

## 1. PLANNING A FLOW CYTOMETRY EXPERIMENT

You want to look at CD25 expression in CD3+ CD4+ T cells in peripheral blood leukocytes. You also need to assess cell death.

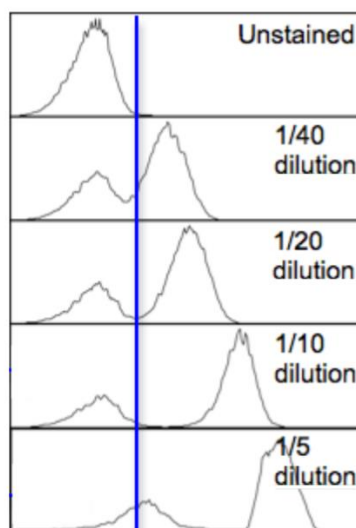
	FITC	PE	PerCP	PE-Cy5	PE-Cy7	APC
anti-CD3	✓	✓		✓	✓	✓
anti-CD4	✓	✓	✓	✓		✓
anti-CD25		✓		✓		✓

Live/Dead: PI, DAPI

- a) Looking at the instruments available at the CEDOC Flow Cytometry Unit, which one would you choose, and suggest a panel to answer the question (Search supplementary information).

## 2. INTERPRETING FLOW CYTOMETRY DATA

- a) What is the goal of a titration?  
-particularly with respect to the positive and negative populations?

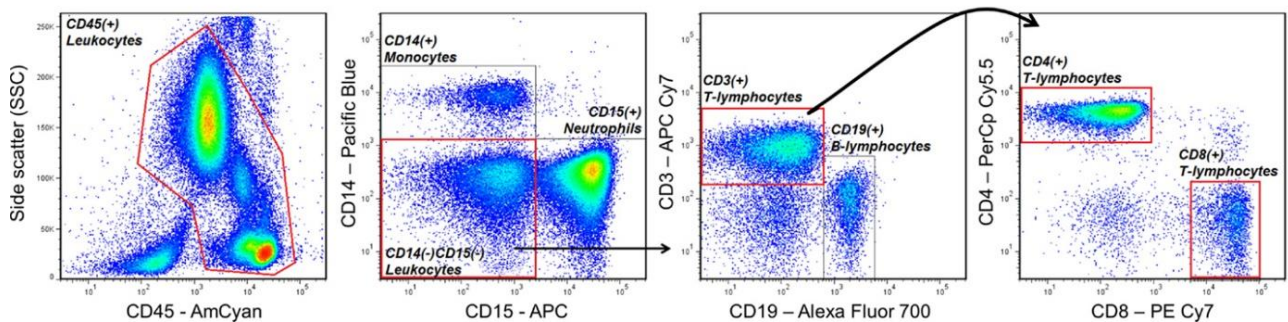


- b) Which concentration of antibody is correct?

## 2. INTERPRETING FLOW CYTOMETRY DATA

One of the most basic principles of FCM analysis is “gating,” which is the sequential identification and refinement of a cellular population of interest using a panel of molecules that are visualized by fluorescence in a unique emission spectrum.

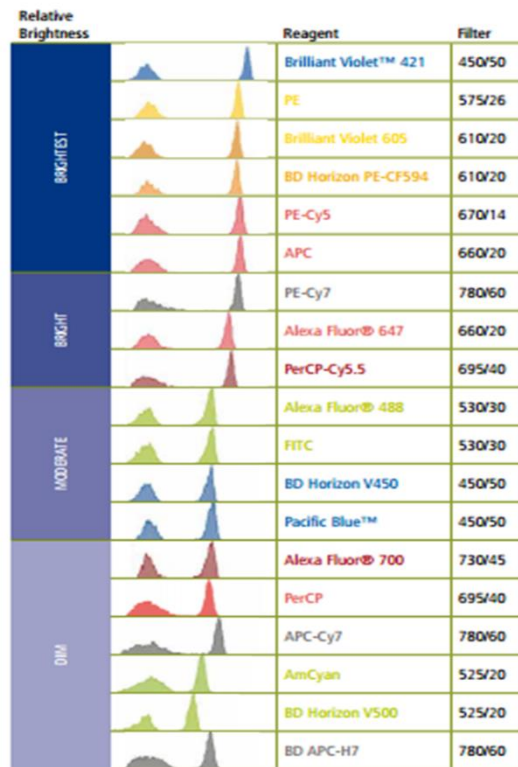
The panel described in this exercise are designed to identify CD4 and CD8 T-lymphocytes in human peripheral blood.



- a) Make an interpretation of the results and describe the gating strategy shown in this figure.



## Brightest Fluorochrome (Stain Index)



## Antigen Density

Cell	Antigen	Molecules per Cell
T cell	TCR	100,000
	CD2	55,000
	CD3	124,000
	CD5	90,000
	CD7	20,000
	CD45	>200,000
CD4+ T cell	CD4	100,000
	CD28	20,000
	CCR5	4,000-24,000
CD8+ T cell	CD8	90,000
	CD28	15,000
B cell	CD19	18,000
	CD20	109,000
	CD21	210,000
	CD22	14,000
	HLA-DR	85,000
	CD11a	10,000
	CD40	2,000
	CD86	16,000
Dendritic cell	CD80	2,000
	CD11a	27,000
	CD40	17,000
	CD86	132,000
Monocyte	CD86	208,000
	CD14	110,000
	CD32	21,000
Neutrophil	CD64	13,000
	CD14	3,500
NK cell	CD16	225,000
	CD56	10,000
Red Blood Cell	Glycophorin A	340,000
Basophil	CD23	15,000
IL2RA	CD25	3,000