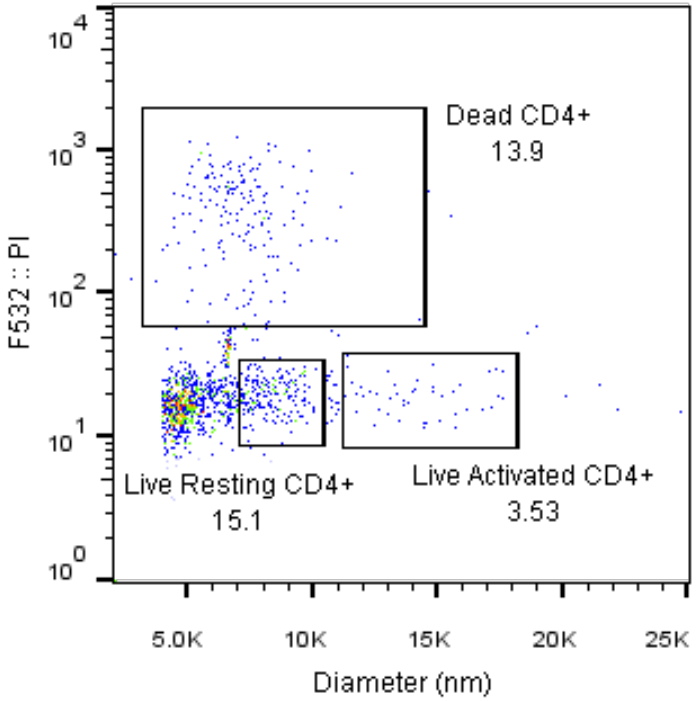
Size gating allows observation of distinct antibiotic effects on Activated and Resting populations

Both Gates (FCS files)

Untreated CD4+



CD4+ with 100ug/mL Zeocin



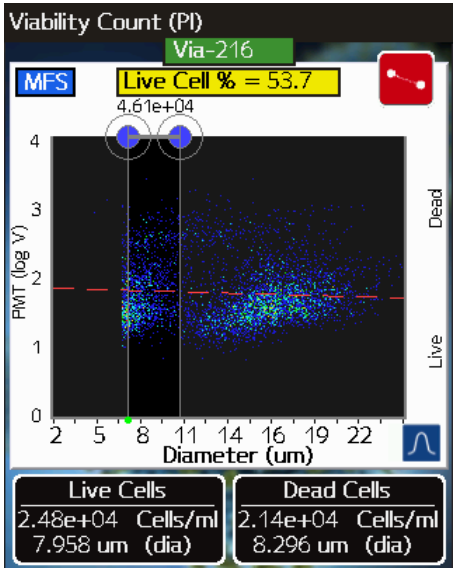
This concentration of Zeocin causes intermediate cell death in Resting population large scale death in Activated population

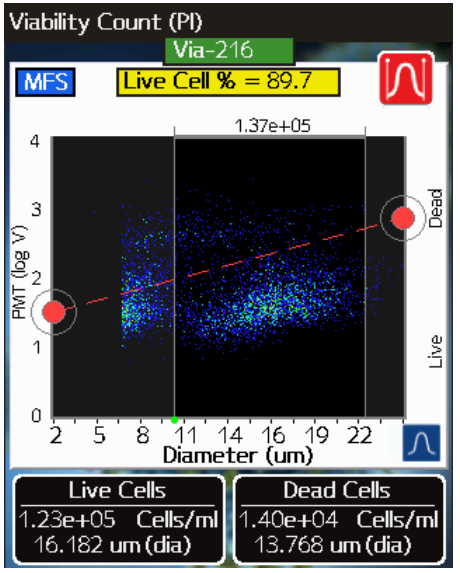
Observing Cell Death with Antibiotics (Resting and Activated populations)

Resting Gate

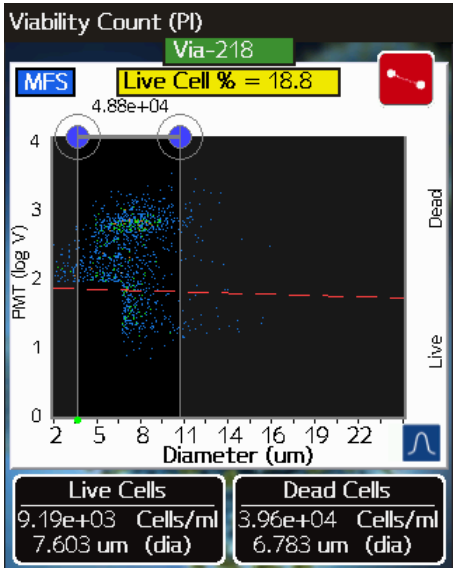
Activated Gate

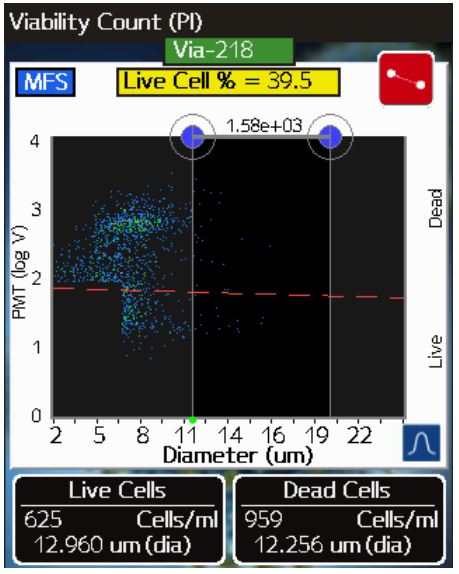
Untreated CD4+ (3 Days)





CD4+ with 1ug/ mL Puromycin (3 Days)

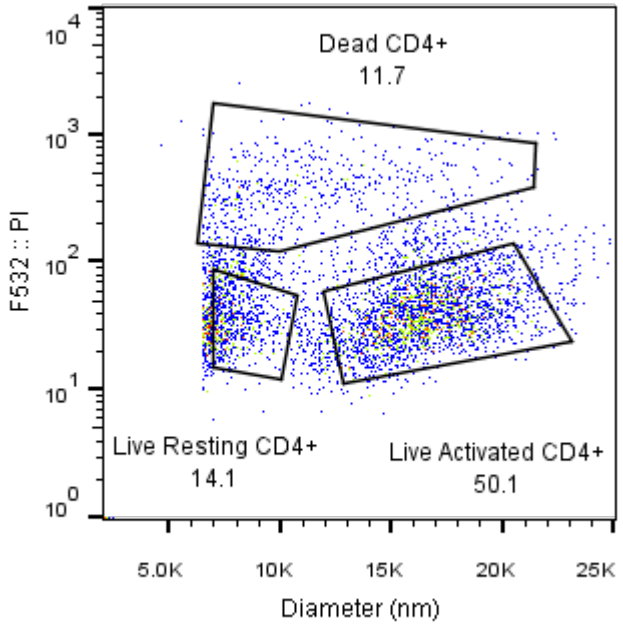




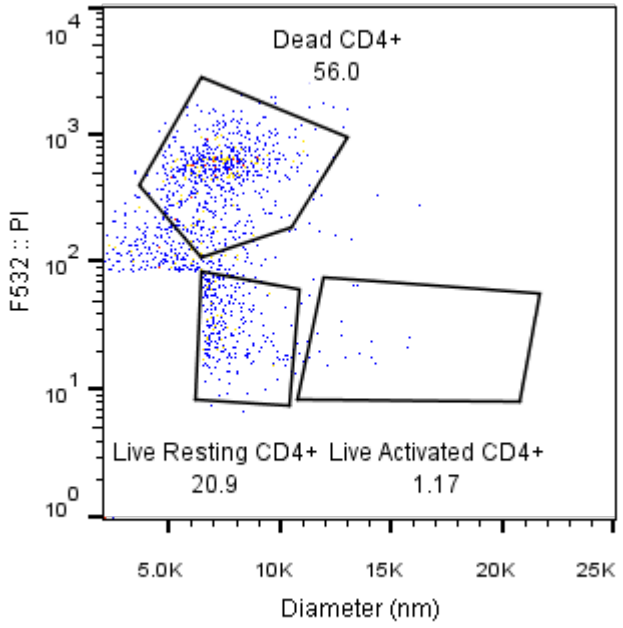
Size gating allows observation of distinct antibiotic effects on Activated and Resting populations

Both Gates (FCS files)

Untreated CD4+

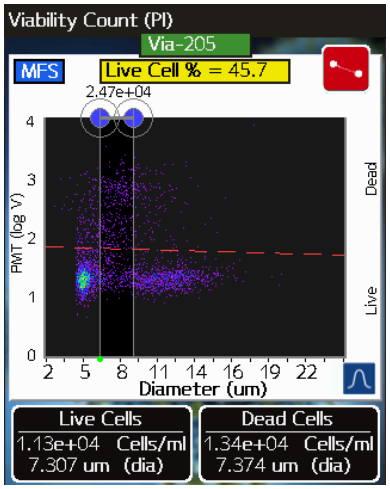
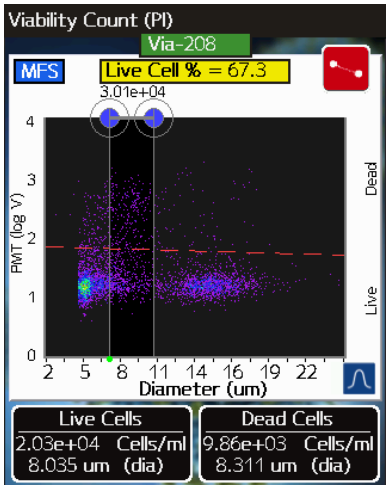
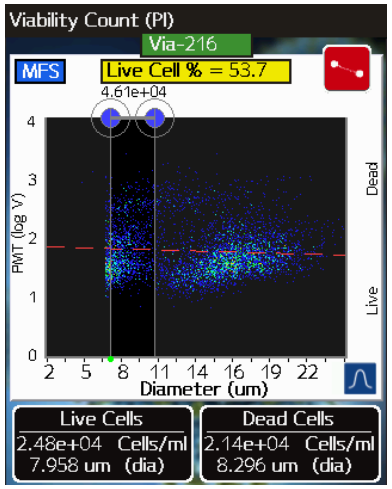
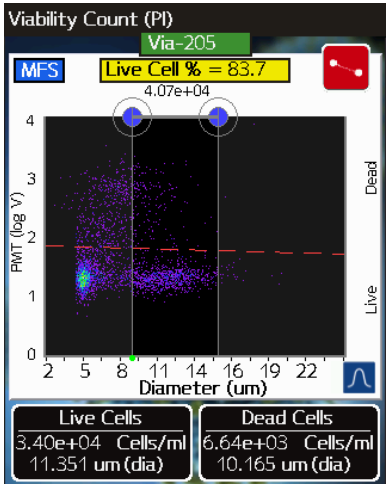
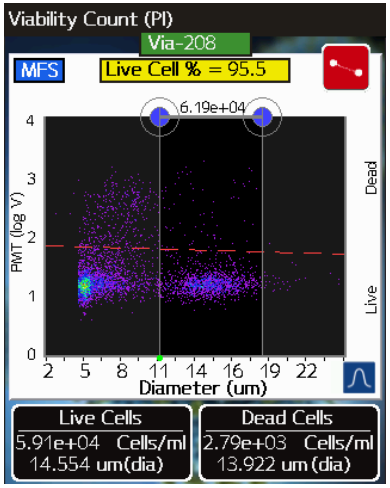
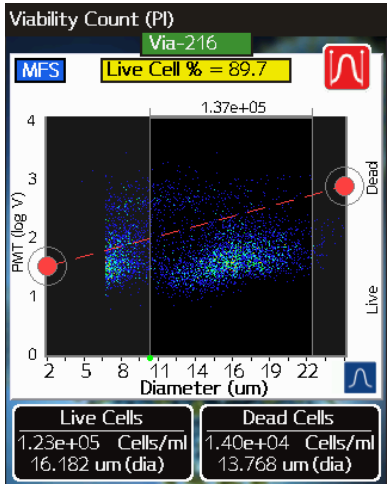


CD4+ with 1ug/mL Puromycin



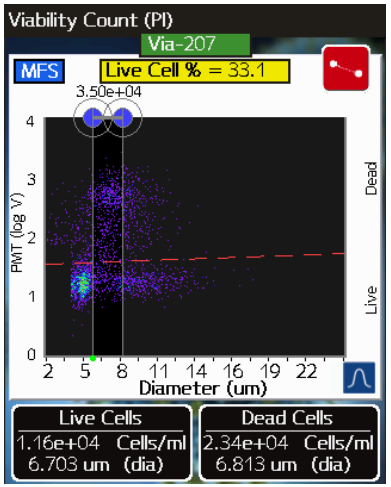
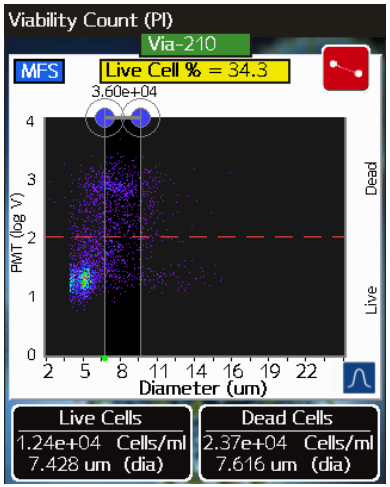
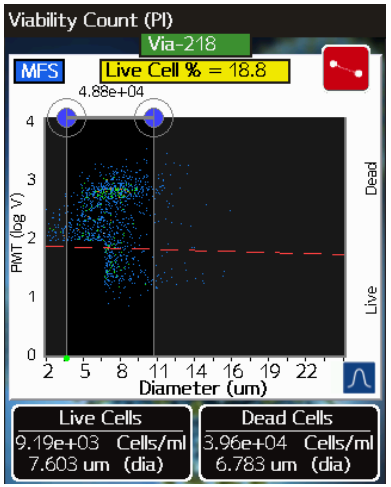
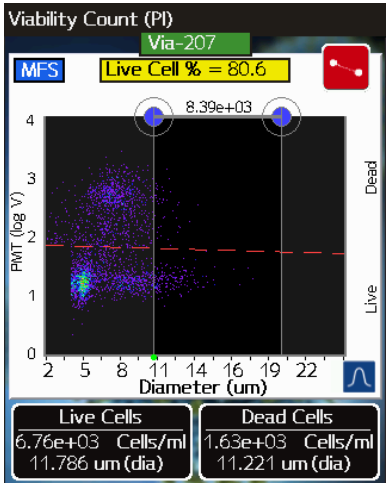
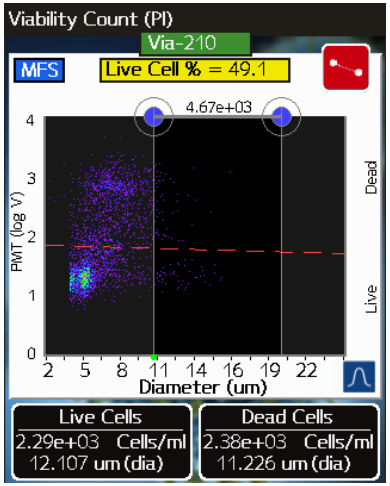
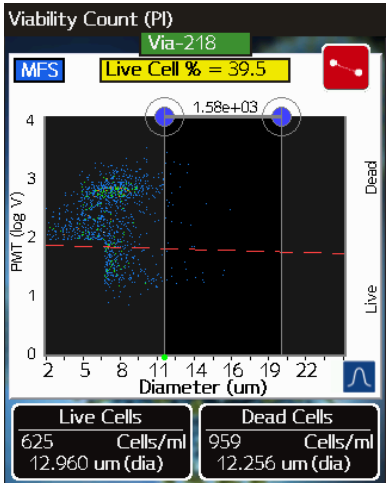
This concentration of causes total death in Activated population with less effect o
Resting population

Tracking CD4+ Activation Over Consecutive Days (Untreated)

	24 hours	48 hours	72 hours
Resting Gate			
Activated Gate			

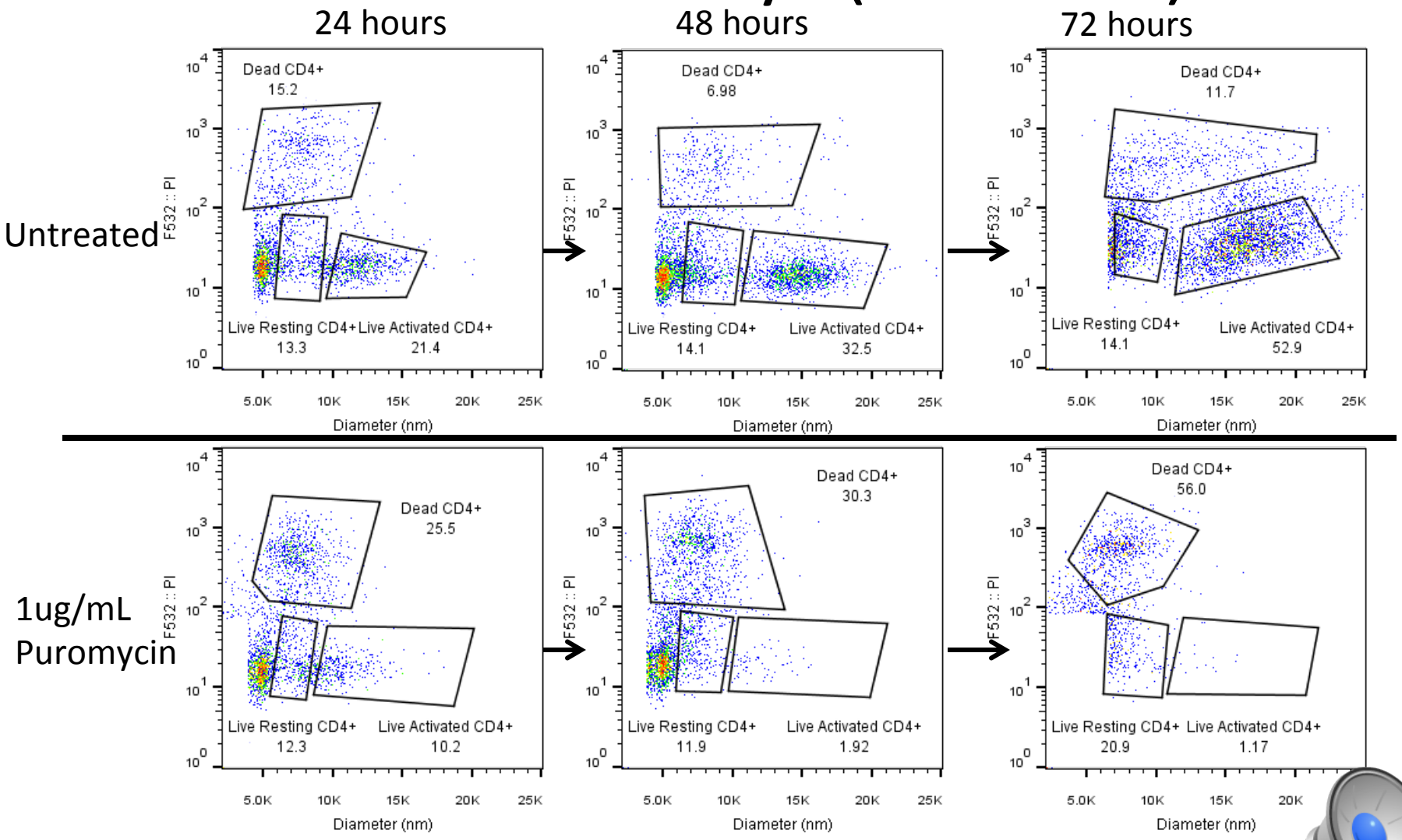
Exponential increase of the Activated cell population is evident from Moxi Flow data.

Tracking CD4+ Activation Over Consecutive Days (1ug/mL Puromycin)

	24 hours	48 hours	72 hours
Resting Gate			
Activated Gate			

Note relative survival of the Resting population while Activated population decreases significantly a t of Puromycin selection.

Tracking CD4+ Activation Over Consecutive Days (FCS view)



Note relative survival of the Resting population while Activated population decreases significantly a t of Puromycin selection.

Conclusions

- Moxi flow can be used to distinguish Resting and Activated CD4+ populations by Coulter principle.
- Treatment of CD4+ cells with different antibiotics has differential effects on Resting and Activated populations.
- These differential effects can be observed on the Moxi Flow through viability counting on specific Resting and Activated size gates.
- These data are useful for determining which antibiotics to use for selection in different scenarios involving CD4+ T cells.