SCIENCE MEETS LIFE

Translating computational cytometry to the clinic

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VIB

Saeyslab
Data mining and Modelling for Biomedicine

GHENT UNIVERSITY

UZ GENT

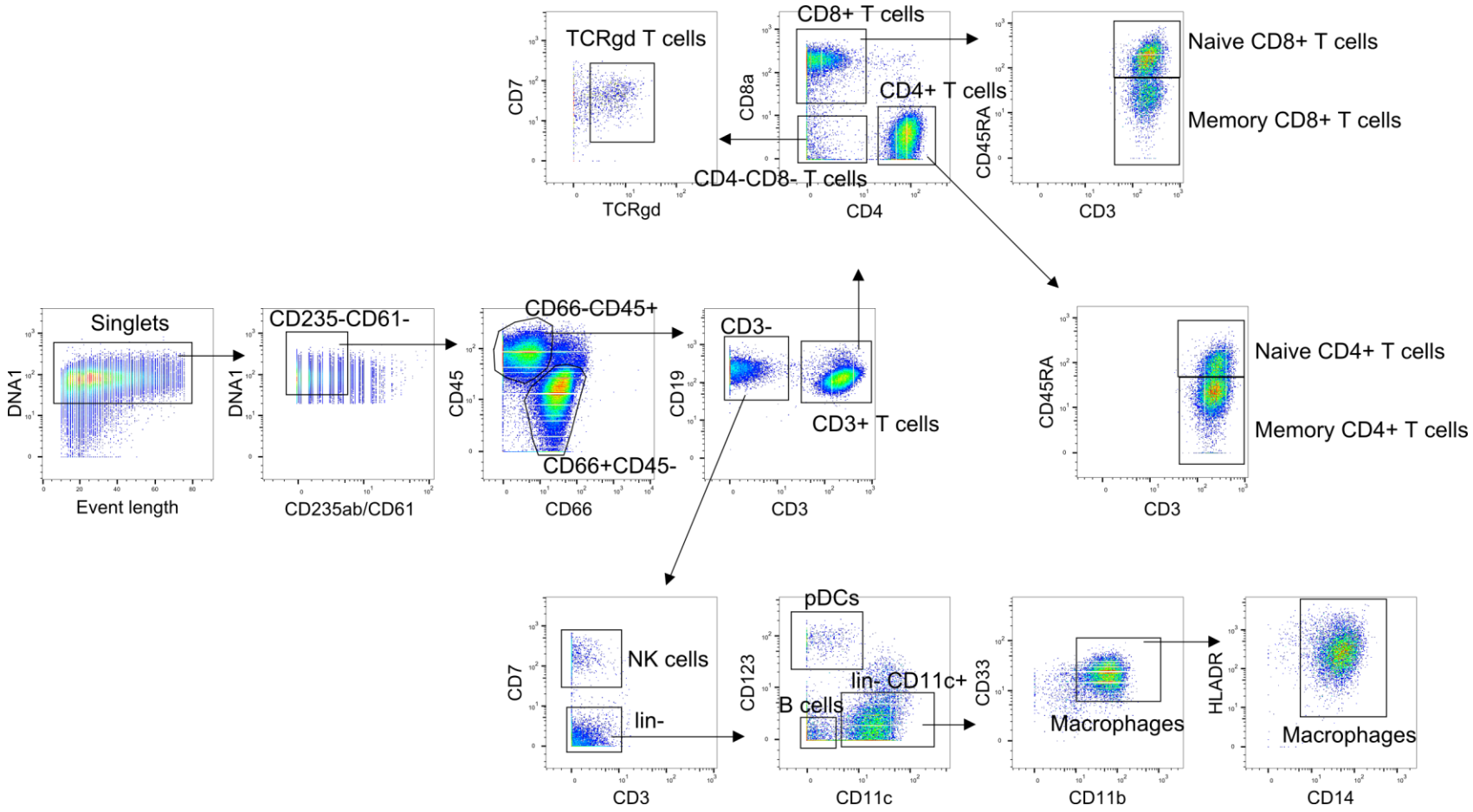
EuroFlow

MB&C course 2024 Diepenbeek

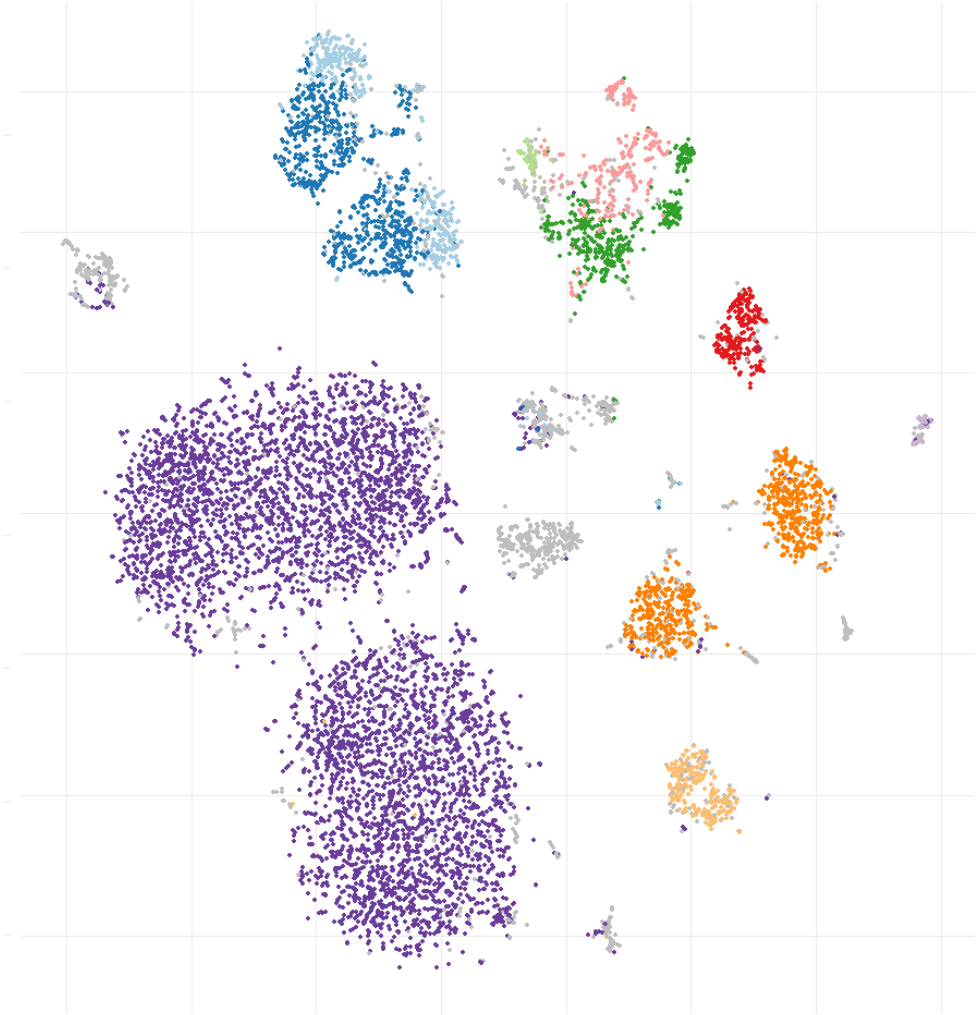
08/02/2024

Computational cytometry?

Typically, you look at cytometry data like this …



... or maybe sometimes like this ...



... but this is what computational cytometry looks like

cytometer channels

1 row = 1 event measured

	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	4117.680
[5,]	136510.203	85375	91446.5781	827.85999	612.01996	593.56000	92.30000	699.399963	44.199997	2373.600	4880.500
[6,]	78543.898	55446	47748.9180	1404.38000	576.51996	651.77997	408.95999	881.399963	20485.398438	1369.980	3677.360
[7,]	181355.391	135280	47900.8594	570.83997	391.91998	852.00000	136.31999	941.199951	204.099991	1736.340	4580.360
[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
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[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
[16,]	190094.391	139592	51612.7383	819.33997	494.15997	565.15997	59.64000	570.699951	50.699997	1637.440	3556.100
[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
[23,]	154710.891	91185	26857.8789	404.69998	332.28000	592.13995	435.93997	22634.298828	19925.099609	14140.980	13489.101
[24,]	151389.891	113838	32080.6387	2335.89990	509.78000	528.23999	66.74000	293.799988	218.399994	1927.260	6317.560
[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
[28,]	262143.000	155869	117181.2344	1625.89990	1033.76001	1252.43994	276.89999	1370.199951	629.199951	3552.660	9155.561
[29,]	15000.300	10133	3662.1799	2827.21997	367.78000	191.70000	178.92000	106.599998	1921.399902	117.820	340.560
[30,]	90050.398	57656	46617.1797	850.57996	505.51999	1187.12000	619.12000	492.699982	12064.000000	1302.900	8154.520
[31,]	170381.703	128415	36090.7188	51757.57812	6458.15967	2797.39990	143.42000	436.799988	244.399994	1245.280	4130.580
[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	1811.91992	575.09998	962.75995	373.45999	1131.000000	366.599976	2670.300	8816.720
[35,]	138026.703	102190	33558.8594	816.50000	242.81999	773.89996	211.57999	568.099976	383.500000	1569.500	6247.900

Manual analysis vs computational cytometry

manual analysis

- subjective
- difficult to standardize
- biased

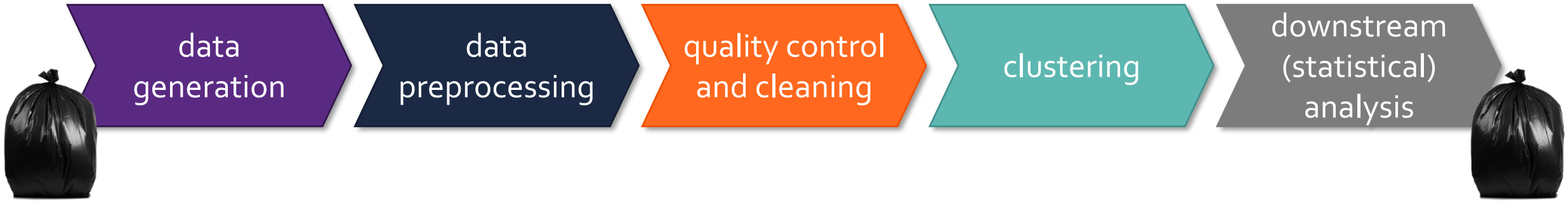
- time consuming
- not feasible for analysis of high-dimensional data

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

Automated computational analysis pipeline



garbage in = garbage out

Automated computational analysis pipeline

data generation → data preprocessing → quality control and cleaning → clustering → downstream (statistical) analysis

standardization

marker names

machine settings

cy 79a
Cy 79a
CD79 CITO
CD79A CITO
CD79a
cyCD79a
cCD79a
CITO79A
CD79
c79A
cCD79A
CD79 A-CITO

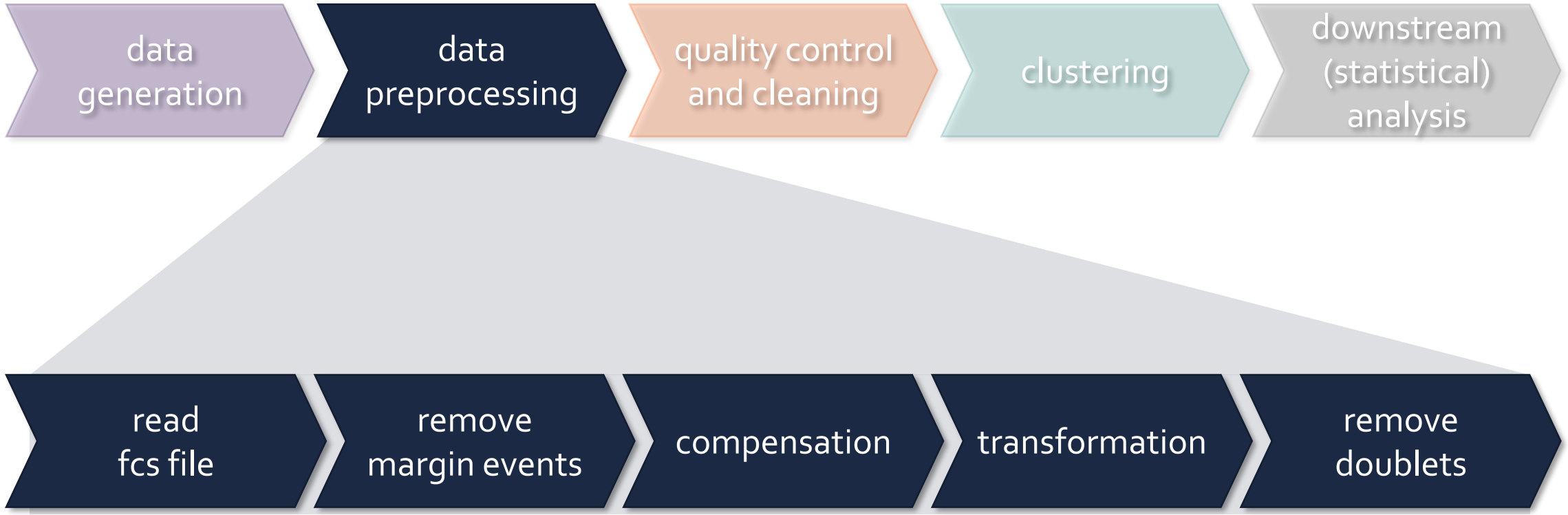
fluorochrome names

compensation controls

A488
AF_488
AlexaFluor 488
AF 488
AF488
Alexa_488
AlexaFluor_488
Alexa-Fluor-488

Blenman et al 2021 Cytometry A

Automated computational analysis pipeline



FLOWJO®

FCS Express 7 Cytometry

Dotmatics OMIQ

R

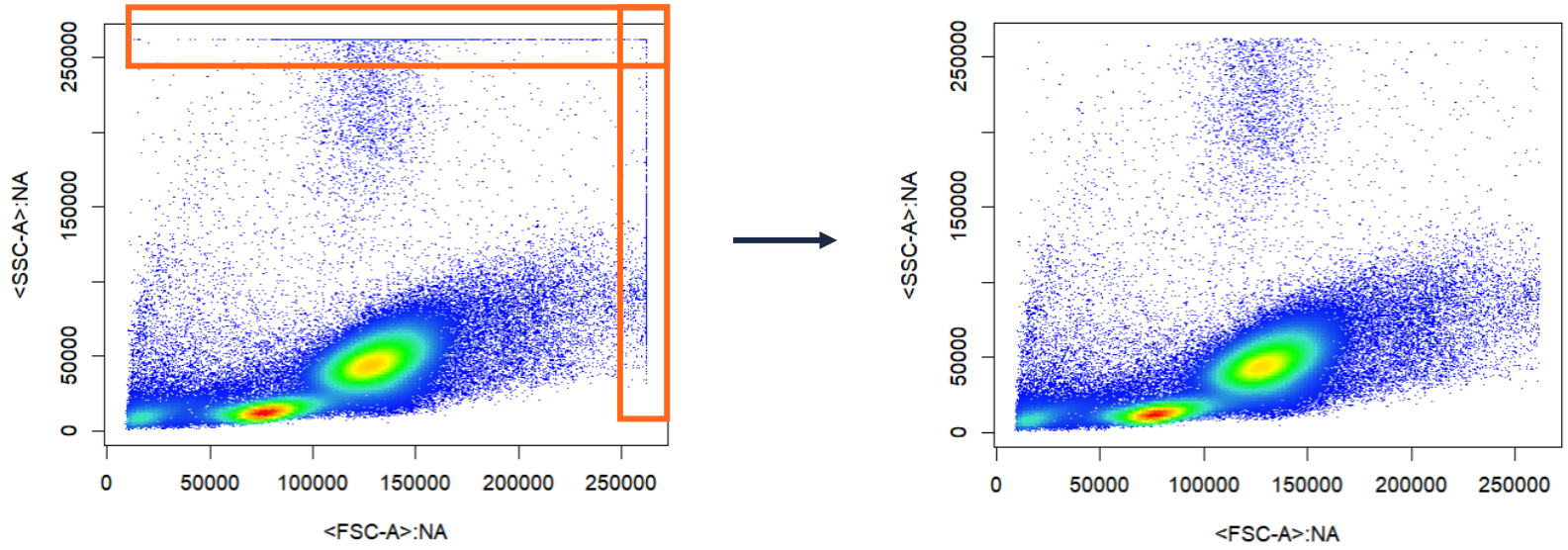
Infinicyt™

Kaluza Analysis Software

Preprocessing overview

read fcs file → **remove margin events** → compensation → transformation → remove doublets

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

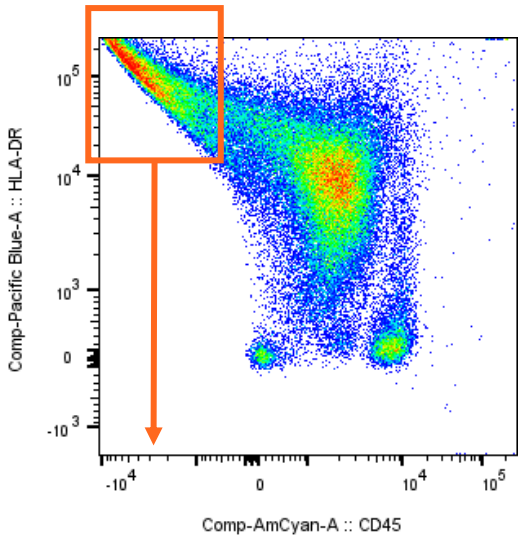


PeacoQC

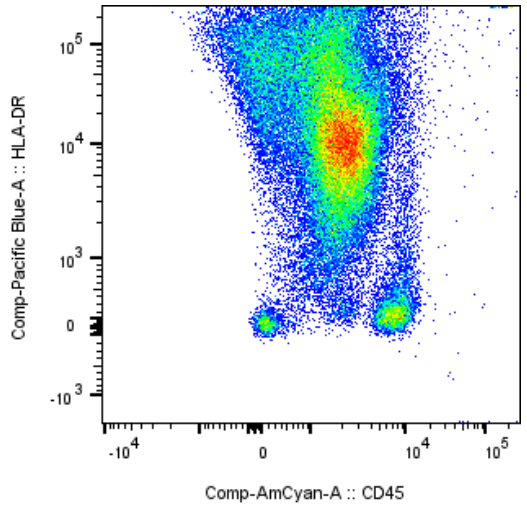
Preprocessing overview

read fcs file → remove margin events → **compensation** → transformation → remove doublets

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



e.g. overcompensation

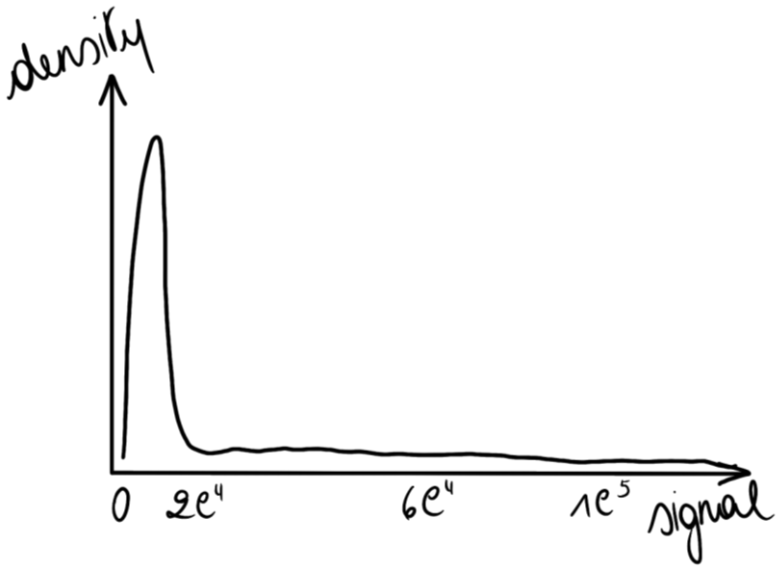


correct compensation matrix applied

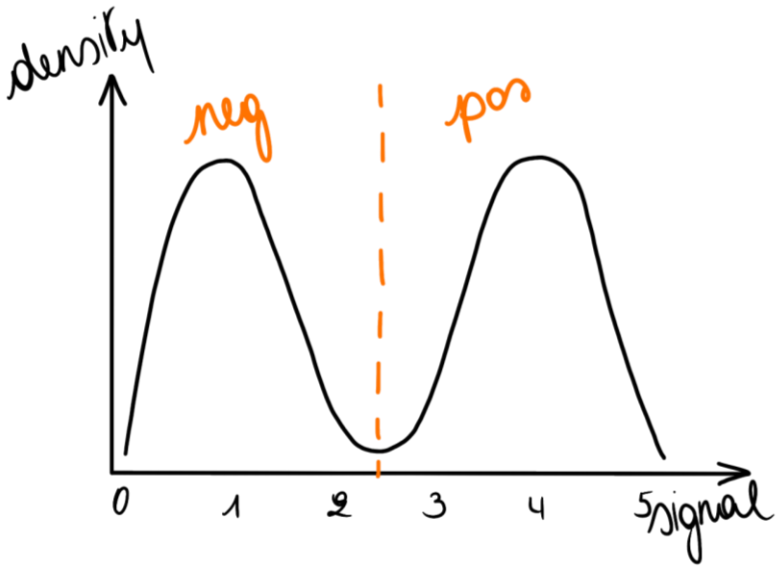
Preprocessing overview

read fcs file → remove margin events → compensation → **transformation** → remove doublets

without transformation

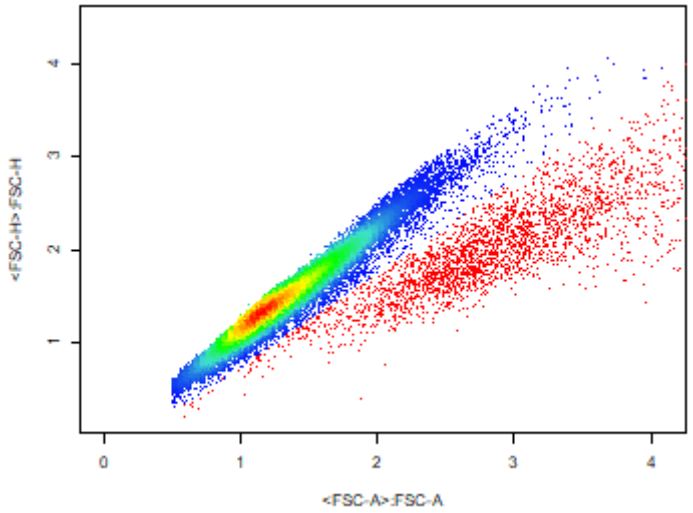


biexponential transformation (e.g. logicle)



Preprocessing overview

read fcs file → remove margin events → compensation → transformation → **remove doublets**



PeacoQC

Automated computational analysis pipeline

Quality control on two levels

per file

e.g.
- clogs during acquisition
- changes in flow rate

check the signal consistency over time

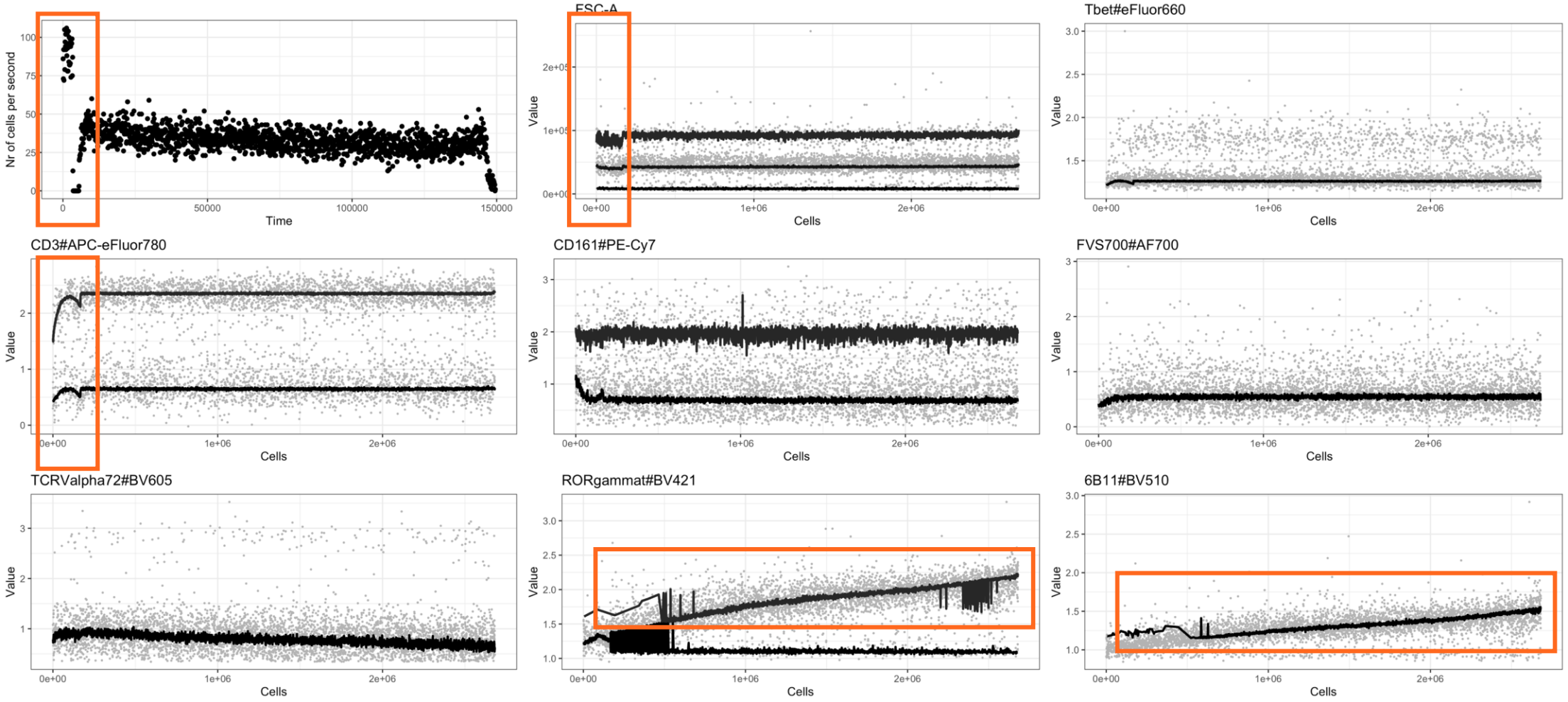
between files

e.g.
- new antibody batch
- change in machine settings

check for batch effects between samples

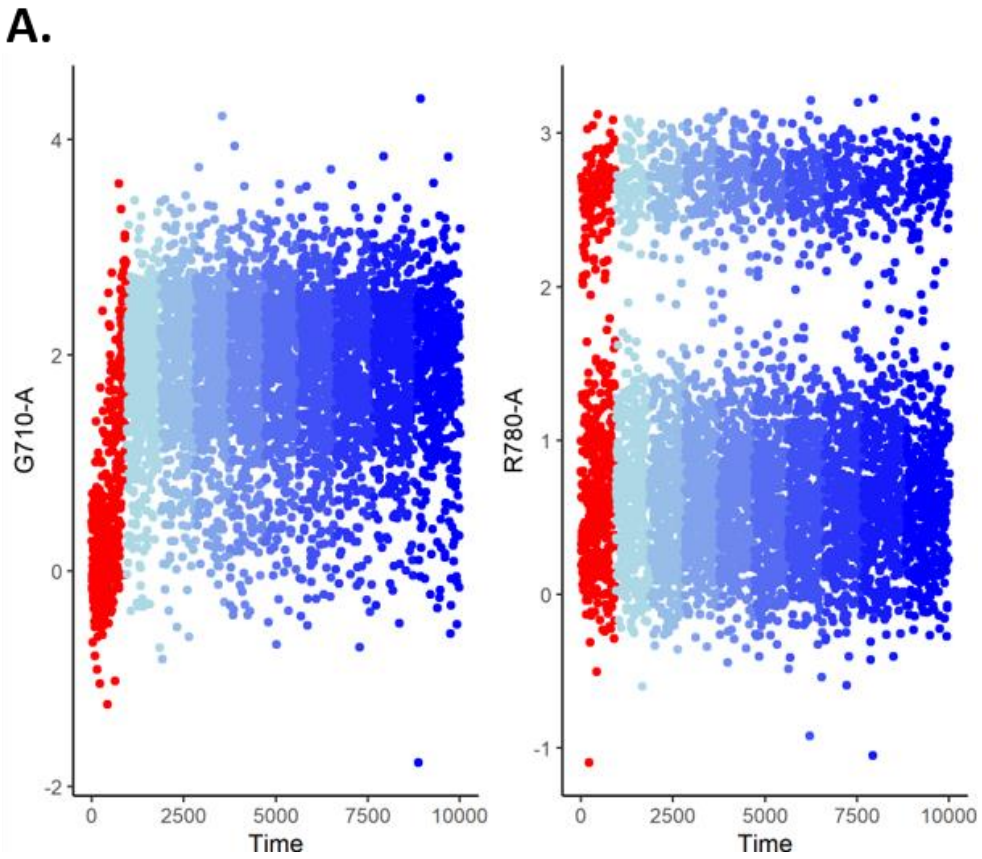
Within-file quality

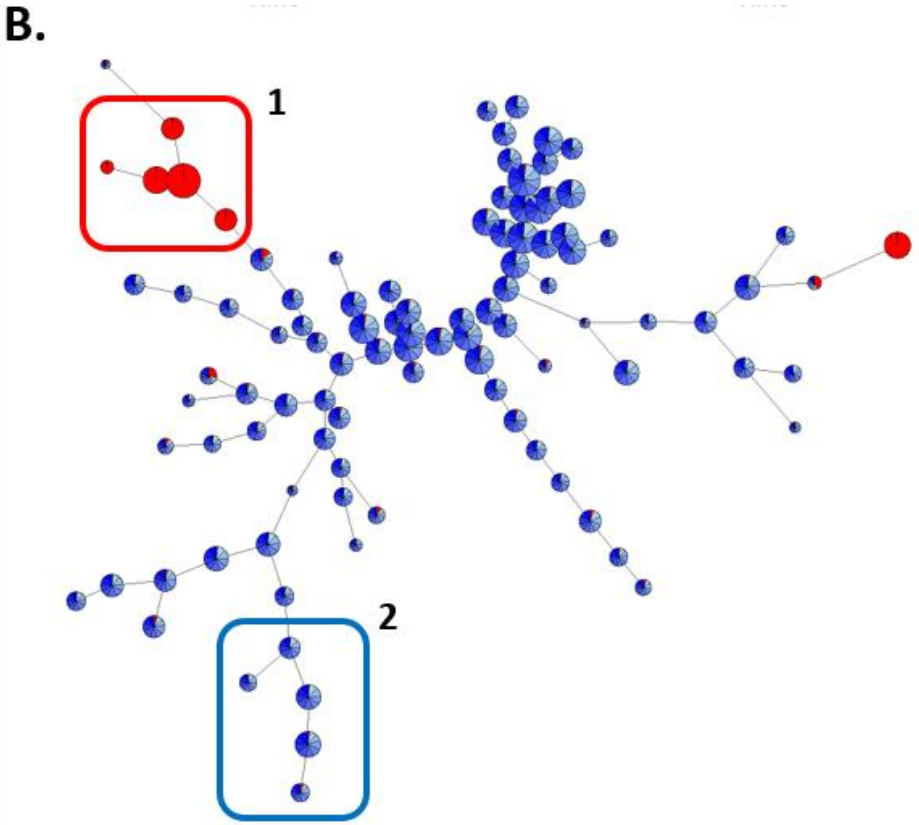
changes in flow rate and signal



changes in signal

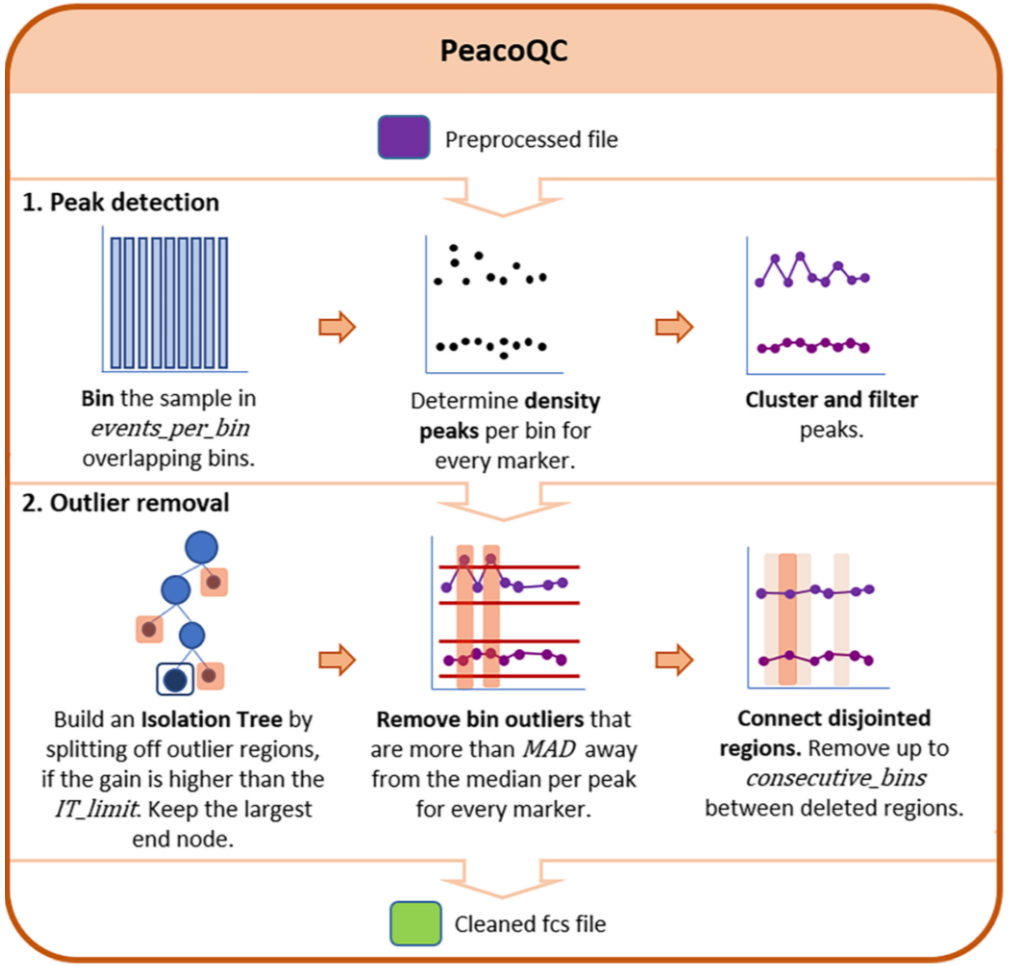
Impact of no quality filtering





Emmaneel et al, 2022, Cytometry A

PeacoQC for automated quality control



Emmaneel et al, 2022, Cytometry A

PeacoQC for automated quality control

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[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	4117.680
[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
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[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
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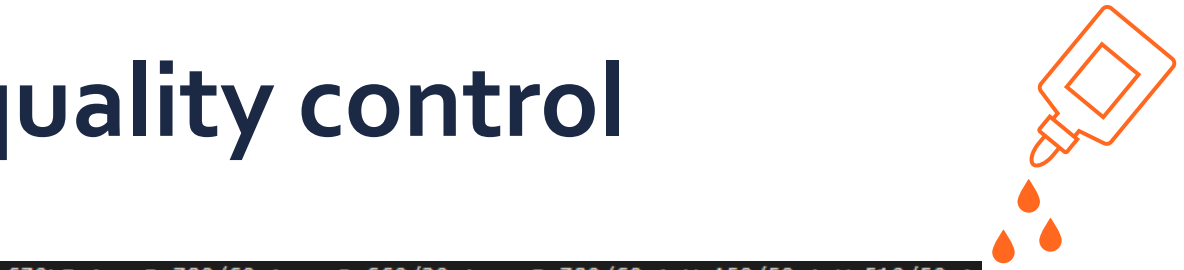
Emmaneel et al, 2022, Cytometry A

PeacoQC for automated quality control



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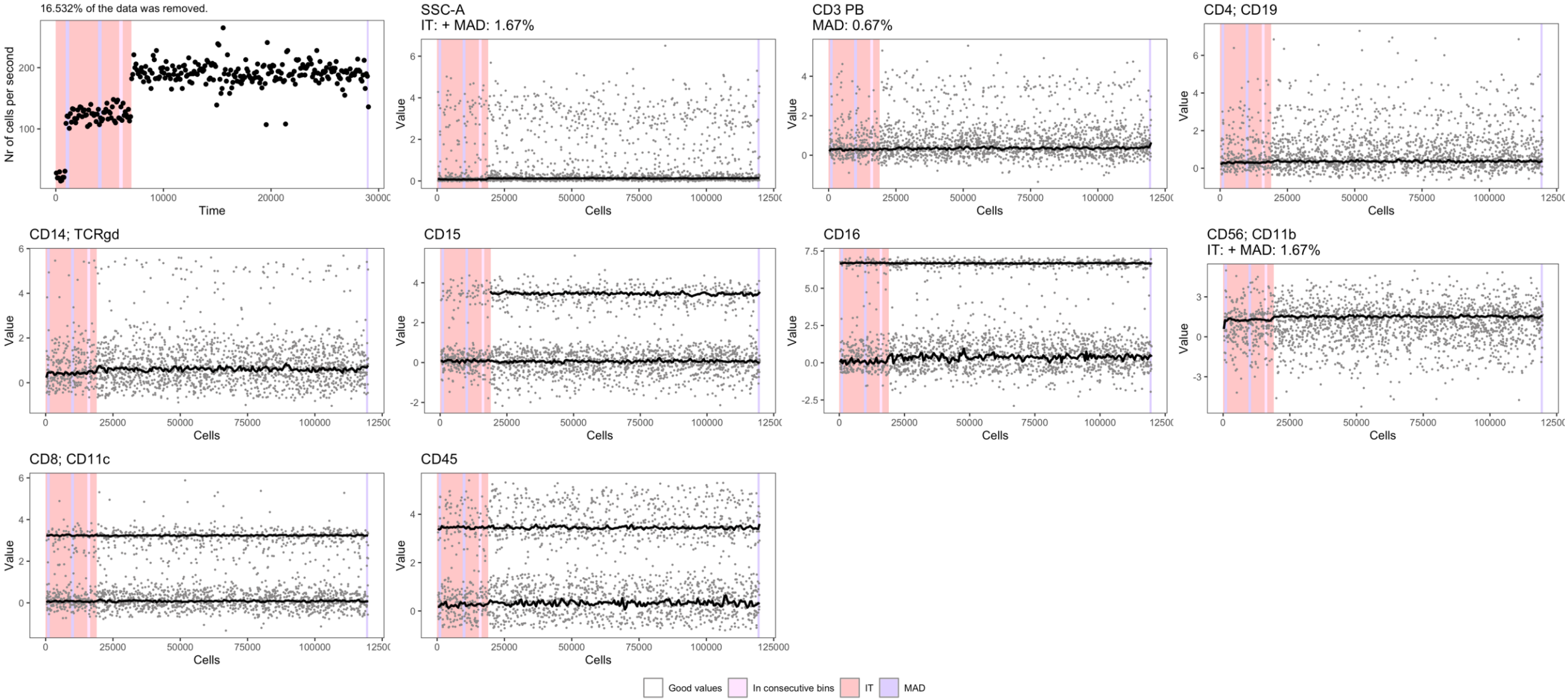
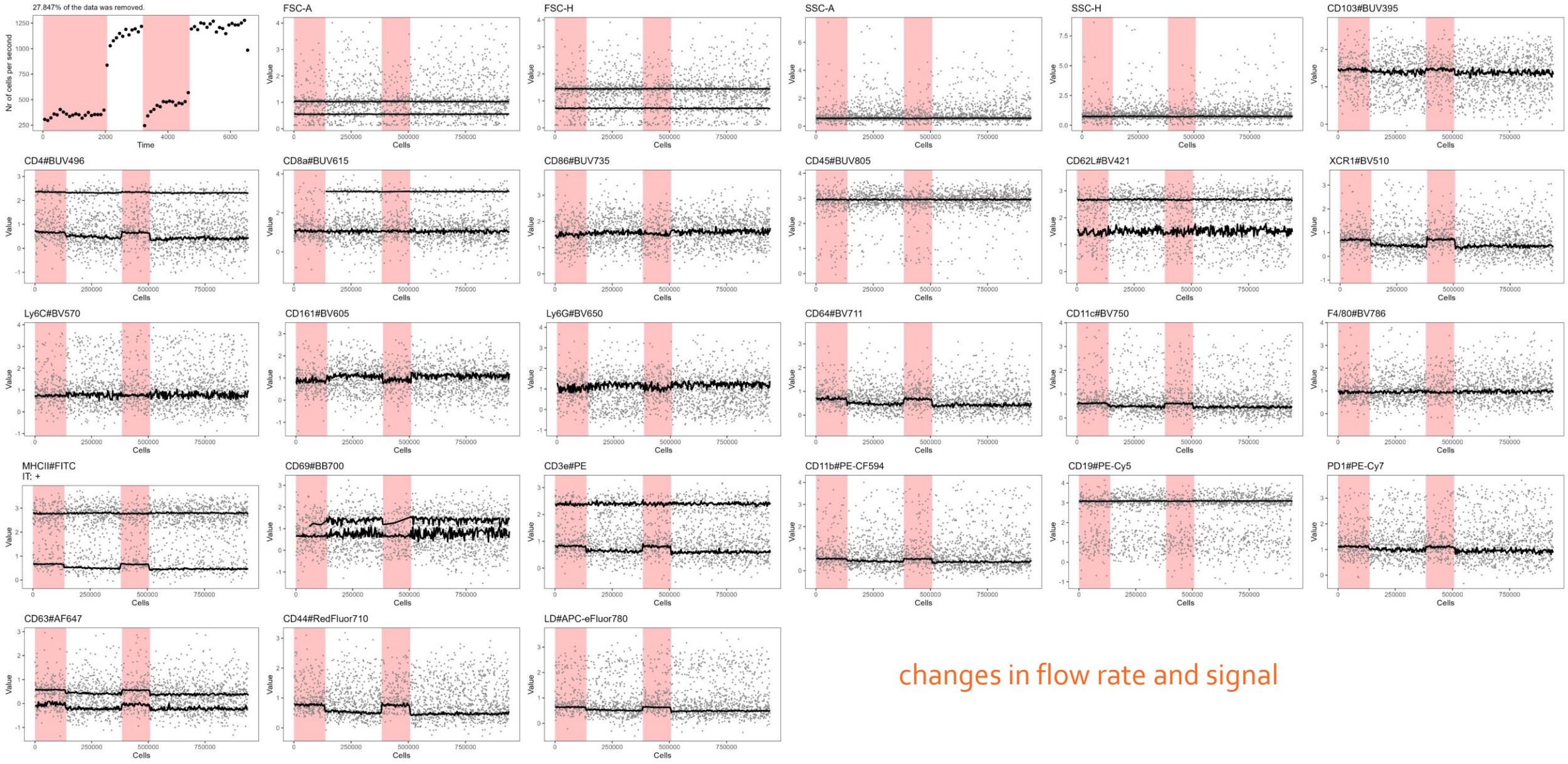
[18,]	35729.098	31493	33698.0195	306.72000	122.12000	452.97998	222.93999	317.199982	209.299988	473.000	836.780
[19,]	128565.898	106740	39812.5391	17013.01953	2379.91992	1581.88000	668.82001	339.299988	1622.399902	1571.220	3267.140
[20,]	176066.094	134946	46135.7969	573.67999	400.44000	479.95999	39.76000	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
[23,]	154710.891	91185	26857.8789	404.69998	332.28000	592.13995	435.93997	22634.298828	19925.099609	14140.980	13489.101
[24,]	151389.891	113838	32080.6387	2335.89990	509.78000	528.23999	66.74000	293.799988	218.399994	1927.260	6317.560
[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
[30,]	90050.398	57656	46617.1797	850.57996	505.51999	1187.12000	619.12000	492.699982	12064.000000	1302.900	8154.520
[31,]	170381.703	128415	36090.7188	51757.57812	6458.15967	2797.39990	143.42000	436.799988	244.399994	1245.280	4130.580
[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	1811.91992	575.09998	962.75995	373.45999	1131.000000	366.599976	2670.300	8816.720
[35,]	138026.703	102190	33558.8594	816.50000	242.81999	773.89996	211.57999	568.099976	383.500000	1569.500	6247.900

Emmaneel et al, 2022, Cytometry A

PeacoQC for automated quality control

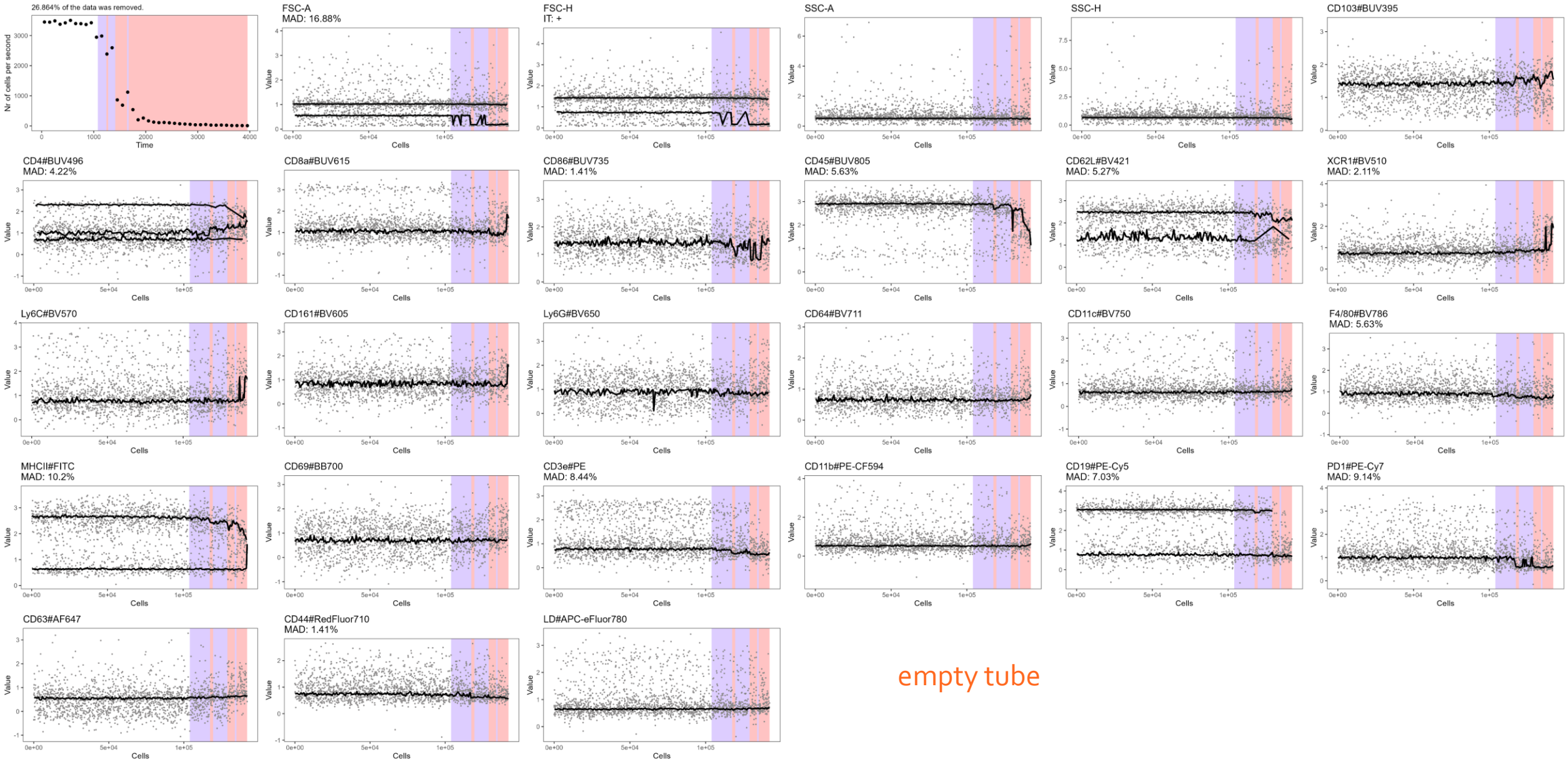


PeacoQC for automated quality control



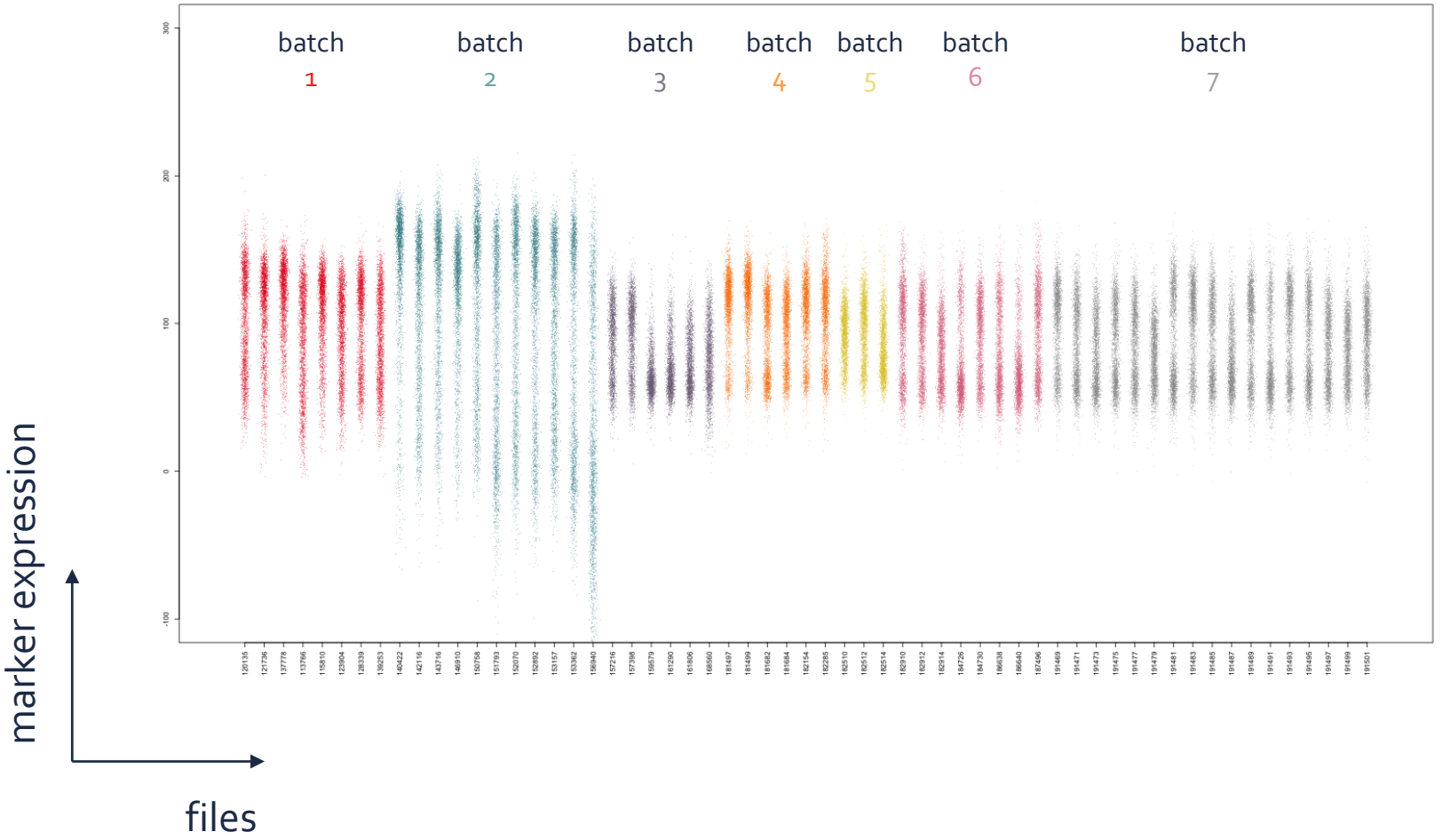
changes in flow rate and signal

PeacoQC for automated quality control



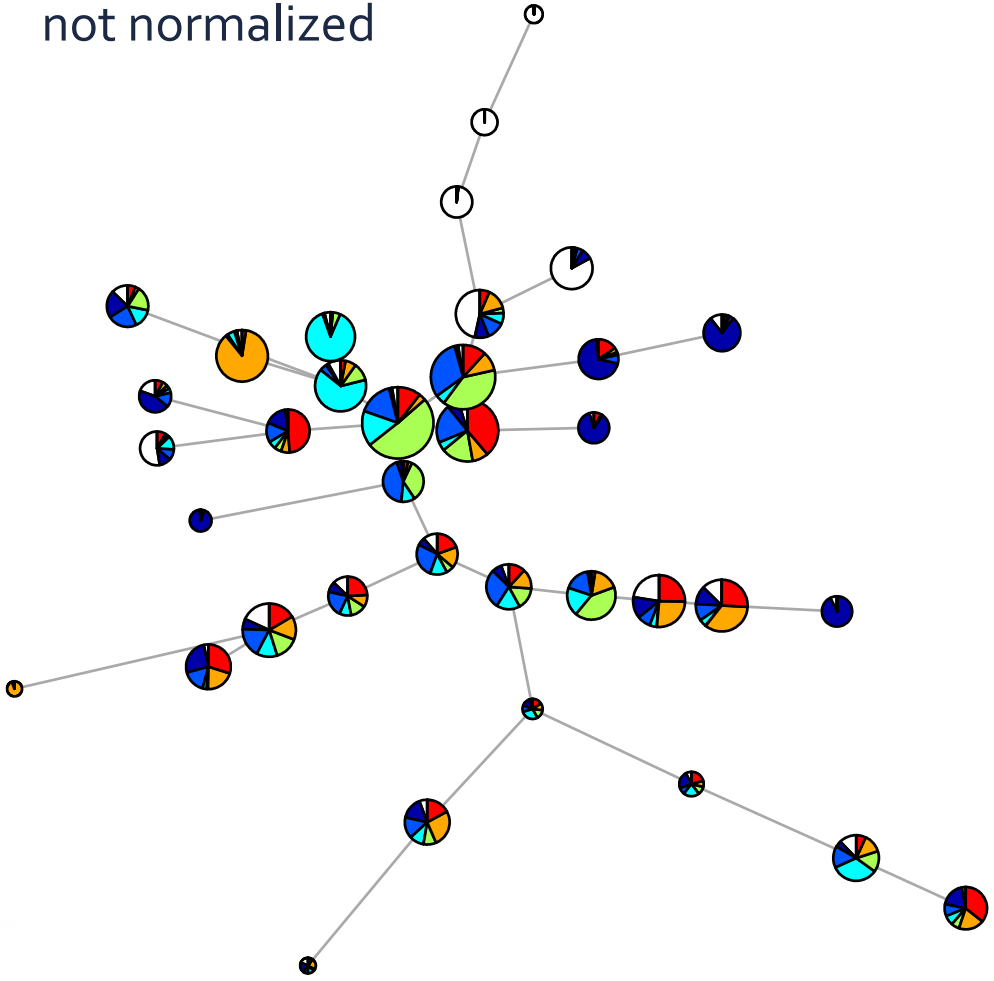
empty tube

Between-file consistency



Impact of no batch effect correction

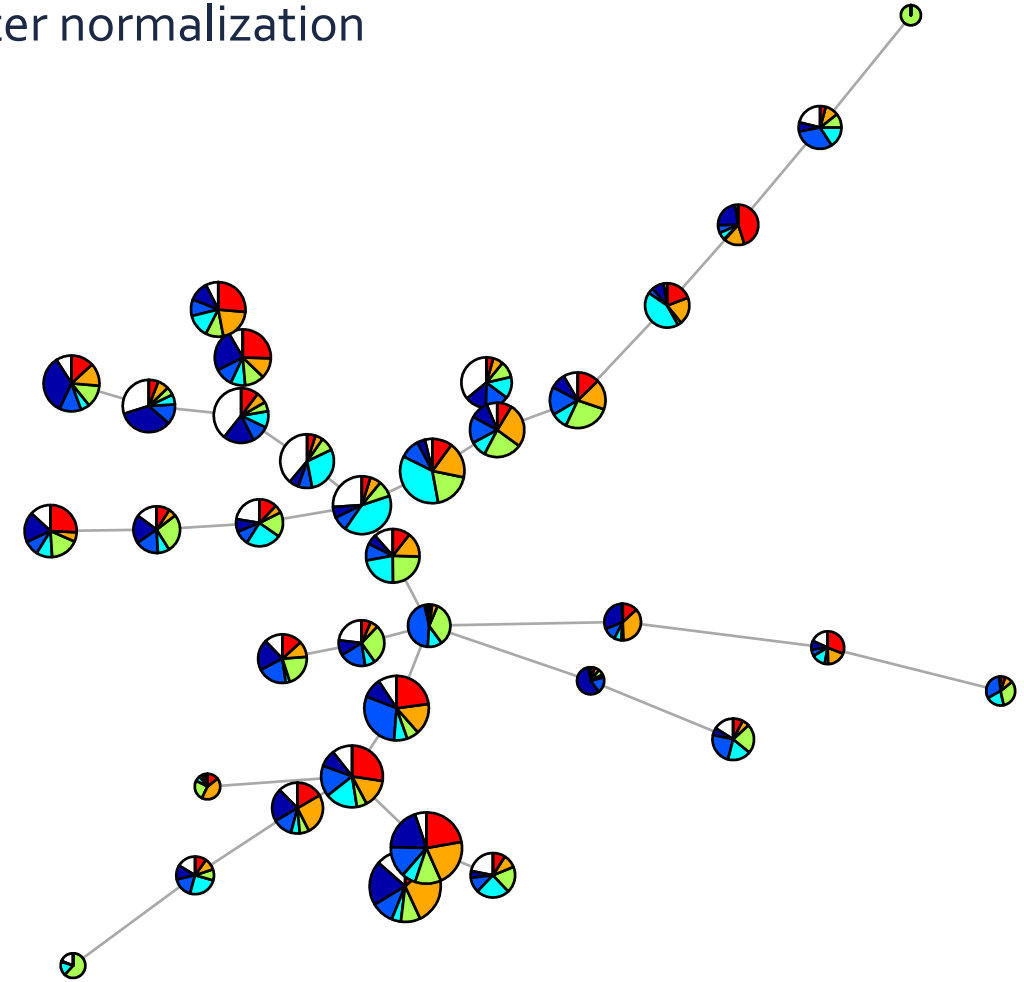
not normalized



batches

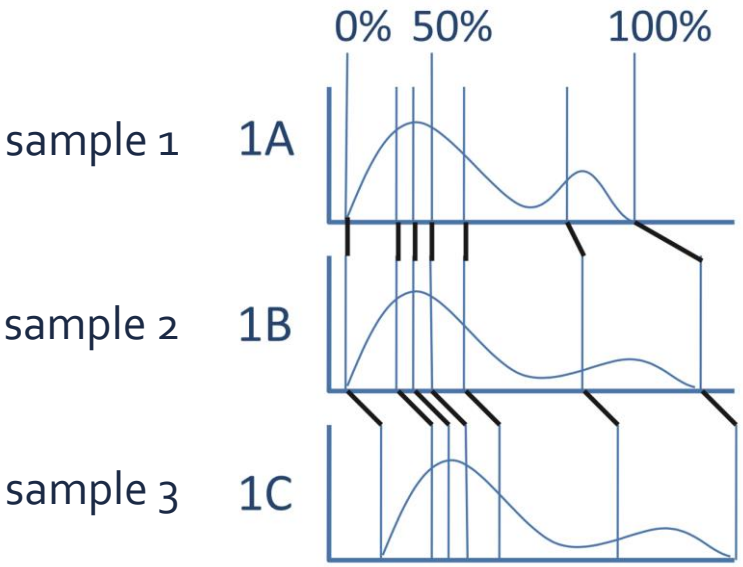
- 1
- 2
- 3
- 4
- 5
- 6
- 7

after normalization



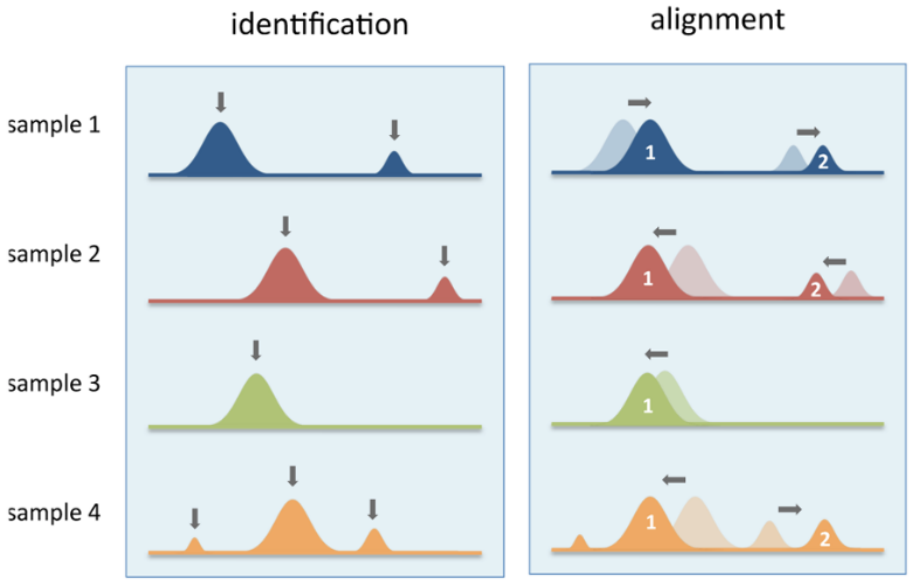
Batch effect correction approaches

aligning quantiles or percentiles



e.g. min-max normalization, percentile normalization

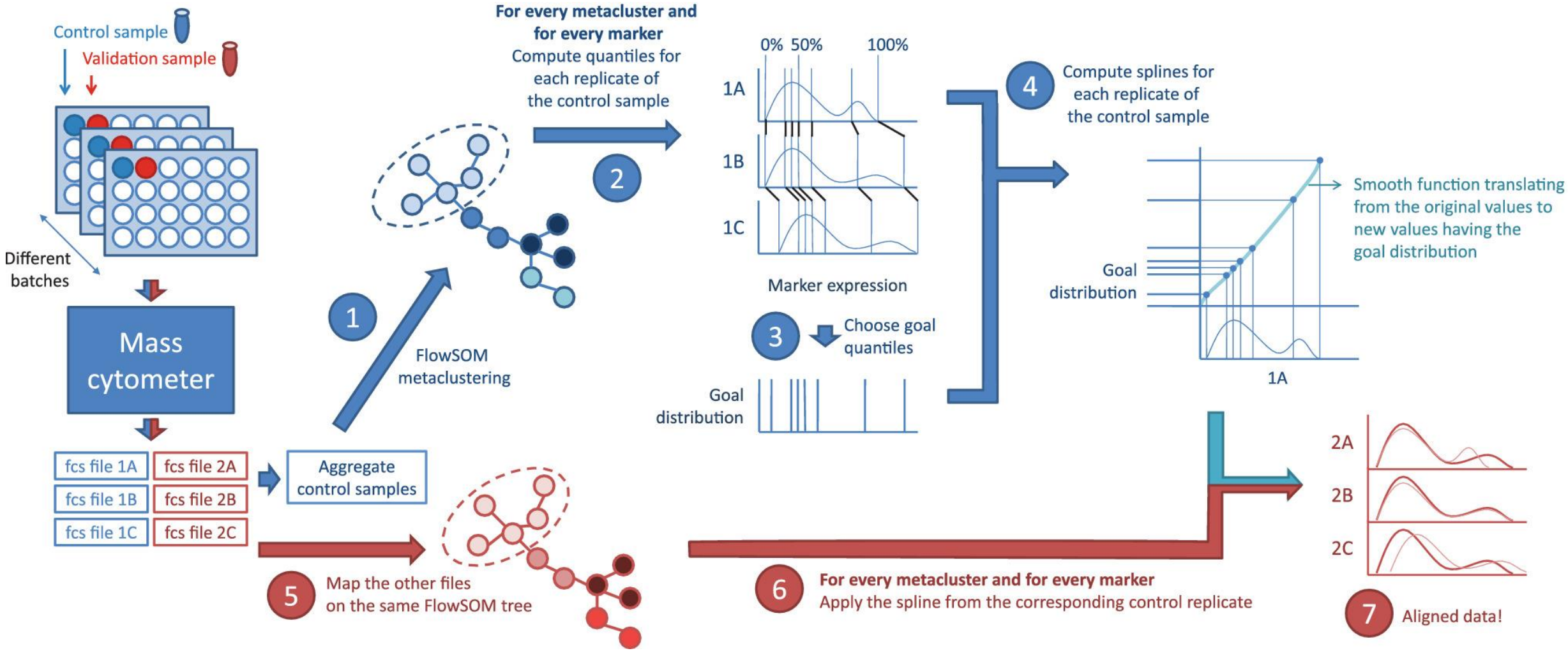
aligning density peaks



e.g. gaussNorm, fdaNorm

Hahne et al, 2009, Cytometry A

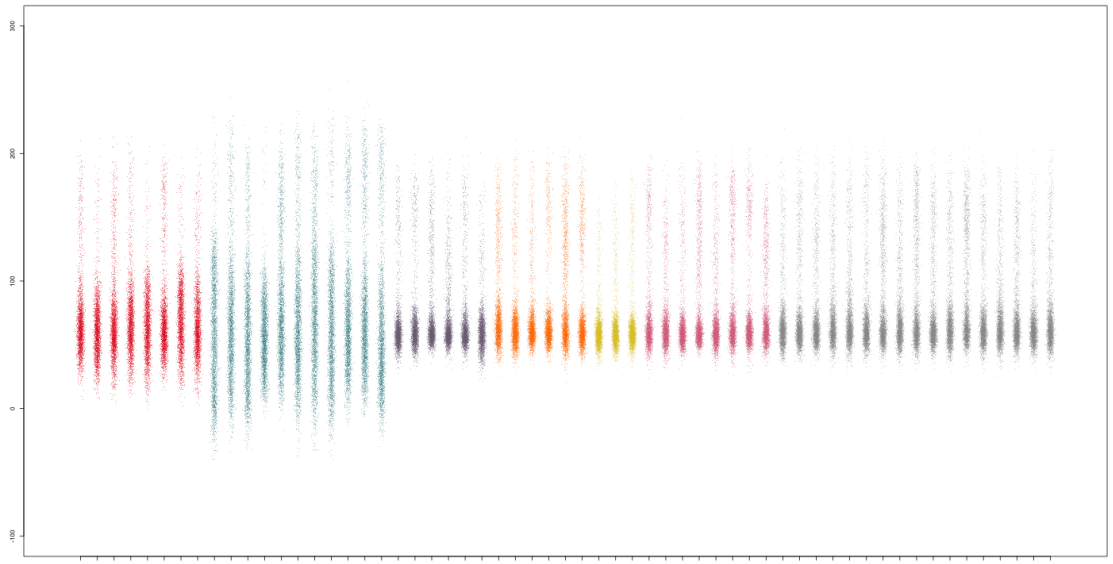
CytoNorm for batch effect correction



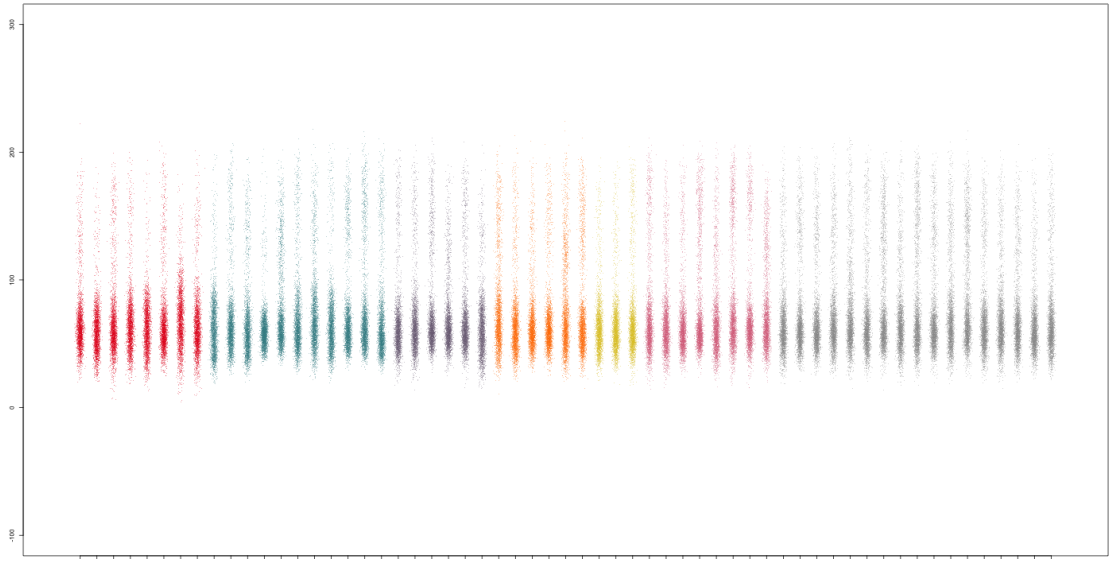
Van Gassen et al, 2019, Cytometry A

CytoNorm for batch effect correction

not normalized

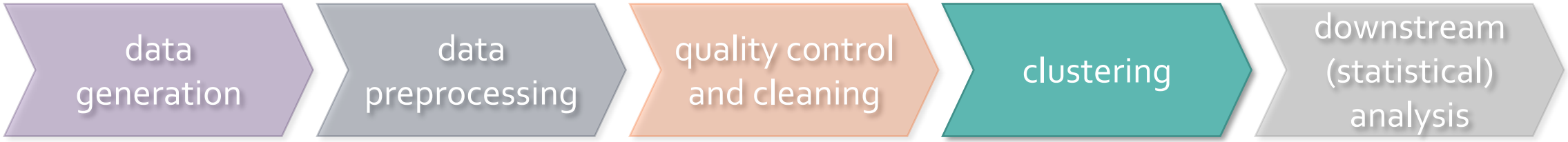


CytoNorm normalized



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

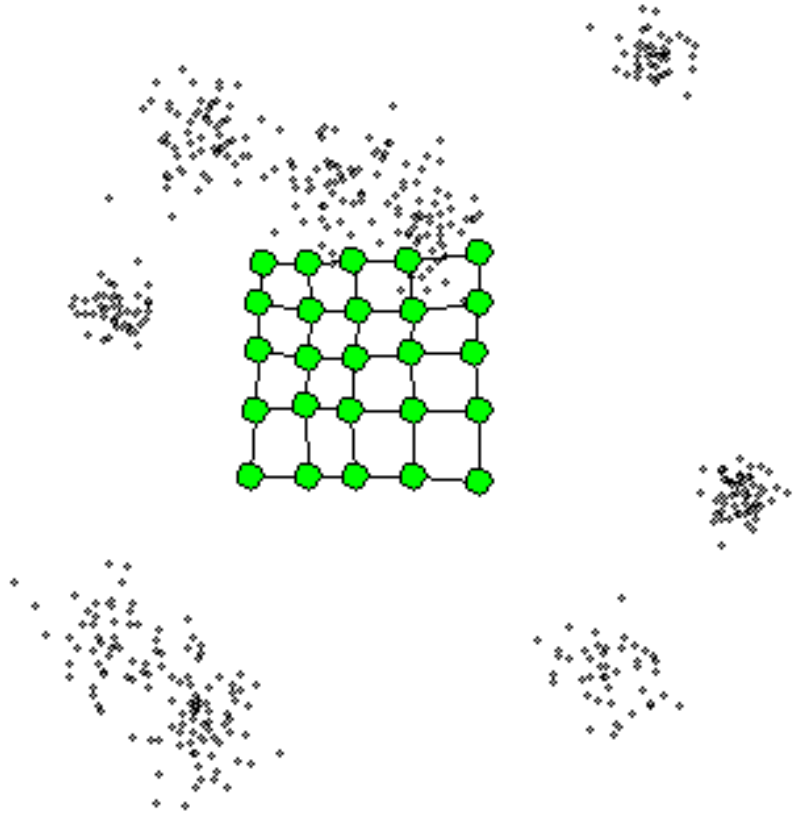
Automated computational analysis pipeline



Automated gating using FlowSOM

Two-level clustering

- simple but fast
  Self-Organizing Map (SOM)
  overclustering



Van Gassen et al, 2015, Cytometry A
Quintelier et al, 2021, Nature Protocols

Automated gating using FlowSOM

Two-level clustering

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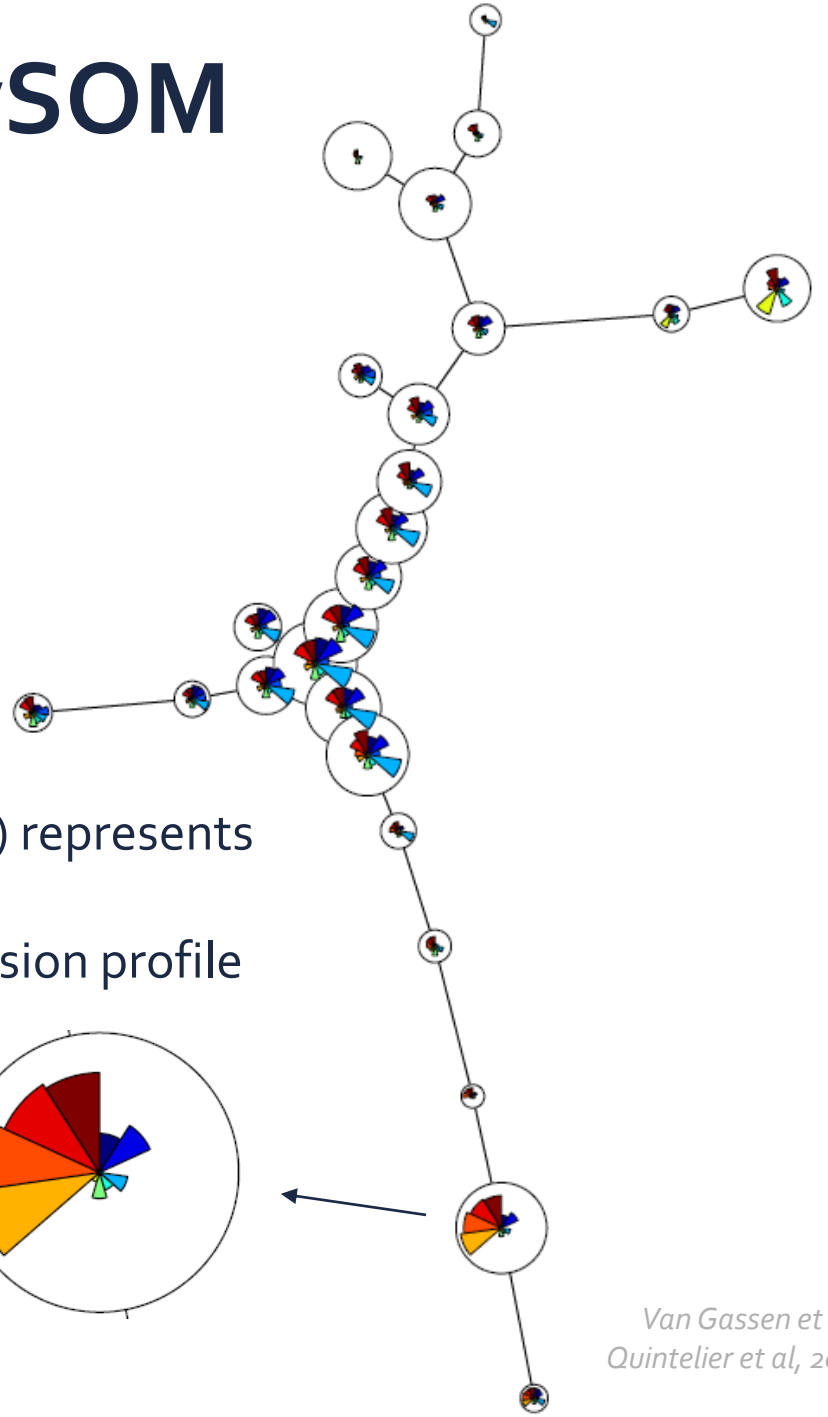
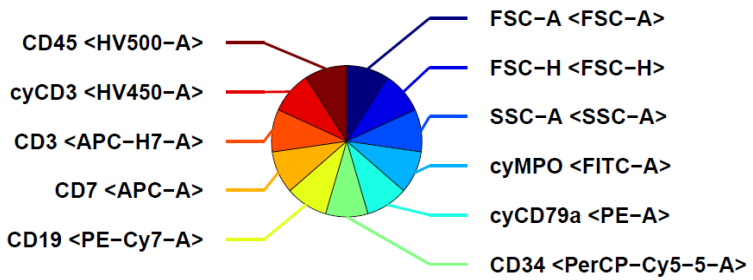


every circle ("cluster") represents
a group of cells with a
similar marker expression profile



Van Gassen et al, 2015, Cytometry A
Quintelier et al, 2021, Nature Protocols

Automated gating using FlowSOM

Two-level clustering

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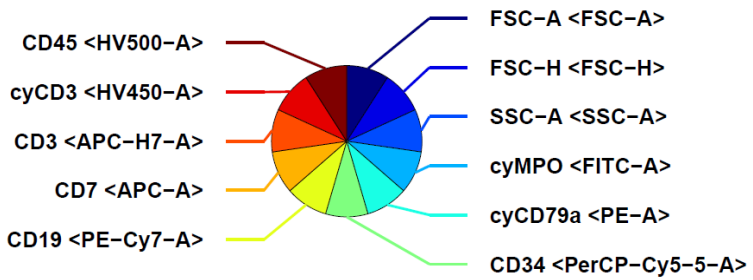
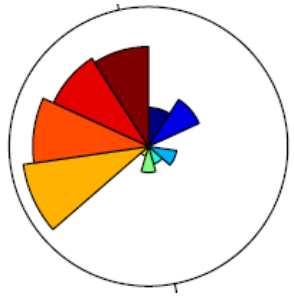


high expression of
CD7, CD3, cyCD3, CD45
(low expression of other markers)
→ T cells



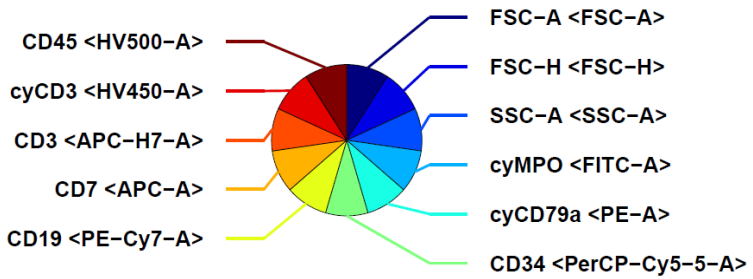
Van Gassen et al, 2015, Cytometry A
Quintelier et al, 2021, Nature Protocols

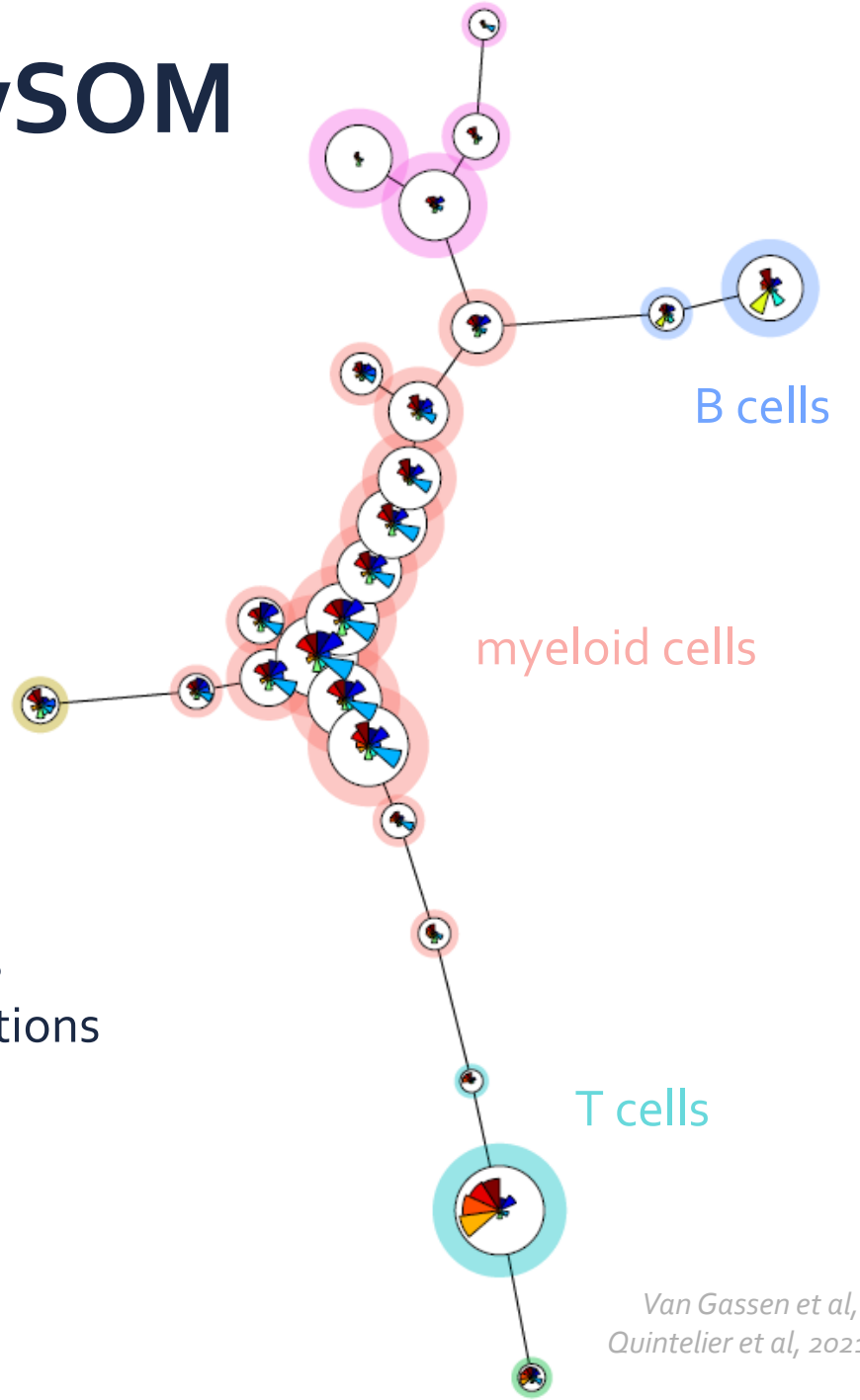
Automated gating using FlowSOM

Two-level clustering

- simple but fast
  Self-Organizing Map (SOM)
  overclustering

- more sensitive
  hierarchical consensus clustering
  on the SOM nodes

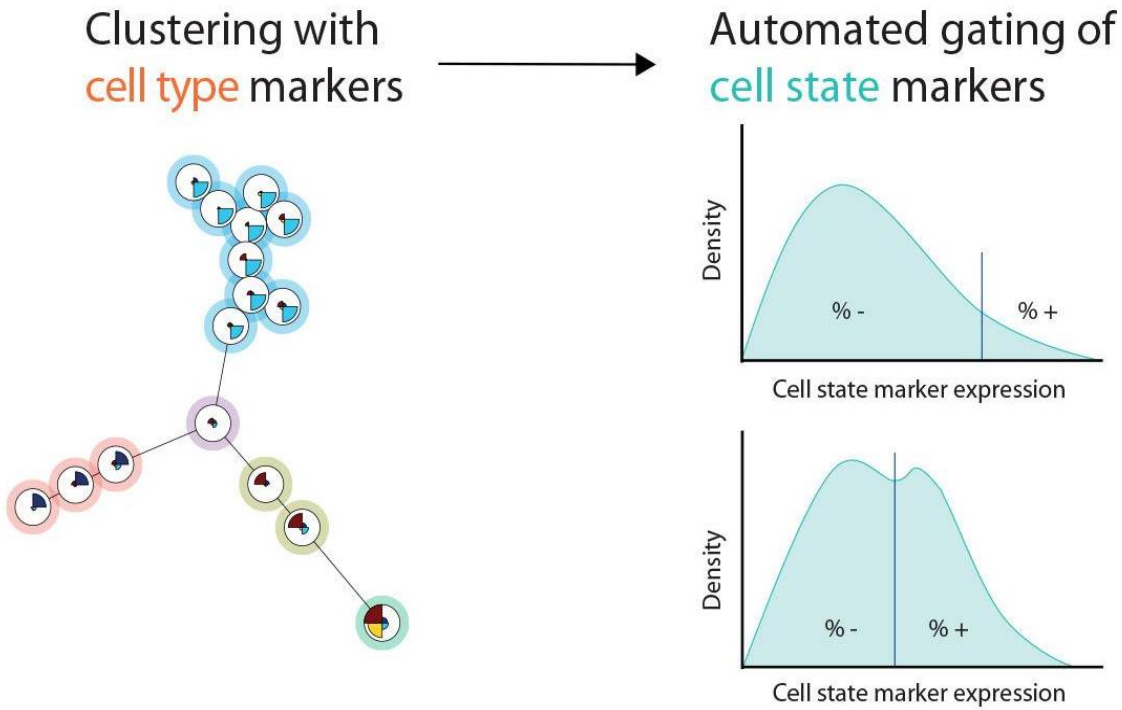




metaclusters
~ cell populations

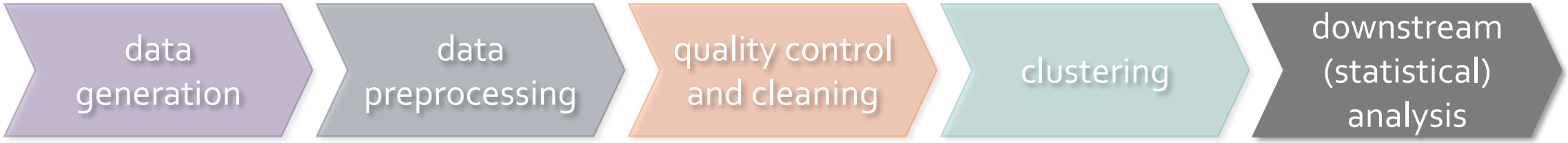
Van Gassen et al, 2015, Cytometry A
Quintelier et al, 2021, Nature Protocols

Cell type vs cell state markers



Dietz, Quintelier, et al, 2023, J Immunother Cancer

Automated computational analysis pipeline



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

Amsterdam UMC
Universitair Medische Centra

Universitätsklinikum Carl Gustav Carus
DIE DRESDNER.

UZ GENT

UZ LEUVEN

KSA Kantonsspital Aarau

Erasmus MC
Universitair Medisch Centrum Rotterdam

EuroFlow

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

training cohort

controls

MDS patients

validation cohort

computational preprocessing
- QC
- removal of doublets
- compensation
- transformation

FlowSOM

features

patients

features per metacluster
- percentage
- MFI per parameter
- CV per parameter

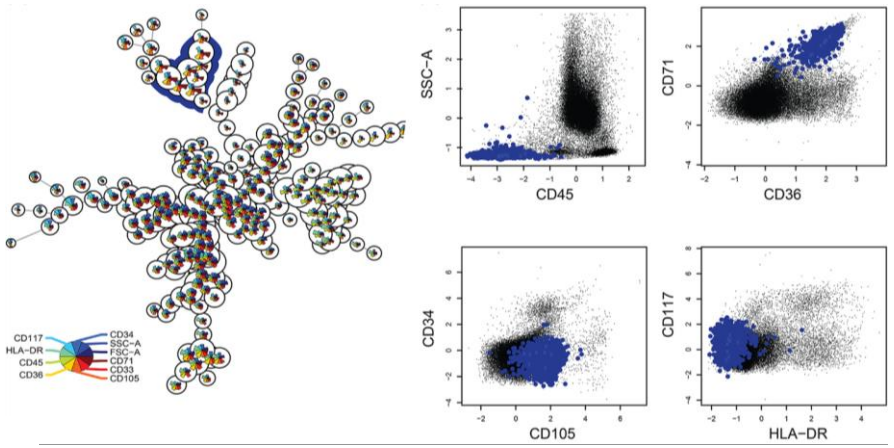
machine learning classifier
(random forest)

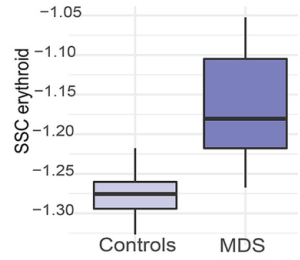
MDS

no MDS

independent validation

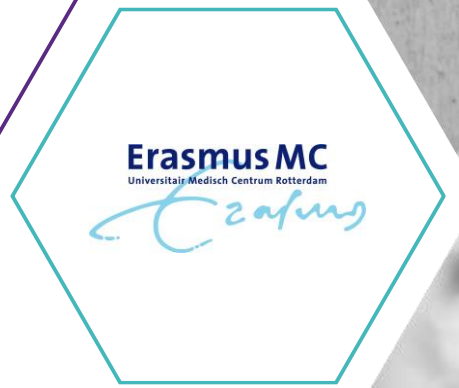
identification of the cellular features most relevant for diagnosis





	sensitivity	specificity	time
manual analysis	81%	86%	1 hour
automated analysis	97%	95%	30 sec

Duetz et al 2021 Cytometry A

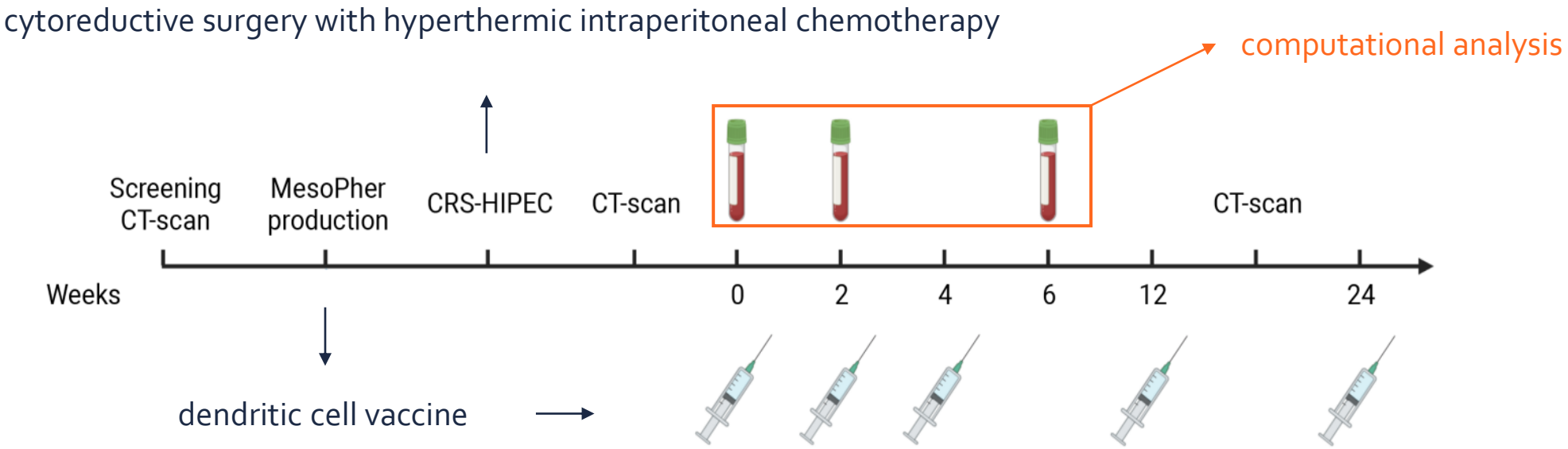


Immune response after immunotherapy for malignant peritoneal mesothelioma

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

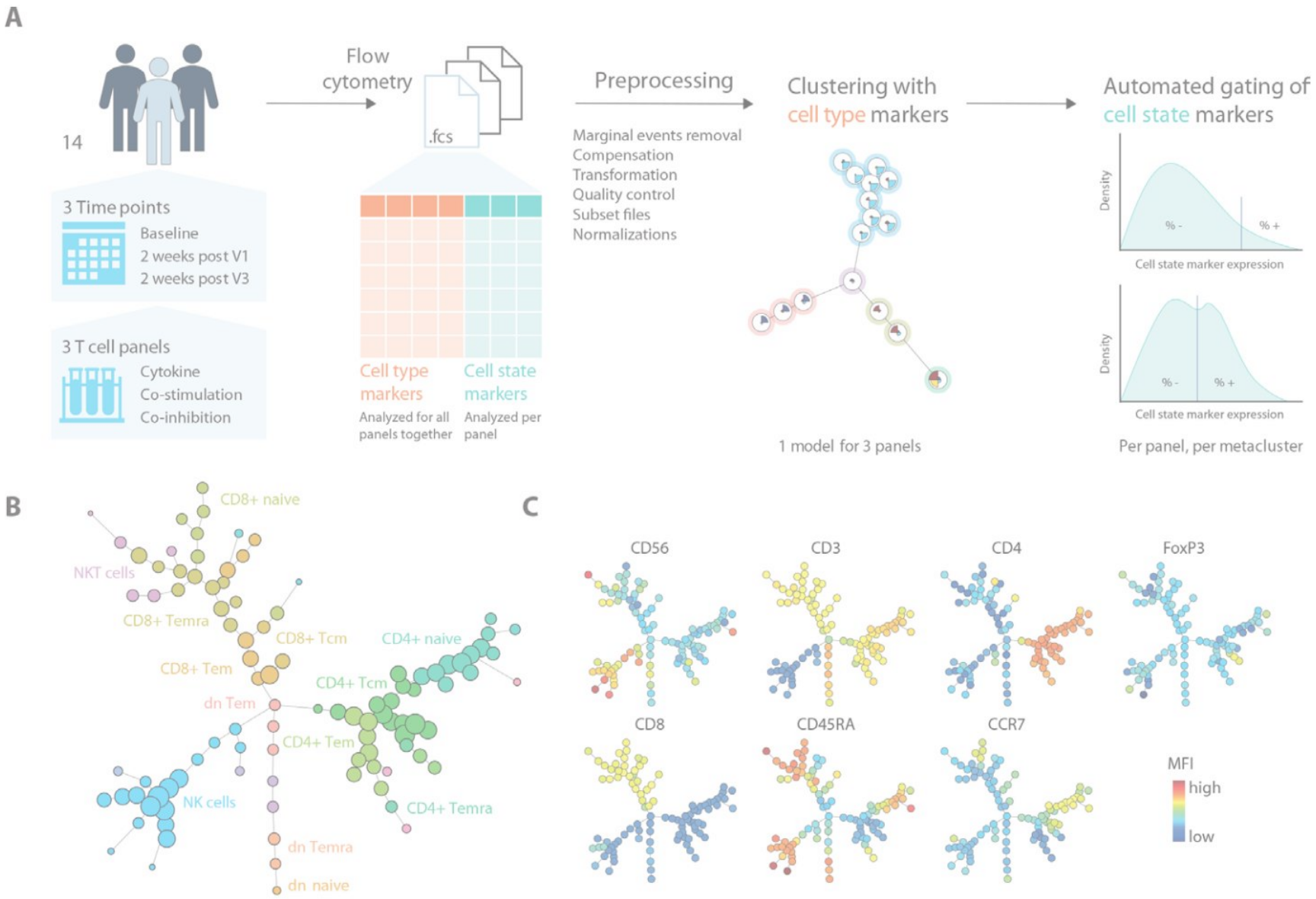
Clinical trial for malignant peritoneal mesothelioma

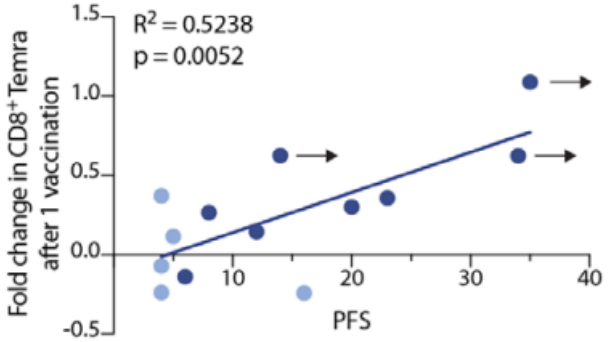
clinical trial design



Dietz MV et al, 2023, J Immunother Cancer

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





increase of CD8+ Temra cells after one vaccination is correlated with longer progression-free survival

Dietz MV et al, 2023, J Immunother Cancer

Saeyslab
Data mining and Modelling for Biomedicine

UZ GENT

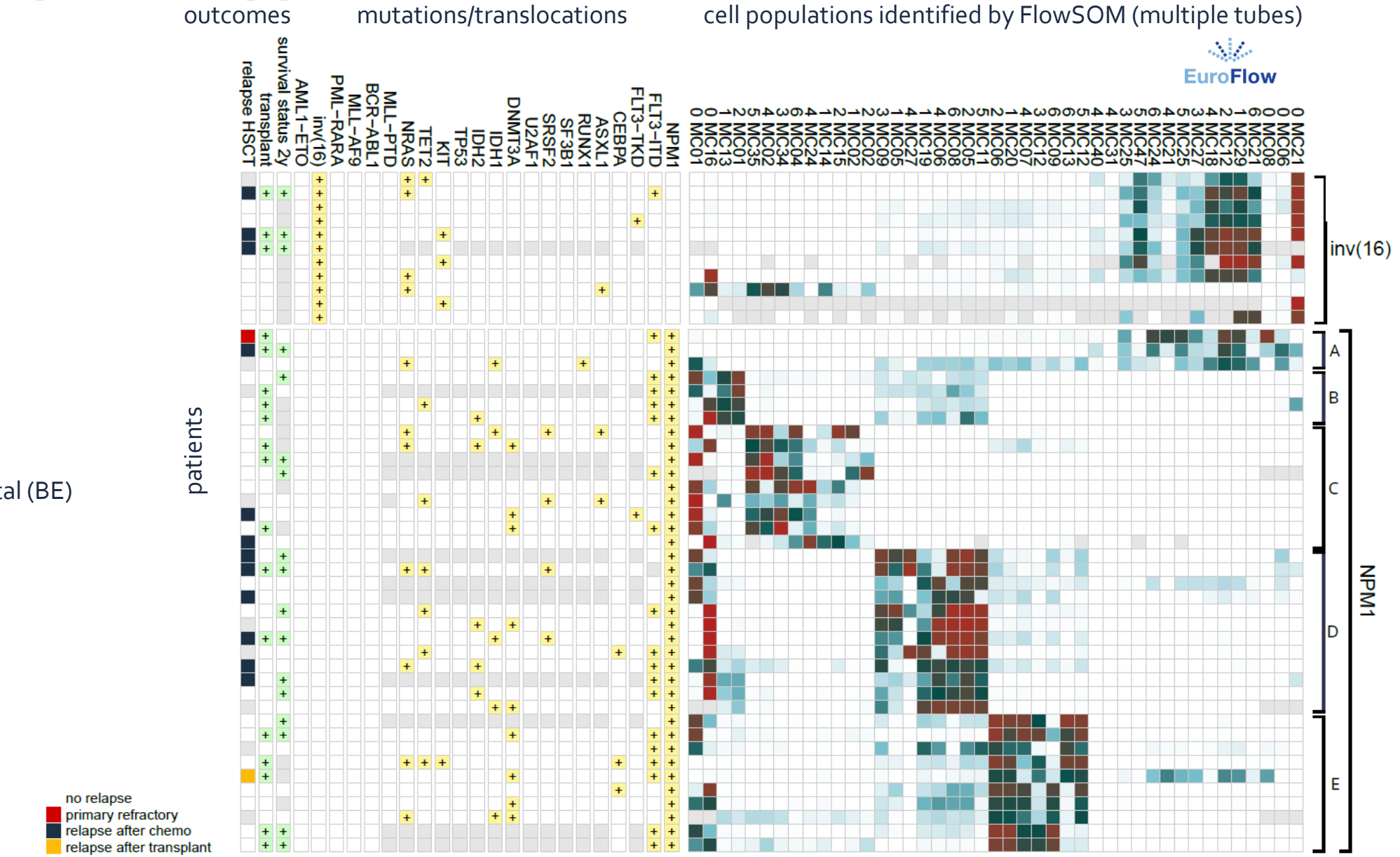
GHENT UNIVERSITY

Unraveling the immunophenotypic landscape in acute myeloid leukemia

Couckuyt A et al (2023) *manuscript submitted*

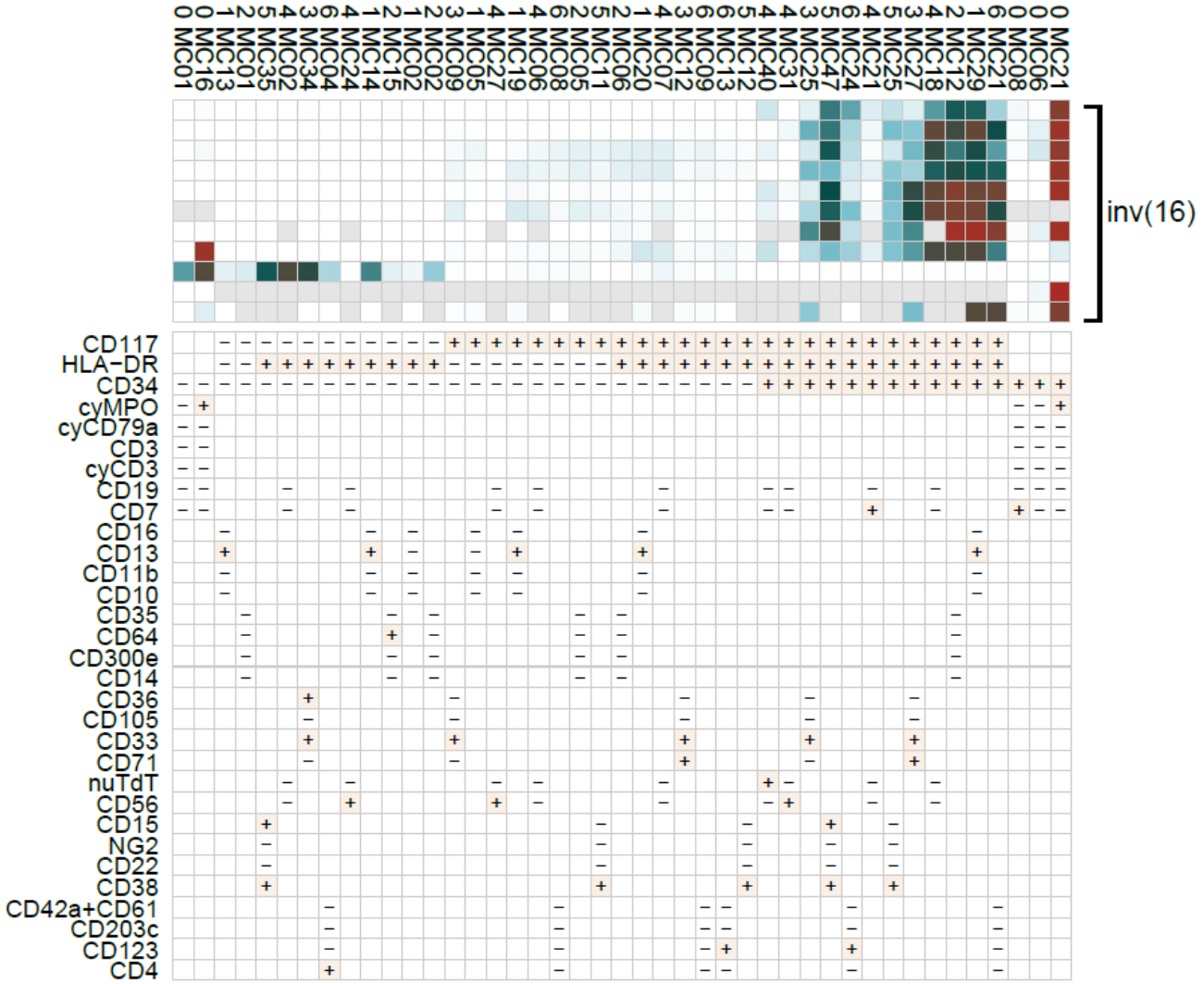
Genotype-phenotype associations

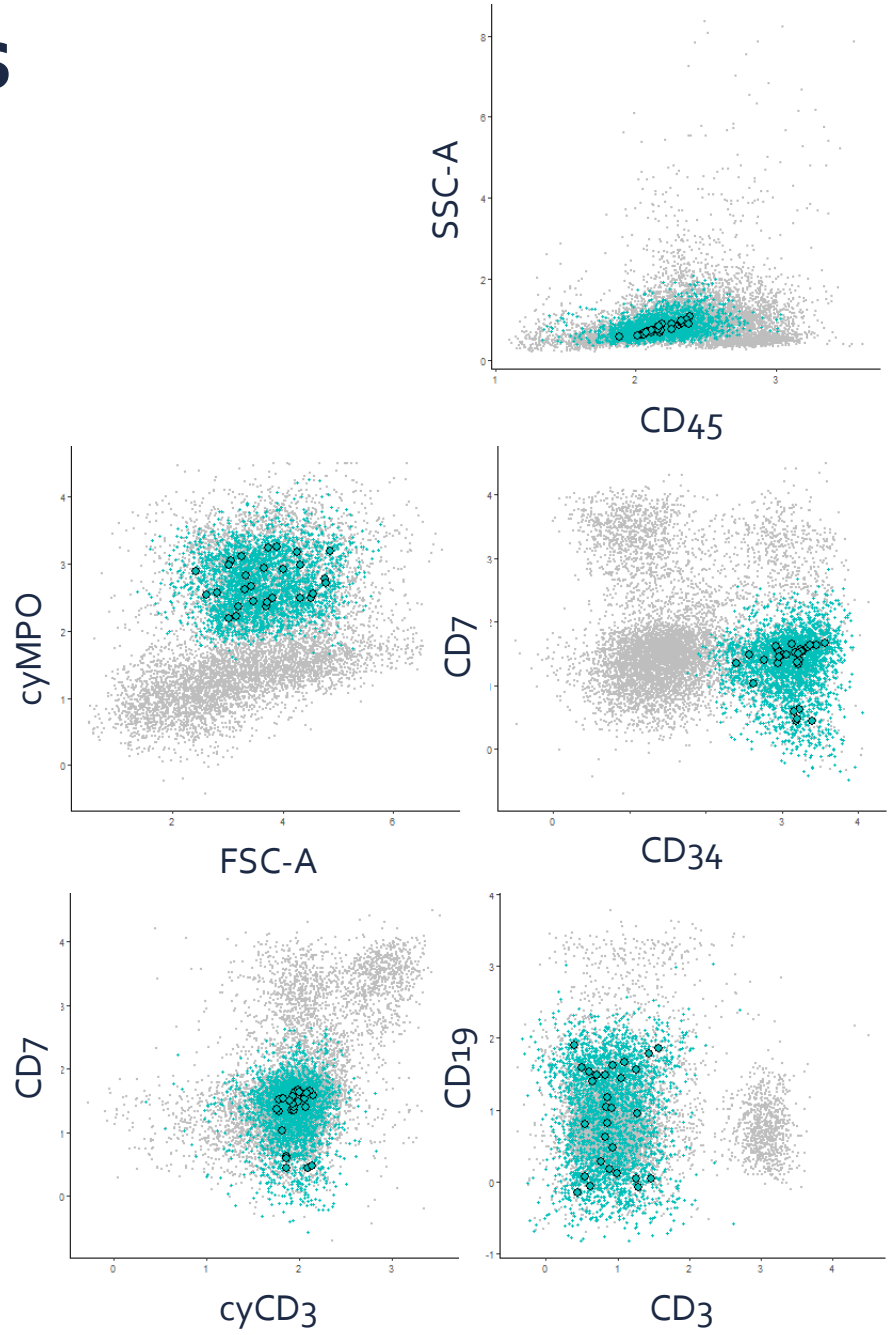
AML patients
Ghent University Hospital (BE)
2015-2019
n = 122



preprint available

Genotype-phenotype associations

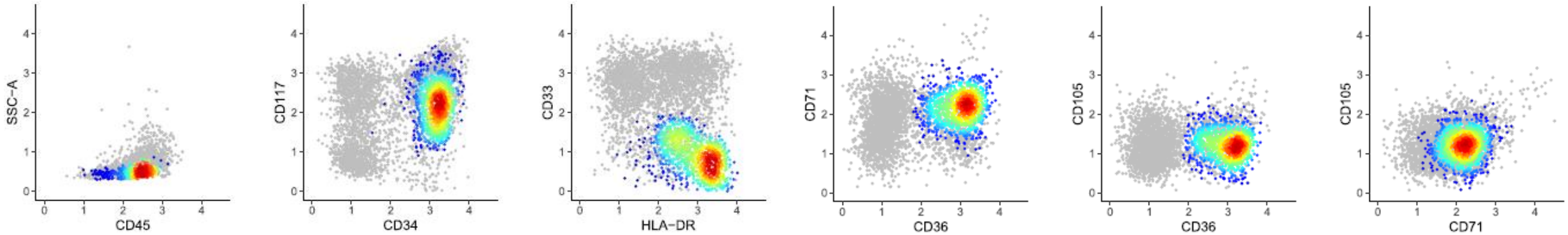


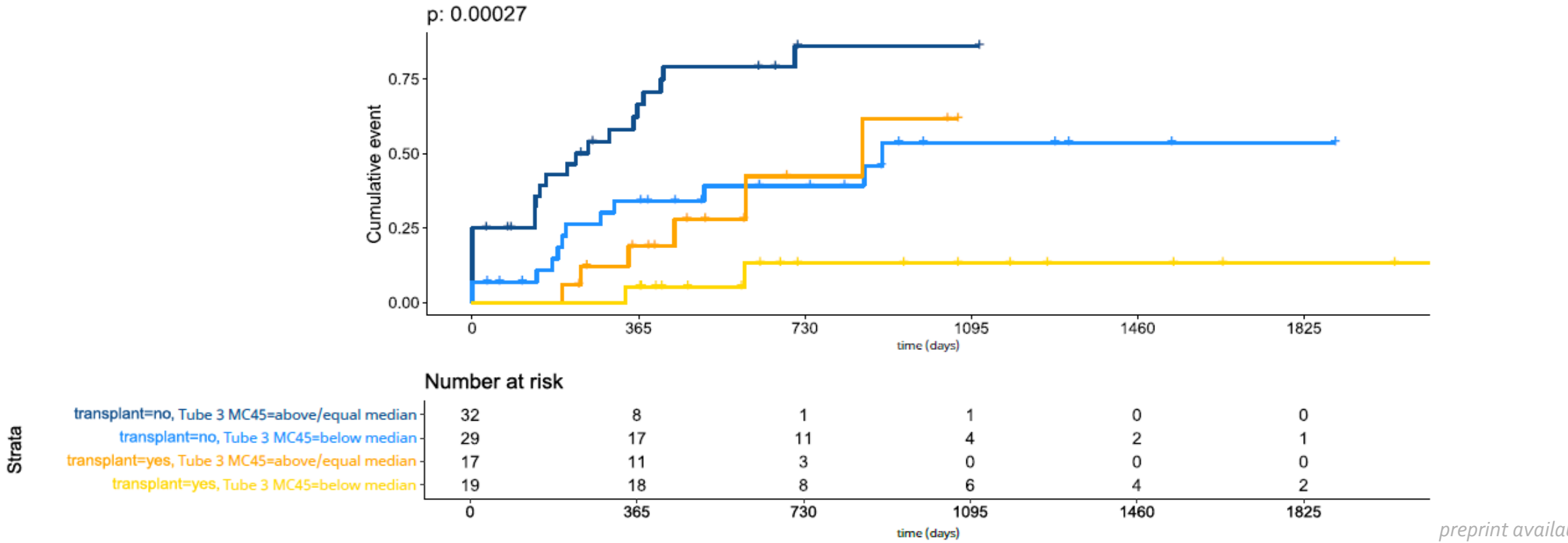


preprint available

Prediction of outcome

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





preprint available

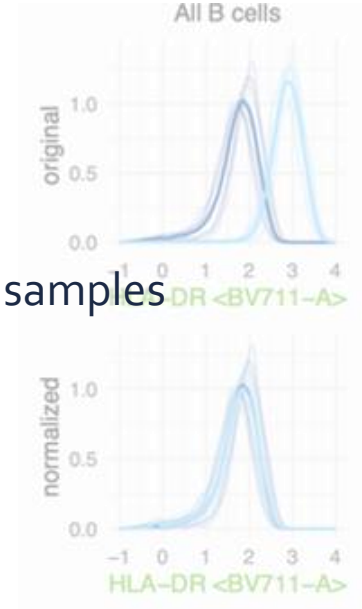
Improved diagnosis of primary immunodeficiences using explainable AI

Emmaneel A et al (2023) *manuscript in preparation*

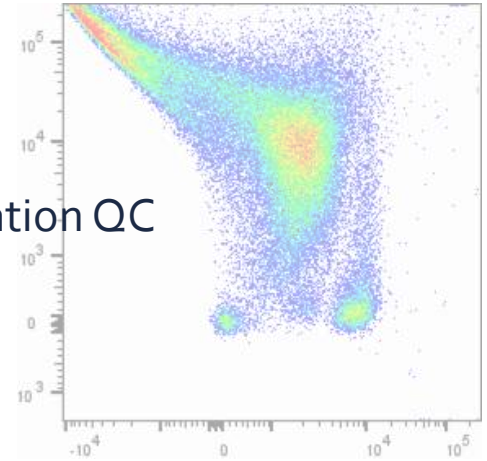
unpublished data (manuscript in preparation)

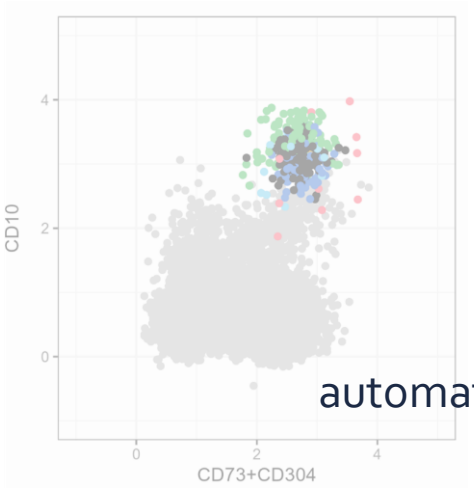
And more…

CytoNorm 2.0
normalization without control samples
manuscript submitted



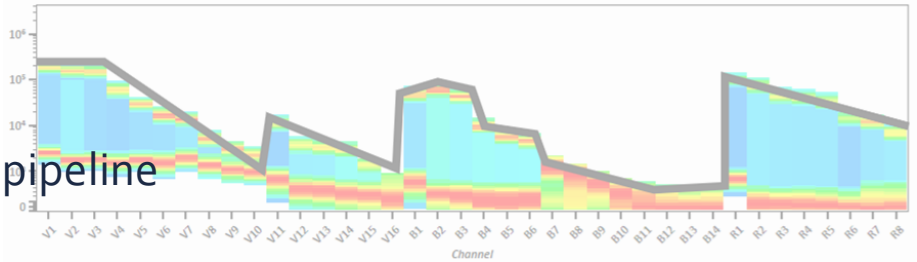
compensation QC





automated MRD detection

spectral flow
preprocessing pipeline



Acknowledgements

VIB

GHENT UNIVERSITY

@saeyslab

✉ SarahM.Bonte@UGent.be