



CYTEK
TRANSCEND THE CONVENTIONAL

Exercise 3

Supplemental Materials

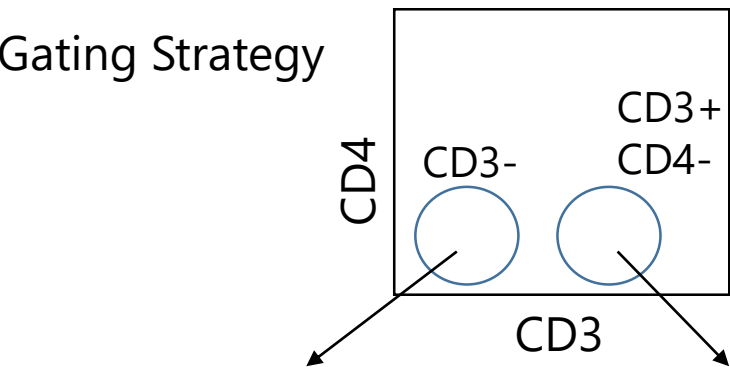
Example for a 4 Laser Aurora

Exercise 3 – Panel Design Steps

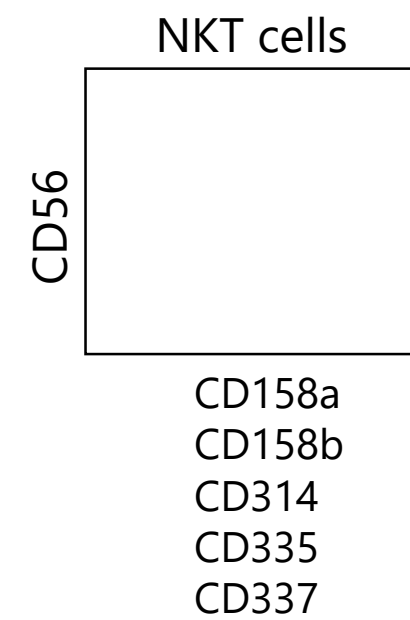
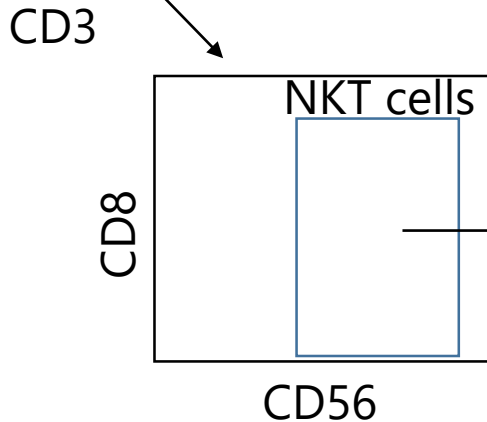
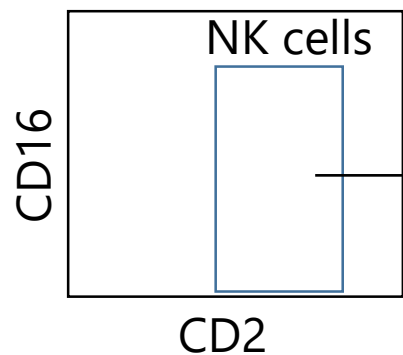
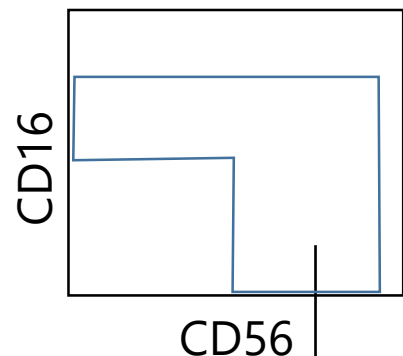
1. Classify the antigens
 - Go to vendor websites, check staining examples
 - Gating strategy
2. Consider co-expression
 - Gating strategy – know your biology
3. Characterize spread of available fluors – use stain index and spread matrix
 - Bright fluors that receive low spread
 - Dim fluors that contribute low spread
4. Assign Fluors
 - Tertiary – very bright/bright fluors that receive low spread
 - Secondary
 - a) bright/mid fluors that receive low spread
 - b) mid fluors that contribute low spread
 - Primary – dim fluors that contribute low spread



Gating Strategy



Exercise 3 NK Cell Panel Design



NK Cell Panel

Classify/Fluor	Specificity	Alt Name	Purpose
	CD2	LFA-2	NK cells
	CD16	FCgRIII	
	CD56	NCAM	
	CD8		non-NK cells
	CD3		
	CD4		NK receptors
	CD158a	KIR2DL1	
	CD158b	KIR2DL2	
	CD314	NKG2D	
	CD335	NKp46	
	CD337	NKp30	



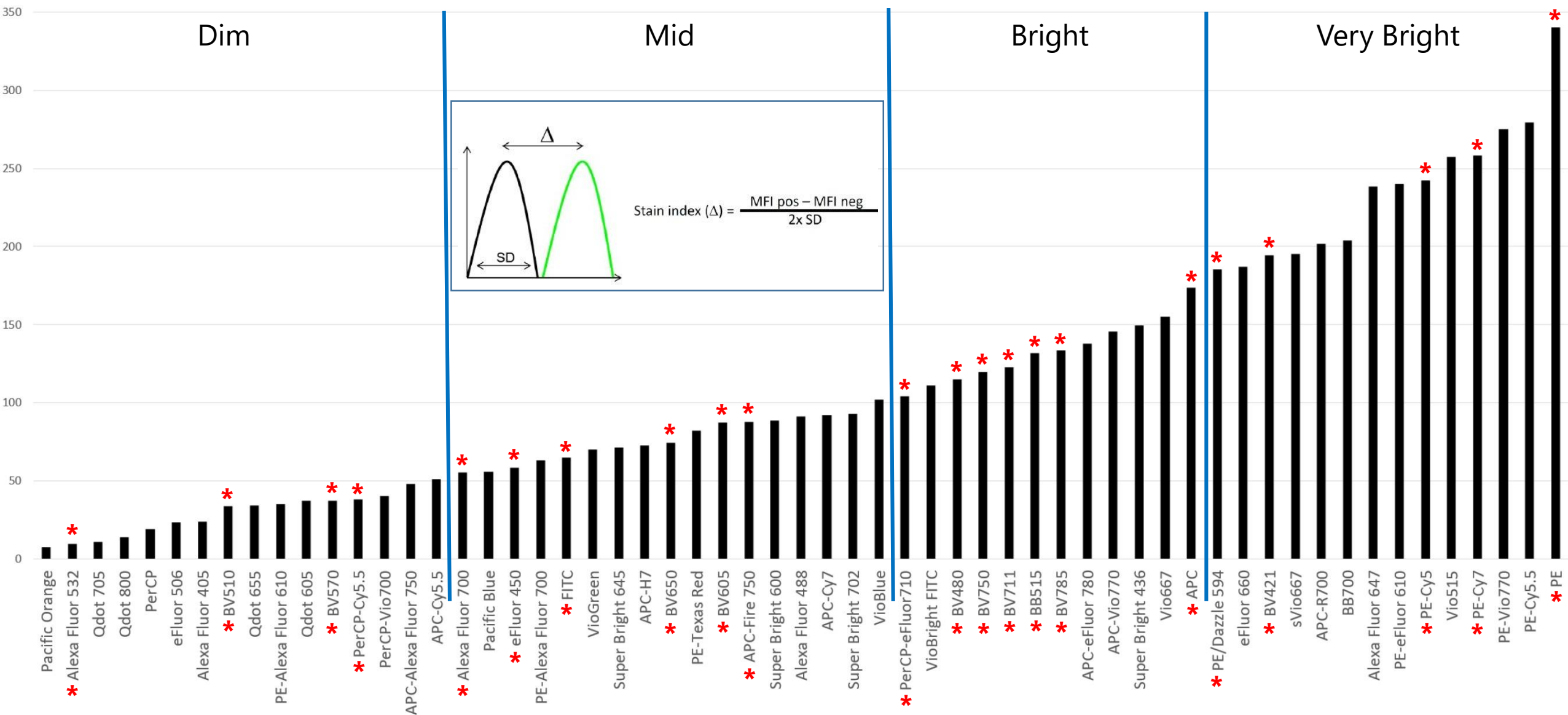
Here is the reagent availability for the markers in the panel:

							1	2	5	3	4
	CD2	CD16	CD56	CD8	CD3	CD4	CD158a	CD158b	CD314	CD335	CD337
BV421	x	x	x	x	x	x	x	x	x	x	x
e450	x	x	x	x	x	x	x			x	
BV480	x	x	x	x	x	x		x	x		x
BV510	x	x	x	x	x	x		x	x	x	x
BV570	x	x	x	x	x	x					
BV605	x	x	x	x	x	x		x	x	x	x
BV650	x	x	x	x	x	x			x	x	x
BV711	x	x	x	x	x	x	x	x	x	x	x
BV750	x	x	x	x	x	x					
BV785	x	x	x	x	x	x		x	x		x
BB515	x	x	x	x	x	x			x	x	
FITC	x	x	x	x	x	x	x	x	x	x	
AF532				x	x	x					
PE	x	x	x	x	x	x	x	x	x	x	x
PE CF594	x	x	x	x	x	x			x	x	
PE Cy5	x	x	x	x	x	x					
PerCP Cy55	x	x	x	x	x	x	x	x			x
PerCPe710	x	x	x	x	x	x			x		
PE Cy7	x	x	x	x	x	x	x	x	x	x	x
APC	x	x	x	x	x	x	x	x	x	x	x
AF700	x	x	x	x	x	x					
APC Fire750	x	x	x	x	x	x			x	x	



* Reagent available

Stain Index Ranking - 59 Dyes



Primary
Secondary
Tertiary

NK Panel Design Exercise 3

(1) = Dim
(2) = Mid
(3) = Bright
(4) = Very Bright

λ	Violet 405nm		Blue 488 nm		Yellow-Green 561 nm		Red 640 nm	
	Antigen	Fluor	Antigen	Fluor	Antigen	Fluor	Antigen	Fluor
390								
430		BV421 (4)						
445								
460		eF450 (2)						
475		BV480 (3)						
510		BV510 (2)		BB515 (3)				
525				FITC (2)				
540				AF532 (1)				
580		BV570 (2)				PE (4)		
615		BV605 (2)				PE-CF594(4)		
660		BV650 (2)						APC (4)
680						PE-Cy5 (4)		
700								
720		BV711 (3)		PerCP-eF710 (3)				AF700 (3)
750		BV750 (3)						
780		BV785 (3)				PE-Cy7 (4)		APC-F750 (2)
780								ZombieNIR
810								

