Translating computational cytometry to the clinic

Sarah Bonte

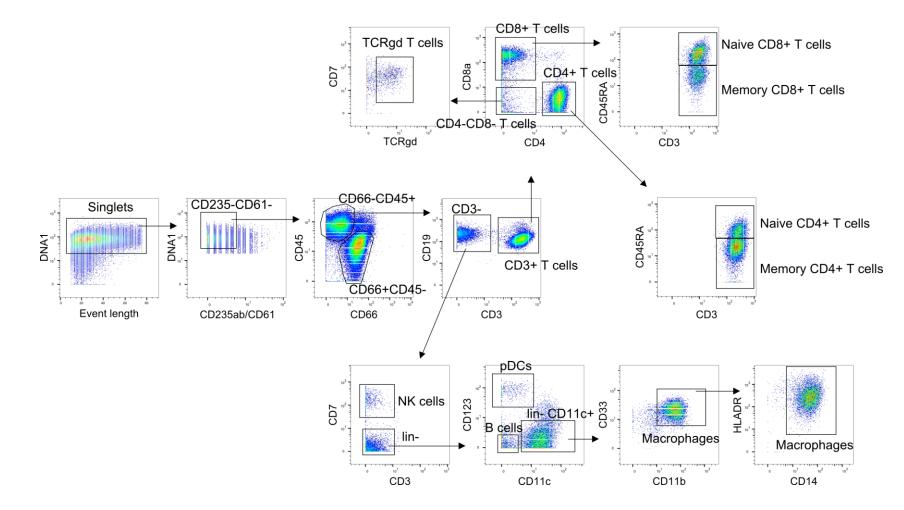


MB&C course 2024 Diepenbeek

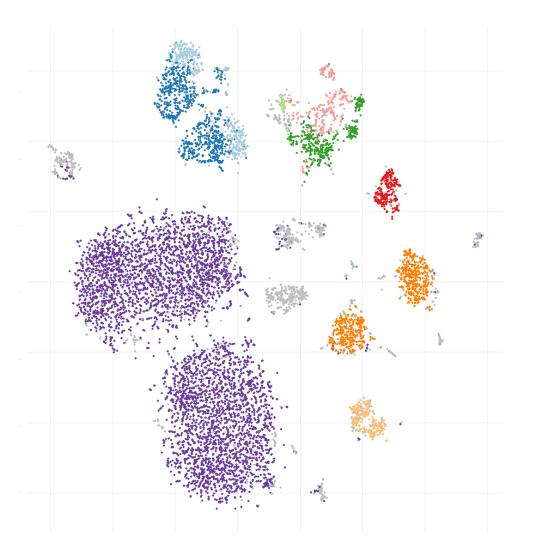
08/02/2024

Computational cytometry?

Typically, you look at cytometry data like this ...



... or maybe sometimes like this ...



3

... but this is what computational cytometry looks like

cytometer channels

FSC-A FSC-A B-530/30-A B-580/60-A R-660/20-A R-780/60-A R-660/20-A R-660/20-A R-660/20-A R-660/20-A R-660/20-A R-660/20-A R-660/20-A		FFFFFFFFFFFFF	F56 11		D 530/30 A	D FOF /43 A	D (70) D (D 700/CO A	D 660/20 A	D 700/60 A	N/ 450/50 A	V F10/F0
$ \begin{bmatrix} 7, 1\\ 16320, 891 121620 57066, 9570 602, 07996 450, 13998 501, 2598 183, 17999 488, 799988 197, 59991 2057, 980 533, 7246 4105, 46 [3, 136547, 594 13573 72303, 5547 8590, 9999 320, 91998 245, 65999 51, 12000 555, 099976 609, 699951 1177, 340 4117, 68 [5, 136510, 203 85375 91446, 5781 827, 85999 612, 01996 593, 56000 92, 30000 699, 399963 44, 19997 2373, 660 4880, 500 [6,] 78543, 898 55446 47748, 9180 1404, 83000 576, 51996 651, 77997 408, 95999 881, 399963 20483, 398438 1360, 980 3677, 36 [7,] 181353, 391 135280 47900, 8594 570, 83997 391, 91998 852, 00000 136, 31999 941, 199951 204, 09991 1736, 340 4580, 36 [7,] 181353, 391 135280 47900, 8594 570, 83997 391, 91998 852, 00000 136, 31999 941, 199951 204, 09991 1736, 340 4580, 36 [7,] 178893, 000 134069 51327, 3203 746, 91998 467, 17999 549, 53998 167, 56000 527, 799988 -28, 59999 11736, 340 4580, 3996 [10,] 247311, 891 167894 55742, 0977 880 39996 533, 79999 312, 24000 445, 89994 1487, 1120 8632, 68 [11,] 170807, 391 131809 36185, 8594 462, 91998 399, 01999 305, 29999 31, 24000 445, 89994 208, 00000 1319, 240 4938, 89 [12,] 182610, 000 136931 65892, 2578 15655, 50000 243, 378788 1226, 88000 1187, 5999 76 -52, 000000 1310, 440 348, 84 [14,] 15761, 703 109292 102329, 4531 958, 50000 498, 41998 633, 3201 79, 52000 1127, 099976 -52, 000000 1262, 040 2174, 94 [14,] 15761, 703 116999 27406, 0000 688, 85999 195, 95999 952, 81995 143, 42000 1601, 599976 -5, 000000 1136, 640 0400 479, 95991 959, 64000 570, 699971 50, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 61400, 836, 78 [14,] 13785, 94 117165 28897, 0000 2054, 25977 2597, 17993 776, 73999 259, 65000 570, 699976 -3, 900000 1131, 760 2724, 48 [14,] 1378, 941 11455 24887, 0000 2054, 25977 2597, 17993 776, 73999 259, 65000 570, 699976 50, 509991 50, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 60997 150, 609997 150, 60997 150, 60997 153, 60000 1131, 760 2724, 48 [14,] $	F+ 1			SSC-A	B-530/30-A	B-585/42-A	B-670LP-A	B-780/60-A	R-660/20-A			
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[33,] 213623.094 155964 64899.6797 626.21997 523.97998 705.73999 53.96000 530.399963 178.099991 1583.260 5214.18 [34,] 262143.000 132466 70965.9141 1811.91992 575.09998 962.75995 373.45999 1131.000000 366.599976 2670.300 8816.72												
[34,] 262143.000 132466 70965.9141 1811.91992 575.09998 962.75995 373.45999 1131.000000 366.599976 2670.300 8816.72												
[35,] 138026.703 102190 33558.8594 816.50000 242.81999 $7/3.89996$ 211.57999 568.099976 383.500000 1569.500 6247.90												
	[35,]	138026.703	102190	33558.8594	816.50000	242.81999	773.89996	211.57999	208.0999/6	383.500000	1569.500	6247.900

Manual analysis vs computational cytometry

manual analysis

- subjective
- difficult to standardize
- biased
- time consuming
- not feasible for analysis of high-dimensional data

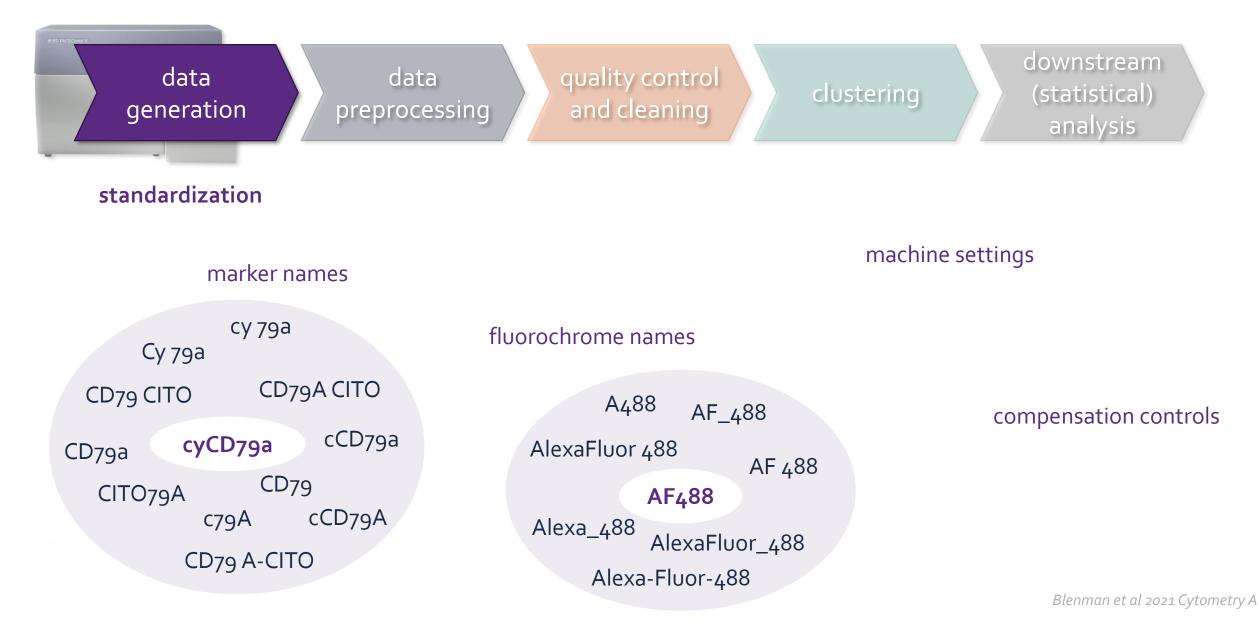


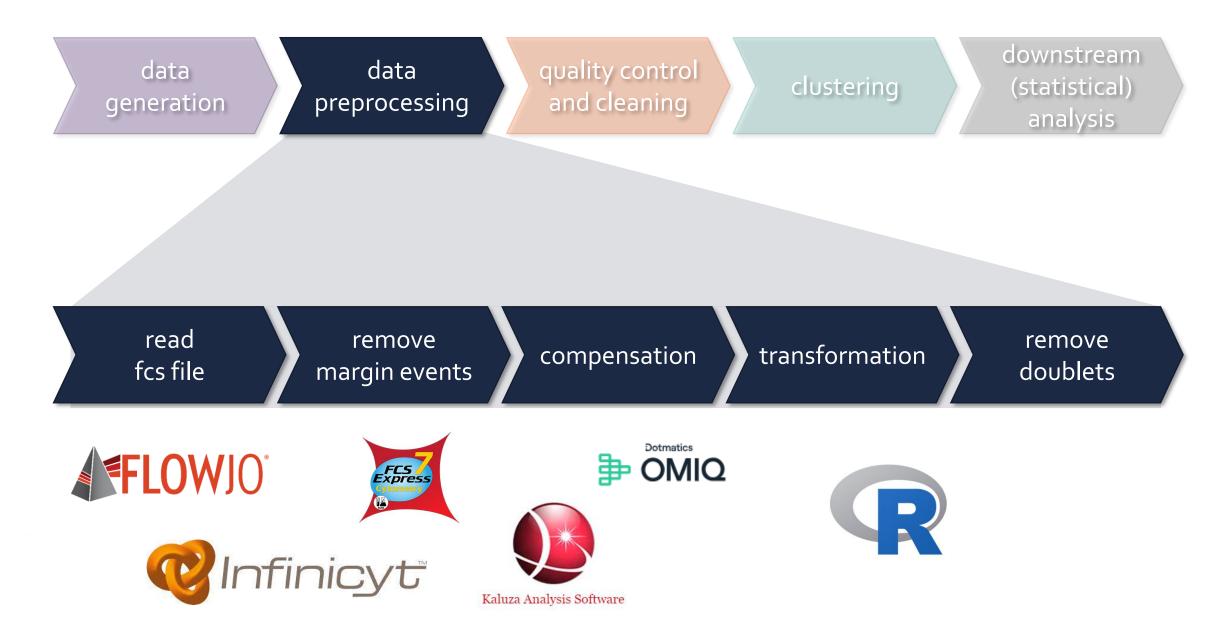
automated analysis

- more objective
- easier to reproduce
 - more unbiased looks at all cells/parameters simultaneously
- faster
- is able to handle high-dimensional data

find optimal synergy between both approaches

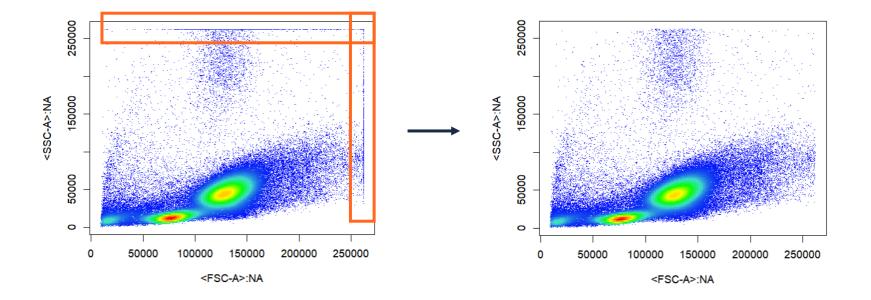




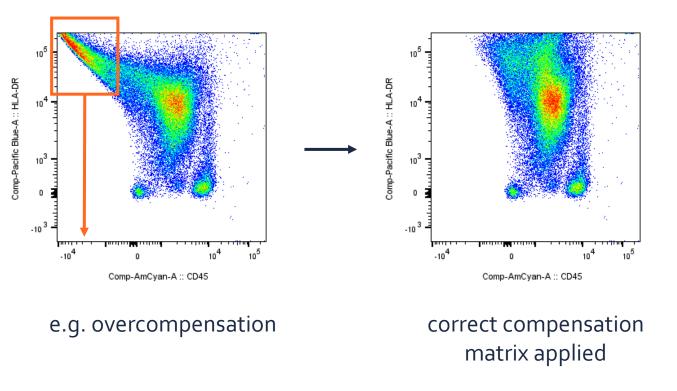




Remove events that are outside, or at the borders of, the detectable range of the cytometer





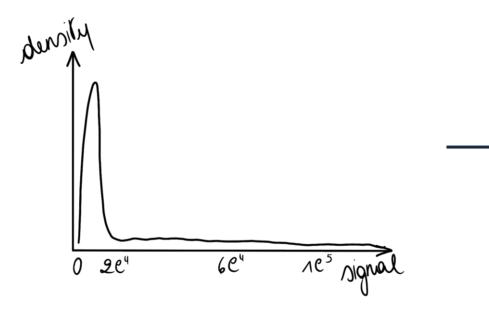


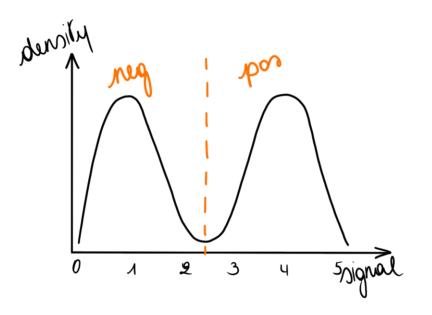
Pacific Blue-A	AmCyan-A
<pre>[1,] 332.28000 [2,] 602.07996 [3,] 859.09998 [4,] 624.79999 [5,] 827.85999 [6,] 1404.38000</pre>	335.12000 450.13998 487.06000 320.91998 612.01996 576.51996
90000	- 10000



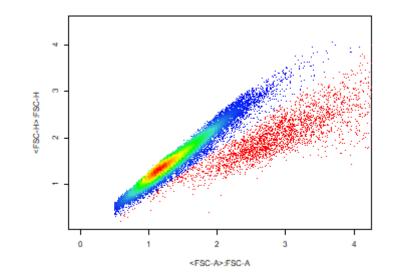
without transformation

biexponential transformation (e.g. logicle)











Quality control on two levels





e.g.

- clogs during acquisition
- changes in flow rate

e.g.

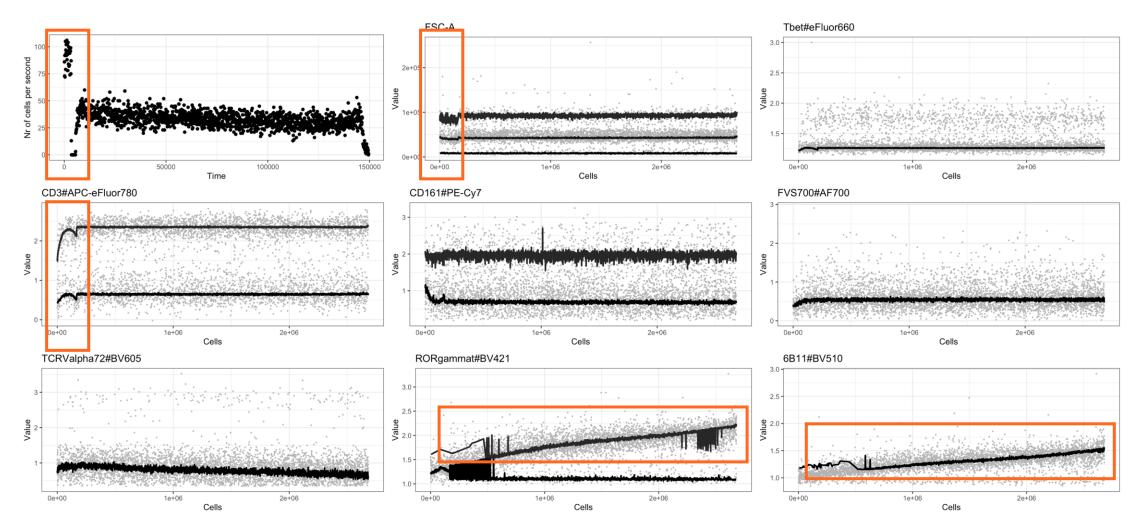
- new antibody batch
- change in machine settings

check the signal consistency over time

check for batch effects between samples

Within-file quality

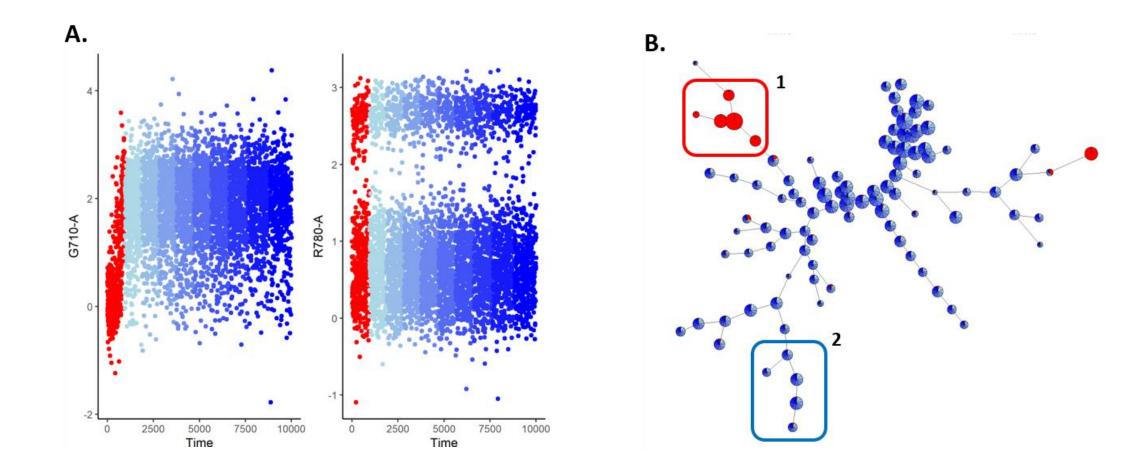
changes in flow rate and signal

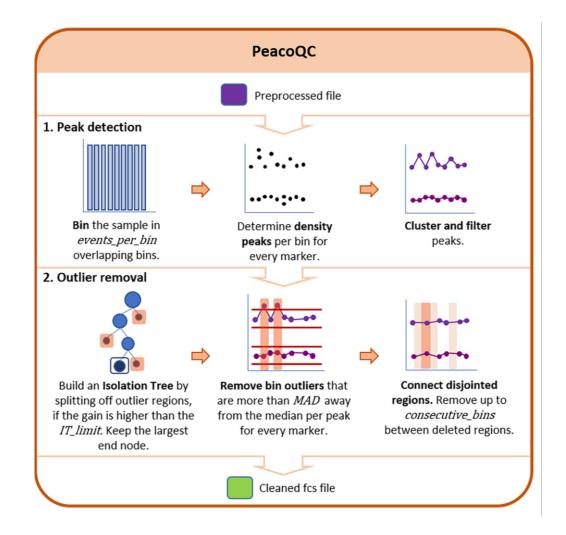


15

changes in signal

Impact of no quality filtering



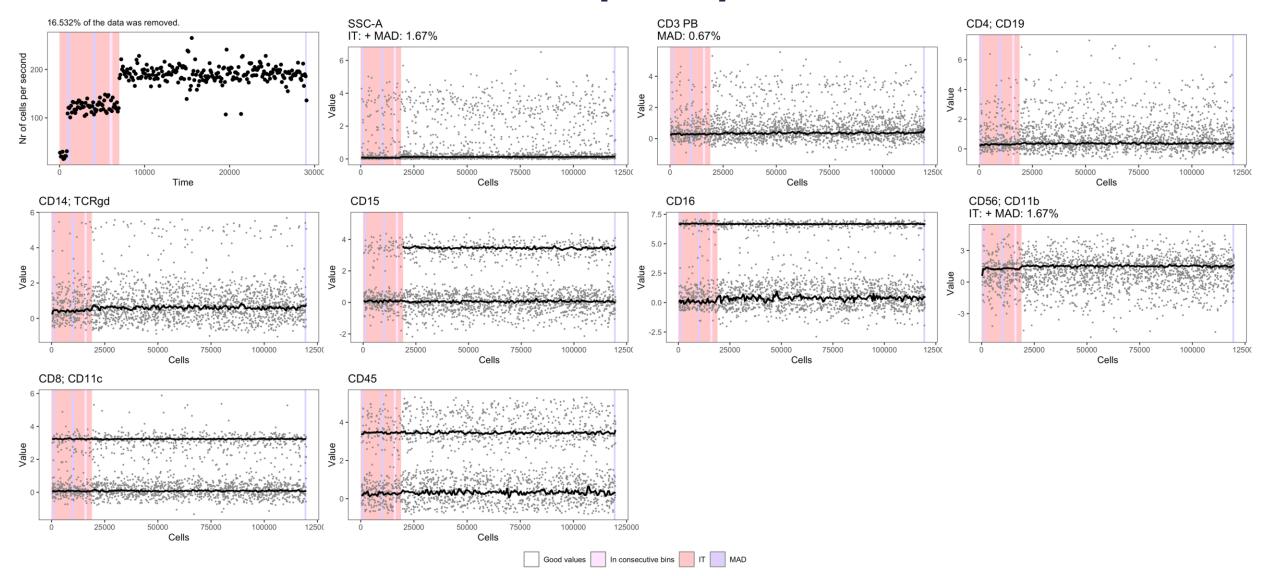


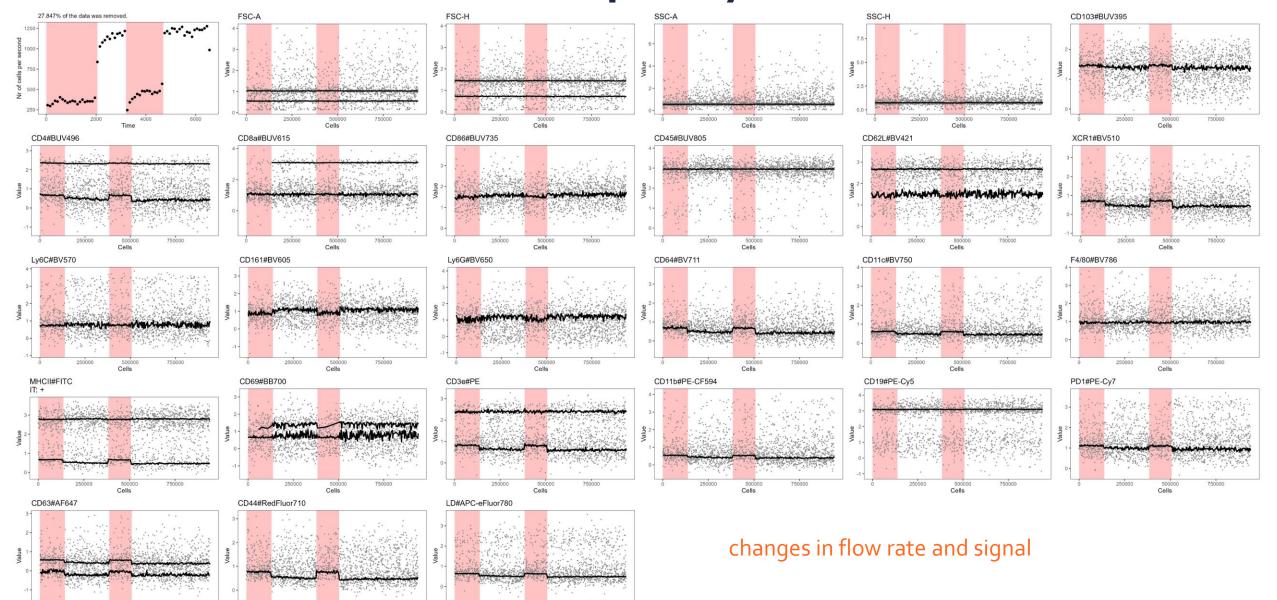




	FSC-A FS	C-H	SSC-A	B-530/30-A	B-585/42-A	B-670LP-A	B-780/60-A	R-660/20-A	R-780/60-A	V-450/50-A	V-510/50-A	1
[1,]] 143804.703 112		37015.1406	332.28000	335.12000	346.47998	53.96000	360.099976	-10.400000	1097.360	3913.000	l
[2,] 166320.891 121	620	57066.9570	602.07996	450.13998	501.25998	183.17999	488.799988	197.599991	2057.980	5333.720	l
[3,	_] 193674.594 138	753	72303.5547	859.09998	487.06000	536.76001	144.84000	673.399963	321.099976	1772.460	4105.640	l
[4,]] 156393.000 122	200	47006.2578	624.79999	320.91998	245.65999	51.12000	555.099976	609.699951	1177.340	4117.680	1
												\sim
												\geq
[8,	-		9542.3994	153.36000	203.06000	323.75998	88.04000	5894.199707	458.899994	14871.120	8632.680	1
] 178893.000 134		51327.3203	746.91998	467.17999	549.53998	167.56000	527.799988	-28.599998	1755.260	3973.200	l
] 247311.891 167		55742.0977	880.39996	542.44000	553.79999	21.30000	821.599976	53.299999	2154.300	5132.480	l
] 170807.391 131		36185.8594	462.91998	399.01999	305.29999	31.24000	445.899994	208.000000	1319.240	4938.980	1
] 182610.000 136		65892.2578	15655.50000	2433.87988	1226.88000	181.75999	756.599976	-16.900000	1520.480	3100.300	1
] 157763.703 109			958.50000	498.41998	633.32001	79.52000	1127.099976	-52.000000	2162.040	2174.940	l
] 157670.094 123		54684.1992	14040.95996	1901.37988	1486.73999	299.62000	513.500000	-26.000000	1587.560	3154.480	
[15,] 149195.703 116	999	27406.0000	685.85999	195.95999	952.81995	143.42000	1601.599976	7.800000	1015.660	1400.080	ı <
F 4 - 7			20007 0000	20504 25077	2507 47003	776 77000	252 25222	045 000075	7 000000	4434 760	2724 400	
] 163785.594 117		28897.0000	20594.25977	2597.17993	776.73999	259.85999	815.099976	-3.900000	1131.760	2724.480	-
] 35729.098 31/		33698.0195	306.72000	122.12000	452.97998	222.93999	317.199982	209.299988	473.000	836.780	l
] 128565.898 106		39812.5391	17013.01953	2379.91992	1581.88000	668.82001	339.299988	1622.399902	1571.220	3267.140	l
] 176066.094 134		46135.7969	573.67999	400.44000	479.95999	39.76000	674.699951	422.499969	1787.080	6928.160	l
] 161051.391 122		45881.6172	474.28000	286.84000	292.51999	-75.25999	460.199982	401.699982	1747.520	4645.720	l
] 164090.703 125] 154710.891 91		29232.1191 26857.8789	4651.91992 404.69998	843.47998 332.28000	931.51996 592.13995	72.42000 435.93997	505.699982 22634.298828	39.000000 19925.099609	2000.360 14140.980	2545.600 13489.101	l
	151389.891 113		32080.6387	2335.89990	509.78000	528.23999	66.74000	293.799988	218.399994	1927.260	6317.560	l
] 165077.094 127		88765.6172	14587.65918	1965.27991	1500.93994	228.62000	916.499939	109.199997	1613.360	2946.360	l
	83719.797 63		23221.2598	502.67999	63.90000	289.67999	-38.34000	349.699982	148.199997	1444.800	6542.880	l
] 190896.297 142		49665.9180	587.88000	265.53998	489.89999	113.60000	384.799988	421.199982	1605.620	4938.980	l
L27,] 190090.297 142		49009.9100	307.00000	201.15550	409.09999	113.00000	504.755500	421.199902	1005.020	4930.900	· ~~
												>
[30.		656	46617.1797	850.57996	505.51999	1187.12000	619.12000	492.699982	12064.000000	1302,900	8154.520	
] 170381.703 128		36090.7188	51757.57812	6458.15967	2797.39990	143.42000	436.799988	244.399994	1245.280	4130.580	l
] 135000.891 105		17315.4785	2093.07983	512.62000	424.57999	-9.94000	5820.099609	412.099976	963.200	1612.500	l
] 213623.094 155		64899.6797	626.21997	523.97998	705.73999	53.96000	530.399963	178.099991	1583.260	5214.180	1
] 262143.000 132		70965.9141	1811.91992	575.09998	962.75995	373.45999	1131.000000	366.599976	2670.300	8816.720	1
] 138026.703 102		33558.8594	816.50000	242.81999	773.89996	211.57999	568.099976	383.500000	1569.500	6247.900	1
												ı

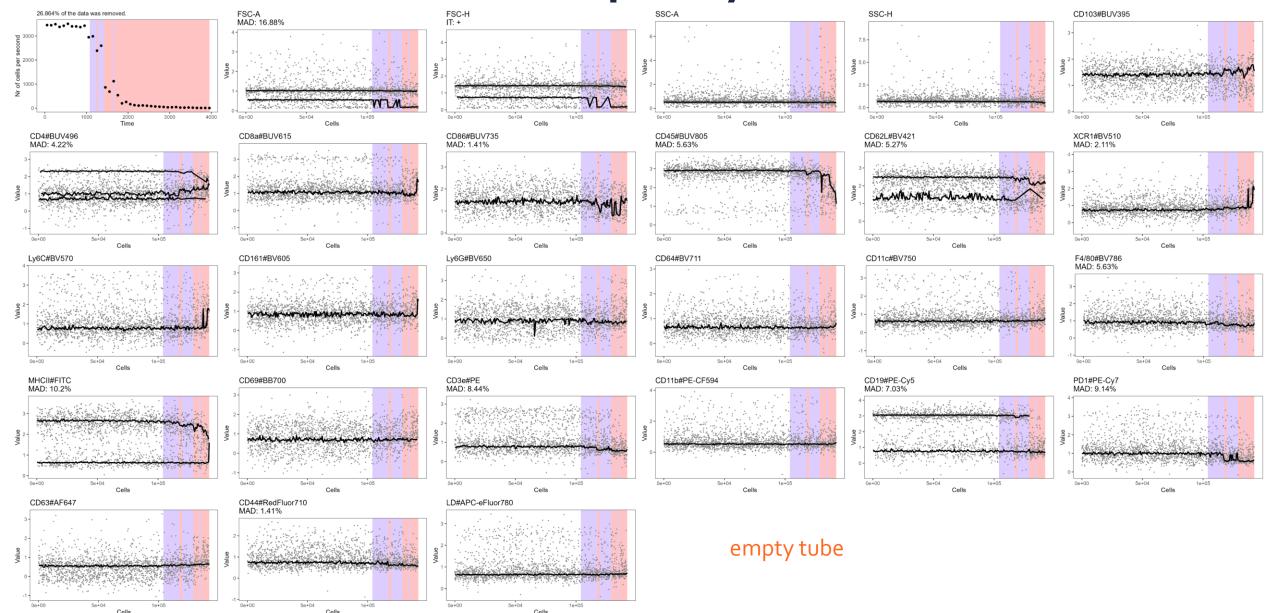
FSC-A FSC-H	SSC-A	B-530/30-A	B-585/42-A	B-670LP-A	B-780/60-A	R-660/20-A	R-780/60-A	V-450/50-A	V-510/50-A
[1,] 143804.703 112032	37015.1406	332.28000	335.12000	346.47998	53.96000	360.099976	-10.400000	1097.360	3913.000
[2,] 166320.891 121620	57066.9570	602.07996	450.13998	501.25998	183.17999	488.799988	197.599991	2057.980	5333.720
[3,] 193674.594 138753	72303.5547	859.09998	487.06000	536.76001	144.84000	673.399963	321.099976	1772.460	4105.640
[4,] 156393.000 122200	47006.2578	624.79999	320.91998	245.65999	51.12000	555.099976	609.699951	1177.340	4103.640
[4,] 130393.000 122200	47000.2378	024.73333	320. 31330	243.03333	51.12000	333.033370	009.099931	11/7.340	4117.000
[8,] 86222.695 74144	9542.3994	153.36000	203.06000	323.75998	88.04000	5894.199707	458.899994	14871.120	8632.680
[9,] 178893.000 134069	51327.3203	746.91998	467.17999	549.53998	167.56000	527.799988	-28.599998	1755.260	3973.200
[10,] 247311.891 167984	55742.0977	880.39996	542.44000	553.79999	21.30000	821.599976	53.299999	2154.300	5132.480
[11,] 170807.391 131809	36185.8594	462.91998	399.01999	305.29999	31.24000	445.899994	208.000000	1319.240	4938.980
[12,] 182610.000 136931	65892.2578	15655.50000	2433.87988	1226.88000	181.75999	756.599976	-16.900000	1520.480	3100.300
[13,] 157763.703 109292	102329.4531	958.50000	498.41998	633.32001	79.52000	1127.099976	-52.000000	2162.040	2174.940
[14,] 157670.094 123955	54684.1992	14040.95996	1901.37988	1486.73999	299.62000	513.500000	-26.000000	1587.560	3154.480
[15,] 149195.703 116999	27406.0000	685.85999	195.95999	952.81995	143.42000	1601.599976	7.800000	1015.660	1400.080
[17,] 163785.594 117165	28897.0000	20594.25977	2597.17993	776.73999	259.85999	815.099976	-3.900000	1131.760	2724.480
[18,] 35729.098 31493	33698.0195	306.72000	122.12000	452.97998	222.93999	317.199982	209.299988	473.000	836.780
[19,] 128565.898 106740	39812.5391	17013.01953	2379.91992	1581.88000	668.82001	339.299988	1622.399902	1571.220	3267.140
[20,] 176066.094 134946	46135.7969	573.67999	400.44000	479.95999	39.76000	674.699951	422.499969	1787.080	6928.160
[21,] 161051.391 122387	45881.6172	474.28000	286.84000	292.51999	-75.25999	460.199982	401.699982	1747.520	4645.720
[22,] 164090.703 125696	29232.1191	4651.91992	843.47998	931.51996	72.42000	505.699982	39.000000	2000.360	2545.600
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[25,] 165077.094 127851	88765.6172	14587.65918	1965.27991	1500.93994	228.62000	916.499939	109.199997	1613.360	2946.360
[26,] 83719.797 63033	23221.2598	502.67999	63.90000	289.67999	-38.34000	349.699982	148.199997	1444.800	6542.880
[27,] 190896.297 142590	49665.9180	587.88000	265.53998	489.89999	113.60000	384.799988	421.199982	1605.620	4938.980
	10017 1707	050 57005		1107 10000	C10 10000	402 600002	12054 000000	1202 000	0154 530
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[32,] 135000.891 105994	17315.4785	2093.07983	512.62000	424.57999	-9.94000	5820.099609	412.099976	963.200	1612.500
[33,] 213623.094 155964	64899.6797	626.21997	523.97998	705.73999	53.96000	530.399963	178.099991	1583.260	5214.180
[34,] 262143.000 132466	70965.9141 33558.8594	1811.91992	575.09998	962.75995	373.45999	1131.000000	366.599976	2670.300	8816.720
[35,] 138026.703 102190	55556.8594	816.50000	242.81999	773.89996	211.57999	568.099976	383.500000	1569.500	6247.900





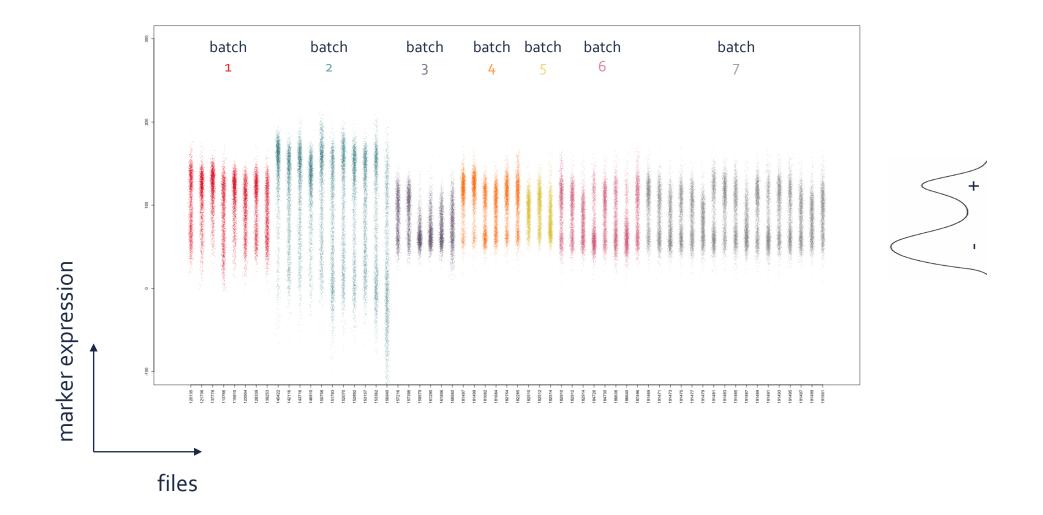
Good Values IT

Cells

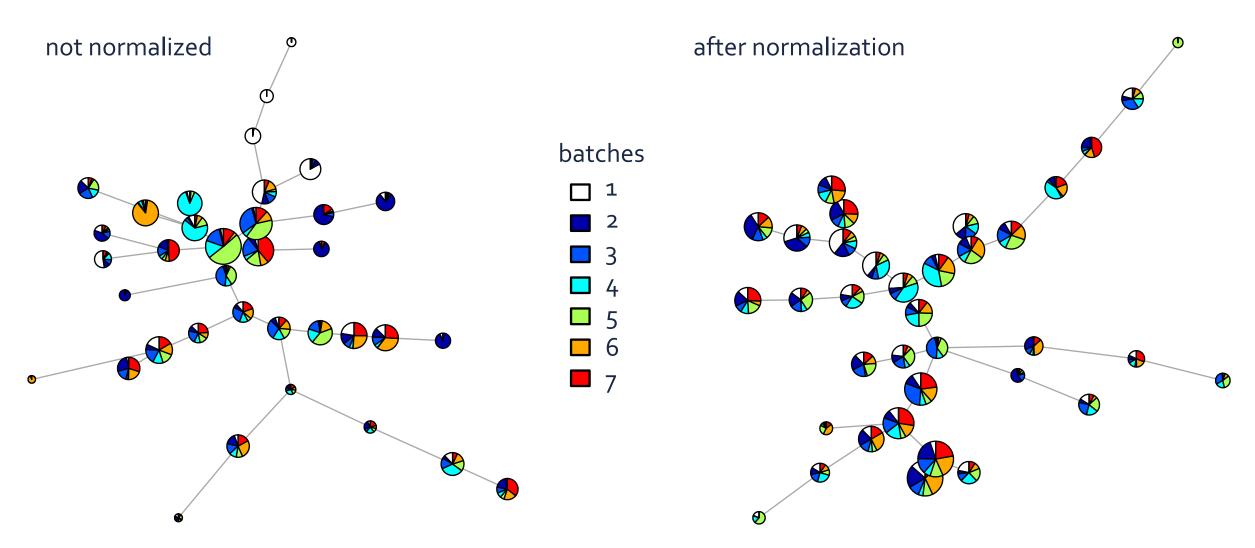


Good Values IT MAD

Between-file consistency



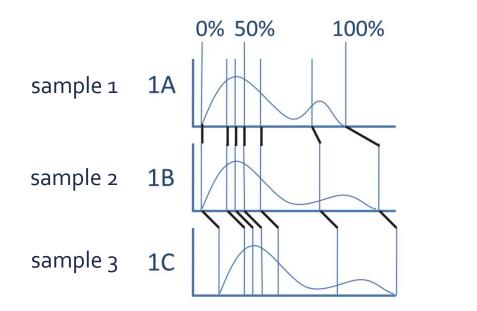
Impact of no batch effect correction



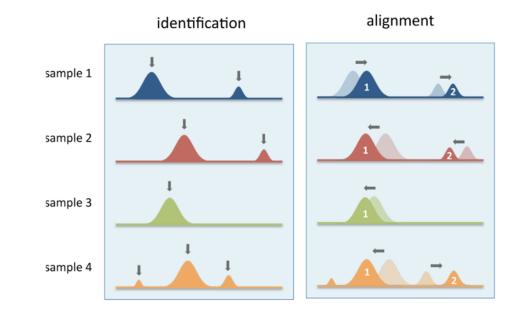
Batch effect correction approaches

aligning quantiles or percentiles

aligning density peaks

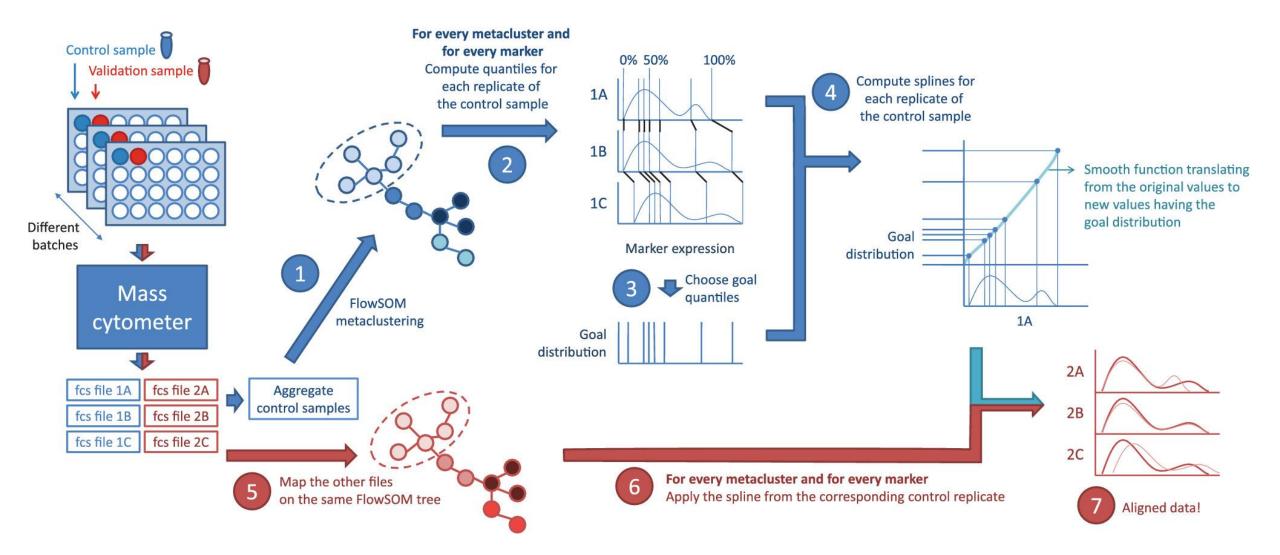


e.g. min-max normalization, percentile normalization



e.g. gaussNorm, fdaNorm

CytoNorm for batch effect correction



CytoNorm for batch effect correction

not normalized

A de de la construcción de la

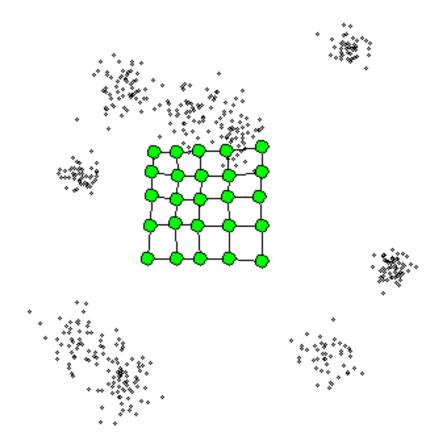
CytoNorm normalized

keep track of possible batch effects be careful not to lose biologically relevant differences



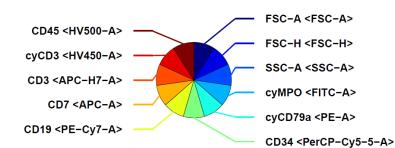
Two-level clustering

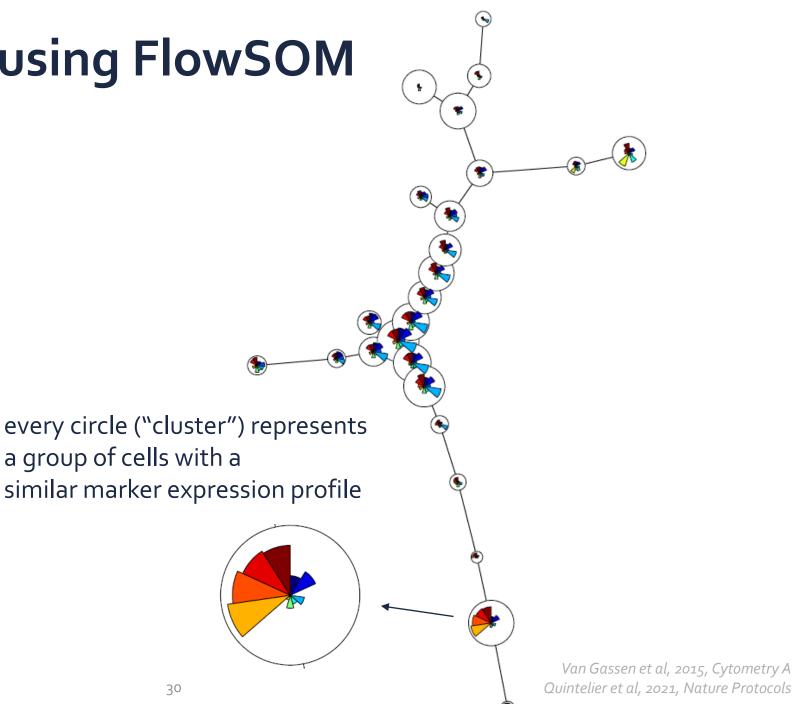
simple but fast
 Self-Organizing Map (SOM)
 overclustering



Two-level clustering

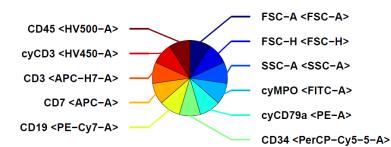
simple but fast
 Self-Organizing Map (SOM)
 overclustering

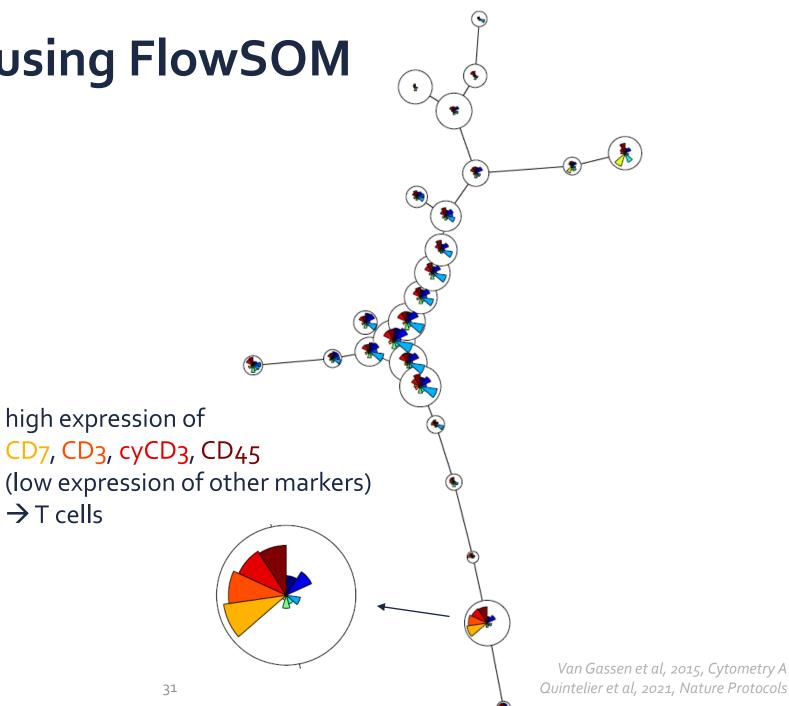




Two-level clustering

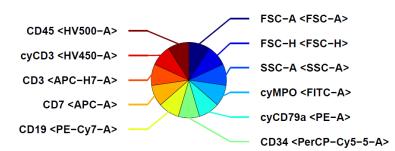
simple but fast
 Self-Organizing Map (SOM)
 overclustering

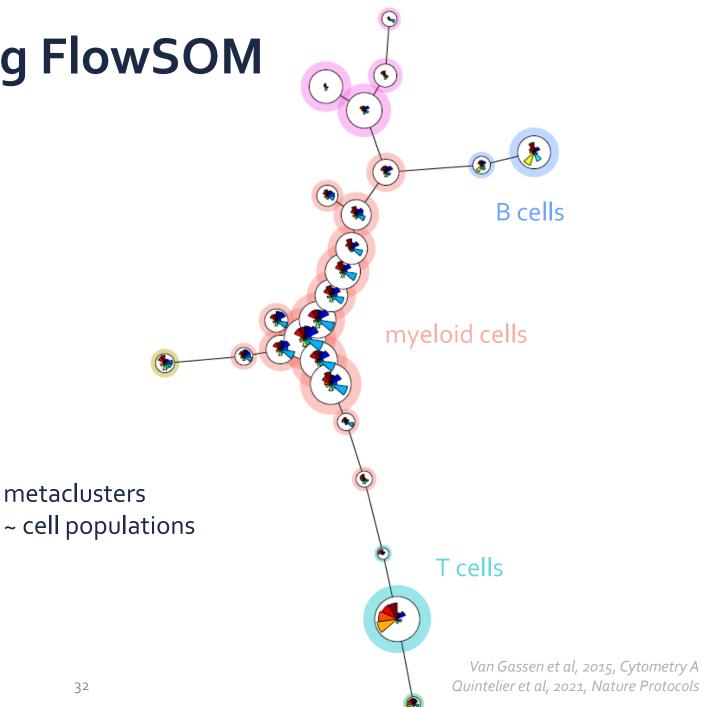




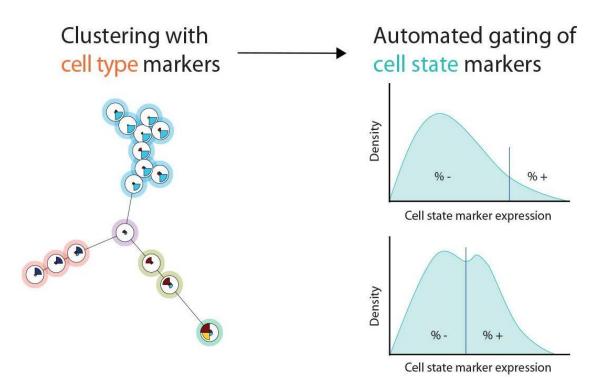
Two-level clustering

- simple but fast Self-Organizing Map (SOM) overclustering
- more sensitive hierarchical consensus clustering on the SOM nodes





Cell type vs cell state markers





FlowSOM output level: cluster and metacluster features: counts, percentages, MFIs

What's next?

compare abundances/population MFIs between outcomes of interest predictive modeling

• • •

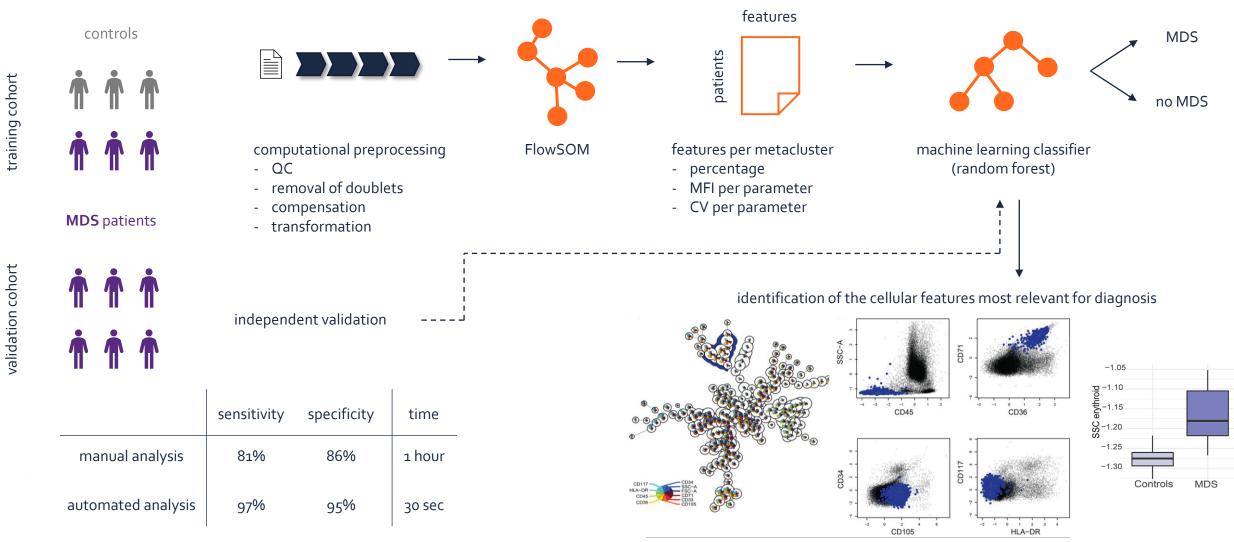
Translating computational cytometry to the clinic



Computational flow cytometry as a diagnostic tool in suspected-myelodysplastic syndromes Duetz C, Van Gassen S, et al (2021) Cytometry A



Automated analysis for more accurate and faster distinction between MDS and non-neoplastic cytopenias



Duetz et al 2021 Cytometry A



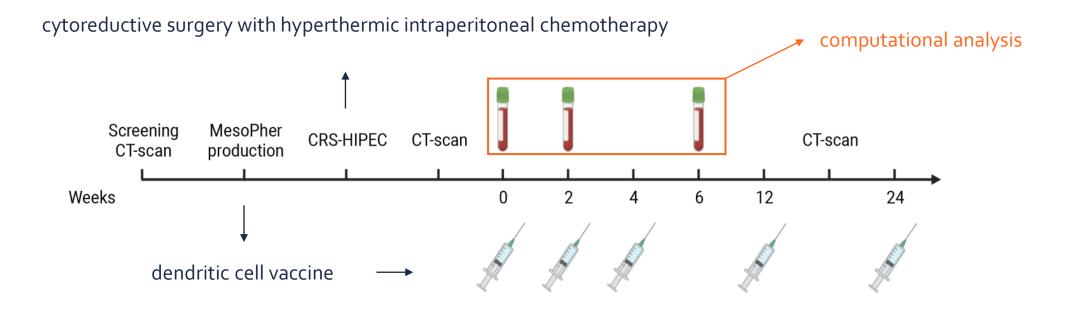
Immune response after immunotherapy for malignant peritoneal mesothelioma

Dietz M, Quintelier K, et al (2023) Journal for ImmunoTherapy of Cancer

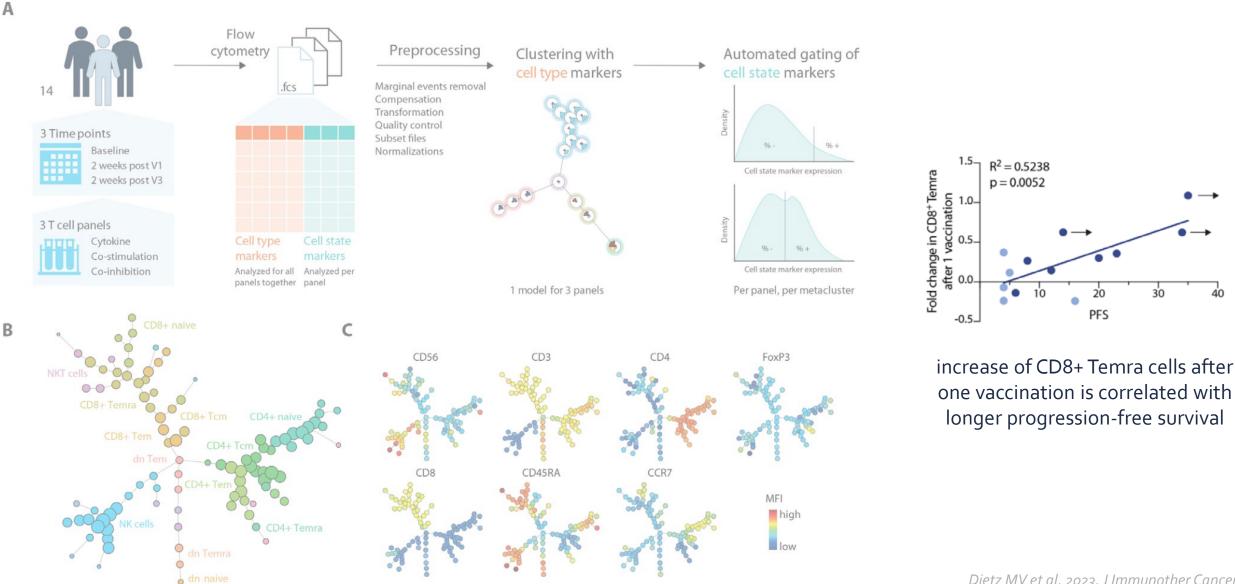


Clinical trial for malignant peritoneal mesothelioma

clinical trial design



Computational analysis allowed for a comprehensive and complete analysis where co-expression of all phenotypic markers could be studied on the cell-type level

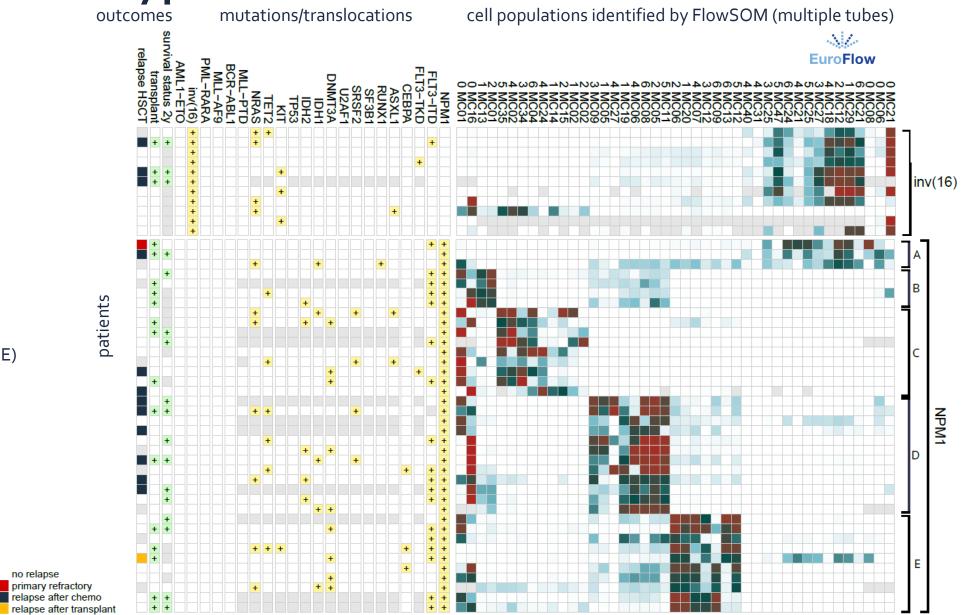


Unraveling the immunophenotypic landscape in acute myeloid leukemia

Couckuyt A et al (2023) *manuscript submitted*



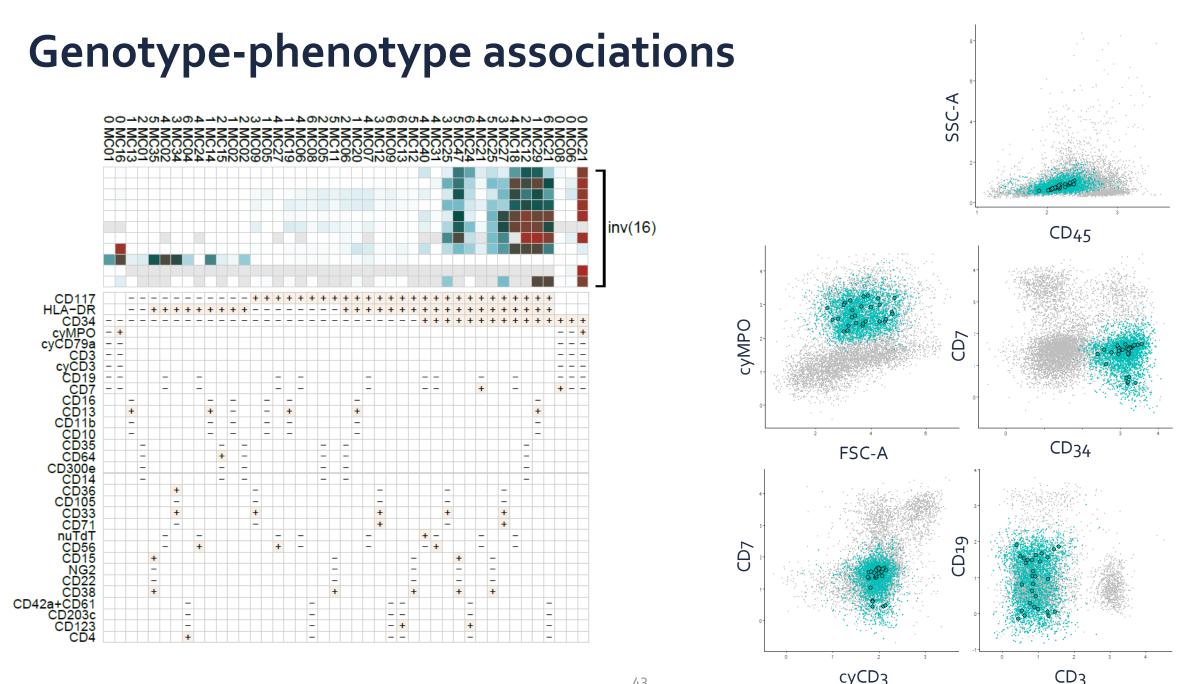
Genotype-phenotype associations



AML patients Ghent University Hospital (BE) 2015-2019 n = 122

no relapse

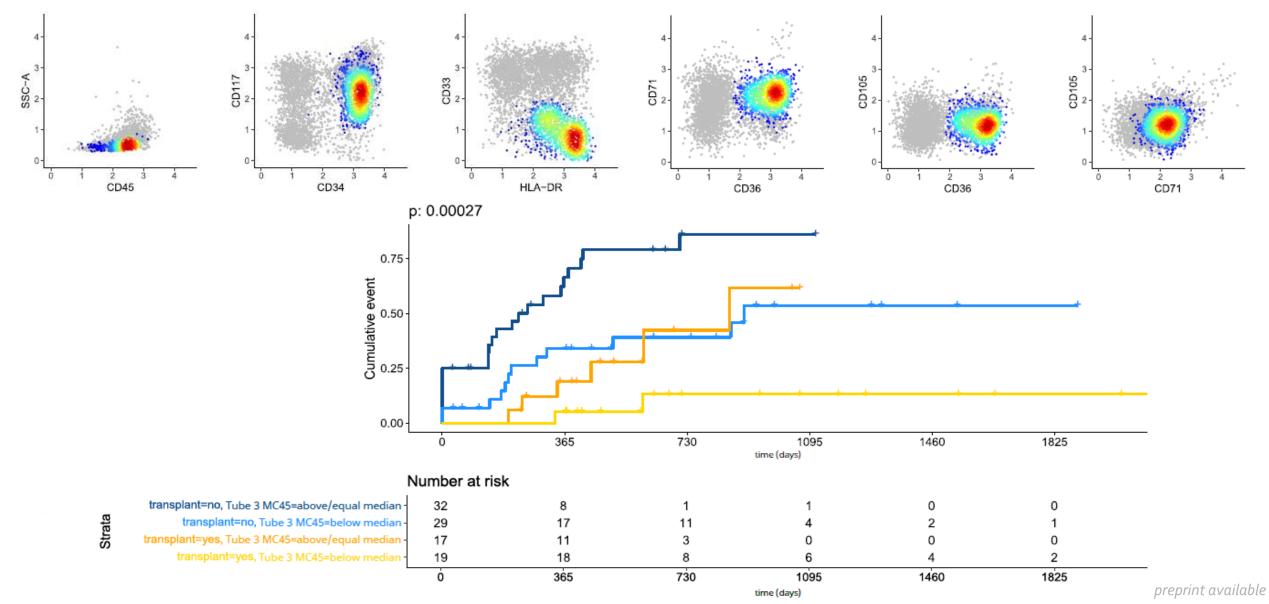
preprint available



preprint available

Prediction of outcome

Patients with above-median expression levels of CD₃₄+ CD₁₁₇+ HLA-DR+ cell populations showed the shortest time-to-relapse





Improved diagnosis of primary immunodeficiences using explainable AI

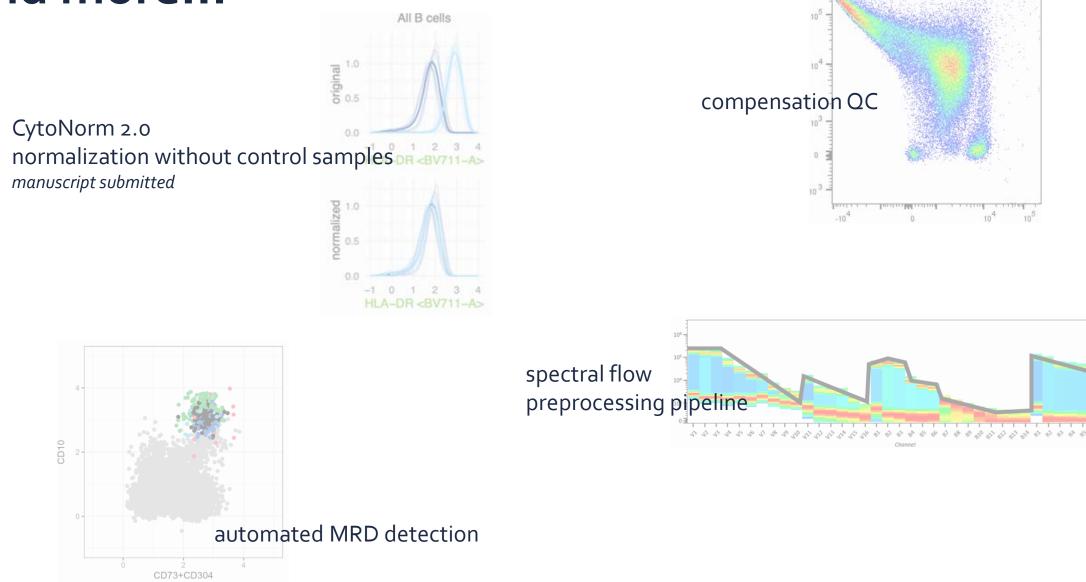
Emmaneel A et al (2023) manuscript in preparation



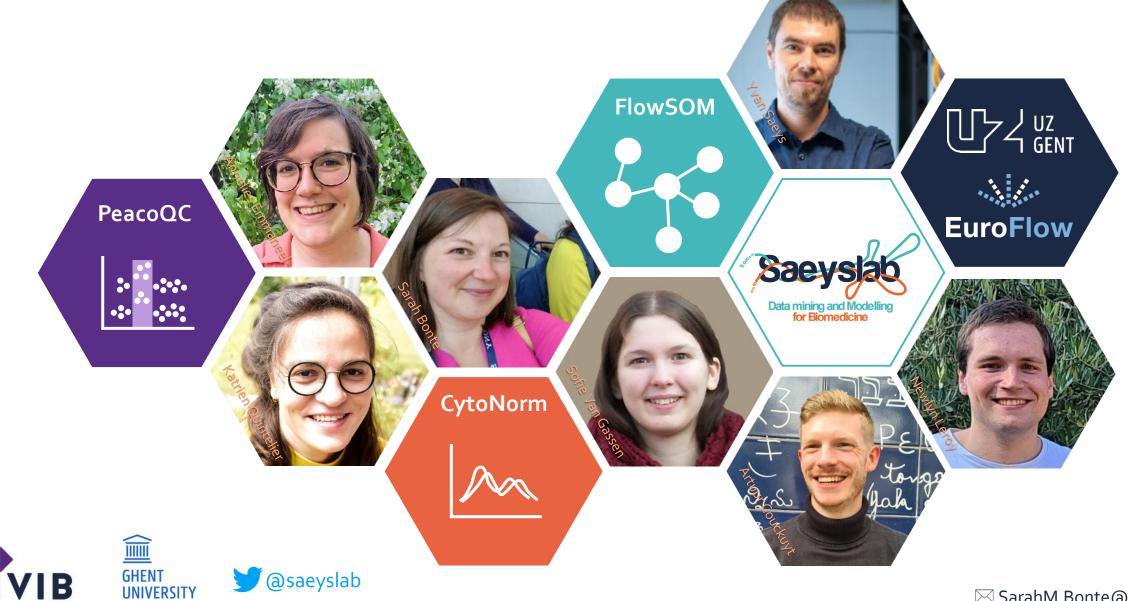


unpublished data (manuscript in preparation)

And more...



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