July 21-22nd, 2022

Fresh-thawed PBMCs
Spectral Panel Optimization #2

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Monocytes Total	2E+6	1.5E+6

Aurora UV - they've fixed the UV3 detector! nice

FSC - 60 20K events unstained

Setting width is available under instrument control adj to height.

CD18 - CD86

CD3+ CD20 (most CD20 stained)

16 x 5 bits

CCR5 by CD80

struggling

HLA-DR+ 88

Doublets hide above FSC 3.0M

Time → ND050 monocytes rough run

My samples CD20 unmixing was badly screwed -10^5 → 10^4

HastTings 10^-4 → 10^4 … not great spot?

my HLA-DR unmixing vs CD20 is especially poor…

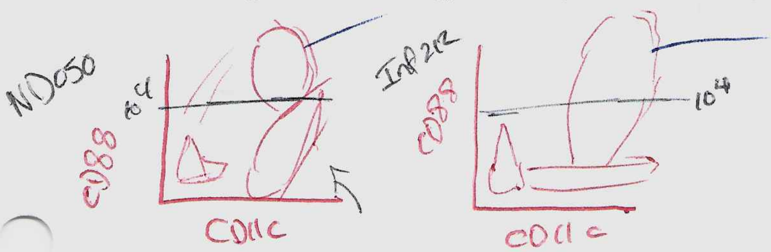
May be a CD20 // higher exp than CD19

basophil contam minor in cord.

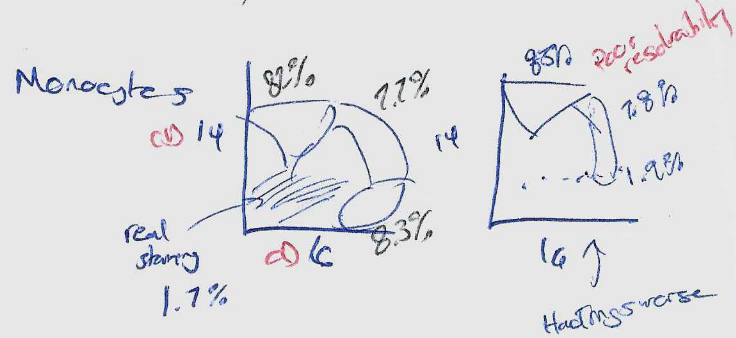
pDCs → "3.5%" ND050, 0.96% Inf212

cDc1/2 no markers to delineate CD11c+ CD141+/- CD1c +/-

CD88 unmixing bt diffs ND050 (imp for nc & iMon)



Not as easy delineate.



CD11c is a poor definer for monocytes

✯ pdc's in adult – monocyte CD88 ✯ → 14/16 negative

not ease for CBMCs //

CCR5 - PBMCs CCR5+ cbmcs

CD88

July 21st, 2022 Monocyte SFC panel

#	Filter	Single color (ul)	Ref ctrl type	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	L/D + FC blocker 3ul/rxn	37oC (RT) for 30min	1	4oC for 30min	1
1	UV2	1.25	Cells		BUV395	CD18	(6.7)			0.8	0.8		
	UV7				BUV496								
2	UV9	1.25	Cells		BUV563	CCR2	(Is132.1D9)			0.8	0.8		
	UV10				BUV615								
3	UV11	1.25	Cells		BUV661	CX3CR1	(2A9-1)			1	1		
	U14				BUV737								
4	UV16	1.25	Cells		BUV805	CD14	(M5E2)					1.0	1
	V1	-	-		BV421								
	V2				Super Bright 436								
	V2				cFluor 420								
	V3				Pacific Blue								
5	V5	1.25	Cells		BV480	CD11a	(HI111)					0.6	0.6
6	V7	1.25	Cells		BV510 (dim)	CD20	(2H7)			0.8	0.8		
7	V8	1.25	Cells		BV570	CD16	(3G8)					1.5	1.5
	V10				cFluor 610 better								
8	V10	1.25	Cells		BV605	CD56	(5.1H11)					1.0	1
9	V11	1.25	Cells		BV650	CD11c	(B-ly6)					1.0	1
10	V13	1.25	Cells		BV711	CD80	(L307.4)			1.5	1.5		
11	V14	1.25	Cells		BV750	CCR5	(2D7)			1.5	1.5		
	V15		Cells		BV786								
	B1				BB515 / VioBright B515								
12	B2	1.25	Cells		FITC	CD11b						0.8	0.8
	B3		Cells		Spark blue 550								
13	B4	1.25	Cells		PE	CD88	(S5/1)			0.6	0.6		
14	B6	1.25	Cells		PE-Dazzle594	CD3						0.6	0.6
15	B8	1.25	Cells		PE-Cy5	CD86	(IT2.2)			0.5	0.5		
	B9				BB700 better								
16	B10	1.25	Cells		PerCP-Cy5.5	CD123	(7G3)			2	2		
17	B13	1.25	Cells		PE-Vio770	HLA-DR	(REA805)					0.5	0.5
	R1	-	Cells		APC								
18	R2	1.25	Cells		Alexa Fluor 647	CD163	(GHI/61)					1.0	1
	R3				Spark NIR 685								
19	R4	1.25	Cells		APC-R700	CD40	(5C3)			1.0	1		
20	R6	1:2500	Cell		Zombie NIR	L/D			<1:2500>				
21	R7	1.3	Cells		APC/Fire 750	CD64	(10.1)			1	1		
22	R8	1.3	Cells		APC/Fire 810	CD38	(HIT2)			1.0	1		
					True Stain FcX					0.5	0.5	0.5	0.5
And UNSTAINED CONTROLS !!!					Antibody Total					13	13	8.5	8.5
					PBS					50	50	50	50
					Pippette draw volume/sample					62		57.5	

Handwritten on table: "PE-CF594 in exp" (near PE-Dazzle594); "which is it?" (near CD88/CD3); "ckc 2/3" (near Spark NIR 685); "custom" (near CD64)

Simplified Protocol

Aliquot cells 1.2E+6 cells/tube

Wash with 1ml PBS, 1300rpm, 8min

2500x diluted Zombie NIR, 1E+6/1ml + 3ul Fc blocker at RT for 15min

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

x ul of 37oC Ab mix, at 37oC for 30min

Remove RBC with 1ml lysing solution for 3min

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

x ul of 4oC Ab mix, at 4oC for 30min

Treat with 600ul of 1x Lysing solution at RT for 3min

Wash with 2ml 2%FBS-PBS-2mM EDTA

Spin at 1300rpm for 8min

Resuspend in 0.4%PFA-PBS

Should you add more ab to controls or leave just smaller conc to Rodney (?)

4.16 / 20 mL / 4/1 × 20 = 80 μL EDTA

Notes: TrueStain FcX (Biolegend 422301) final 0.1ug/ml for 24hr

InvivoGen tlrl-3pelps Lipopolysaccharides from Escherichia coli O111:B4

LPS stock 5mg/ml, 10ul; take one aliquot out and add 490ul R10 to make 100ug/ml working solution

Wash with 2mM EDTA

gate to [illegible] or steepest?

Single color controls gates

capping @ 10^6 unless good reason

Monocytes SFC Panel 7/8/2022

UV

Spectrum	Channel	Fluor	Rank	Marker
373	UV1			
388	UV2	BUV395	[2]	CD18
428	UV3			
443	UV4			
458	UV5			
473	UV6			
508				
514	UV7	BUV496	[2]	
525				
542	UV8			
582	UV9	BUV563	[3]	CCR2
598				
613	UV10	BUV615	[3]	
664	UV11	BUV661	[3]	CX3CR1
679				
697	UV12			
717	UV13			
738				
750	UV14	BUV737	[3]	
760				
783	UV15			
812	UV16	BUV805	[1]	CD14

Violet

Channel	Fluor	Rank	Marker
V1	BV421	[4]	
V2			
V3	PacBlue	[1]	
V4			
V5	BV480	[3]	CD11a
V6			
V7	BV510	[1.5]	CD20
V8	BV570		CD16
V9			
V10	BV605	[3]	CD56
V11	BV650	[3.5]	CD11c
V12			
V13	BV711	[4]	CD80
V14	BV750	[2.5]	CCR5
V15	BV786	[3]	
V16			

Blue

Channel	Fluor	Rank	Marker
B1			
B2	FITC	[1.5]	CD11b
B3	SparkBlue 550	[1]	
B4	PE	[4]	CD88
B5			
B6	PE-CF594 (Dazzle)	[4]	CD3
B7			
B8	PE-Cy5	[4.5]	CD86
B9	PerCP-Cy5.5	[2]	CD123
B10			
B11			
B12			
B13	Pe-Vio770	[3]	HLA-DR
B14			

Red

Channel	Fluor	Rank	Marker
R1	APC	[3.5]	
R2	AF647	[3.5]	CD163
R3			
R4	APC-R700	[3]	CD40
R5			
R6	Zombie NIR	[ ]	~~L/D~~ Live Dead
R7	APC-Fire 750	[2]	CD64
R8	APC-Fire 810	[3]	CD38

- BUV395: mostly +
- CD18: 10^4.5 to 10^5 half
- BUV496: mostly +
- 10^5.5 edge; some oddballs include to 10^6
- CCR2: some [illegible]
- CX3CR1: long tail 10^5 → 10^6
- BUV737 / CD14: Removed this; narrow shift 10^5.5
- V4 circled; BV510: gen issue?
- BV650: most near
- BV711: very weak stain
- CD11a: some negative, 10^5–10^6
- CD20: spiked in CD3 edge 10^5–10^5.5
- CD16: to brightest 10^5–10^5.5
- CD56: 10^5–10^5.5
- CD11c: 10^5–10^6
- CD80: below 10^5
- CCR5: all @ intermediate, some very bright, 10^4.5 → 10^6; shoulder & brightest; no red AF
- PE-CF594: Dazzle, 0.95
- CD11b: 10^5–10^5.5
- CD88: 10^5 → 10^6
- CD3: 10^5 → 10^6
- CD86: 10^5 → 10^6, lower
- CD123: 10^4 → 10^5, 30K cells
- HLA-DR: most middle, 10^5.5 → 10^6
- CD163: 10^4 → 10^6
- CD40: 10^4.5 → 10^5.5; 10^4–10^5
- CD64: 10^5 → 10^6
- CD38: 10^6 3/4 → 10^6.1

50,000 monocytes unstained gated solidly

15,000 events for positives

high shift in negative (from AF or stain?)

FSC @ 60 centers the monocytes on FSC-A

AF: B3A high, UV8, V5 – V11

CD80 is a very weak stain; 30 to 15 → 45K; ↑ 15,000 cells

~~BV480~~ CD80 at 10^3.5/8th → 10^4

best we've got.

Spiked in edge markers; CD20,

CD56 → up to 10^6.2 (10^4.5 →) in case

CD3

CD123 → 45K?

CD40 w/in monocytes @ edge.

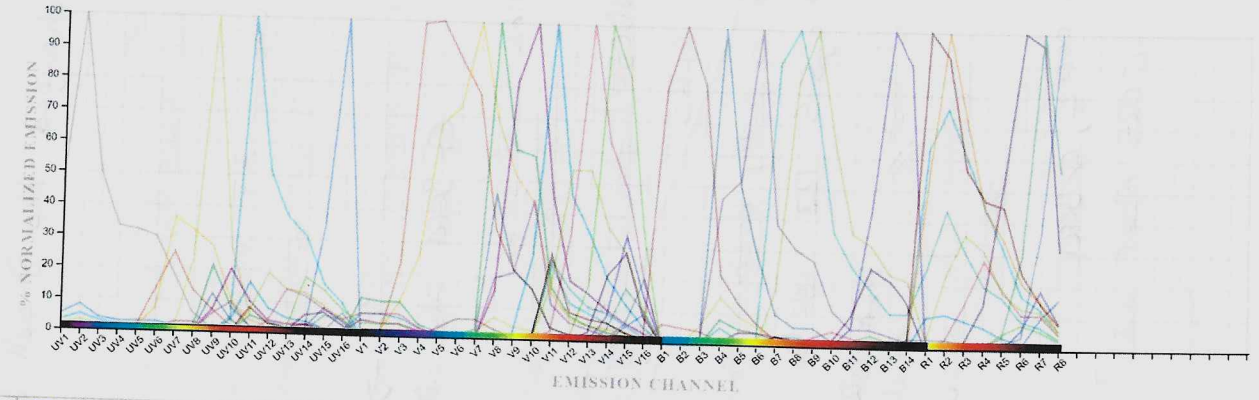
*CCR5 have a peak 10^4, spots too! up to 10^5 but some way way brighter

Compromise CCR5 split

Date: May 12, 2022

Monocyte SFC Reference Library

#	Filter	Fluorochrome	Marker	Clone	Manufacturer	Catalog #	Cell Location	Abundance	Lot #	Control type	Fixed?	Condition	Panel ul	Panel brightest MFI	Total #	Single color (ul)	Negative MFI	Positive MFI	Comments	Reference Library Name
1		BUV395	CD18	(6.7)												1.5 1 0.5				
2		BUV563	CCR2	(Is132.1D9)												1.5 1 0.5				
3		BUV661	CX3CR1	(2A9-1)												1.5 1 0.5				
4		BUV805	CD14	(M5E2)												1.5 1 0.5				
5		BV480	CD11a	(HI111)												1.5 1 0.5				
6		BV510	CD20	(2H7)												1.5 1 0.5				
7		BV570	CD16	(3G8)												1.5 1 0.5				
8		BV605	CD56	(5.1H11)												1.5 1 0.5				
9		BV650	CD11c	(B-ly6)												1.5 1 0.5				
10		BV711	CD80	(L307.4)												1.5 1 0.5				
11		BV750	CCR5	(2D7)												1.5 1 0.5				
12		FITC	CD11b													1.5 1 0.5				
13		PE	CD88	(S5/1)												1.5 1 0.5				
14		PE-Dazzle594	CD3													1.5 1 0.5				
15		PE-Cy5	CD86	(IT2.2)												1.5 1 0.5				
16		PerCP-Cy5.5	CD123	(7G3)												1.5 1 0.5				
17		PE-Vio770	HLA-DR	(REA805)												1.5 1 0.5				
18		Alexa Fluor 647	CD163	(GHI/61)												1.5 1 0.5				
19		APC-R700	CD40	(5C3)												1.5 1 0.5				
20		Zombie NIR	L/D													1.5 1 0.5				
21		APC/Fire 750	CD64	(10.1)												1.5 1 0.5				
22		APC/Fire 810	CD38	(HIT2)												1.5 1 0.5				
23																1.5 1 0.5				
24																1.5 1 0.5				
25																1.5 1 0.5				
26																1.5 1 0.5				
27																1.5 1 0.5				
28																1.5 1 0.5				
29																1.5 1 0.5				
30																1.5 1 0.5				
31																1.5 1 0.5 1.5				



BUV395	BUV563	BUV661	BUV805	BV480	BV510	BV570
BV605	BV650	BV711	BV750	FITC	PE	PE-Dazzle594
PE-Cy™5	PerCP-Cy™5.5	PE-Vio®770	Alexa Fluor® 647	APC	Zombie NIR™	APC-Fire™ 750
APC-Fire 810						

AFs:

BV510 CD16 ←
Fitc CD115 ←
PE PE CD28 ←
PerCP-eF594 CD3

interlaser spillover from CD20

Hsu's compensations: CD56 510 AF BV480 BV605

AF by CD20 −100
↖ only changed the B cell lines (not enough to overcome crap)

Is the (matters) AF impact what driving the look, or clash elsewhere?

CD20/16 — changing AF makes your horrid unmixing error only look slightly less crappy. it's still wrong.

// ~ Hao Ting's panel ≠ unmixing CD56 → ~~BV4~~ BV480 vs CD56 BV605
Cx3CR1 also affected?

CD56 + CD16a odd stripe
→ minor adjustment for ND050

(No CD80 in ND050) stimulation? or an issue?
no CCR5
86 is pretty blobbish for both — fitc spread ✓
PerCP-cy5-5 123 ugh for ND050
CD40 weak at best // — HLA-DR way better for Inf212 stretch vs ablobs
CD38 better ND050. odd look
BV480 streaking

Adult CD3 have CCR2, Cx3CR1
CCR5 absent adult?

The aurora UV is down

Fitc ≈ BUV563 CD80/56 understoss CD16 CCR2 CD163 vs BUV563

Similarity™ Indices

Configuration:4L 16UV-16V-14B-8R

	BUV395	BUV563	BUV661	BUV805	BV480	BV510	BV570	BV605	BV650	BV711	BV750	FITC	PE	PE-Dazzle594	PE-Cy5	PerCP-Cy5.5	PE-Vio770	Alexa Fluor 647	APC	Zombie NIR	APC-Fire 750	APC-Fire 810
BUV395	1																					
BUV563	0.05	1																				
BUV661	0.04	0.05	1																			
BUV805	0.11	0.01	0.1	1																		
BV480	0.02	0.08	0.01	0	1																	
BV510	0.02	0.24	0.04	0	0.84	1																
BV570	0.01	0.25	0.04	0	0.27	0.58	1															
BV605	0.01	0.14	0.12	0.02	0.17	0.43	0.71	1														
BV650	0	0.03	0.37	0.02	0.07	0.16	0.26	0.54	1													
BV711	0	0	0.25	0.09	0.03	0.06	0.07	0.17	0.45	1												
BV750	0	0	0.1	0.14	0.02	0.04	0.04	0.1	0.24	0.69	1											
FITC	0.01	0.07	0	0	0.1	0.06	0.04	0.02	0	0	0	1										
PE	0.01	0.28	0.01	0	0.11	0.23	0.48	0.27	0.06	0.01	0	0.17	1									
PE-Dazzle594	0.01	0.2	0.04	0	0.07	0.18	0.37	0.4	0.17	0.05	0.02	0.12	0.69	1								
PE-Cy5	0	0.03	0.34	0.02	0.01	0.02	0.05	0.1	0.25	0.19	0.09	0.02	0.13	0.4	1							
PerCP-Cy5.5	0	0.01	0.33	0.07	0.01	0.02	0.05	0.13	0.36	0.53	0.31	0	0.06	0.28	0.81	1						
PE-Vio770	0	0	0.03	0.11	0	0.01	0.01	0.02	0.04	0.16	0.25	0	0.01	0.05	0.13	0.27	1					
Alexa Fluor 647	0	0	0.71	0.02	0	0	0	0	0.16	0.17	0.02	0	0	0	0.38	0.27	0.02	1				
APC	0	0.01	0.76	0.03	0	0.02	0.03	0.07	0.33	0.23	0.07	0	0.01	0.03	0.4	0.3	0.03	0.93	1			
Zombie NIR	0	0	0.21	0.14	0	0.01	0.01	0.02	0.09	0.31	0.31	0	0	0.02	0.16	0.26	0.36	0.27	0.28	1		
APC-Fire 750	0	0	0.13	0.21	0.01	0.01	0	0.01	0.05	0.19	0.2	0	0	0	0.08	0.13	0.21	0.16	0.17	0.79	1	
APC-Fire 810	0	0	0.1	0.25	0	0	0	0.01	0.04	0.12	0.12	0	0	0	0.06	0.09	0.15	0.12	0.13	0.43	0.74	1

Complexity™ Index: 10.21

CVs: CX3CR1, CD14, CD11a, ~~CD57~~

↑ Δ pps expression

CD11c?, CD80, CCR5, ~~CD7~~ ~~CD8~~?, CD123, CD40, <1D, CD64?

saturday

14:30 pm → machine still on, I was still last one logged in. where the fuck was Alexa?

CD20, CD56 CD3 // today's acquisitions
DR ~~CD8~~ unF Lyflurad

for the unstained sample

Day 2 (monocytes ↓ FSC-A vs yesterday...) [definitely not good]

Lymphocytes UV7, V7, B3 (minor autoF)

20,000 unstained

AF pos ≠ AF negative

// CD20 monocytes looked normal
10^{5} ~> $10^{5\frac{1}{2}}$ (upper bound @ 10^6)
detector V5

CD56 ditto monocytes
brightest $10^{5-5\frac{1}{2}}$

$10^{4\sim 8}$ tail → 10^6
$\wedge 10^{4\sim 5}$

CD3 (pretty dilute) 10^5 → 10^6
↓ CD3 MFI drop vs yesterday's.
wrong name, adjusting it.

Try # 2 (PE CF-594 not PE dazzle.)

Sooo... it looks better w/ the current monocyte spilled controls....