Journées Pratiques

Analyses non supervisées en cytométrie

Du 5 au 7 Février 2020
Sophia-Antipolis

Présidées par :

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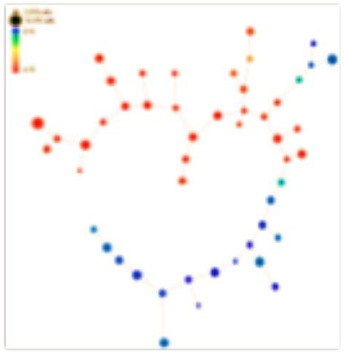
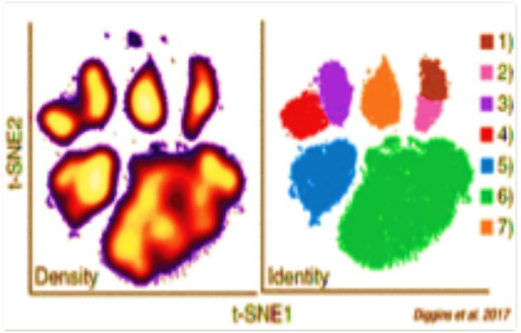
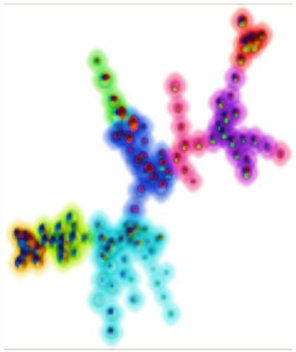
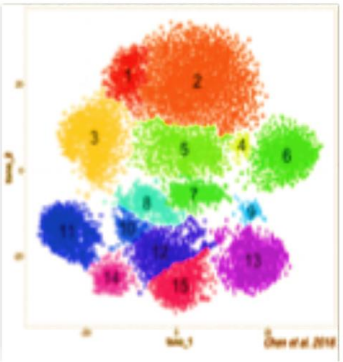
Julie Cazareth (IPMC, CNRS Valbonne) et **Sierra Barone** (Vanderbilt University, Nashville USA)







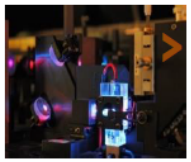


Formation
Provence-Alpes-Côte d'Azur et Corse

Inserm

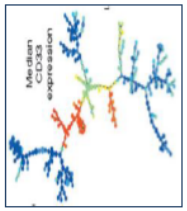
Passeport cytométrie Marseille

Édition 2020



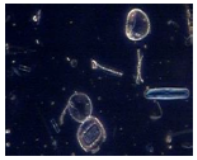
Cytométrie multiparamétrique avancée : théorie & pratique

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

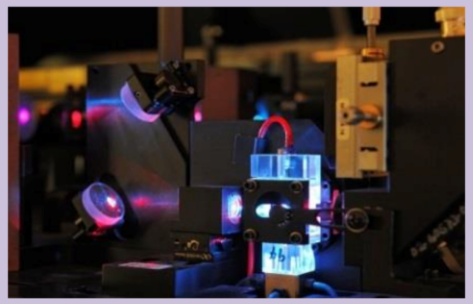
novembre 2020

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

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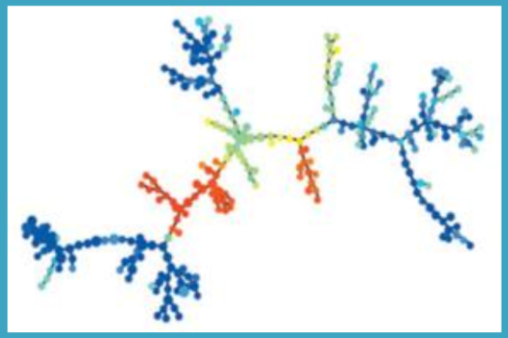
Passeport cytométrie Marseille



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

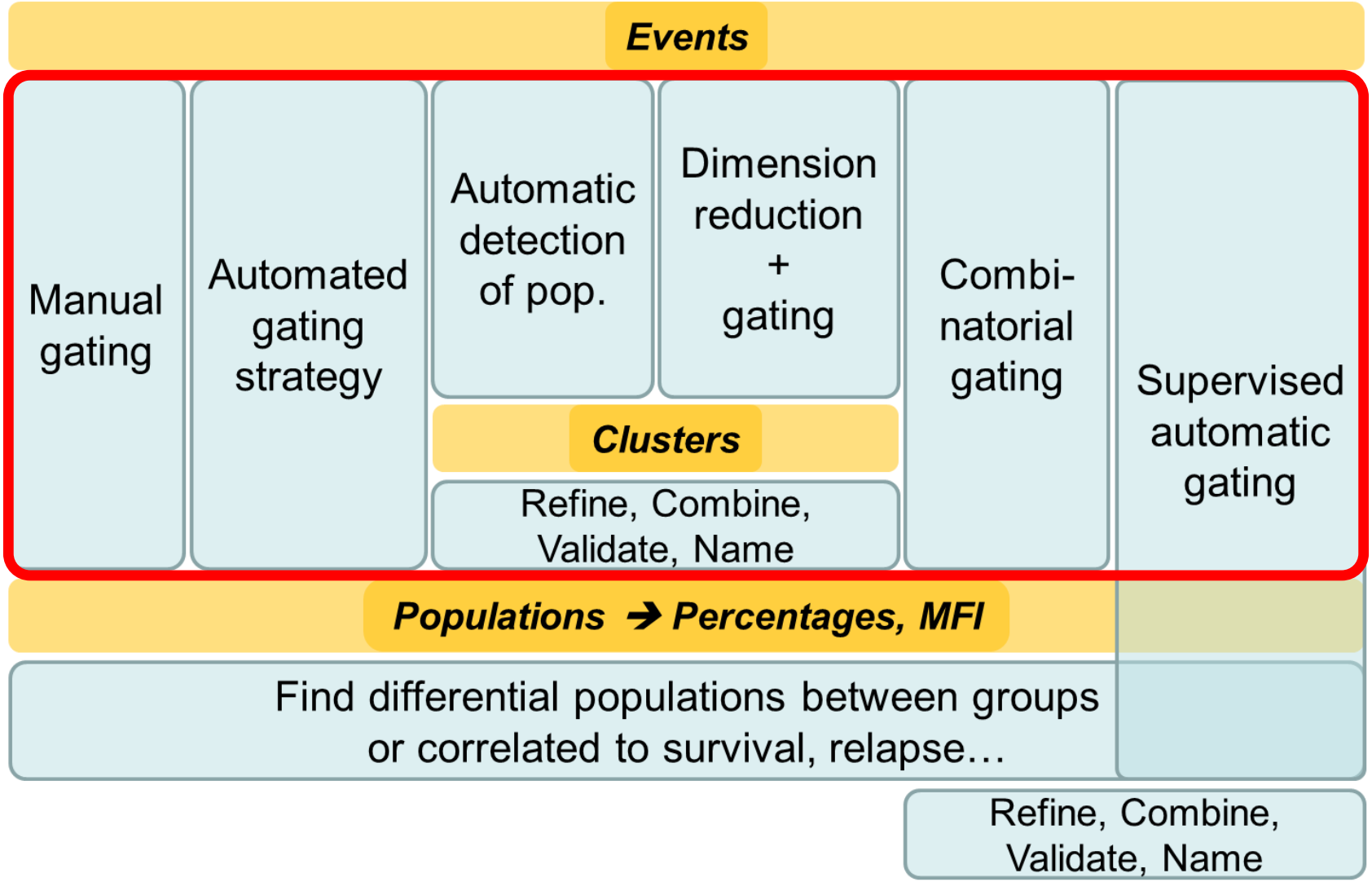
- Connaître les outils actuels d'analyse de données avancées, les mettre en œuvre sur des jeux de données tests afin d'apprendre à les maîtriser
- Permettre aux participants d'identifier la méthode d'analyse de choix appropriée pour une question définie et extraire le maximum d'informations à partir 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

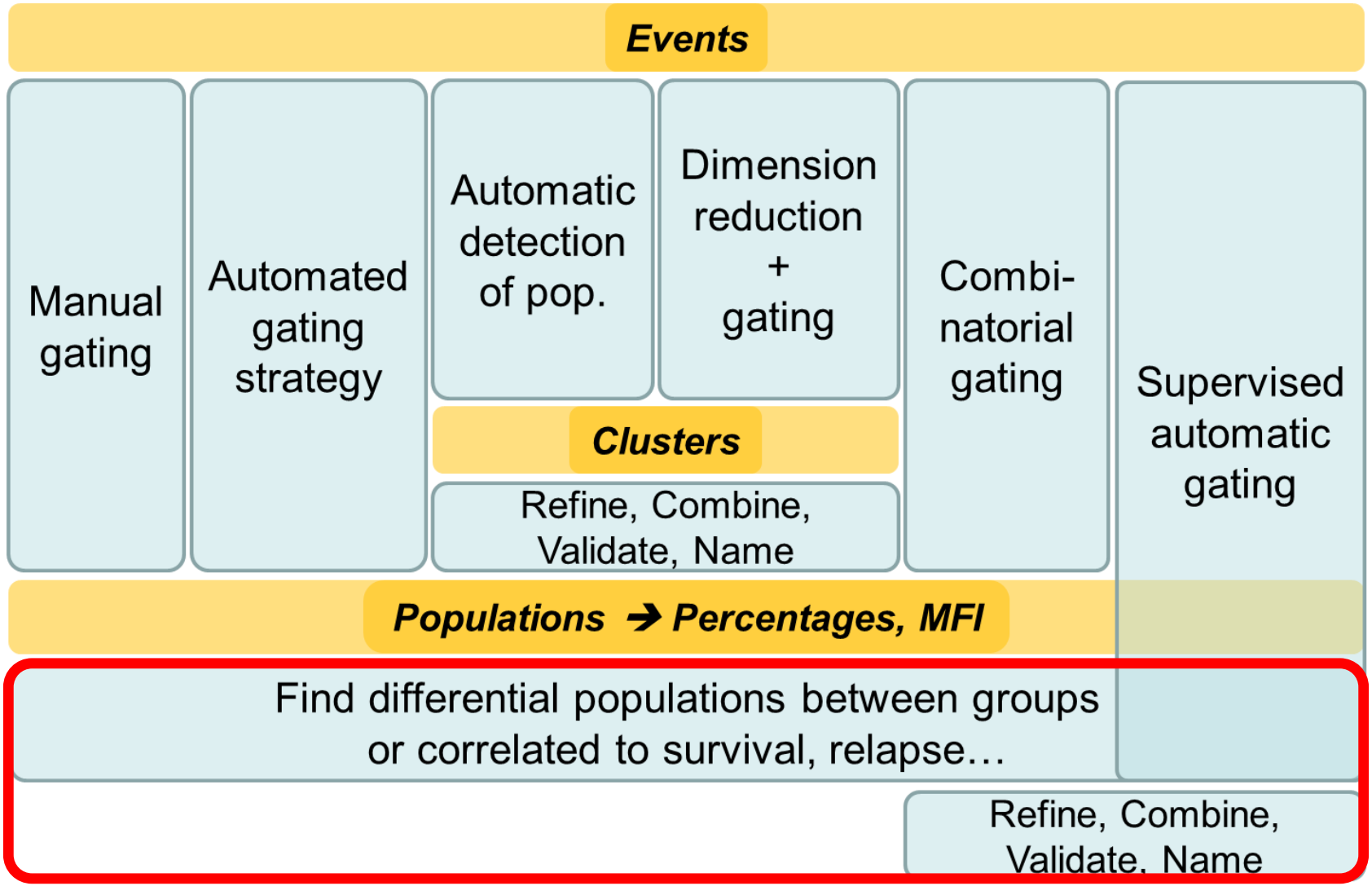
Participants : 8 personnes

PIPELINES OVERVIEW AND DETAILS

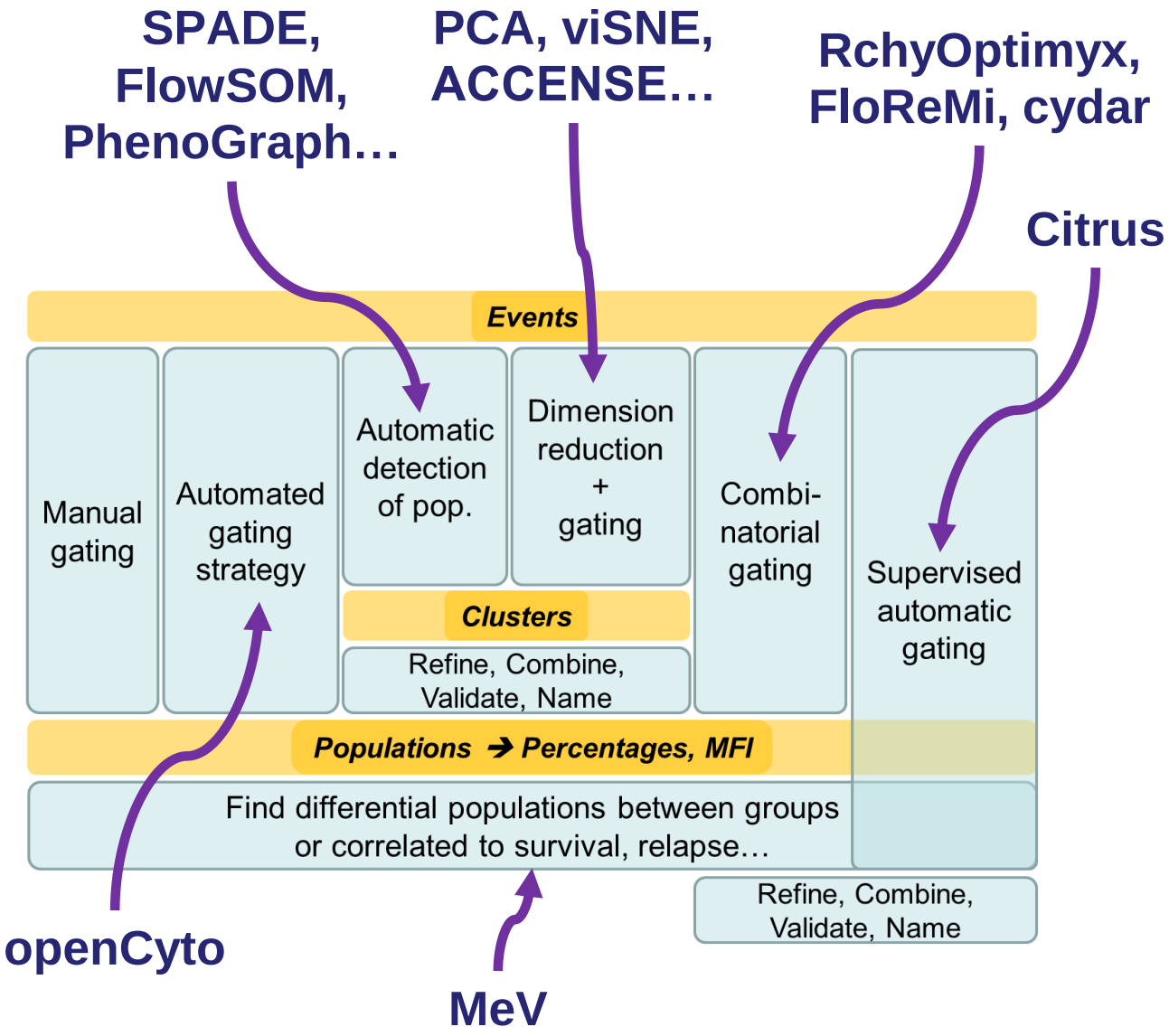
Strategies


Events
Manual gating
Automated gating strategy
Automatic detection of pop.
Dimension reduction + gating
Clusters
Refine, Combine, Validate, Name
Combi-natorial gating
Supervised automatic gating
Populations → Percentages, MFI
Find differential populations between groups or correlated to survival, relapse…
Refine, Combine, Validate, Name

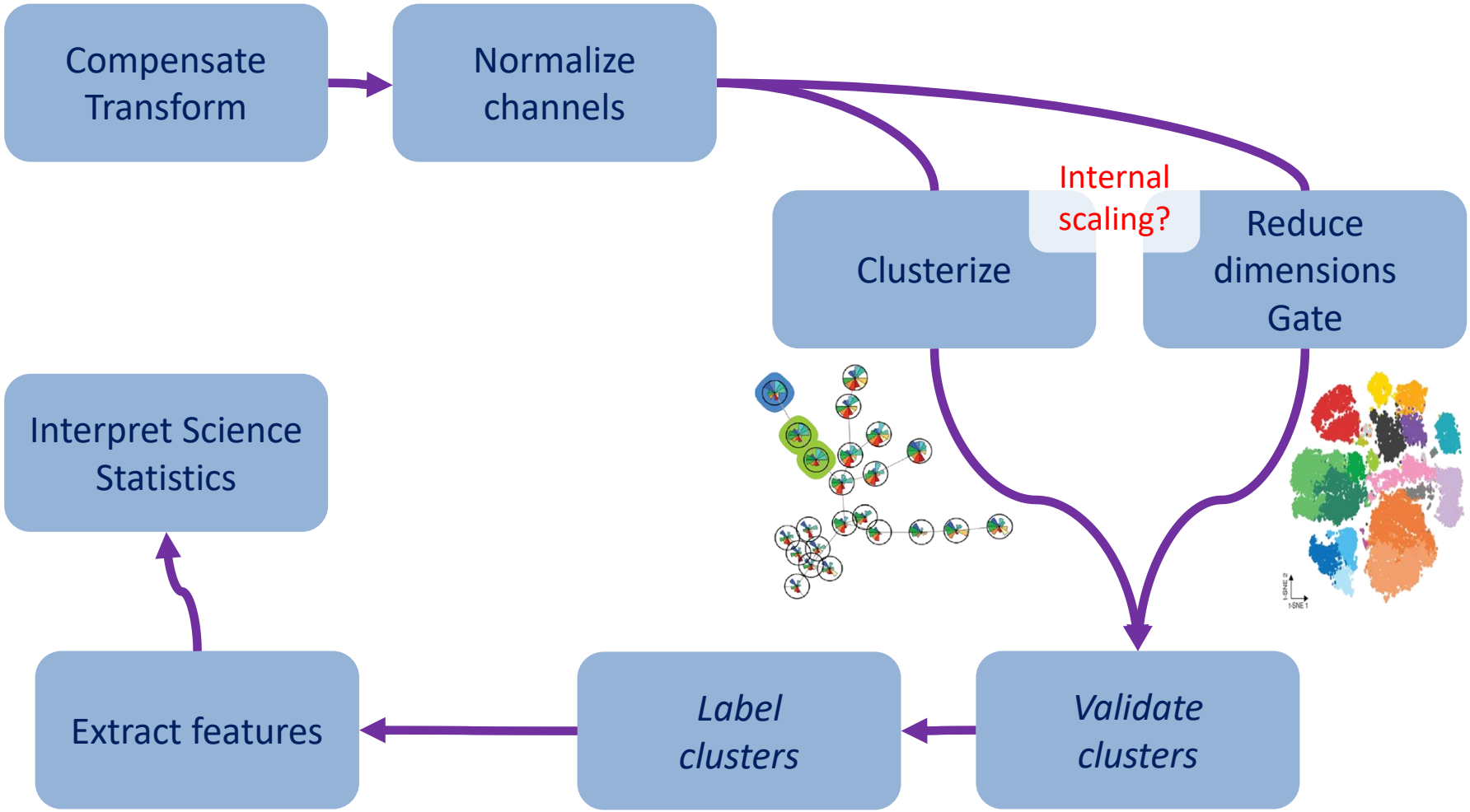
Features analysis



Free tools

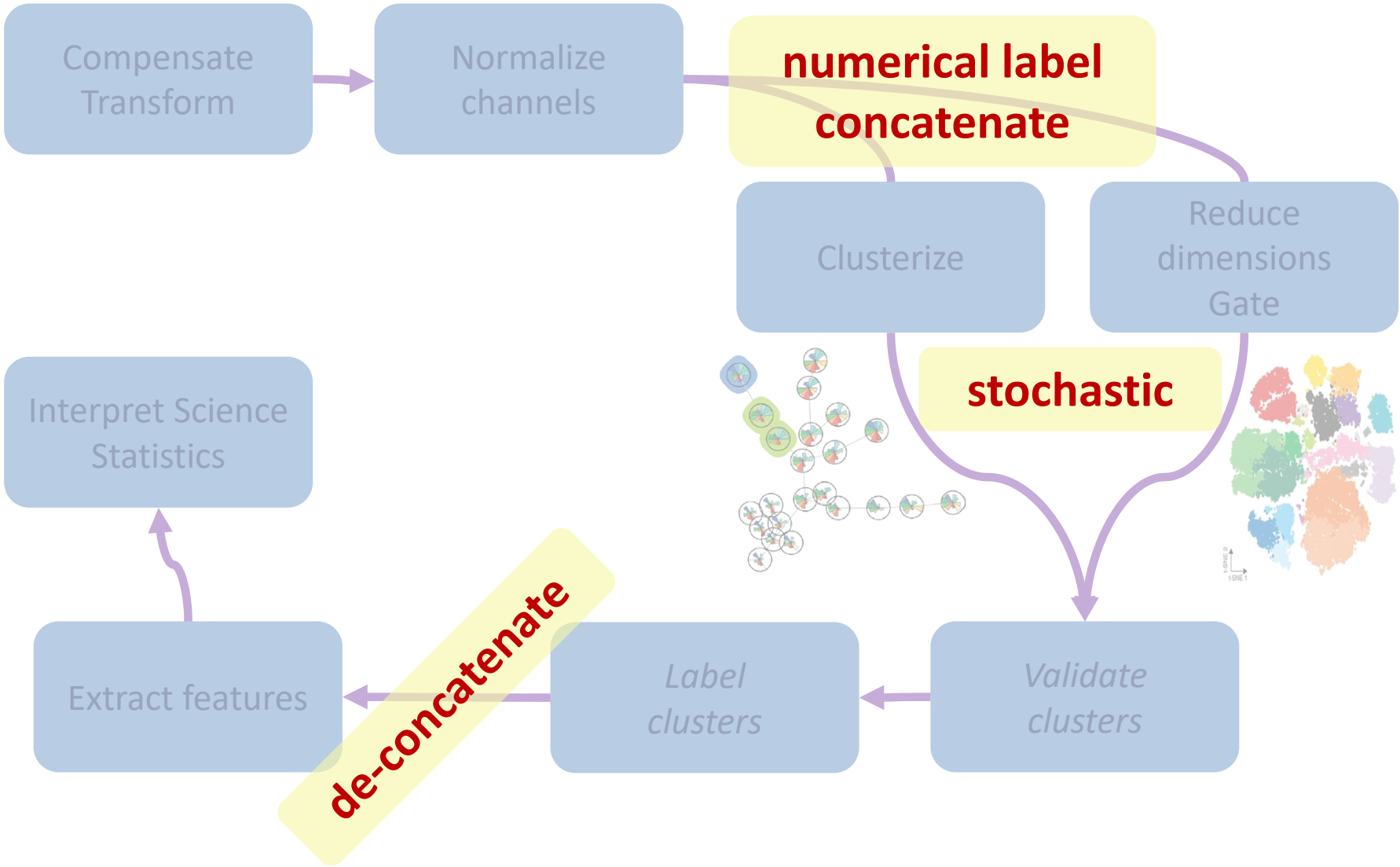

SPADE, FlowSOM, PhenoGraph…
PCA, viSNE, ACCENSE…
RchyOptimyx, FloReMi, cydar
Citrus
Events
Manual gating
Automated gating strategy
Automatic detection of pop.
Dimension reduction + gating
Combi-natorial gating
Supervised automatic gating
Clusters
Refine, Combine, Validate, Name
Populations ➔ Percentages, MFI
Find differential populations between groups or correlated to survival, relapse…
Refine, Combine, Validate, Name
openCyto
MeV

Pipeline (one sample)

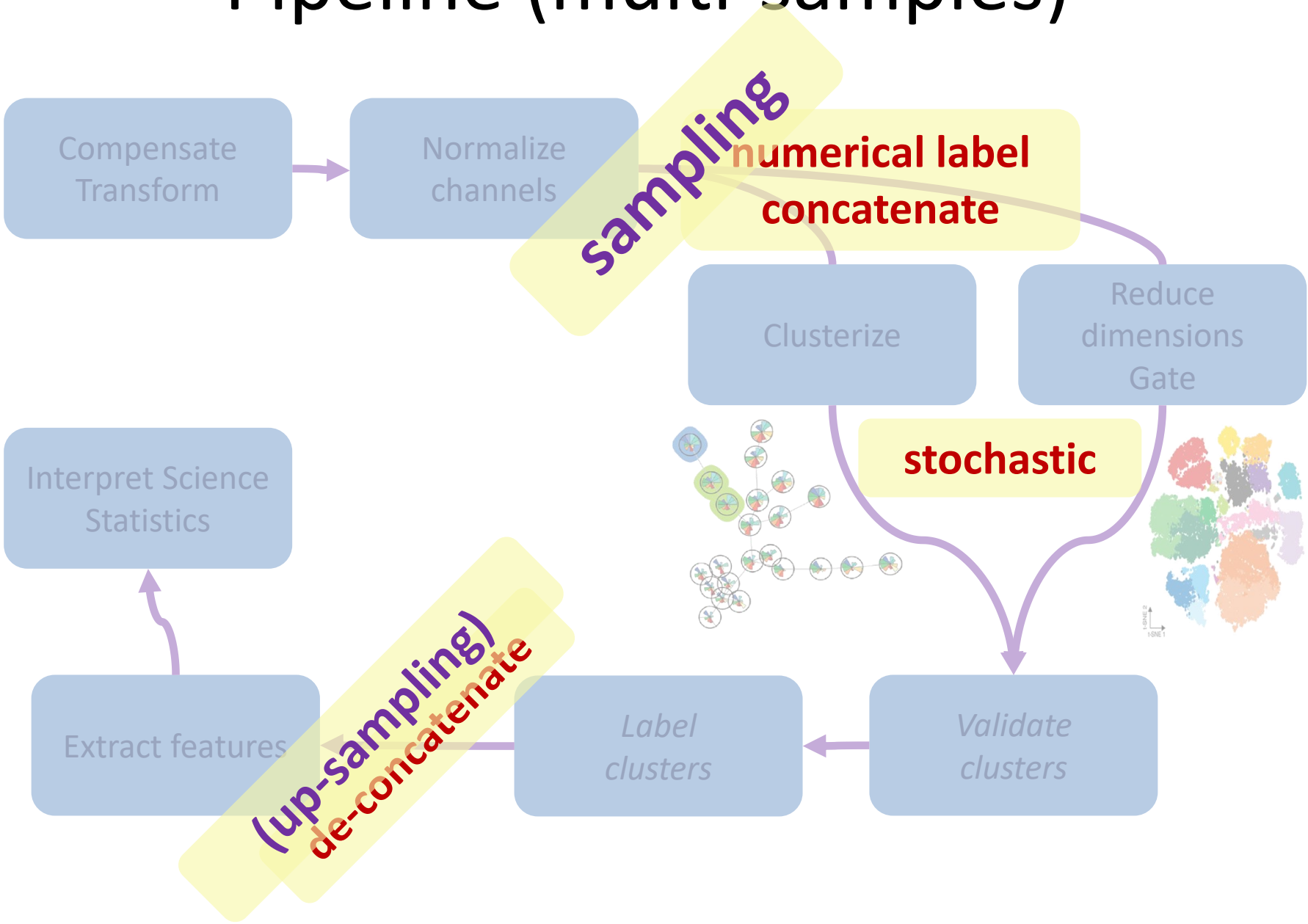


strength = weakest step

Pipeline (multi-samples)

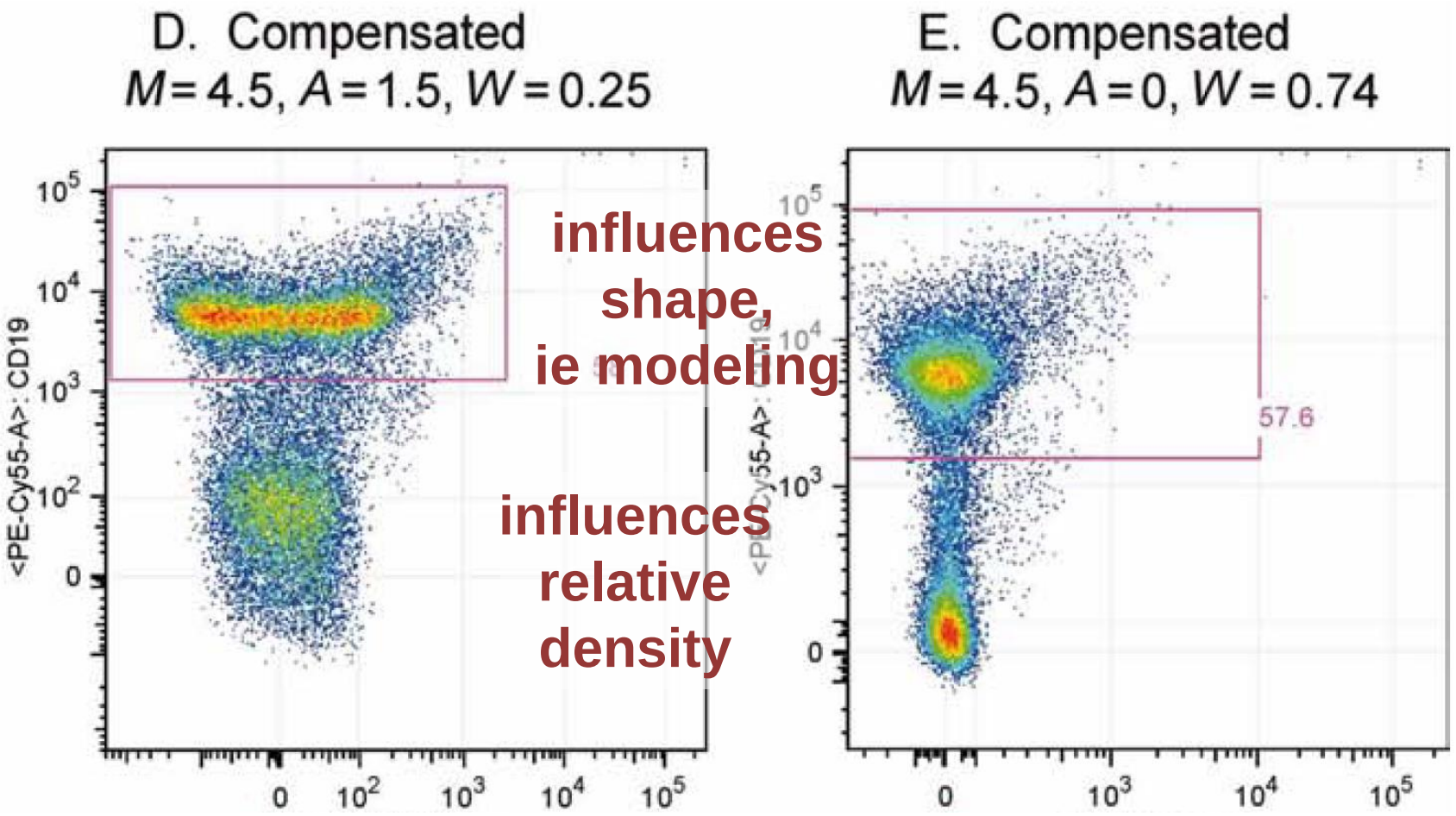


Pipeline (multi-samples)



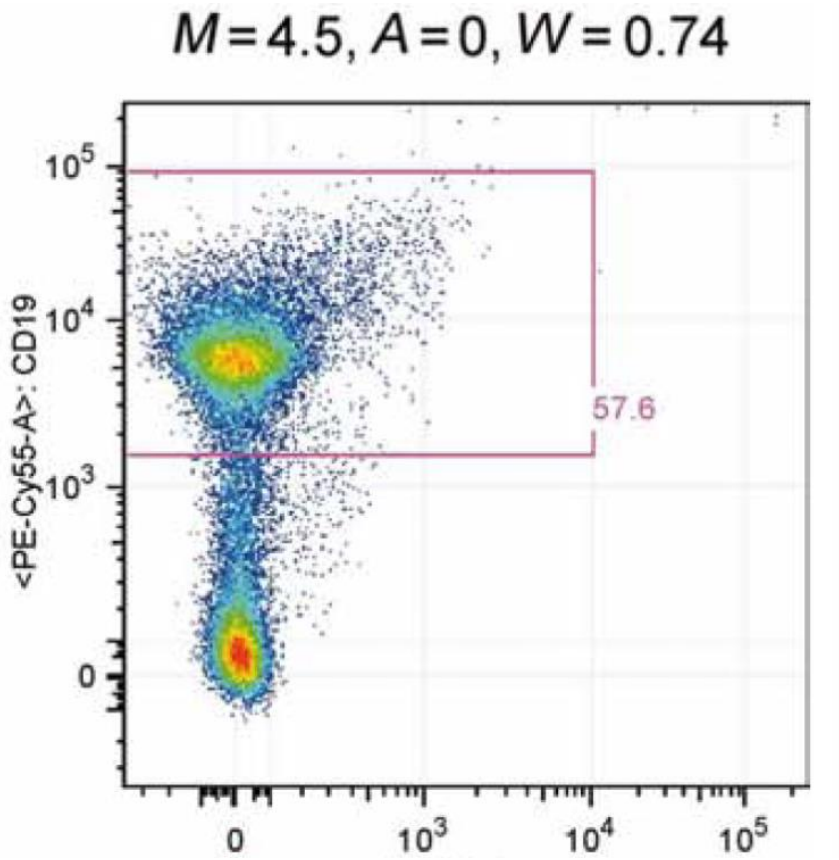
TRANSFORMATIONS: ASINH VS BI-EXP

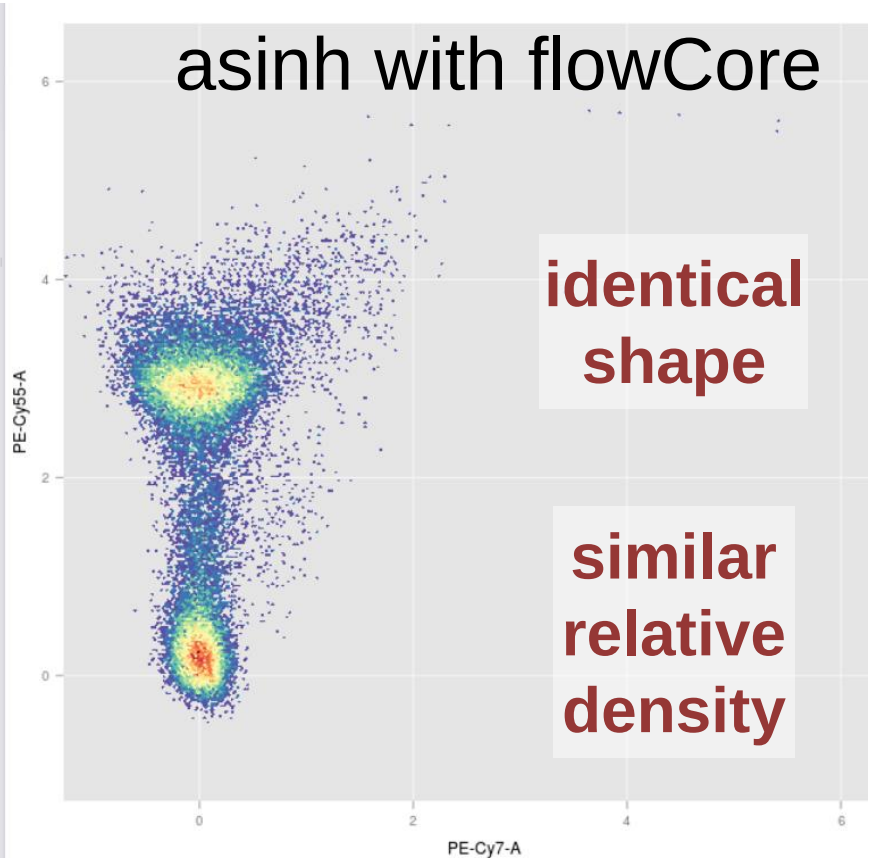
Transformation parameters



Moore WA, Parks DR. Cytometry A. 2012

logicle vs asinh



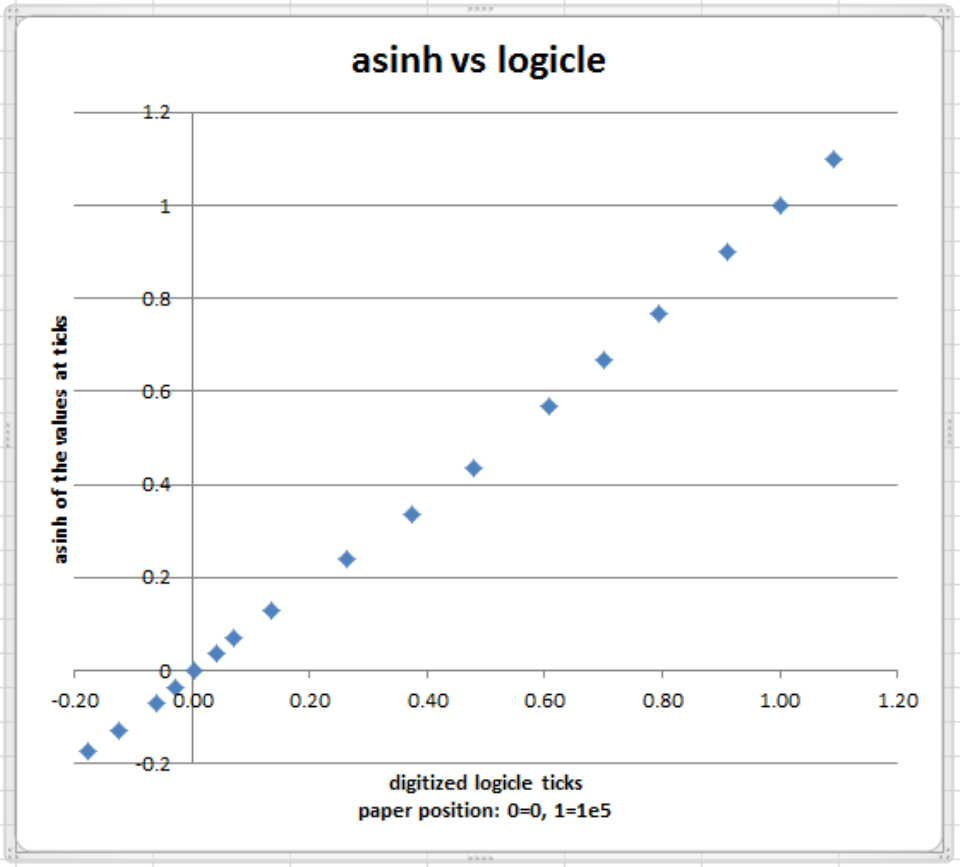


Moore WA, Parks DR. Cytometry A. 2012

logicle vs asinh

one coefficient

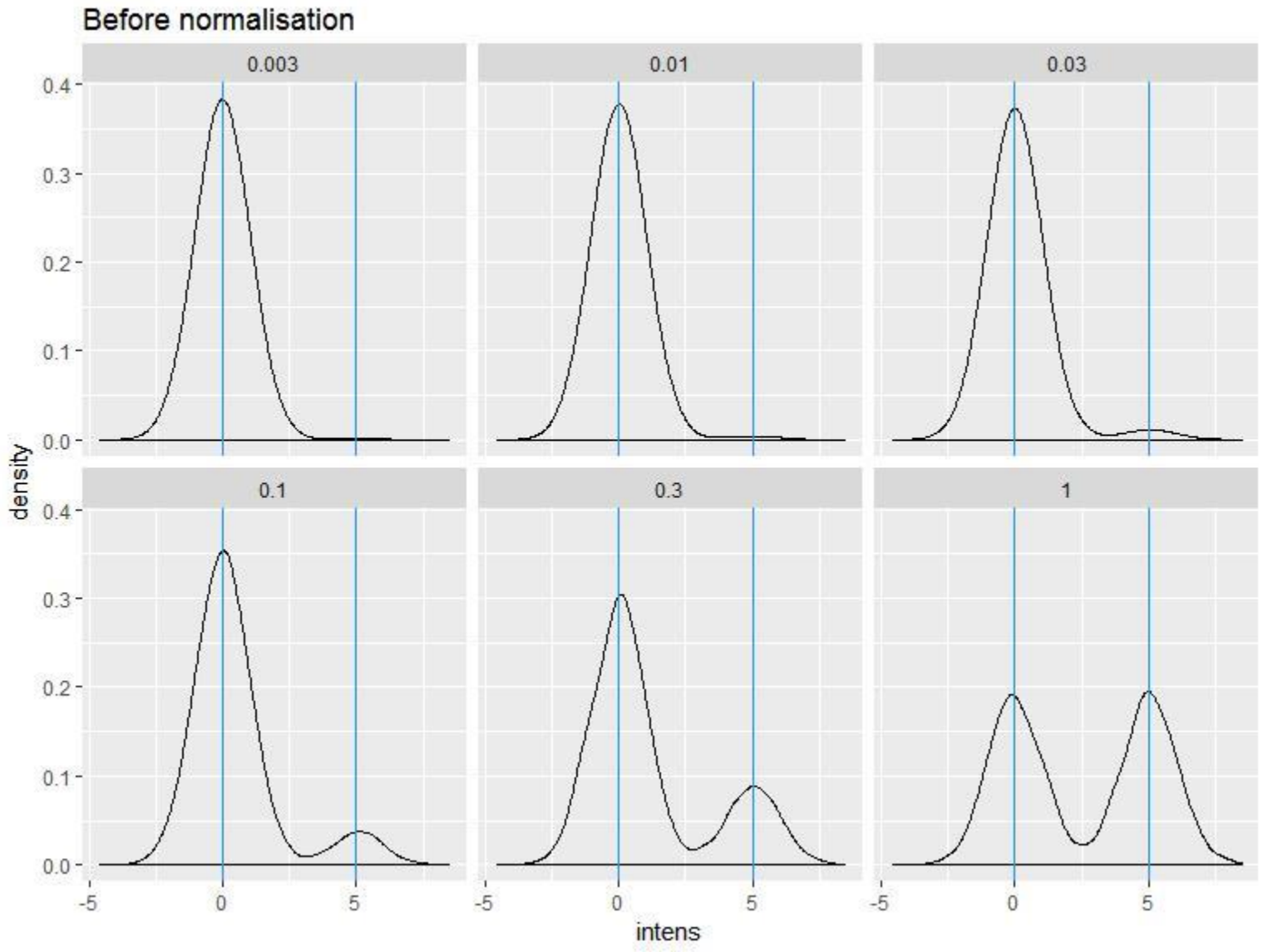
		scaling	200	
log10	units	digitized	asinh	delta%
1.06	2.00E+05	1.09	1.10	-1
1.00	1.00E+05	1.00	1.00	0
0.94	5.00E+04	0.91	0.90	1
0.86	2.00E+04	0.79	0.77	4
0.80	1.00E+04	0.70	0.67	5
0.74	5.00E+03	0.61	0.57	7
0.66	2.00E+03	0.48	0.43	10
0.60	1.00E+03	0.37	0.33	12
0.54	5.00E+02	0.26	0.24	10
0.46	2.00E+02	0.13	0.13	6
0.40	1.00E+02	0.07	0.07	2
0.34	5.00E+01	0.04	0.04	17
0.00	0.00E+00	0.00	0.00	#DIV/0!
0.34	-5.00E+01	-0.03	-0.04	-20
0.40	-1.00E+02	-0.06	-0.07	-11
0.46	-2.00E+02	-0.13	-0.13	-1
0.50	-3.00E+02	-0.18	-0.17	2



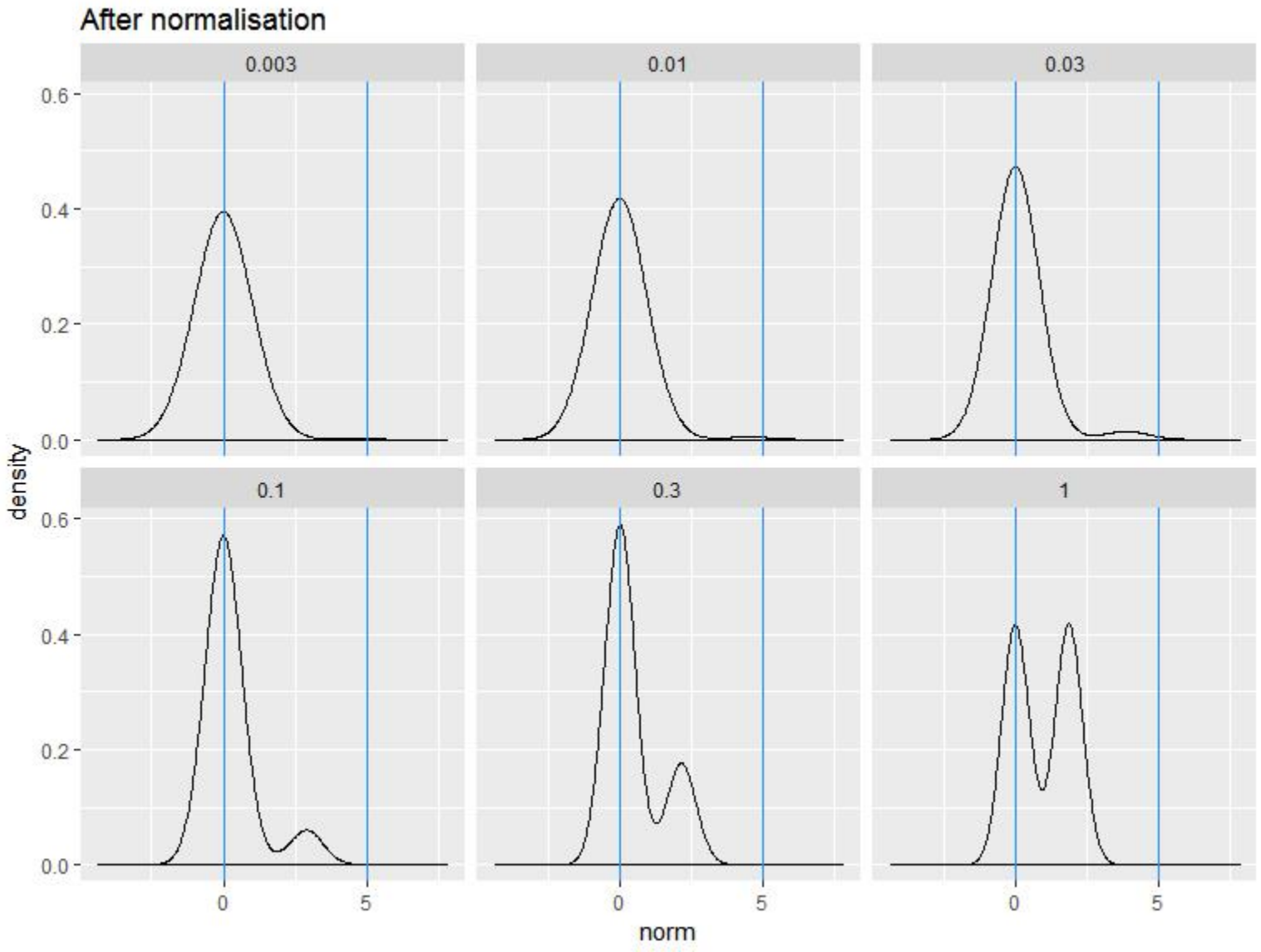
available in Excel ;-)

NORMALIZATION DIFFICULTIES

Normalisation, scaling…

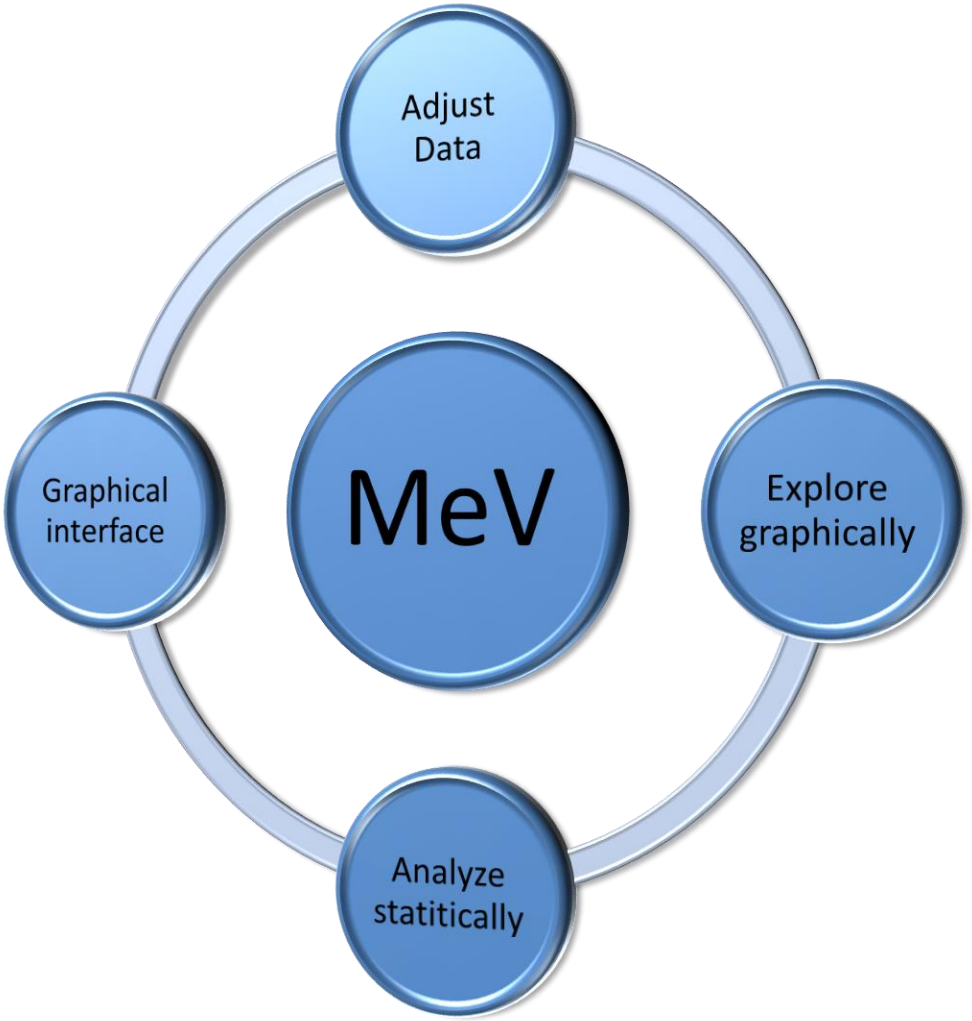


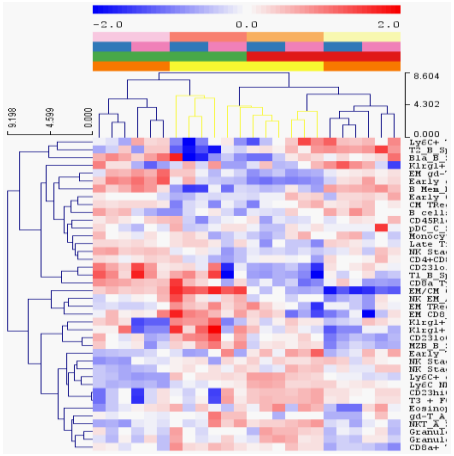
Normalisation, scaling…

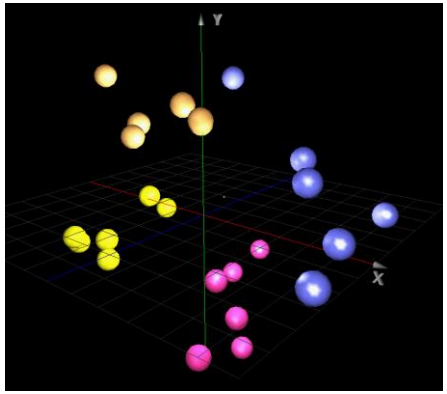


PERCENTAGES ANALYSIS

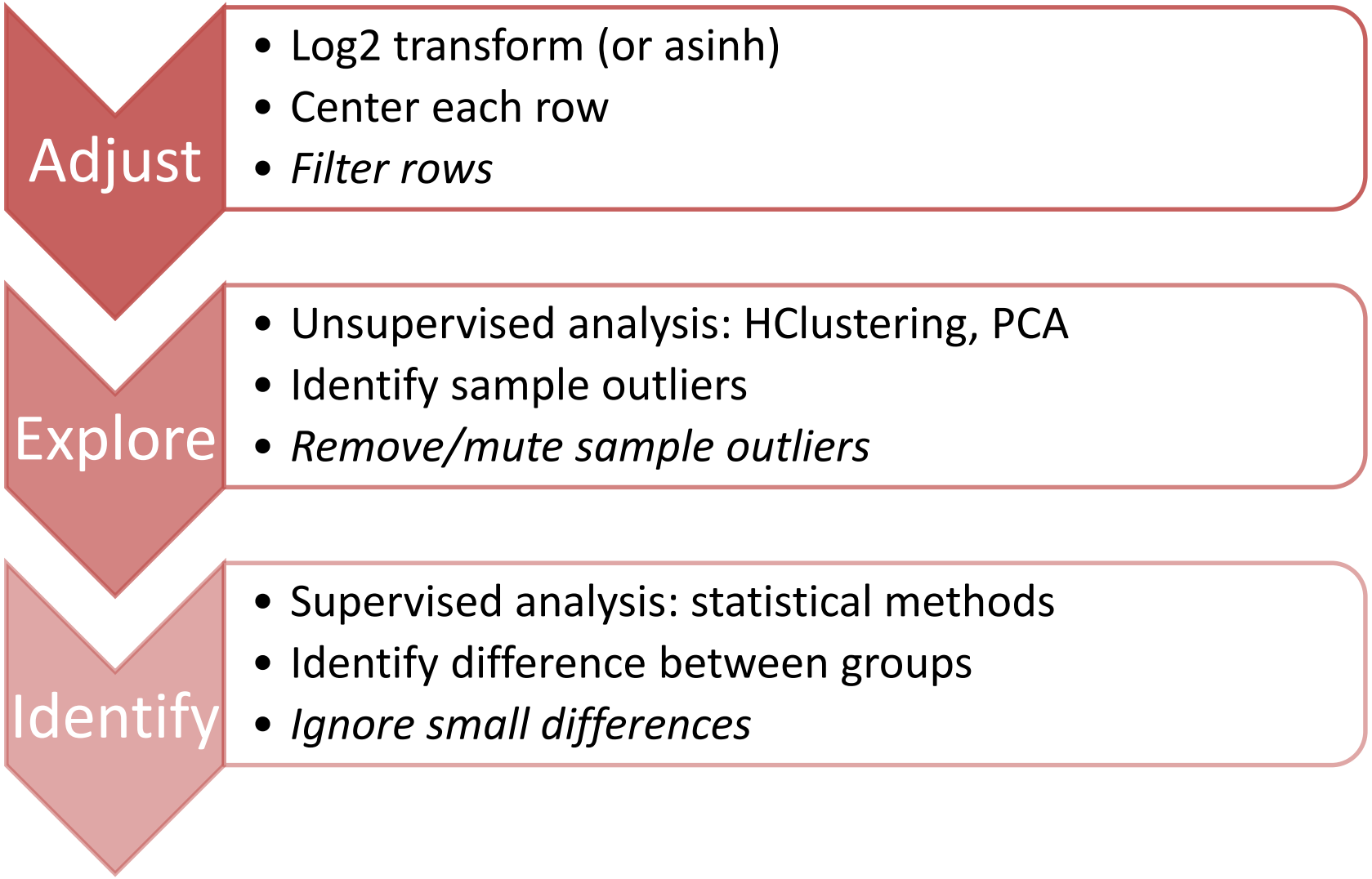
MeV capabilities



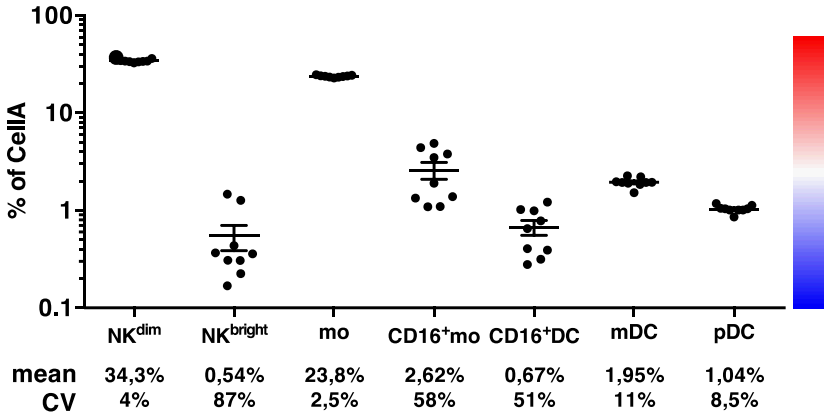


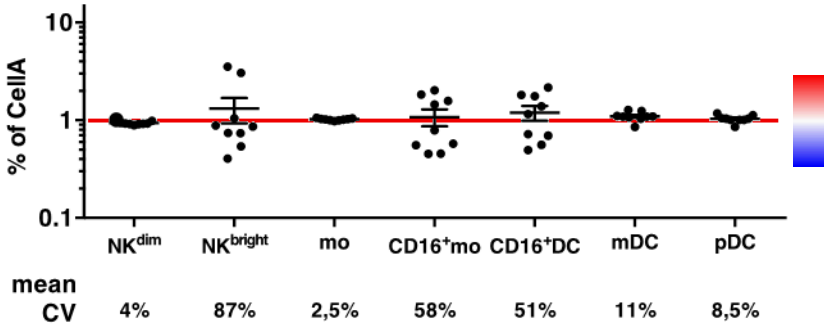


Analysis Pipeline



Adjust





- Percentages are usually displayed in a log scale
- some MFI also
- Luminex concentr.

- Centering focus on differences between groups within populations

Log2 properties

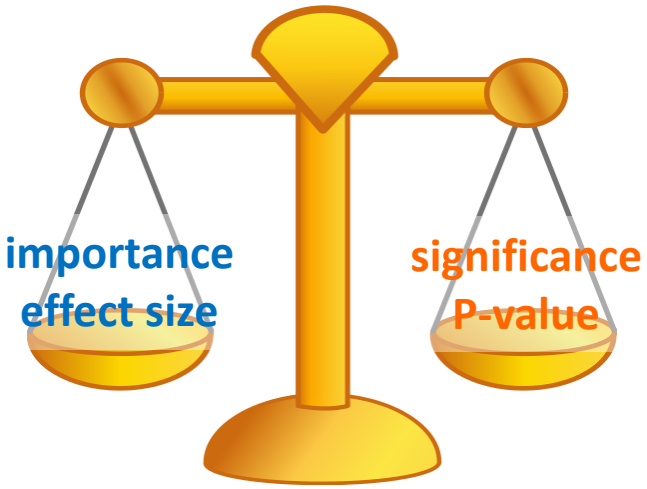
- log2 is proportional to log10
- 1 qRT-PCR cycle ~ x 2
- ratio => addition
  x 2 => + 1

- is symmetric: +100% = x 2 = +1
  - 50% = / 2 = -1
- stabilizes the dispersion
- log2(a / b) = log2(a) - log2(b)

Statistics

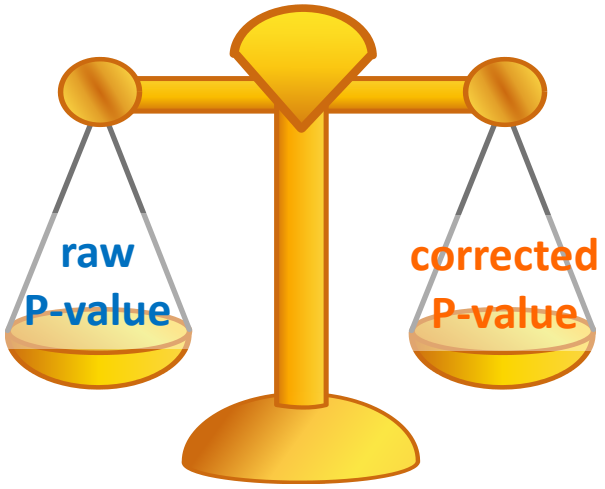
- Important, Unavoidable
- Abusive or abused
  - » Statistics does not tell us whether we are right. It tells us the chances of being wrong.
- Point Of Significance in Nat. Meth.
  - » http://mkweb.bcgsc.ca/pointsofsignificance/
  - » http://blogs.nature.com/methagora/2013/08/giving_statistics_the_attention_it_deserves.html

Statistical trade-offs



Volcano Plot

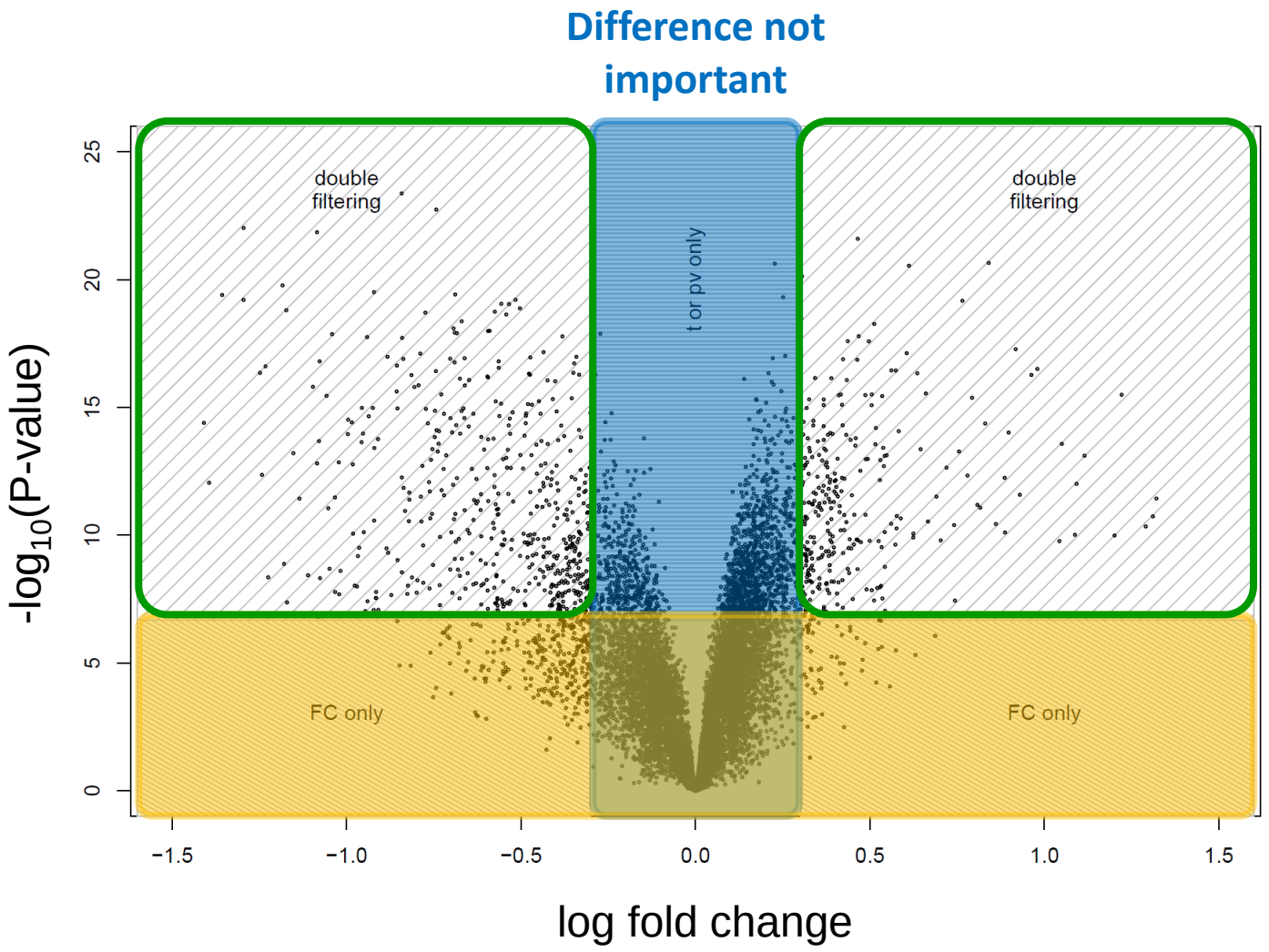
report important and significant



False Discovery Rate

correct multiple tests

FC vs P: Volcano plot



Volcano Plots in Analyzing Differential Expressions with mRNA Microarrays

Wentian Li

More about multiple testing

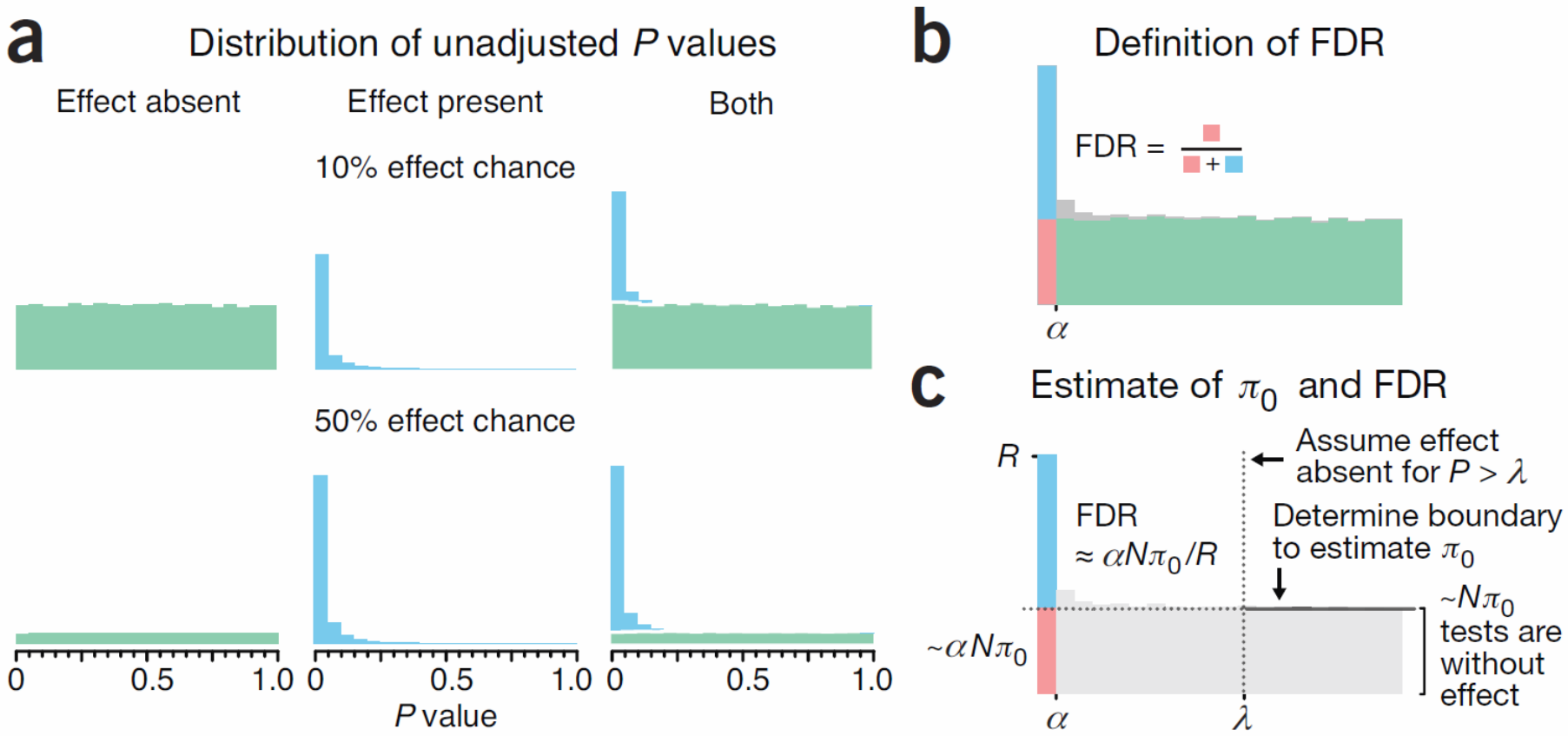


Figure 3 | The shape of the distribution of unadjusted *P* values can be used to infer the fraction of hypotheses that are null and the false discovery rate (FDR).

http://mkweb.bcgsc.ca/pointsofsignificance/

Nat. Methods

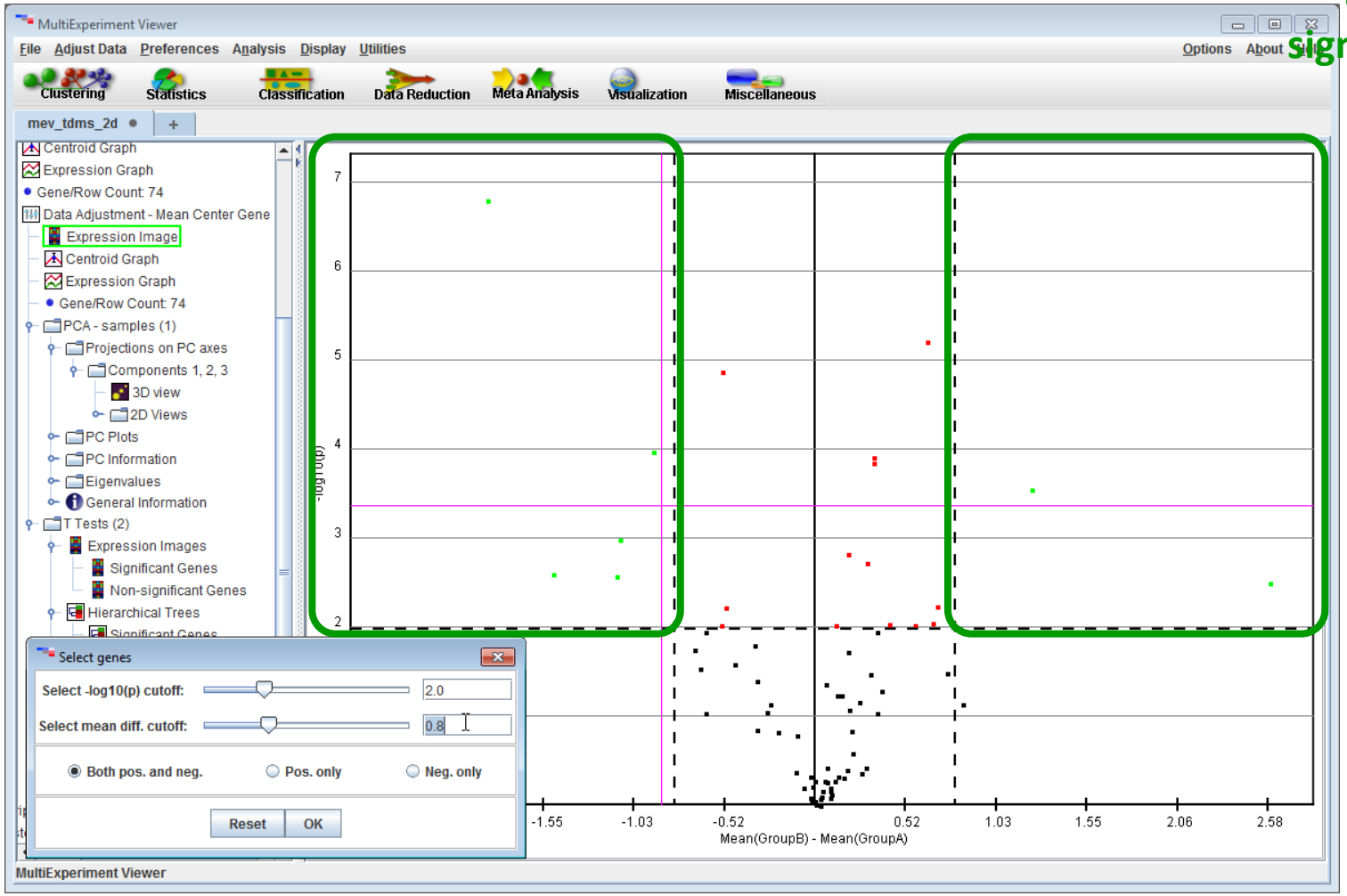
What is "significance"?

- Statistical significance is not the same as practical importance.
- P-value does not tell whether the result is of a **practical importance**.
- Statistics does not tell us whether we are right. It tells us the chances of being wrong.
- Any particular threshold for declaring significance is arbitrary.

A Difference, to Be a Difference, Must Make a Difference
Gertrude Stein

Difference threshold

important and significant



CLUSTERING ALGORITHMS IN CYTOFKIT

ClusterX

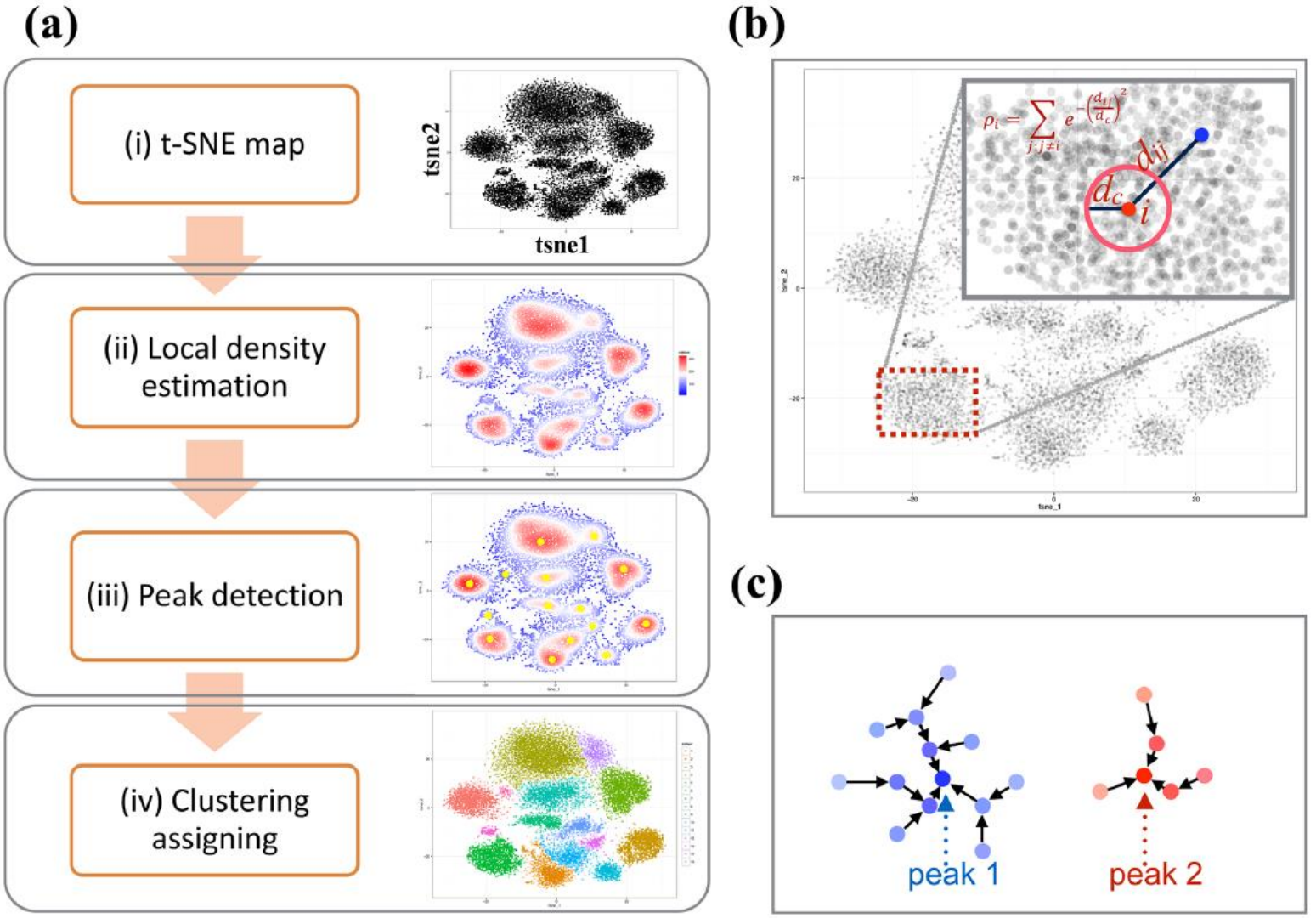
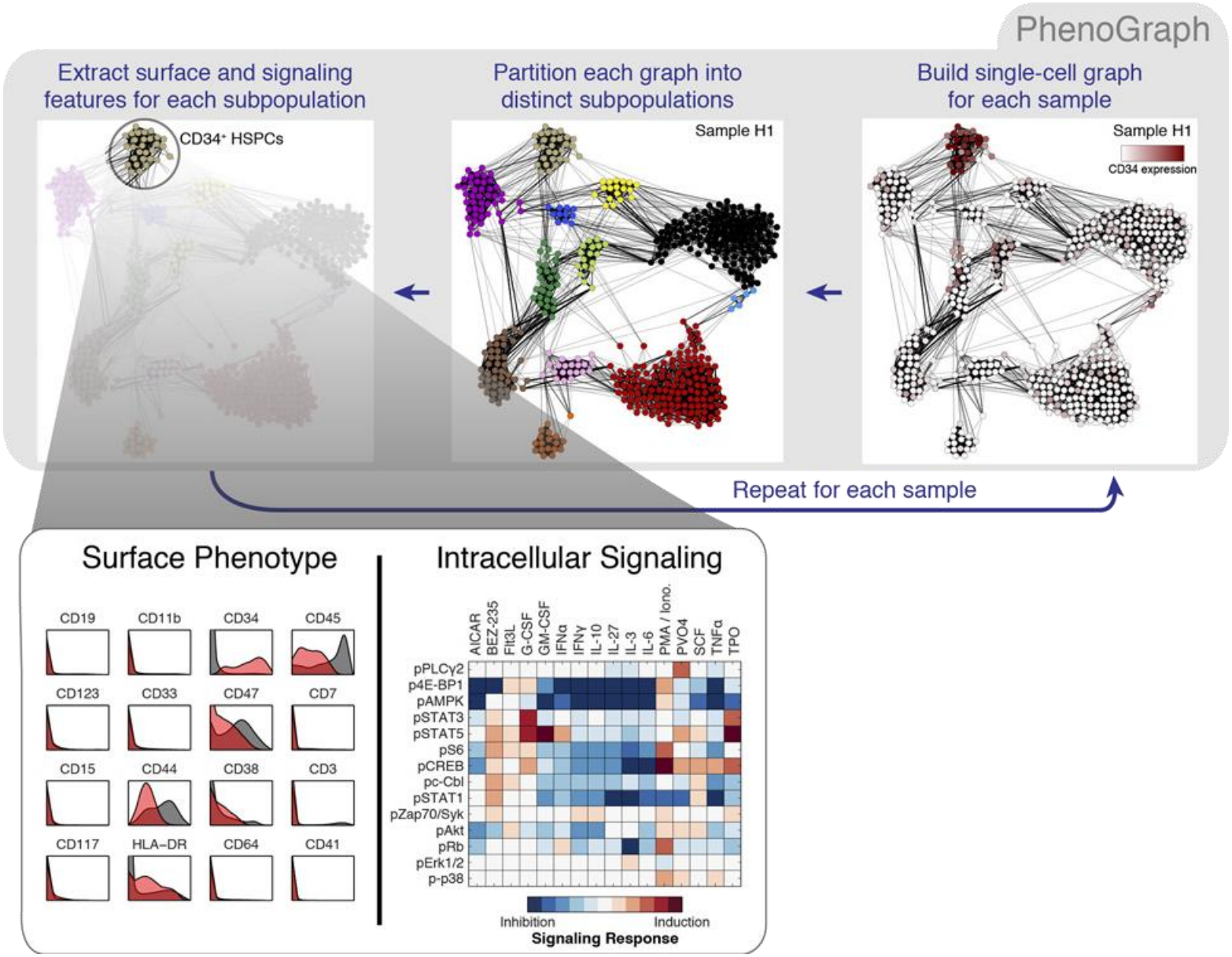


Fig 2. Workflow of ClusterX for mass cytometry data clustering. (a) depict the workflow of ClusterX for mass cytometry data clustering, which contains four steps: (i) t-SNE dimensionality reduction (ii) estimate the local density on the t-SNE map (iii) detect the density peaks represented as cluster centers and (iv) assign the remaining cells to clusters. (b) Explains the local density estimation method. (c) Illustrate the cluster assigning step using two peaks, peak1 and peak 2. Each point is a cell and the color intensity represents the local density of the cell. Then each cell is assigned to be the same cluster as its nearest neighbor cell which has higher density than it.

Phenograph


PhenoGraph
Extract surface and signaling features for each subpopulation
Partition each graph into distinct subpopulations
Build single-cell graph for each sample
CD34⁺ HSPCs
Sample H1
Sample H1
CD34 expression
Repeat for each sample
Surface Phenotype
CD19
CD11b
CD34
CD45
CD123
CD33
CD47
CD7
CD15
CD44
CD38
CD3
CD117
HLA-DR
CD64
CD41
Intracellular Signaling
AICAR
BEZ-235
Flt3L
G-CSF
GM-CSF
IFNα
IFNγ
IL-10
IL-27
IL-3
IL-6
PMA / Iono.
PVO4
SCF
TNFα
TPO
pPLCγ2
p4E-BP1
pAMPK
pSTAT3
pSTAT5
pS6
pCREB
pc-Cbl
pSTAT1
pZap70/Syk
pAkt
pRb
pErk1/2
p-p38
Inhibition
Induction
Signaling Response

Résumé

- ClusterX
  - recherche de sommets et de montagnes
  - Rodriguez et Laio Science 2014
- Phenograph
  - recherche de groupes dans les réseaux sociaux
  - Levine et al. Cell 2015 Web
- FlowSOM
  - déformation d'une grille pour l'adapter aux données
  - van Gassen et al. Cyto A 2015 Web