Journées Pratiques

Analyses non supervisées en cytométrie

Du 5 au 7 Février 2020
Sophia-Antipolis

Présidées par :

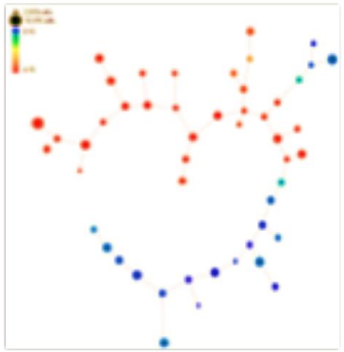
Jonathan M. IRISH

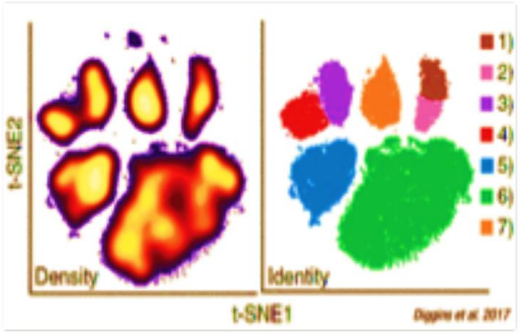
Mass Cytometry Center of Excellence (MCCE), Vanderbilt University, Nasheville, (USA)

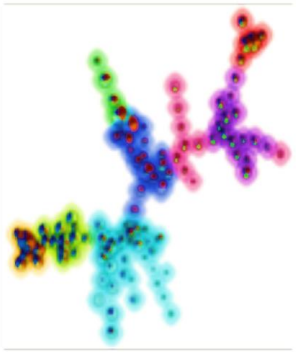
Co-animées par :

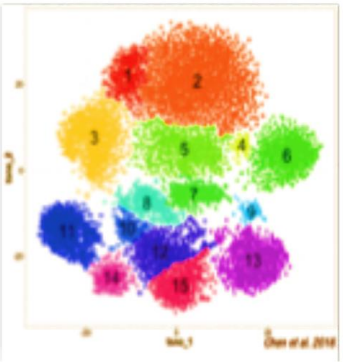
Aïda Meghraoui-Kheddar (IPMC, CNRS Valbonne) et **Samuel Granjeaud** (CRCM, INSERM Marseille)

Julie Cazareth (IPMC, CNRS Valbonne) et **Sierra Barone** (Vanderbilt University, Nashville USA)







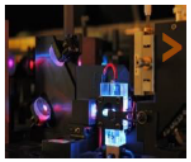


Formation
Provence-Alpes-Côte d'Azur et Corse

Inserm

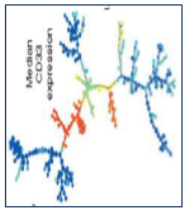
Passeport cytométrie Marseille

Édition 2020



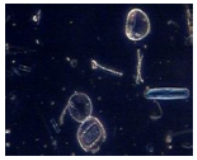
Cytométrie multiparamétrique avancée : théorie & pratique

du 24 au 27 mars 2020



Outils d'analyse de données avancées en cytométrie de flux et de masse : théorie & pratique

du 9 au 12 juin 2020



Cytométrie en flux : du photon à la cellule : théorie & pratique

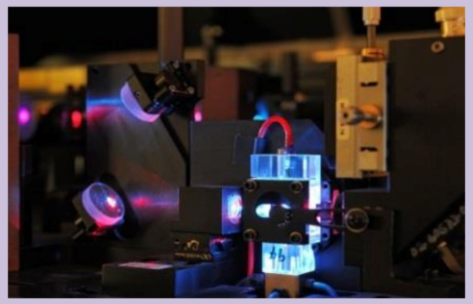
novembre 2020

Formation en partenariat avec le CNRS Délégation Provence et Corse

Formation continue
Provence-Alpes-Côte d'Azur et Corse

Inserm

Passeport cytométrie Marseille



Cytométrie multiparamétrique avancée

Du 24 au 27 mars 2020, à Marseille

Public

Tout public intéressé par l'application de la cytométrie à son champ expérimental et désireux d'augmenter le nombre de paramètres étudiés simultanément sur ses cellules d'intérêt

Prérequis

Connaissances de base en cytométrie de flux conventionnelle (4-6 paramètres)

Objectif

Mieux appréhender les approches multiparamétriques en cytométrie (Flux, Masse et Spectrale)

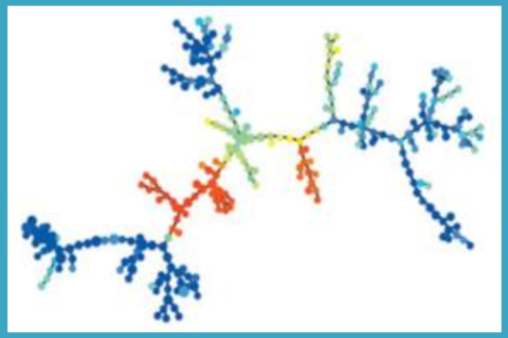
Programme

- Rappels théoriques sur la cytométrie en flux multiparamétrique
- Réglages, optimisation et standardisation des cytomètres
- Notions sur les microparticules : optimisation des paramètres d'acquisitions
- Mise au point de panel à façon, marquage 15 couleurs
- Acquisition sur BD LSR2 et Fortessa
- Initiation à la cytométrie de Masse sur Helios
- Initiation à la cytométrie Spectrale sur Cytek Aurora
- Notions de signalisation intracellulaire

Formation continue
Provence-Alpes-Côte d'Azur et Corse

Inserm

Passeport cytométrie Marseille



Outils d'analyse de données avancées en cytométrie de flux et de masse - Théorie & pratique

Du 9 au 12 juin 2020, Marseille

Public

Chercheurs et ingénieurs effectuant des analyses en cytométrie multiparamétrique.
Cette offre de formation s'adresse aux biologistes de préférence.
Prérequis : notions d'anglais

Objectifs

- Connaître les outils actuels d'analyse de données avancées, les mettre en œuvre sur des jeux de données tests afin d'apprendre à les maîtriser
- Permettre aux participants d'identifier la méthode d'analyse de choix appropriée pour une question définie et extraire le maximum d'informations à partir d'un set de données
- Présenter des solutions logicielles simples permettant de visualiser et de synthétiser les résultats autrement qu'en histogrammes ou en cytogrammes bivariants classiques
- Réaliser des analyses intégratives de données issues aussi bien de plusieurs analyses complexes en cytométrie de flux que d'autres types de tests (multiplex immuno-assay, formule sanguine…)

Lieu : Délégation régionale Inserm - 13009 Marseille

Participants : 8 personnes

Cytofkit – Cytofast Analysis

Journées Pratiques
Analyse non supervisées
Sophia 2020

Cytofkit – Cytofast Analysis

- http://i-cyto.github.io

- cytofkit
  - Cytofkit: A Bioconductor Package for an Integrated Mass Cytometry Data Analysis Pipeline

- Cytofast
  - Cytofast: A workflow for visual and quantitative analysis of flow and mass cytometry data to discover immune signatures and correlations

Outline

- Question: Find a group of cells that differ in abundance between two groups of patients


- Discover cytofkit
- Run calculations
- Use graphical interface to view results
- Use a cytofast script to get nicer figures

Installation

- Open R/RStudio
- Do copy/paste/run commands **line by line**
- Whenever you get a message 'Update all/some/none? [a/s/n]:', answer **n**

```
if (!requireNamespace("BiocManager", quietly = TRUE))
        install.packages("BiocManager")
BiocManager::install("flowCore")
BiocManager::install("uwot")
BiocManager::install("remotes")
BiocManager::install("i-cyto/cytofkit")
BiocManager::install("i-cyto/cytofast")
```

Run calculations with cytofkit

```
## cytofkit analysis

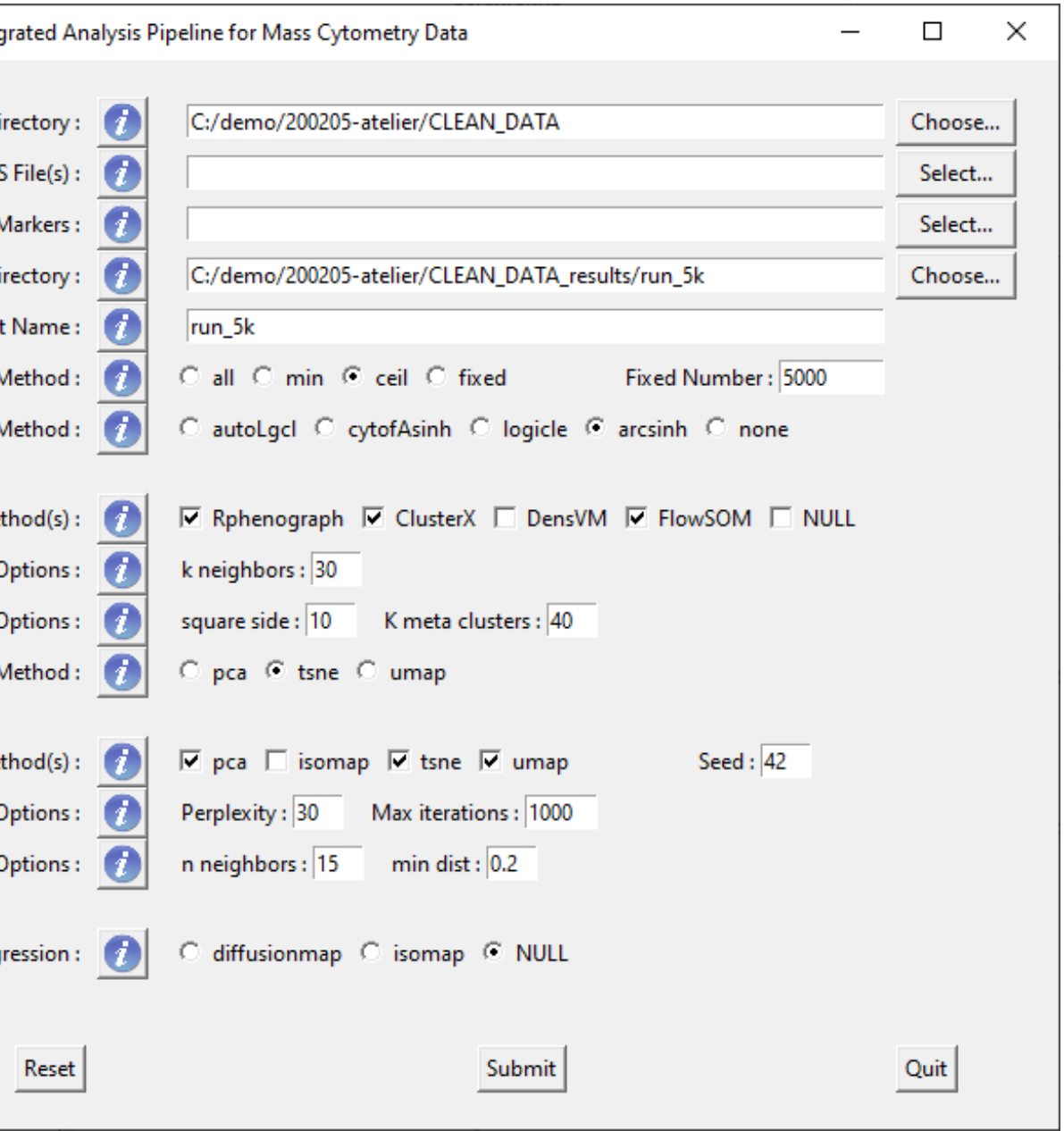

Run by hand

```{r}
library(cytofkit)
# Launch the Graphical User Interface for tuning the run
cytofkit_GUI()
# Note the path to the result file

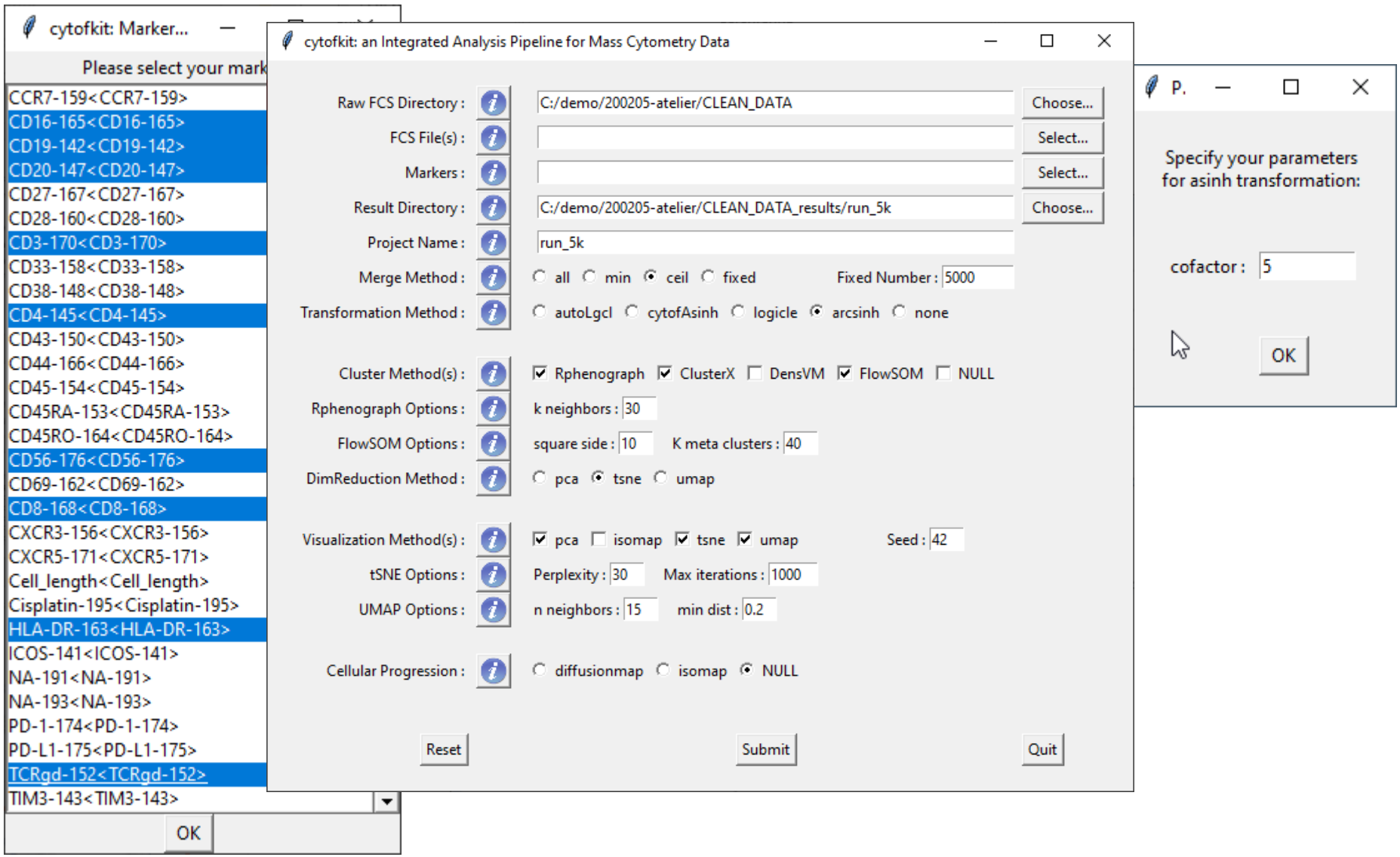
# Launch the Shiny interface to view and annotate the analysis
cytofkitShinyAPP()
```


```{r}
# Launch the Shiny interface using a defined path
analysis_file = "E:/demo/200205-atelier/CLEAN_DATA_results/run_5k/run_5k.RData"
if (file.exists(analysis_file))
  cytofkitShinyAPP(analysis_file)
```
```

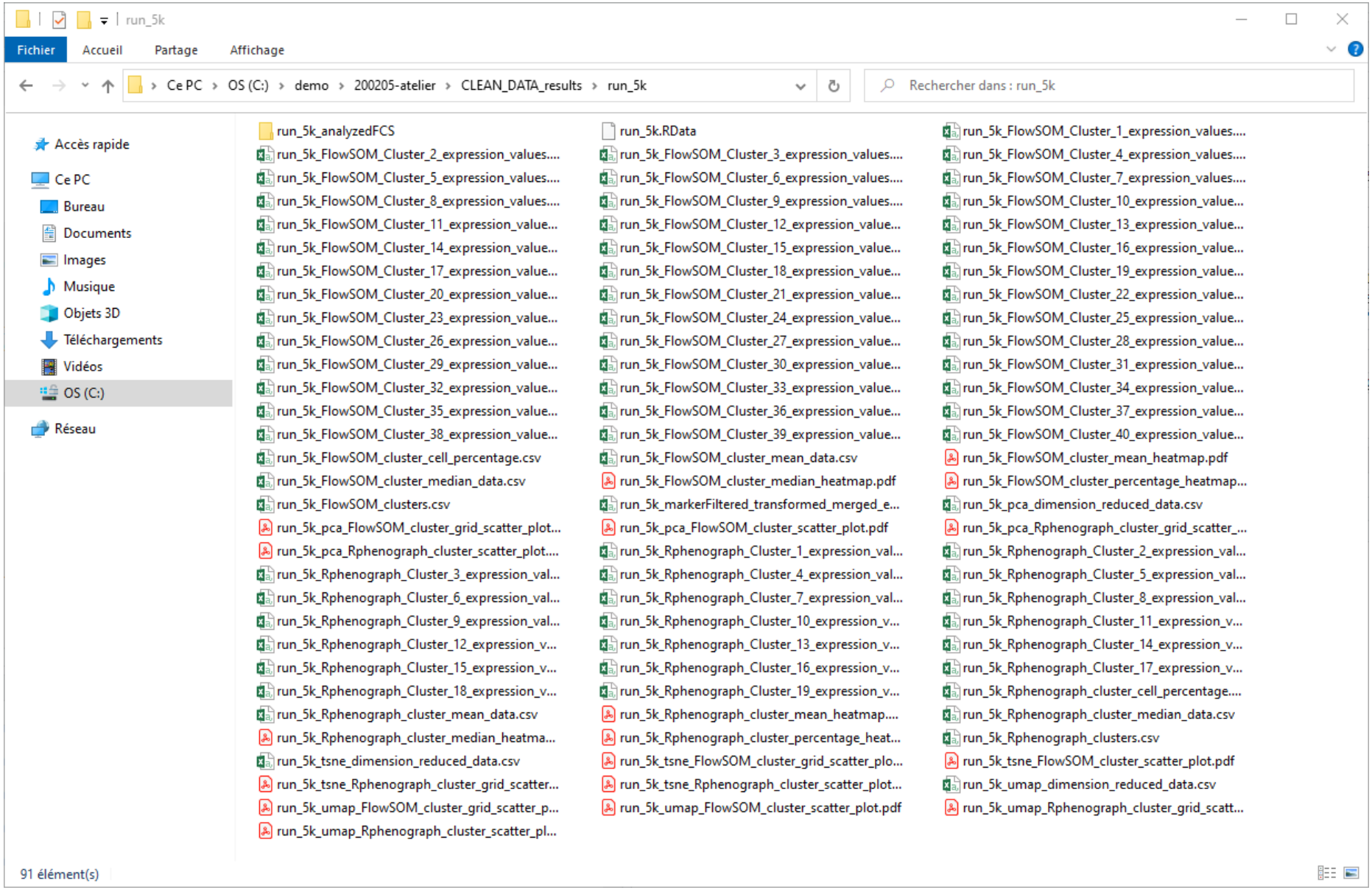
Calculations GUI



Calculations GUI



Results of calculations



Start analysis interface

```
## cytofkit analysis

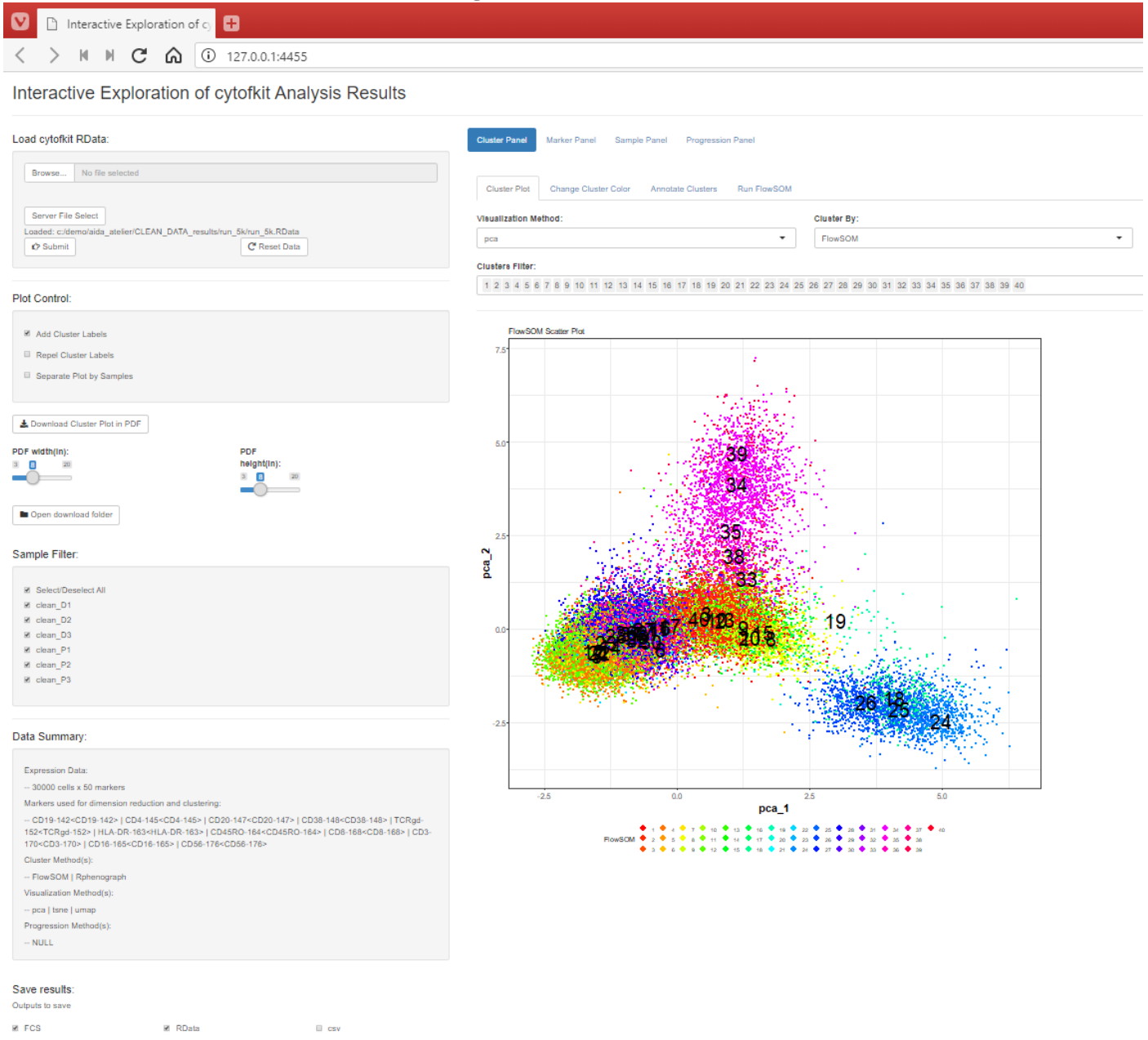

Run by hand

```{r}
library(cytofkit)
# Launch the Graphical User Interface for tuning the run
cytofkit_GUI()
# Note the path to the result file

# Launch the Shiny interface to view and annotate the analysis
cytofkitShinyAPP()
```


```{r}
# Launch the Shiny interface using a defined path
analysis_file = "E:/demo/200205-atelier/CLEAN_DATA_results/run_5k/run_5k.RData"
if (file.exists(analysis_file))
  cytofkitShinyAPP(analysis_file)
```
```

Shiny interface



Load cytofkit RData:

Browse... No file selected

Server File Select

Loaded:
c:/demo/aida_atelier/CLEAN_DATA_results/run_5k/run_5k.RData

Submit Reset Data

Plot Control:

- [x] Add Cluster Labels
- [ ] Repel Cluster Labels
- [ ] Separate Plot by Samples

Download Cluster Plot in PDF

PDF width(in): 3 8 20

PDF height(in): 3 8 20

Open download folder

Sample Filter:

- [x] Select/Deselect All
- [x] clean_D1
- [x] clean_D2
- [x] clean_D3
- [x] clean_P1
- [x] clean_P2
- [x] clean_P3

Data Summary:

Save results:

Outputs to save

- [x] FCS
- [x] RData
- [ ] csv

Save Data

Cluster Panel | Marker Panel | Sample Panel | Progression Panel

Cluster Plot | Change Cluster Color | Annotate Clusters | Run FlowSOM

Visualization Method: pca

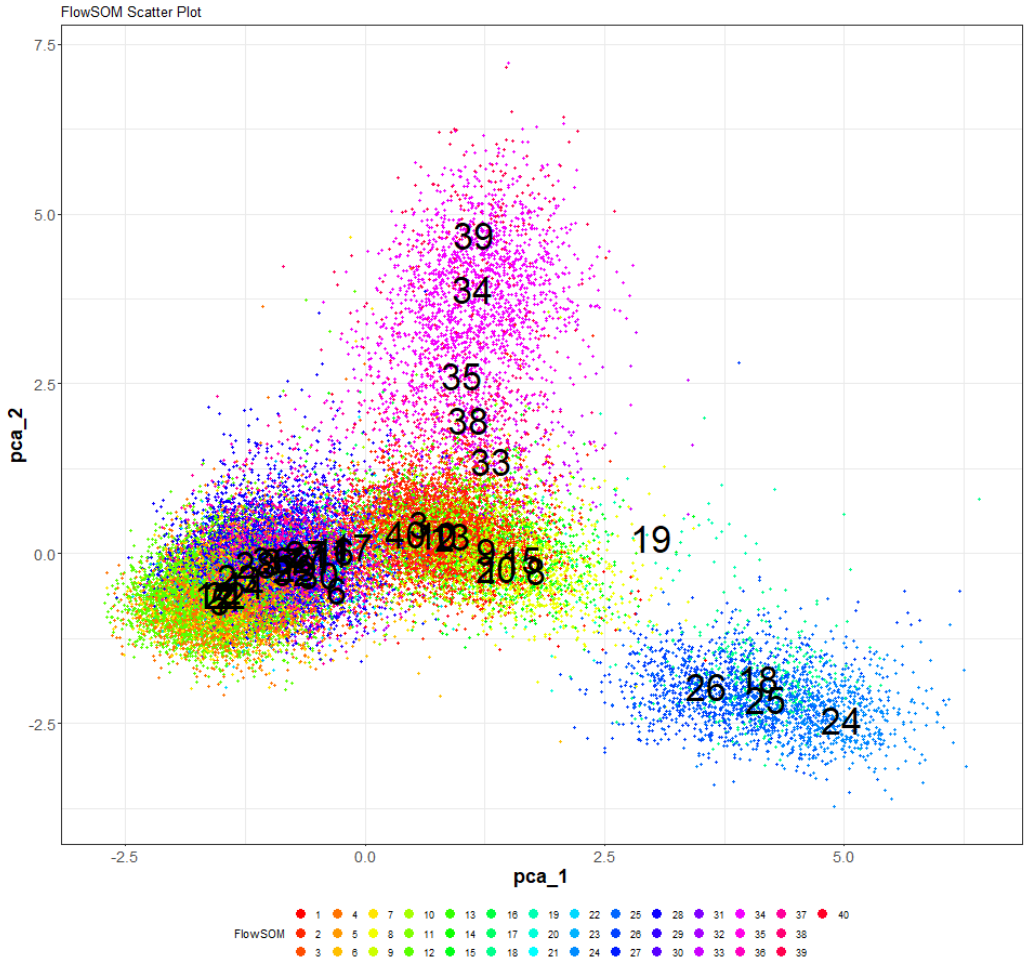
Cluster By: FlowSOM

Point Size: 1

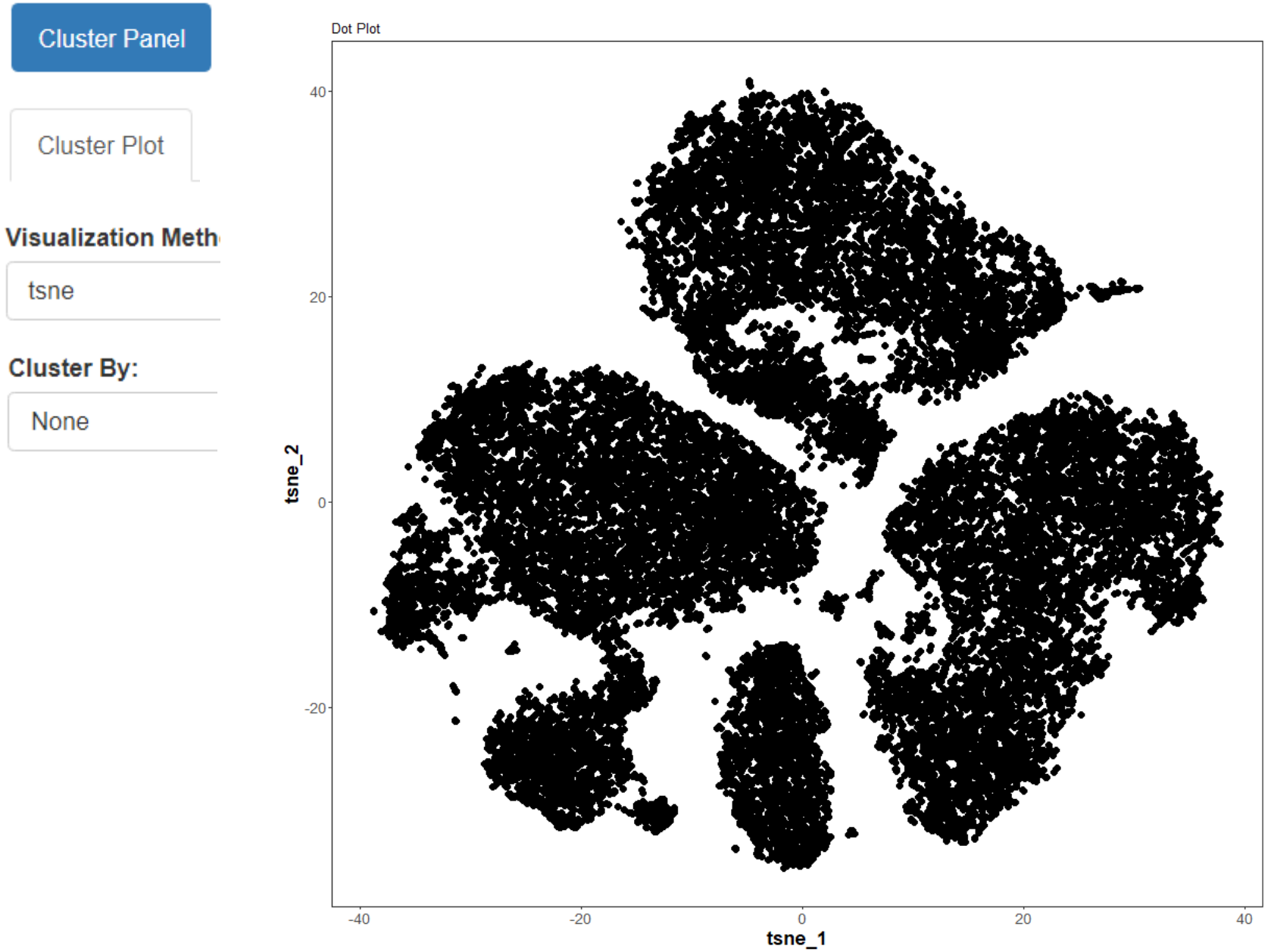
Label Size: 12

Clusters Filter:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40



tSNE reduction into 2D



Cell density on tSNE map

Cluster Panel

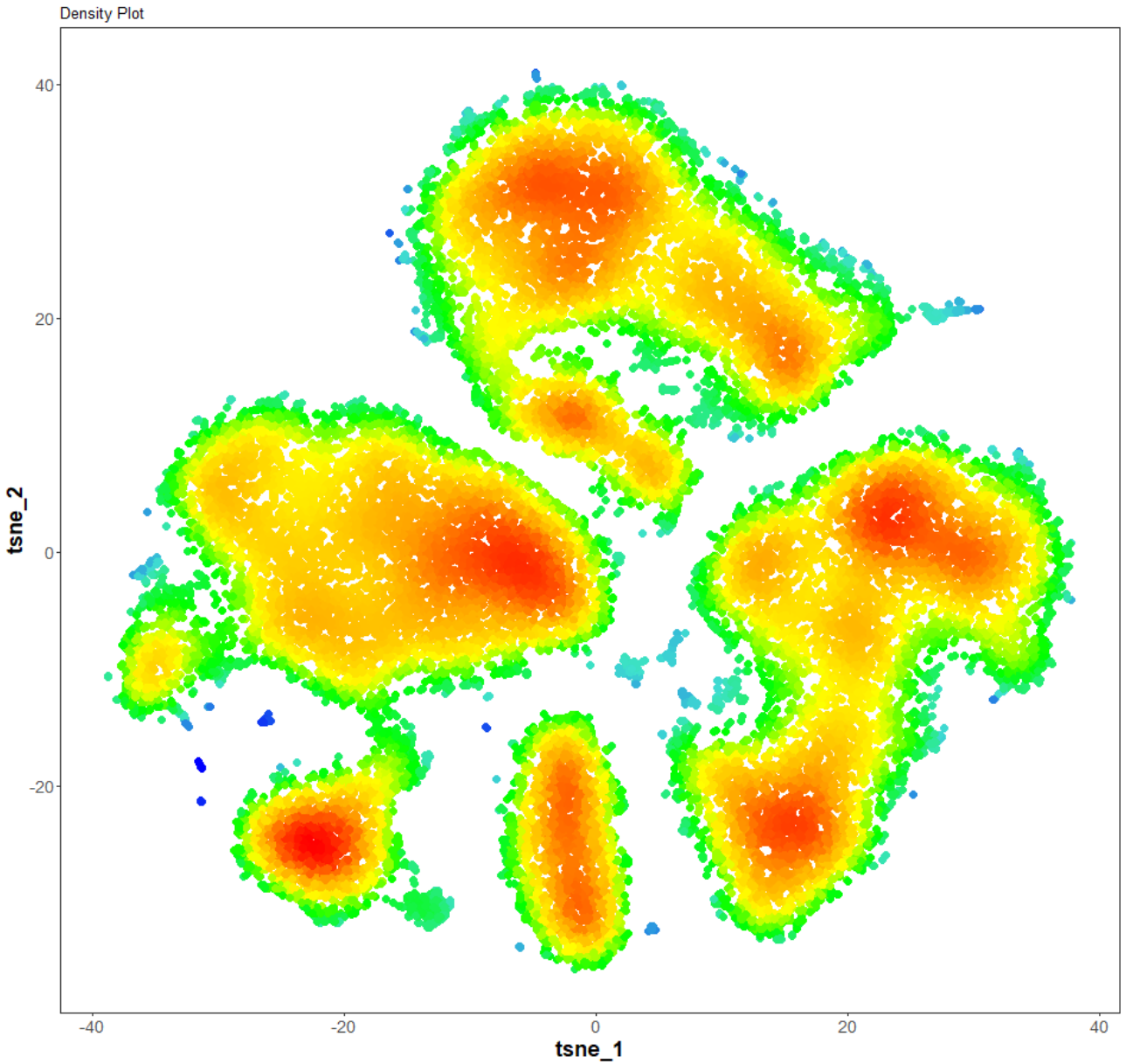
Cluster Plot

Visualization Meth

tsne

Cluster By:

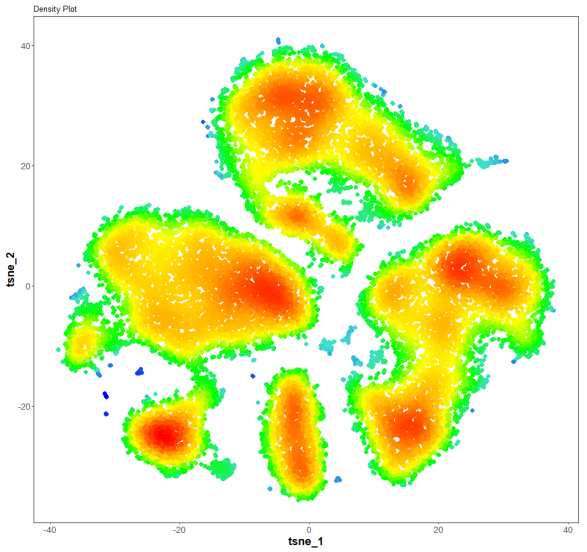
Density



Quizz

- How to make sense of the aggregates?

- Without clustering yet!



Markers on tSNE

Marker Panel

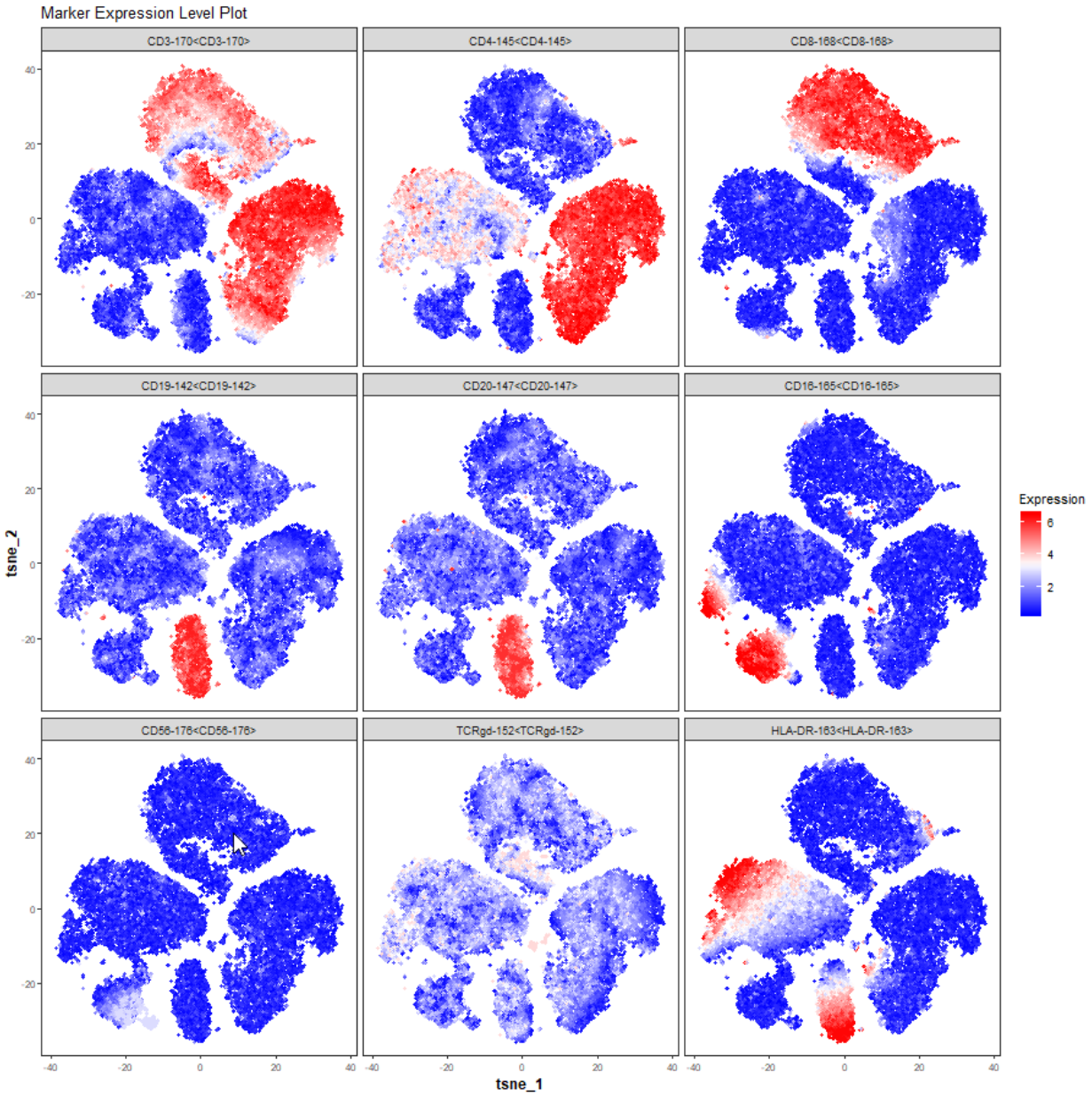
Expression Level Plot

Plot Marker:

- CD3-170<CD3-170>
- CD4-145<CD4-145>
- CD8-168<CD8-168>
- CD19-142<CD19-142>
- CD20-147<CD20-147>
- CD16-165<CD16-165>
- CD56-176<CD56-176>
- TCRgd-152<TCRgd-152>
- HLA-DR-163<HLA-DR-163>

All Markers

Update Plot



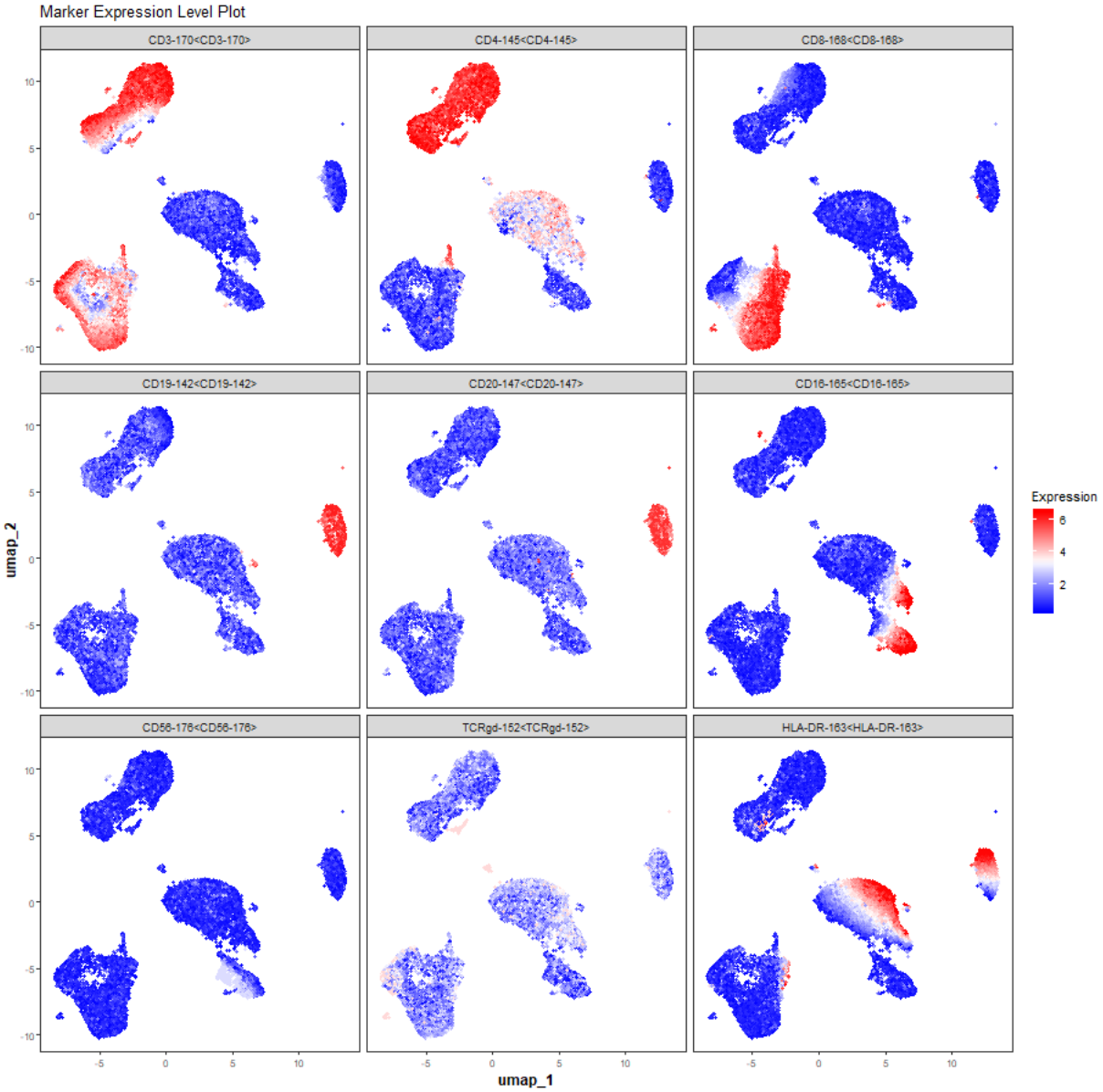
Markers on UMAP

Cluster Panel | Marker Panel | Samp

Expression Heat Map | Expression Le

Visualization Method:

umap



What about clusterings?

- FlowSOM
- Phenograph
- ClusterX

- By definition, in cytofkit, what is the difference of ClusterX vs FlowSOM & Phenograph?

- Let’s overlay FlowSOM on top of tSNE

Clusters on tSNE

Cluster Panel

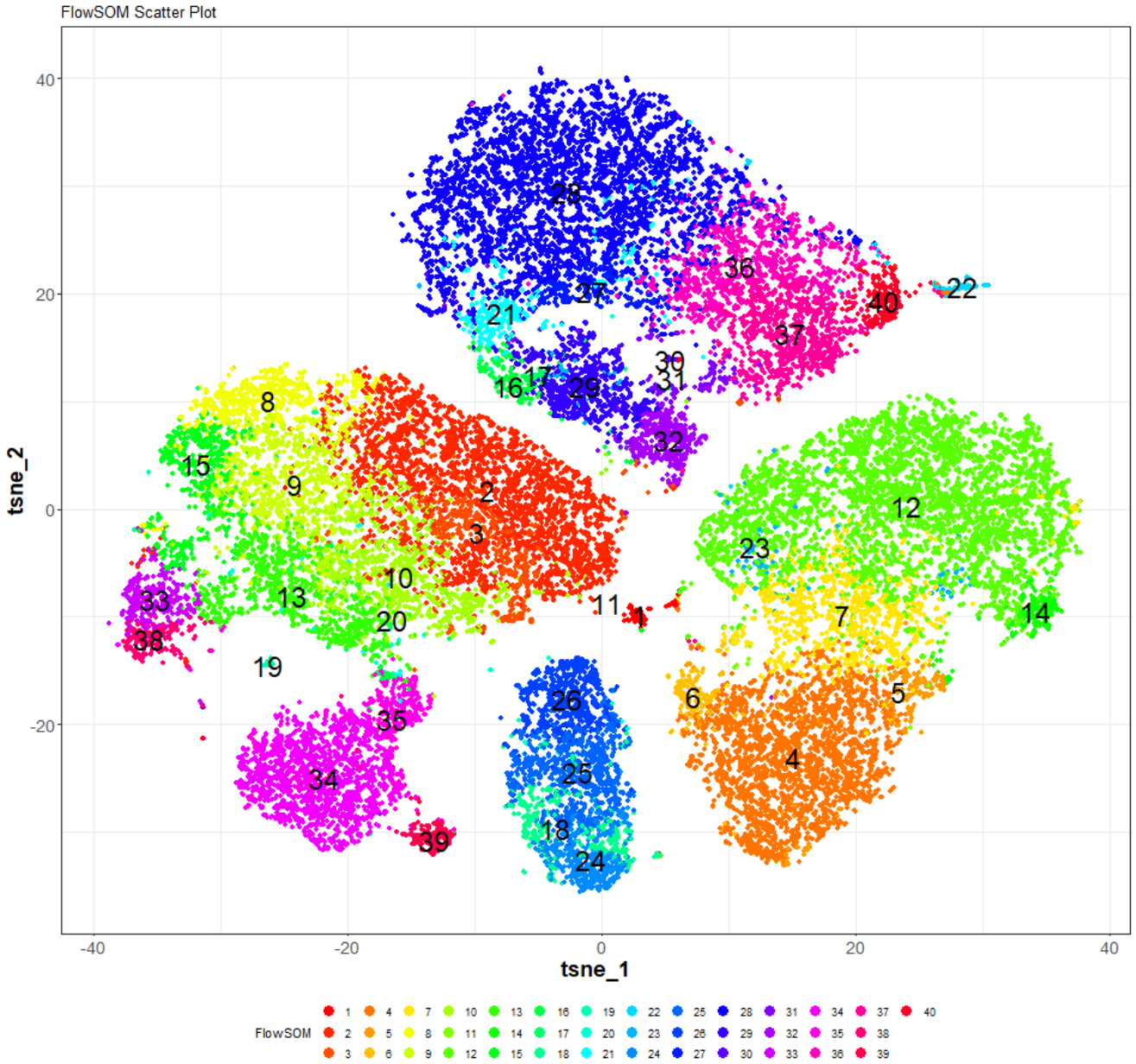
Cluster Plot

Visualization Meth

tsne

Cluster By:

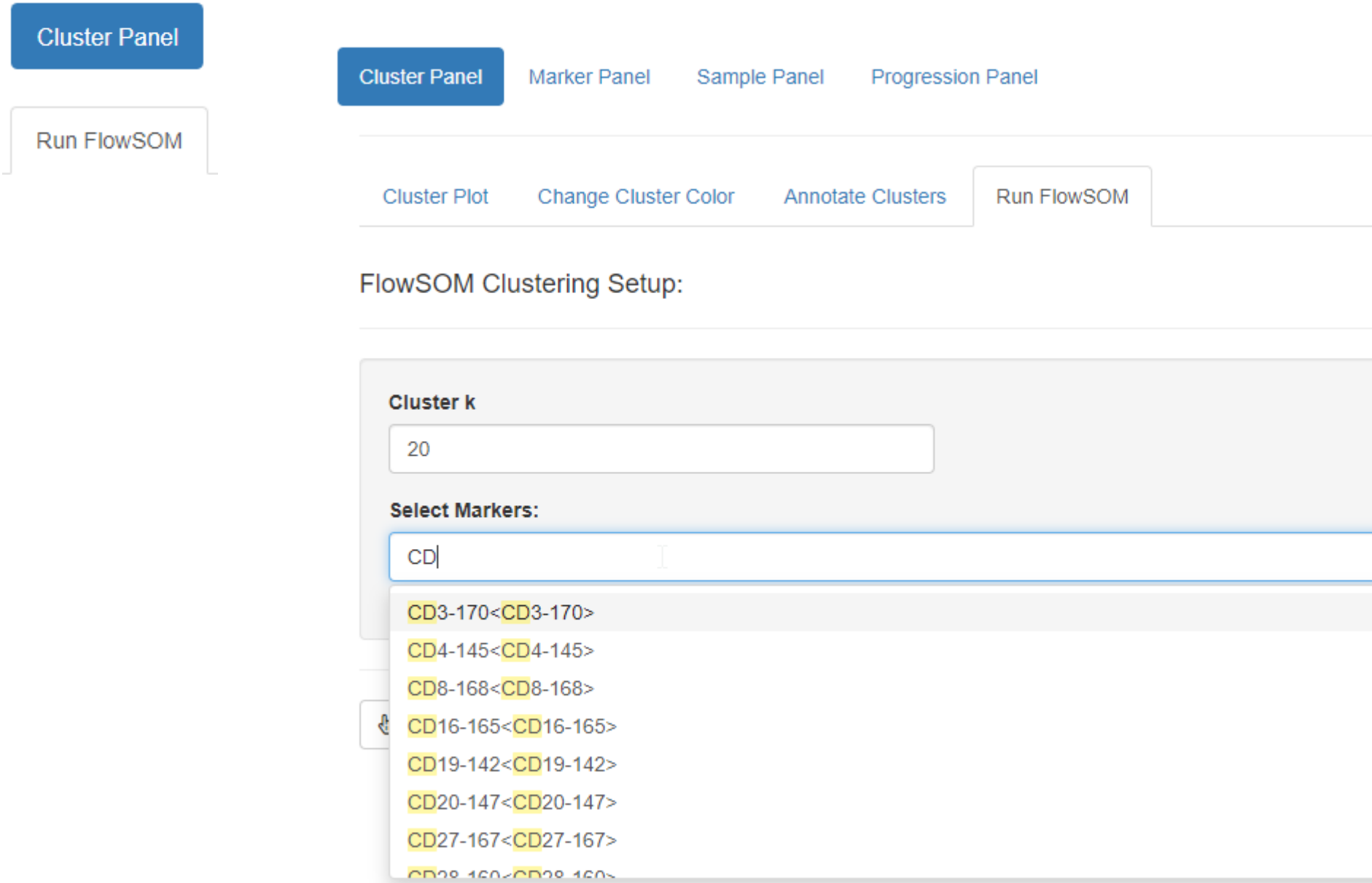
FlowSOM



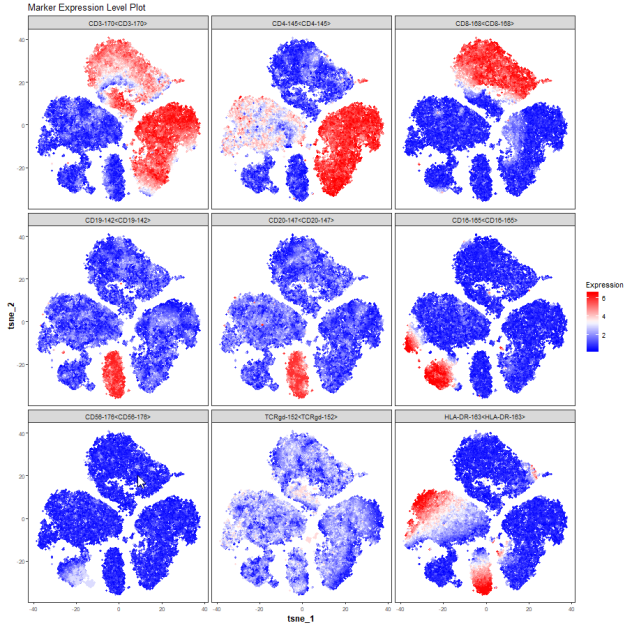
Hands on

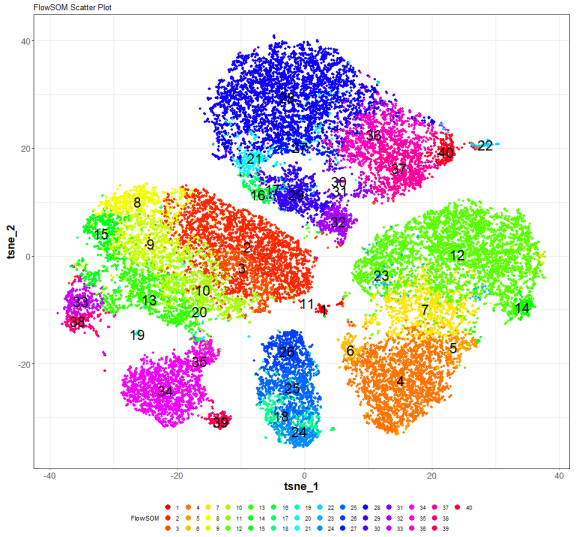
- Overlay other clusterings
- Use UMAP also

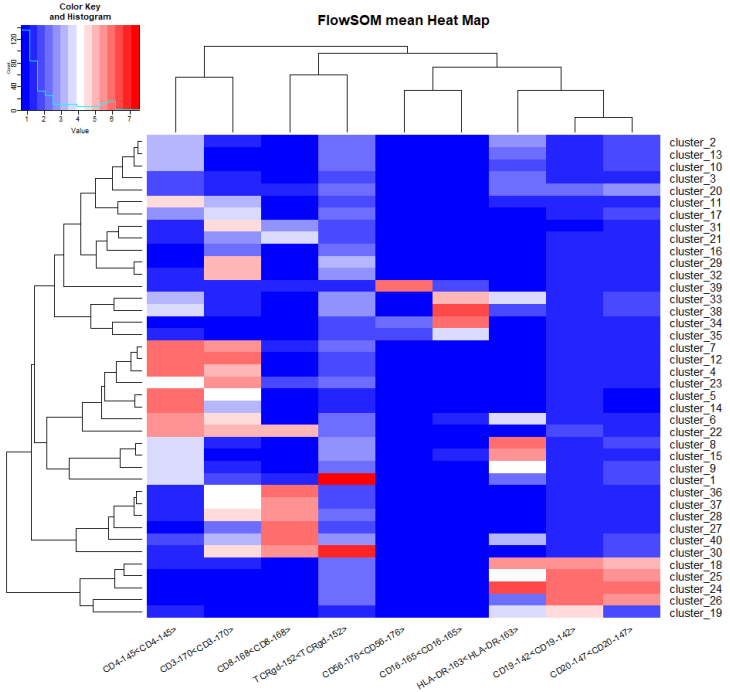
FlowSOM is fast… do it again!



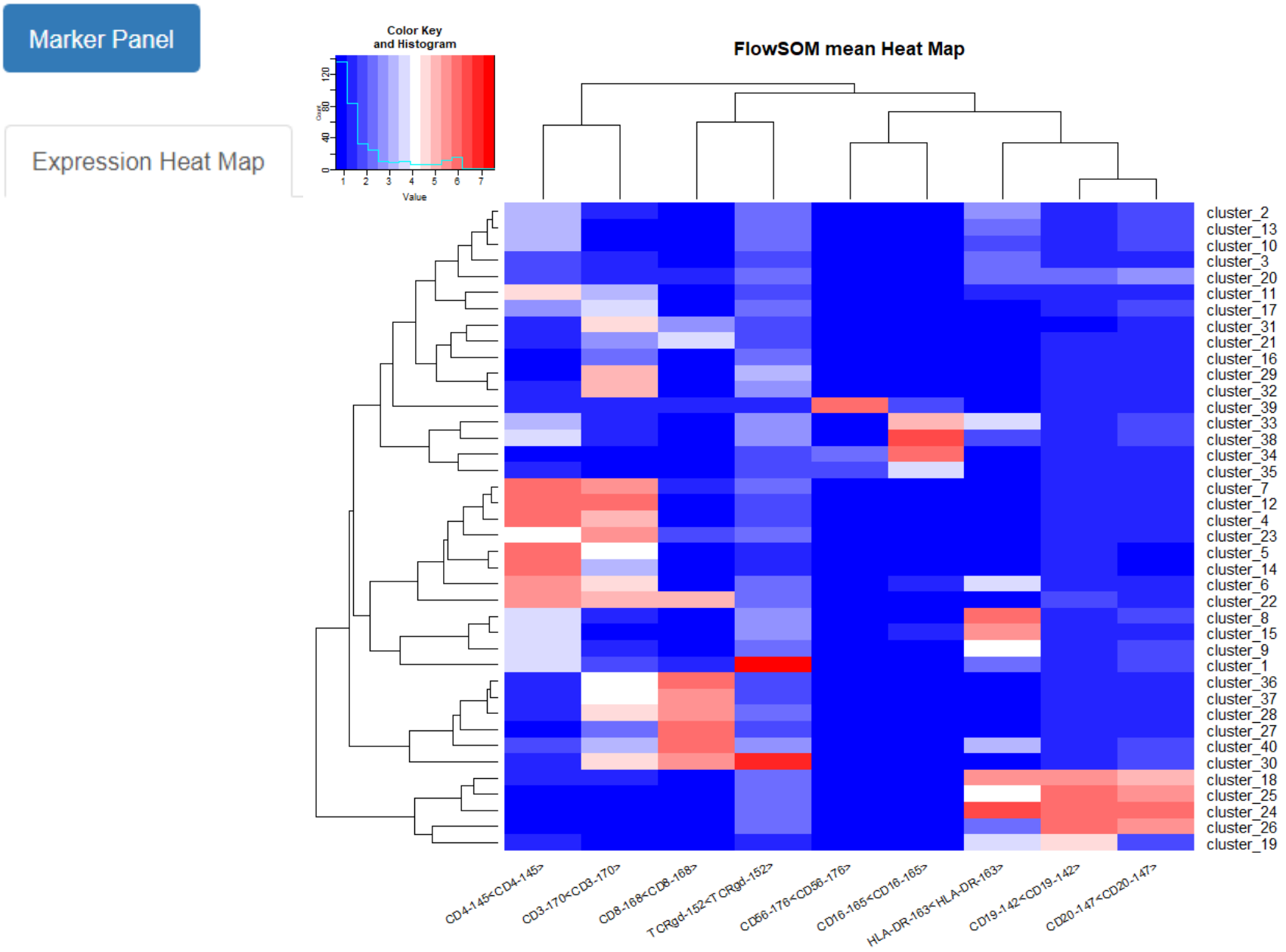
Annotating clusters







Expression heatmap of clusters



Cytofkit - Analysis

Marker Panel

Annotate Clusters

Choose Cluster Results to Annotate:

FlowSOM

Type In Your Name for Annotated Cluster

Annotated_FlowSOM

Cluster 1 :

Type in the cell type

Cluster 2 :

Type in the cell type

Cluster 3 :

Type in the cell type

Cluster 39 :

Type in the cell type

Cluster 40 :

Type in the cell type

Submit Cluster Label

Hands on

- Merge a few clusters

Outline

- ☑ Reduce dimensions
- ☑ Clusterize cells
- ☑ Merge clusters
- ☑ Annotate clusters

What have we done?

Outline

- ☑ Reduce dimensions
- ☑ Clusterize cells
- ☑ Merge clusters
- ☑ Annotate clusters
- ☛ Unsupervised gating
- ☛ Data driven cell populations with expert annotation

What's next?

Back to the question

- Find a group of cells that differ in abundance between two groups of patients

- Try to view differences on tSNE (or UMAP)

tSNE split by samples

Cluster Panel

Cluster Plot

Visualization Meth

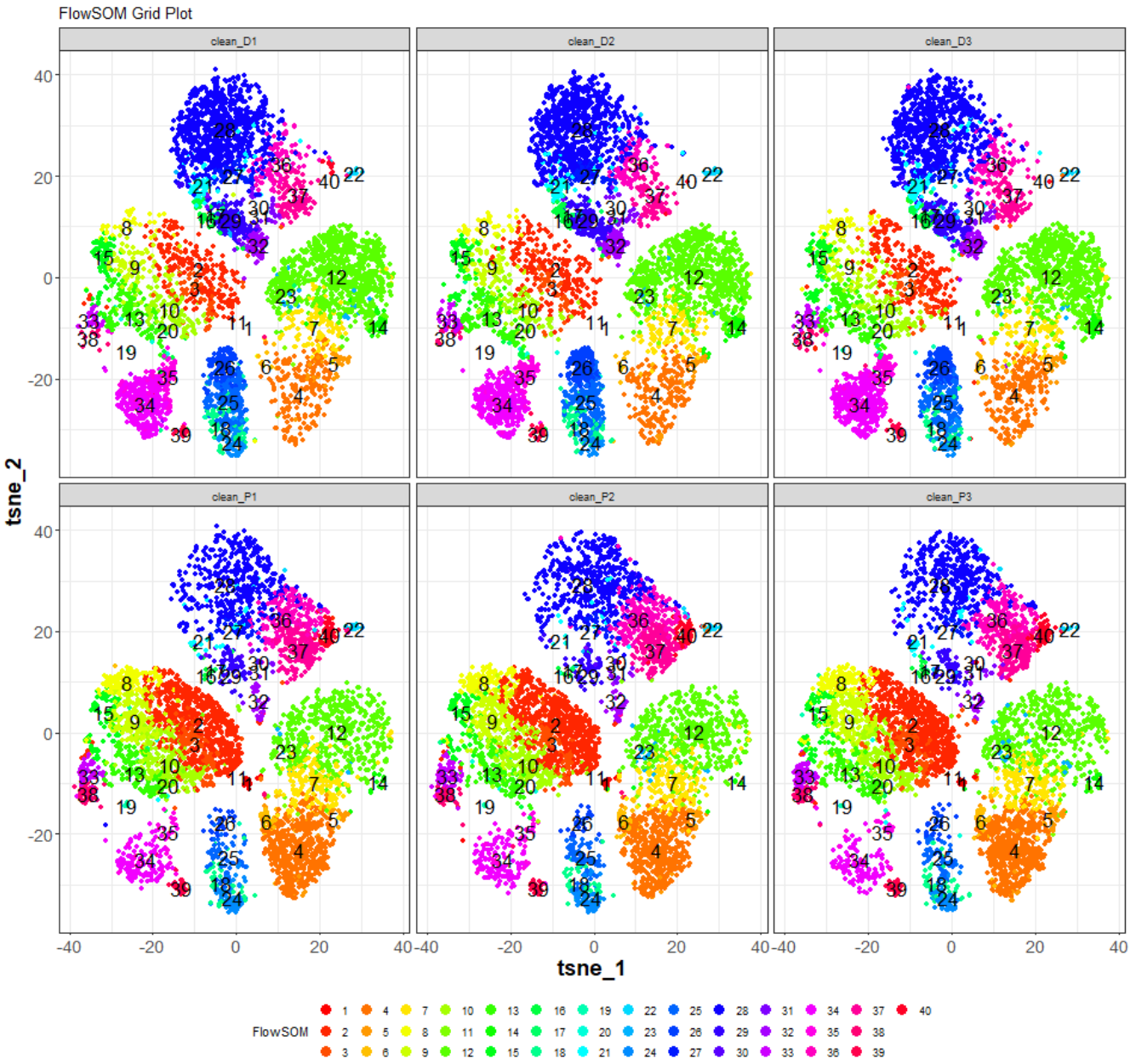
tsne

Cluster By:

FlowSOM

Plot Control:

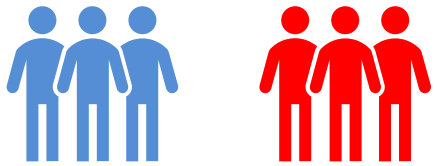
- [x] Add Cluster Labels
- [ ] Repel Cluster Labels
- [x] Separate Plot by Samples



Back to the question

- Find a group of cells that differ in abundance between two groups of patients

- What would you like to see/do?



Pool samples

Sample Panel

Regroup Samples

Type in the Group Name for Each Sample:

clean_D1 :

D_status

clean_D2 :

D_status

clean_D3 :

D_status

clean_P1 :

P_status

clean_P2 :

P_status

clean_P3 :

P_status

Group Name Levels: (to order the group names)

Type in group names in order, seperated by semicolon(;)

Submit New Sample Groups

Revert to Old Sample Names

Hands on

- Pool samples in order to get two meta-samples
- View side-by-side meta-samples

- Visualize differences on tSNE (or UMAP)

Side-by-side tSNE

Cluster Panel

Cluster Plot

Visualization Meth

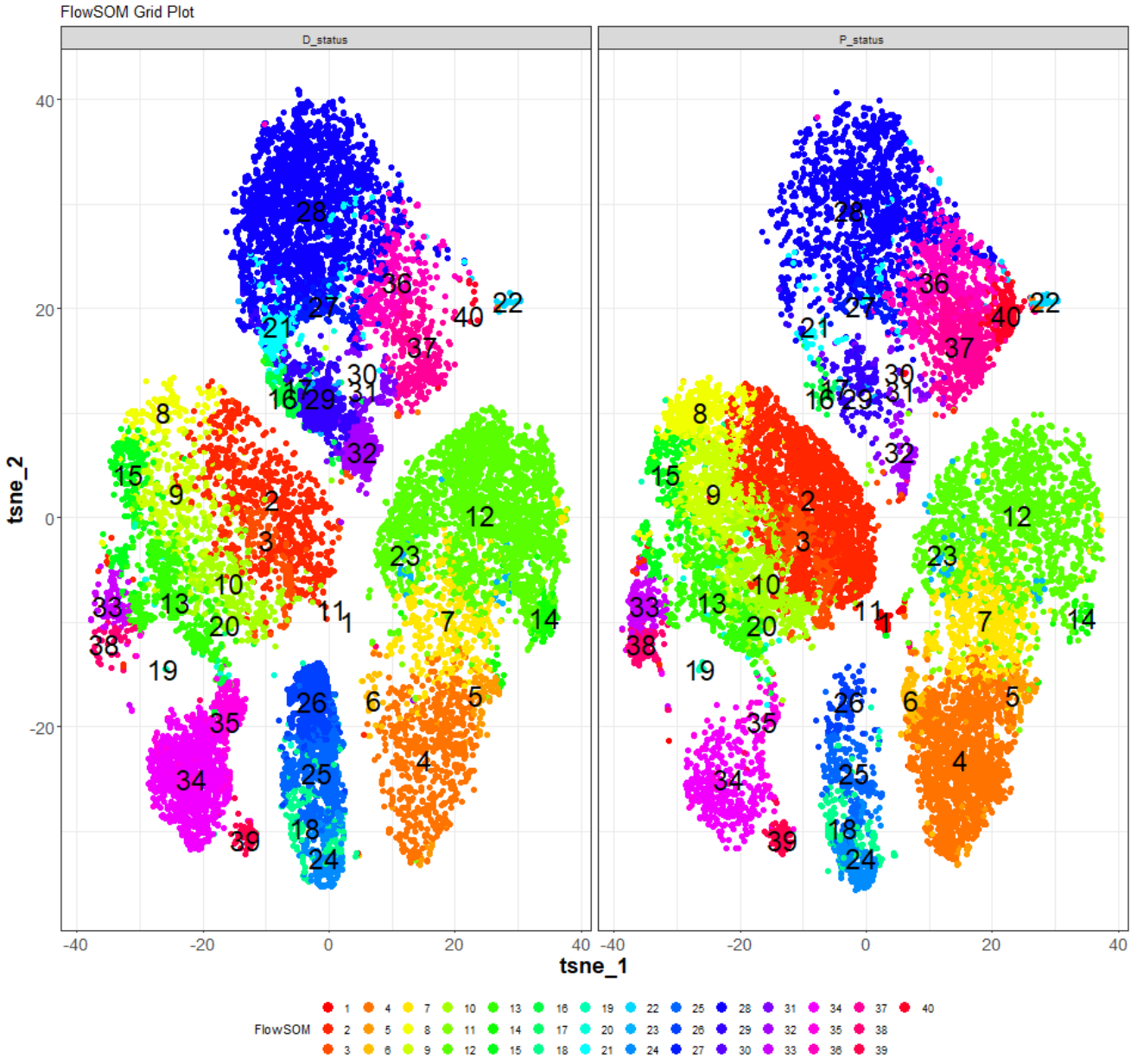
tsne

Cluster By:

FlowSOM

Plot Control:

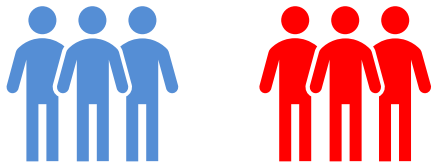
- [x] Add Cluster Labels
- [ ] Repel Cluster Labels
- [x] Separate Plot by Samples



Outline

- ☑ Reduce dimensions
- ☑ Clusterize cells
- ☑ Merge clusters
- ☑ Annotate clusters
- ☛ Unsupervised gating, cell populations

- ☛ What's next?



Back to the objective

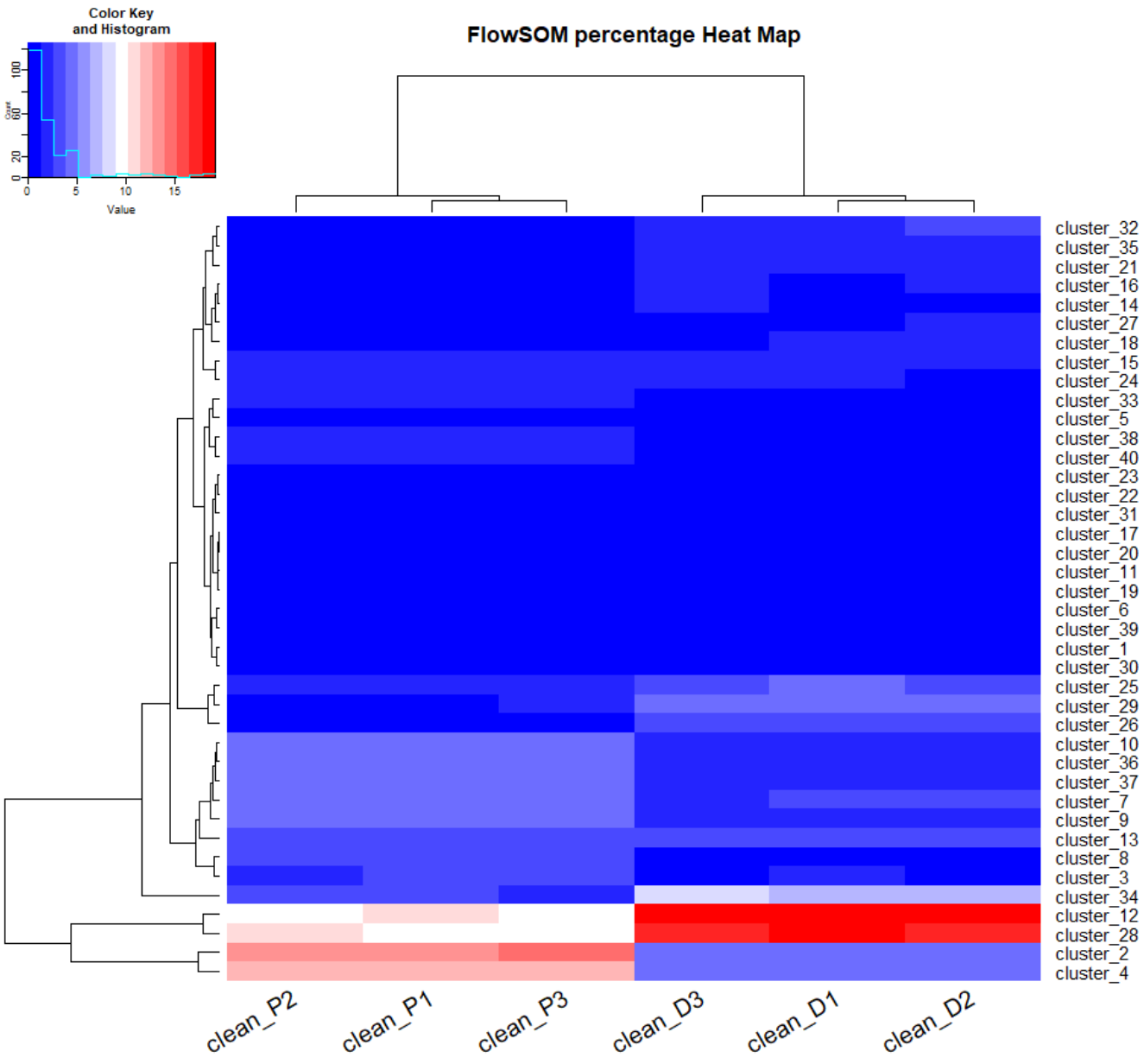
- Find a group of cells that differ in abundance between two groups of patients

- How to quantify the differences?
- Which feature to extract?

Cell percentage heatmap

Sample Panel

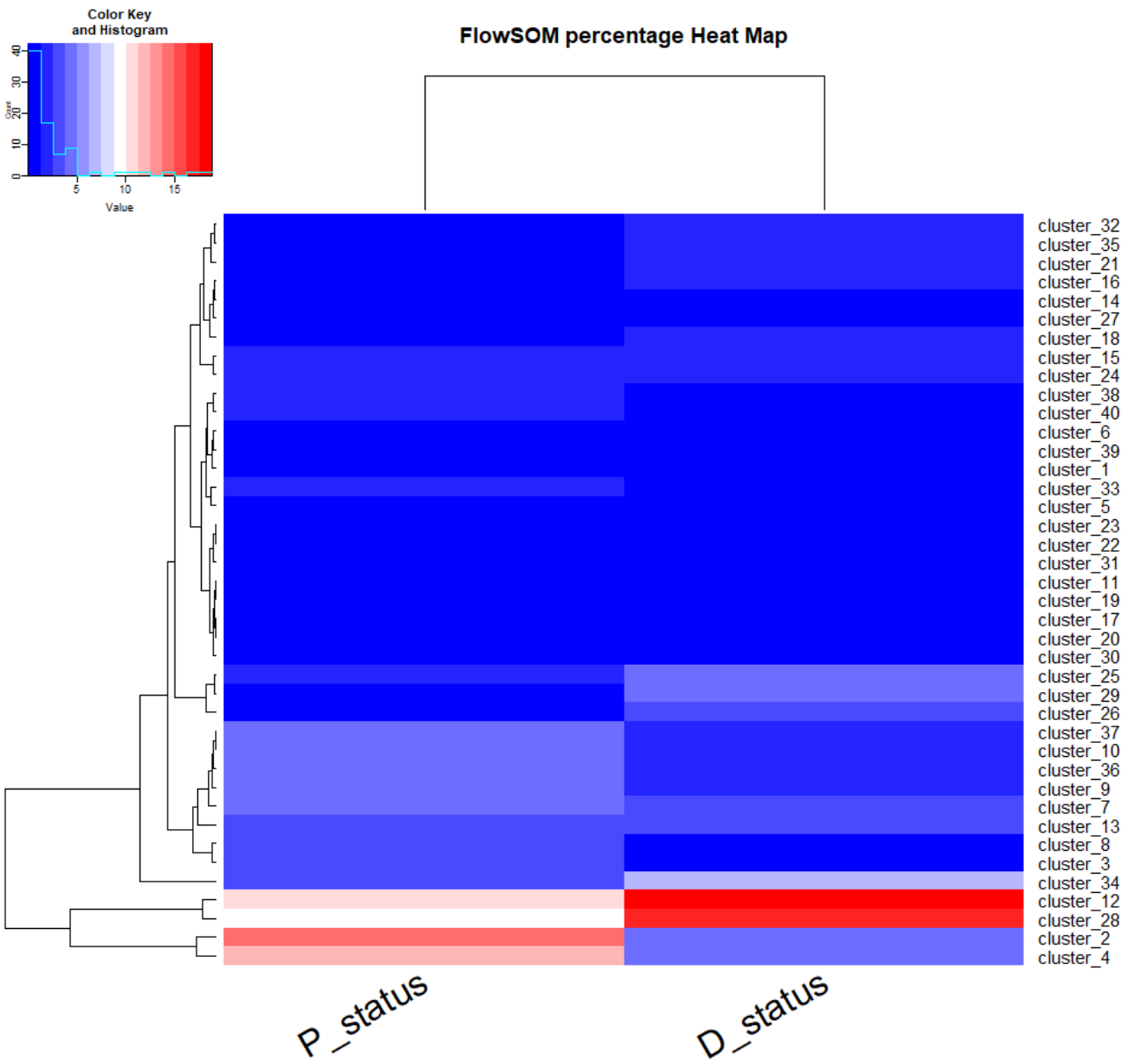
Cell Percentage Heatmap



Cell percentage heatmap

Sample Panel

Cell Percentage Heatmap

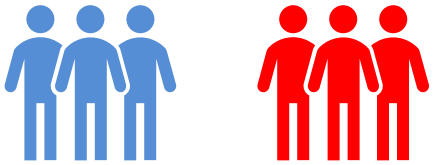


Outline

- ☛ Unsupervised gating, cell populations

- ☛ Cell percentage analysis
- ☑ Extract percentages
- ☑ Visualize percentages as heatmap
- ☑ Clusterize percentages of cell populations

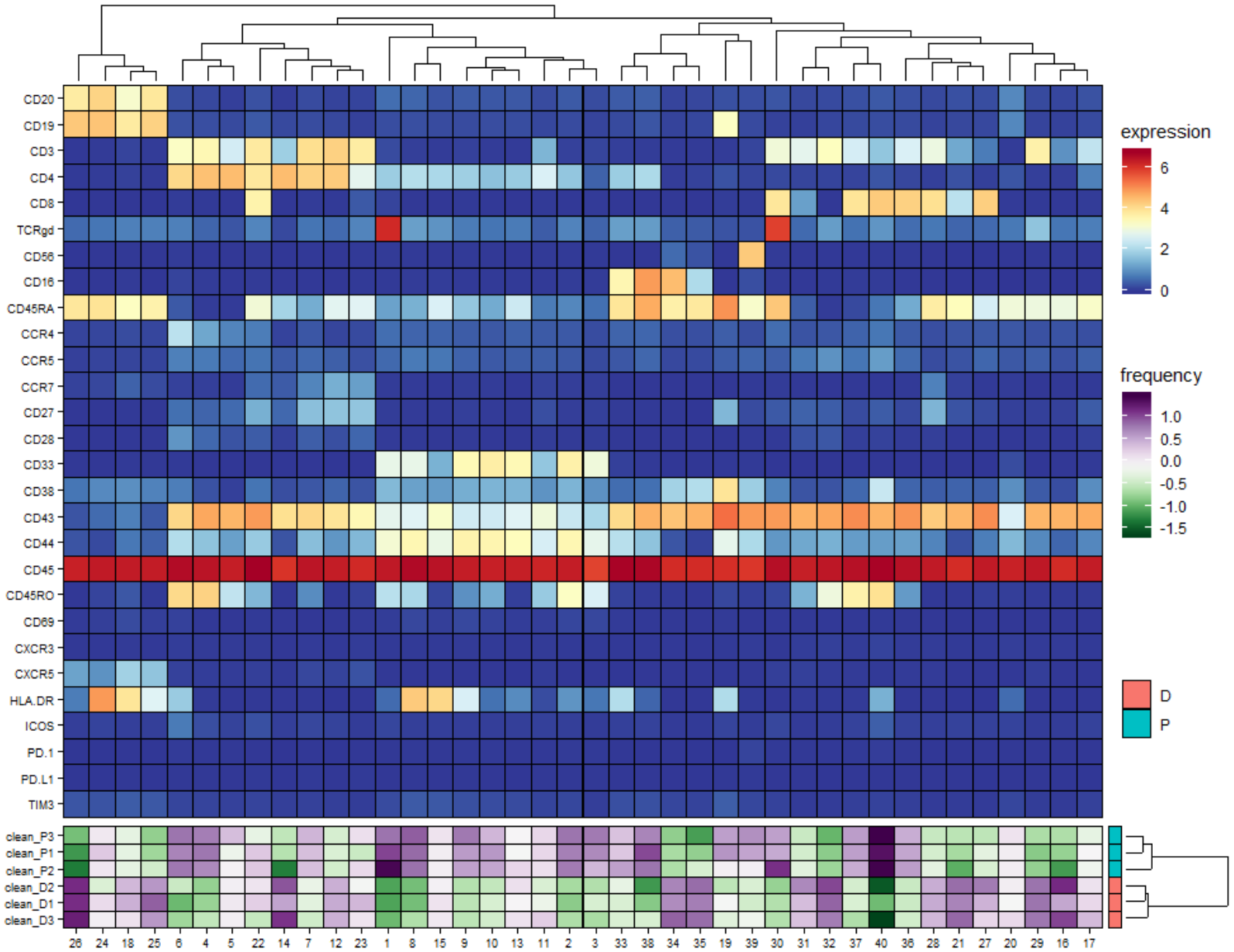
- ☛ What's next?



Outline

- ☛ Unsupervised gating, cell populations
- ☛ Cell percentage analysis
- ☛ What's next?
- ☐ Publication ready figures
- ☐ Box-plots, p-values
- ☐ What else?

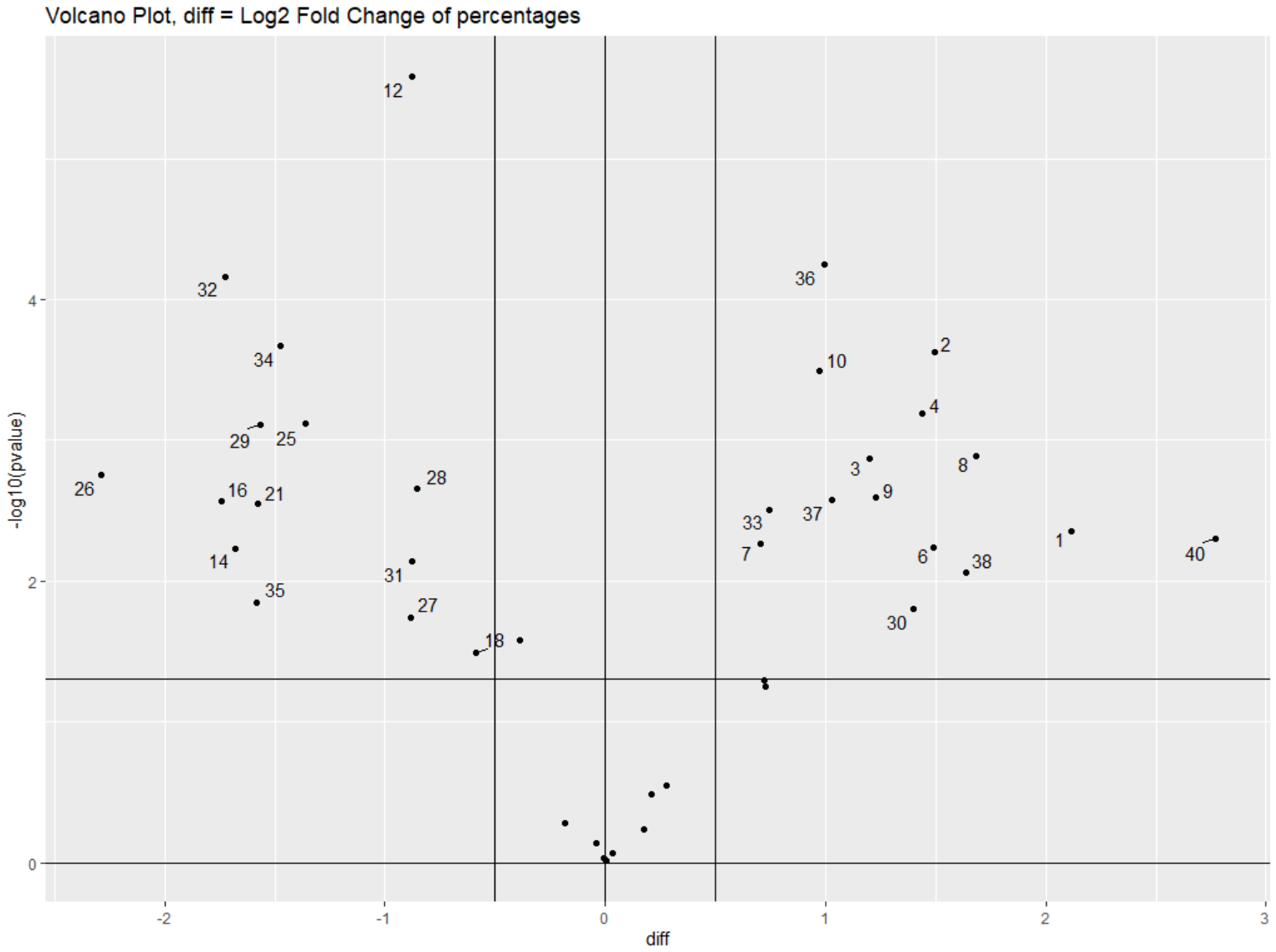
Prettier heatmaps



Boxplots


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
value
grouping
grouping
D
P

Multiple testing



Statistical tests

	pvalue	adjusted	fold	diff	clean_D1	clean_D2	clean_D3	clean_P1	clean_P2	clean_P3
1	0.0044447	0.0093572	4.33	2.1132617	0	0	1	33	46	27
2	0.0002351	0.0018806	2.82	1.4959178	227	247	254	687	701	718
3	0.0013465	0.0048965	2.29	1.1976641	66	55	53	152	124	163
4	0.0006430	0.0034625	2.71	1.4362518	207	205	242	628	580	609
6	0.0057602	0.0103445	2.81	1.4921239	9	16	12	50	53	54
7	0.0054537	0.0103445	1.63	0.7038460	130	138	116	211	211	221
8	0.0012854	0.0048965	3.22	1.6858110	40	40	50	160	156	167
9	0.0025327	0.0066377	2.34	1.2273065	88	85	88	208	204	241
10	0.0003242	0.0021612	1.96	0.9721761	113	103	110	238	224	207
12	0.0000026	0.0001030	-1.84	-0.8776517	956	939	932	517	499	509
14	0.0059481	0.0103445	-3.21	-1.6819130	43	61	69	14	5	15
16	0.0027074	0.0066377	-3.35	-1.7449611	46	77	70	13	8	15
18	0.0321336	0.0428447	-1.50	-0.5888729	79	76	60	44	44	44
21	0.0028210	0.0066377	-2.98	-1.5757889	86	95	97	26	19	29
25	0.0007619	0.0034625	-2.57	-1.3591659	233	187	186	68	85	65
26	0.0017456	0.0058185	-4.88	-2.2860022	175	176	186	28	24	35
27	0.0182757	0.0261081	-1.85	-0.8836710	56	73	46	30	27	23
28	0.0021898	0.0066377	-1.81	-0.8560822	901	890	846	474	525	447
29	0.0007791	0.0034625	-2.96	-1.5641741	204	205	202	59	61	67
30	0.0157821	0.0233808	2.64	1.3991602	1	0	1	14	27	15
31	0.0071710	0.0119516	-1.83	-0.8747789	30	40	29	13	15	12
32	0.0000683	0.0009111	-3.31	-1.7275874	120	132	109	30	32	26
33	0.0031106	0.0069124	1.67	0.7438357	37	42	37	66	81	68
34	0.0002145	0.0018806	-2.78	-1.4731193	346	357	407	129	132	119
35	0.0142354	0.0219006	-2.99	-1.5813353	106	114	114	30	42	22
36	0.0000561	0.0009111	1.99	0.9931444	101	100	104	212	220	205
37	0.0026399	0.0066377	2.04	1.0262677	114	111	94	230	217	232
38	0.0087517	0.0140028	3.11	1.6349654	16	11	25	83	71	66
40	0.0049719	0.0099439	6.81	2.7685644	8	2	1	76	83	84

Cytofast - Analysis

- Designed for cytosplore
- Available as R commands
- Read FCS files with cell annotations
  - new channels holding clustering results
  - new channels holding tSNE, UMAP dimensions

- Adapted to read cytofkit results
- Available as a R MarkDown file

R session

- All information
  - 200204-atelier_afc_2020

- Rmd script
  - JT_AFC_2020.Rmd

- HTML result
  - JT_AFC_2020.html

Final word

- ☛ Unsupervised gating, cell populations
- ☛ Cell percentage analysis
- ☛ Better and deeper analyses with cytofast
- ☐ What else?