

Flow Cytometry Bioinformatics Service

FACS Sorting Core Unit
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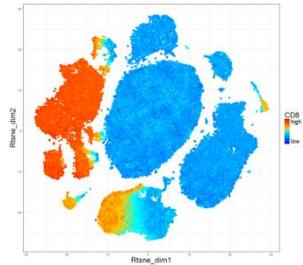
Flow Cytometry Bioinformatics Services:

1. Analysis of data (Excel format) generated after classical manual analysis (t-SNE, heatmaps)
 2. t-SNE analysis of FCS files
 3. Prospective analysis of FCS files using SPADE
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t-SNE and SPADE

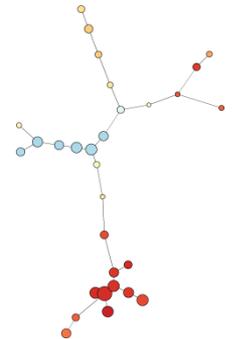
▶ t-SNE:

- t-Distributed Stochastic Neighbor Embedding
- dimensionality reduction algorithm, mapping all dimensions of the original data to two dimensions, resulting in an intelligible representation for the human eye.



▶ SPADE:

- Spanning-tree Progression Analysis of Density-normalized Events
- Used for finding clusters in multidimensional FCS data and assigning cells to clusters based on similarity.
 - Building of SPADE population trees by connecting clusters by edges.



1.

Analysis of data (Excel format)
generated after classical manual
analysis (t-SNE, heatmaps)



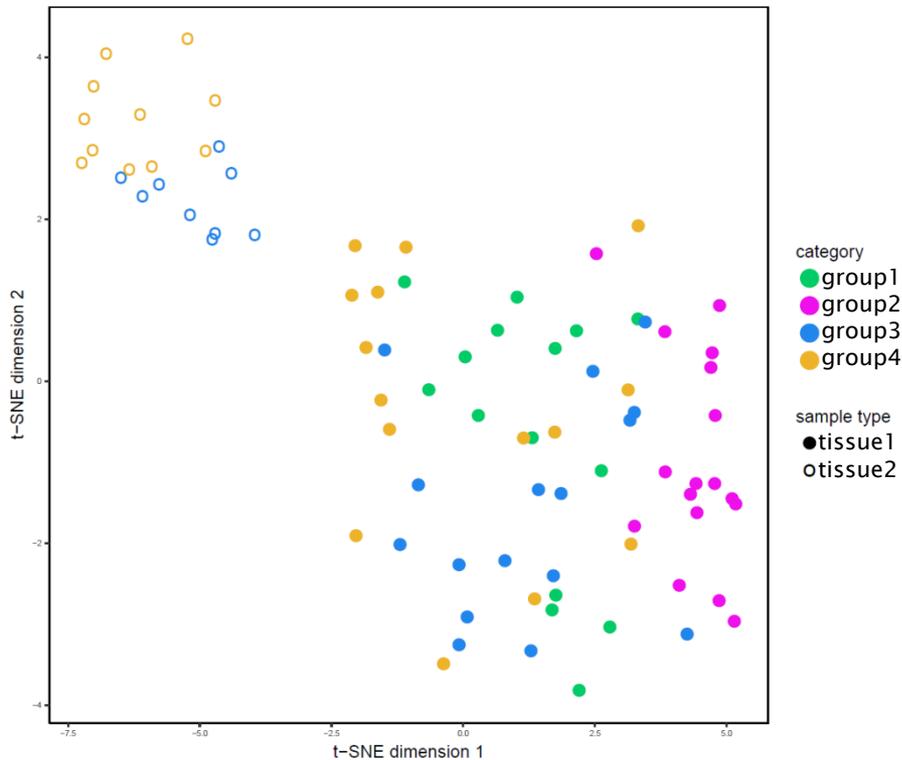
Analysis of data (Excel format) generated after classical manual analysis (t-SNE, heatmaps)

- ▶ Large Excel tables generated after manual gating and data export

	A	B	C	D	E	F	...
1	sample ID	TRX_CD4+_FqCD3	TRX_CD4conv_FqCD3	TRX_Tregs_FqCD3	TRX_CD8+_FqCD3	TRX_DN_FqCD3	...
2	sample1	77.81	73.23	4.41	19.03	2.6	
3	sample2	70.85	67.03	3.72	25.86	2.9	
4	sample3	71.08	66.46	4.50	25.33	3.3	
5	sample4	70.41	64.57	5.73	24.27	4.9	
6	sample5	74.01	67.05	6.89	23.26	2.7	
7	sample6	66.28	62.28	3.84	28.65	4.3	
8	sample7	63.13	59.10	3.74	31.41	3.3	
9	sample8	76.74	72.20	4.39	18.21	3.7	
10	sample9	73.86	69.08	4.66	22.64	1.7	
11	sample10	78.69	72.05	6.74	20.55	0.9	
12	sample11	70.92	65.26	5.55	24.15	2.8	
13	sample12	77.31	72.99	4.17	19.60	2.3	
14	sample13	77.39	73.08	4.26	17.88	4.0	
15	sample14	65.25	61.55	2.65	27.90	2.7	
16	sample15	63.09	58.49	4.24	32.05	2.6	
17	sample16	76.05	71.93	3.60	20.95	1.4	
18	sample17	70.80	64.46	5.86	25.37	1.6	

...

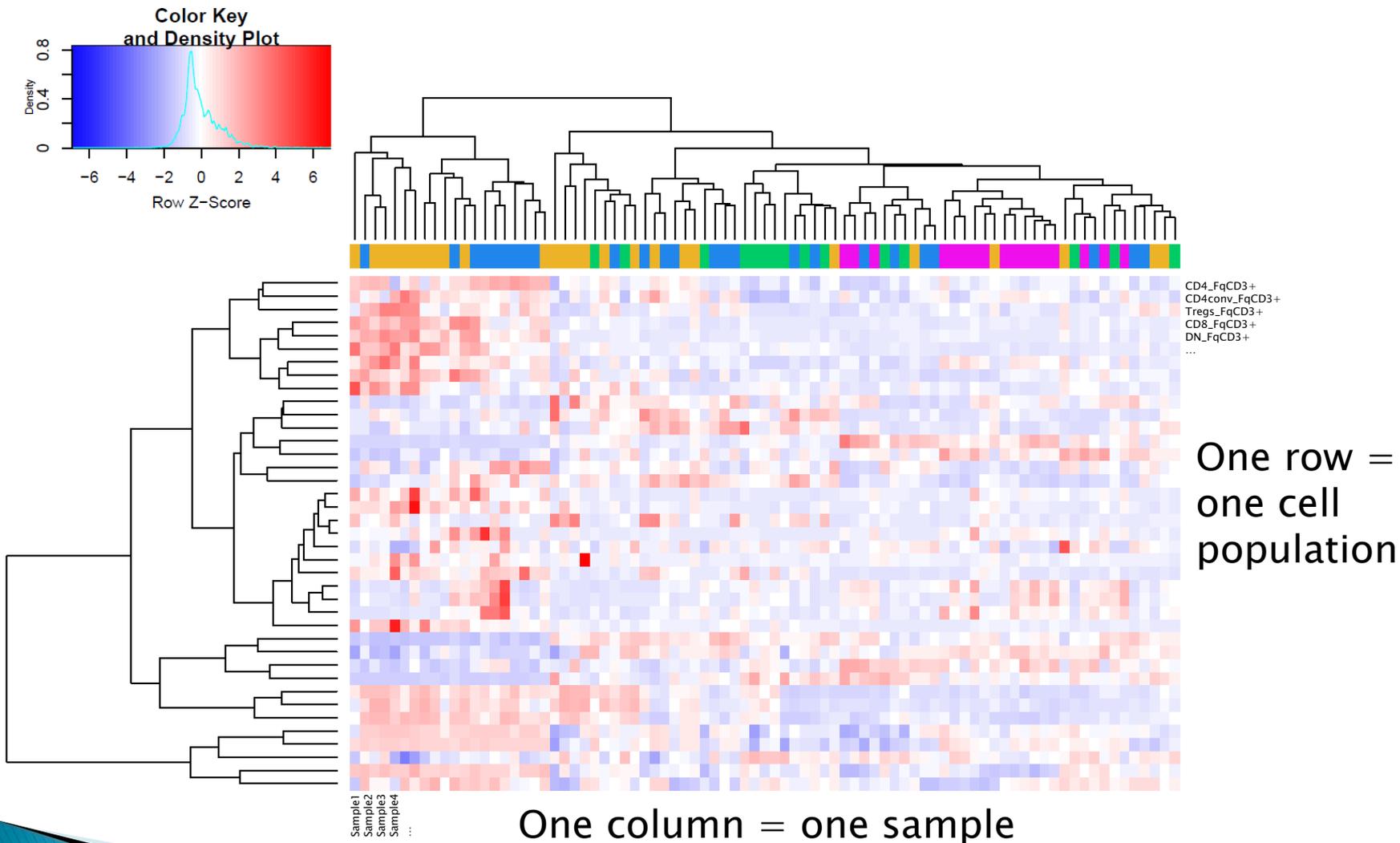
Analysis of data generated after classical manual analysis (t-SNE)



Mohme et al., 2018, Immunophenotyping of Newly Diagnosed and Recurrent Glioblastoma Defines Distinct Immune Exhaustion Profiles in Peripheral and Tumor-infiltrating Lymphocytes, Clin Cancer Res, DOI: 10.1158/1078-0432.CCR-17-2617

- One dot = one sample
- The closer two dots are, the more similar they are in all dimensions/subpopulations.
- Different color and shape codes are possible, based on tissue origin, sample conditions, clinical traits...
- Data from several different panels can be analyzed jointly

Analysis of data generated after classical manual analysis (heatmap)



1. Analysis of data (Excel format) generated after classical manual analysis (t-SNE, heatmaps)

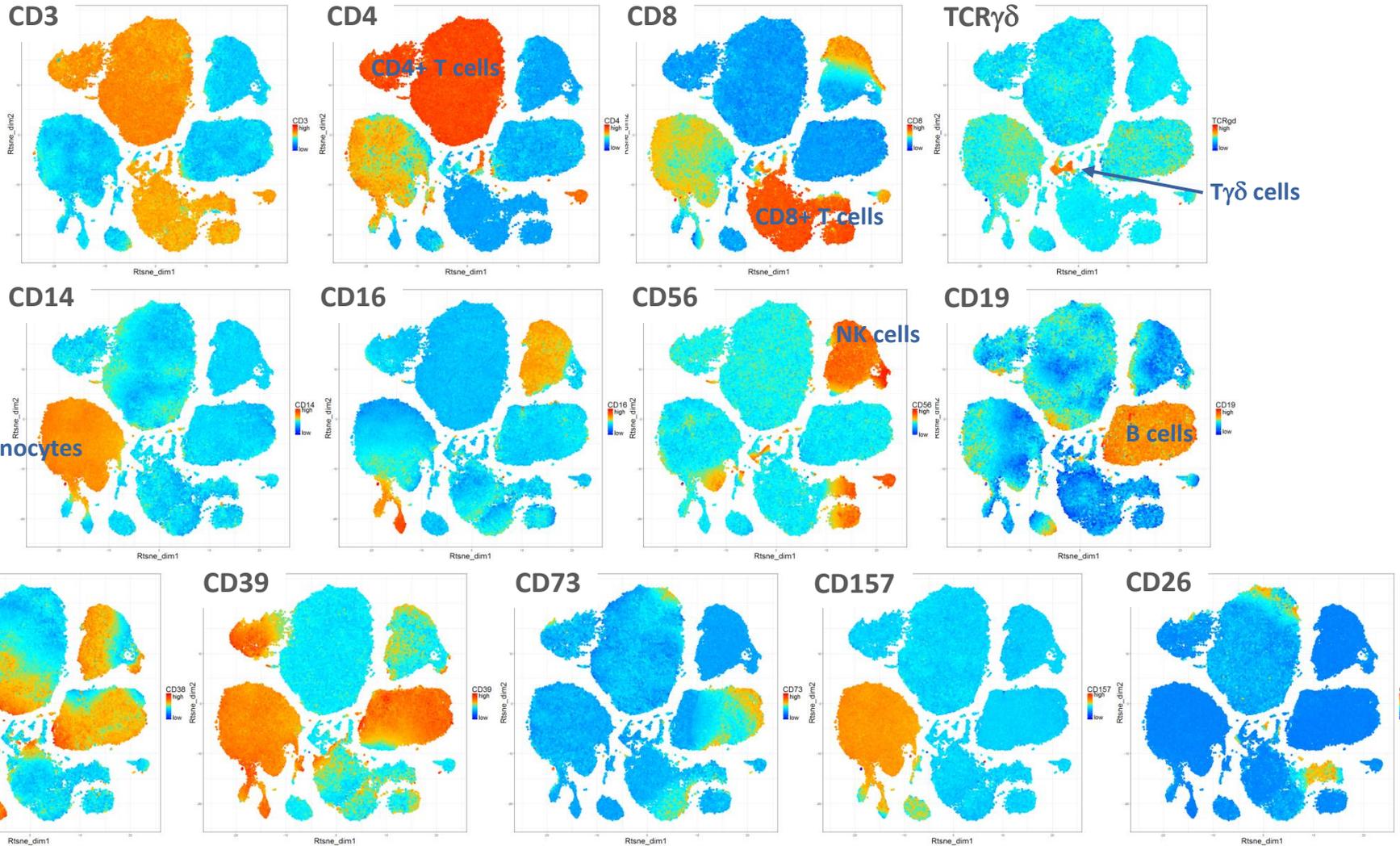
- ▶ **Data used:**
 - (Large) Excel tables which can be derived from different experimental methods, including clinical data.
- ▶ **Data requirements:**
 - R-compatible format for row/column titles.
 - Complete dataset (no missing values) or at least the possibility to exclude columns or rows containing missing values, resulting in a complete dataset.
- ▶ **Possibilities:**
 - Grouping of columns into subsets (selection of populations across panels).
- ▶ **Outcome:**
 - t-SNE dimensionality reduced dot plot showing groups of samples which are similar, color coded by available factors.
 - Heatmap showing which columns make most of the differences.

2. t-SNE analysis of FCS files

t-SNE analysis of FCS files

- ▶ Possibilities:
 - Analysis of a single FCS file for data exploration
 - Analysis of groups of samples for comparison
- ▶ Great for visualization, but not for quantification or finding statistically significant differences.

Data exploration – Expression of ectoenzymes in PBMCs



The closer two dots(= two cells) are, the more similar they are in all dimensions/markers.

Comparison of treatment groups – neonatal thymus

CD3

CD4

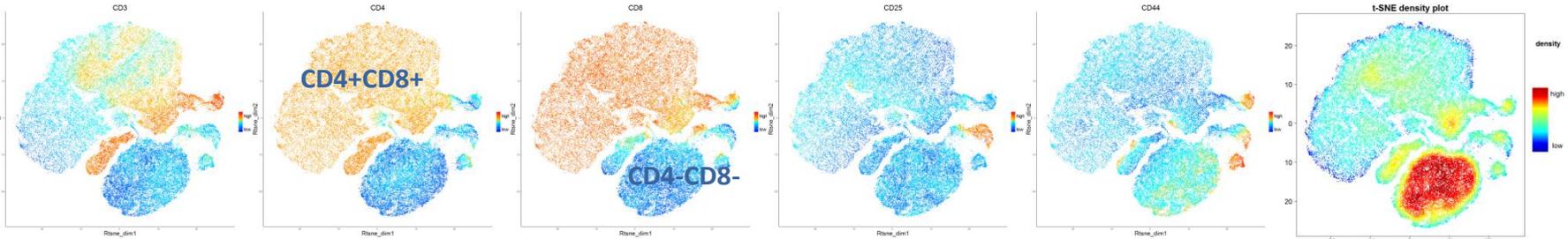
CD8

CD25

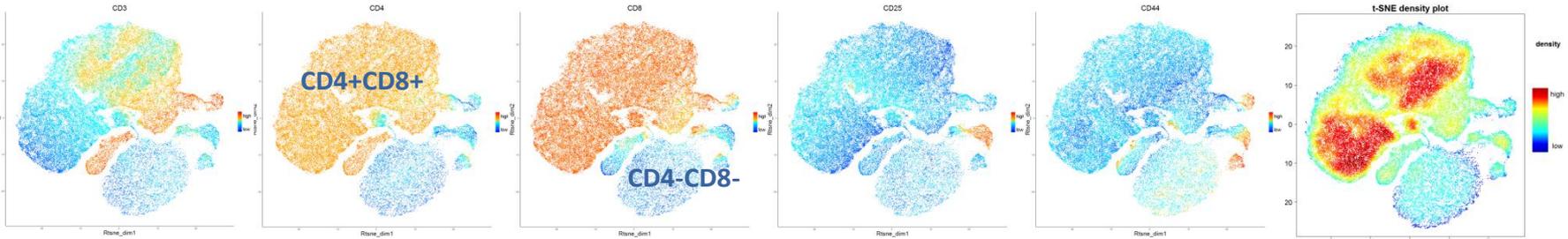
CD44

density

Betamethasone



PBS



2. tSNE analysis of FCS files

- ▶ **Data used:**
 - FCS files
- ▶ **Data requirements:**
 - FCS files have to be perfectly compensated and cleaned (containing only the cell population of interest).
- ▶ **Possibilities:**
 - Single sample analysis for data/panel exploration.
 - Comparison of multiple conditions/sample groups.
 - Only the markers of interest have to be included in the analysis.
- ▶ **Outcome:**
 - t-SNE dot plots showing clusters of cells with similar phenotypes.
 - One t-SNE plot color coded for each marker.
 - Density dot plot to detect differences between groups.
 - Possibility to append t-SNE channels to FCS files.

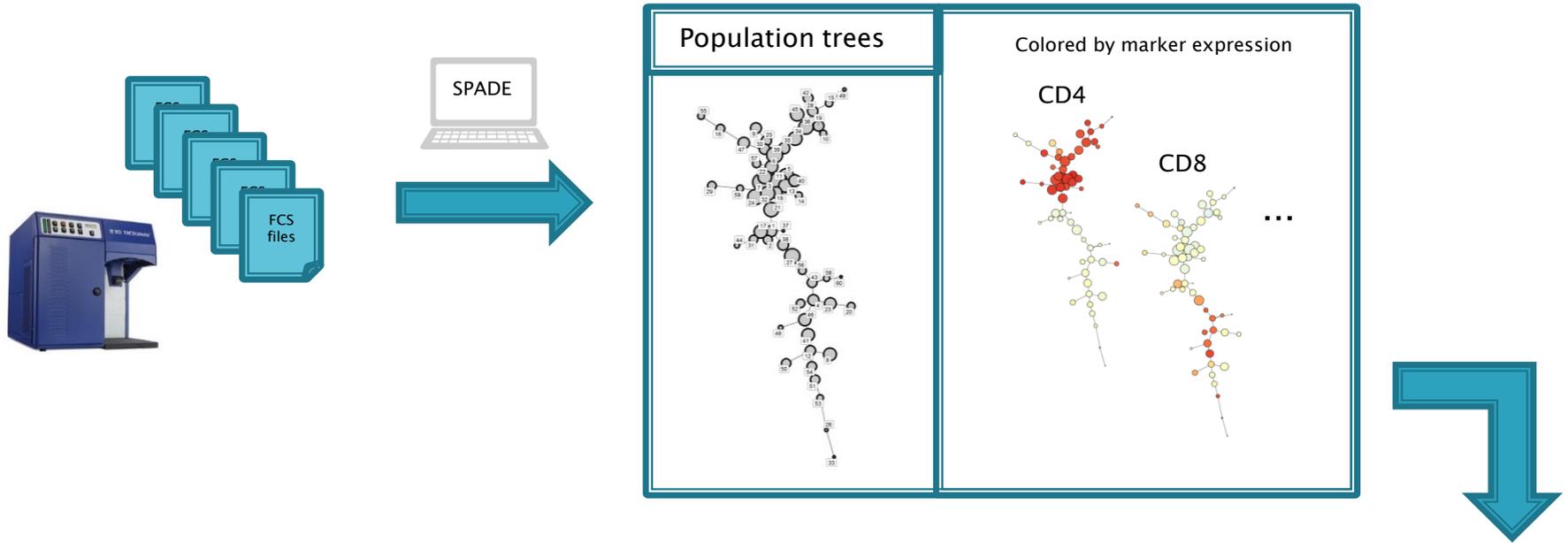
3. Prospective analysis of FCS files using SPADE



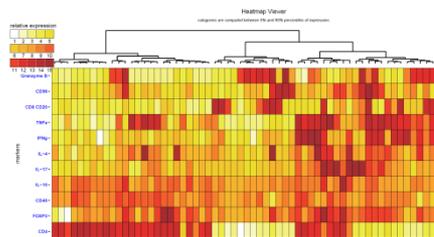
Prospective analysis of FCS files using SPADE

- ▶ No manual gating required → objective analysis
- ▶ Unsupervised
- ▶ Used for comparison of two or more groups of samples
- ▶ Only feasible if:
 - Samples are stained with the same antibody cocktail
 - FCS data was acquired within a short period of time
 - FCS data was acquired at the same cytometer

Prospective analysis of FCS files using SPADE

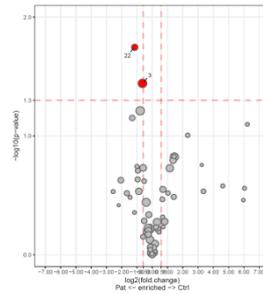


Statistical analysis and identification of the DAC



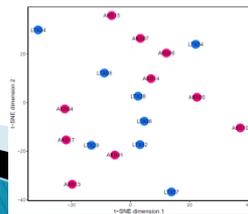
Heatmap showing cluster phenotypes

Differentially abundant clusters (DAC)

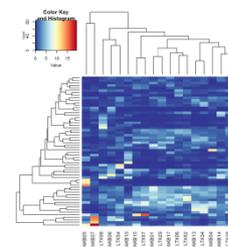


H-SNE
group: ● Cl ● Pe

Cluster sizes

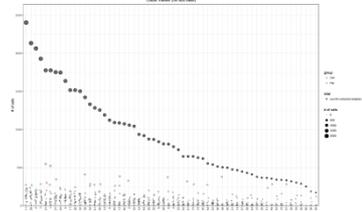


Quality control



Cluster compositions

Cluster sizes



Prospective analysis of FCS files using SPADE

- ▶ **Data used:**
 - FCS files
- ▶ **Data requirements:**
 - FCS files have to be perfectly compensated and cleaned (containing only the cell population of interest).
- ▶ **Possibilities:**
 - Comparison of multiple conditions/sample groups.
 - Only the markers of interest have to be included in the analysis.
- ▶ **Outcome:**
 - A collection of plots describing the population tree clusters in phenotype and metadata (like size, composition,...).
 - Statistically significant differences between conditions (differentially abundant clusters, DACs).