TWENTY-ONE-COLOR FULL SPECTRUM FLOW CYTOMETRY PANEL FOR IMPC IMMUNOPHENOTYPING OF MAJOR CELL SUBSETS IN MOUSE SPLEEN

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Background and Aim

Currently, for IMPC immunophenotyping, splenic cell populations are assayed using two flow cytometry (FCM) panels (Panel A and Panel B) which detect twenty-one individual parameters including cell viability[1].

Using spectral FCM, 21 parameters can be easily assayed in one tube. The use of a single staining panel simplifies and accelerates sample preparation, acquisition and data analysis.

Methods

Mouse splenocytes were stained with a cocktail of fluorescence labeled antibodies and Sytox Blue viability dye and FCM data was acquired using Cytec Aurora full spectrum flow cytometer, analyzed in FlowJO software and visualized using ShinySOM.

Conclusions

All leukocyte populations defined by IMPC Panels A and B were identified using the twenty-one color panel.

References

[1] https://www.mousephenotype.org/impress/ProcedureInfo? action=list&procID=1225&pipeID=7

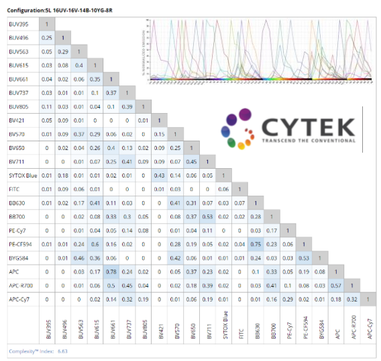
[2] Kratochvíl M, Bednárek D, Sieger T, Fišer K, Vondrášek J. Bioinformatics, Volume 36, Issue 10, 15 May 2020, 3288–3289

Results – panel design

#	param.	Ag level	T	B	NK	NKT	myeloid	neu	mono	MF	DC	pDC
1	viability	lo-hi										
2	CD5	hi										
3	CD4	hi										
4	CD44	int-hi										
5	CD8α	hi										
6	gdTCR	lo										
7	CD161	lo-int										
8	Klrg1	lo-int										
9	GITR	lo										
10	CD25	lo										
11	CD62L	int-hi										
12	Ly6G	hi										
13	CD19	lo										
14	Ly6C	hi										
15	CD21/35	hi										
16	CD11b	int-hi										
17	B220	lo-int										
18	F4/80	lo										
19	CD23	int										
20	CD11c	int-hi										
21	MHCII	hi										

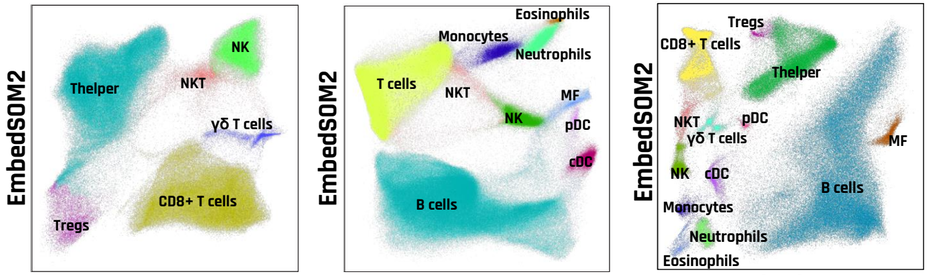
expression level: negative | low/int | positive

Marker list



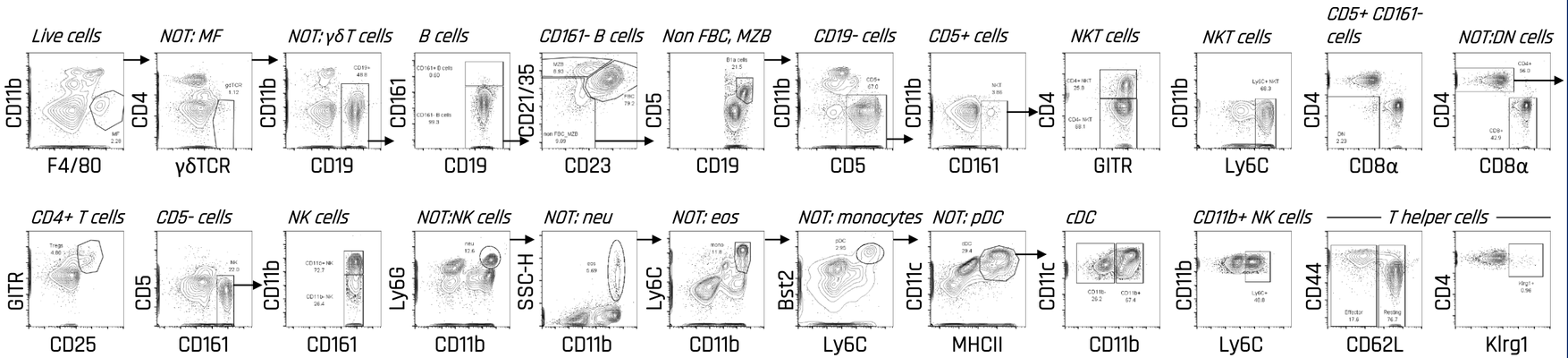
Similarity™ and Complexity™ indices

Results – data visualization in 2D space using ShinySOM[2]



IMPC Panel A | *IMPC Panel B* | *IMPC Panel A+B*

Results – gating strategy



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