High-dimensional single cell analysis by mass cytometry - introduction and call for collaboration

Vinko Tosevski, Ph.D.
Head of Mass Cytometry Facility
University of Zurich

University of Zurich[UZH]

Mass Cytometry Facility

Disclaimer

- I declare no conflict of interest
  - Products
  - Services
  - …

Hypothesis

- You do single cells analysis
- You are open to new technologies that can advance your research
- You didn’t come across mass cytometry before

Outline

- What is mass cytometry?
- What can be done with it?
- What does it take to start doing it?
- How can we assist you in doing it?
  - Who are “we”?

What is mass cytometry?

- Novel high-dimensional analysis technology that allows highly multiplexed measurement of protein and RNA content at the single cell level
- Novel
  - 1st prototype published in 2009
  - Currently around 150 instruments worldwide
- High-dimensional
  - 130 paramters (measurement channels) *possible*

What is mass cytometry?

analytical chemistry

Search | Citation | Subject | Advanced Search

Enter search text / DOI | Anywhere | Search

Anal. Chem. | All Publications/Website

Subscriber access provided by UZH Hauptbibliothek / Zentralbibliothek Zuerich

Browse the Journal | Articles ASAP | Current Issue | Multimedia | Submission & Review | Open Access | About the Journal

Article

Previous Article | Next Article | Table of Contents

Mass Cytometry: Technique for Real Time Single Cell Multitarget Immunoassay Based on Inductively Coupled Plasma Time-of-Flight Mass Spectrometry

Dmitry R. Bandura*†, Vladimir I. Baranov†, Olga I. Ornatsky†, Alexei Antonov‡, Robert Kinach†, Xudong Lou†, Serguei Pavlov‡, Sergey Vorobiev‡, John E. Dick§ and Scott D. Tanner†‡

Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario M5S 3H6, Canada, DVS Sciences, Inc., 70 Peninsula Crescent, Richmond Hill, Ontario L4S 1Z5, Canada, and University Health Network, Toronto Medical Discovery Tower, 101 College Street, Toronto, Ontario M5G 1L7, Canada

Anal. Chem., **2009**, *81* (16), pp 6813–6822

DOI: 10.1021/ac901049w

Publication Date (Web): July 14, 2009

Copyright © 2009 American Chemical Society

openURL

* Corresponding author. Phone: +1 416 946 8420. Fax: +1 416 978 4317. E-mail: Dmitry.Bandura@utoronto.ca., † University of

Article Options

PDF (934 KB) | PDF w/ Links (367 KB) | Full Text HTML

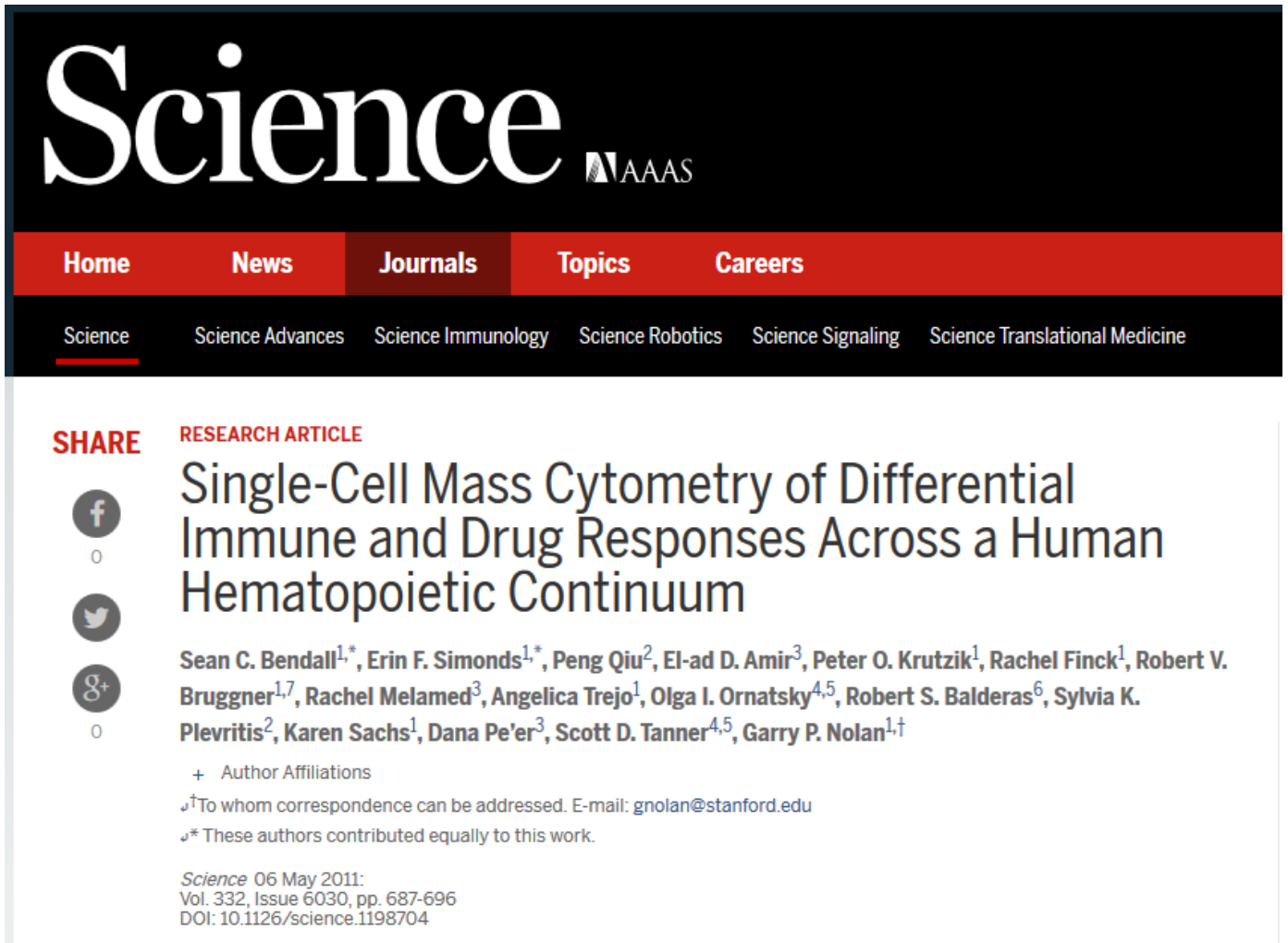
Abstract | Figures | References | Citing Articles

Add to ACS ChemWorx

University of Zurich[UZH]

Mass Cytometry Facility

What is mass cytometry?



Science AAAS

Home News Journals Topics Careers

Science Science Advances Science Immunology Science Robotics Science Signaling Science Translational Medicine

SHARE

RESEARCH ARTICLE

Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum

Sean C. Bendall[1,*], Erin F. Simonds[1,*], Peng Qiu[2], El-ad D. Amir[3], Peter O. Krutzik[1], Rachel Finck[1], Robert V. Bruggner[1,7], Rachel Melamed[3], Angelica Trejo[1], Olga I. Ornatsky[4,5], Robert S. Balderas[6], Sylvia K. Plevritis[2], Karen Sachs[1], Dana Pe'er[3], Scott D. Tanner[4,5], Garry P. Nolan[1,†]

+ Author Affiliations

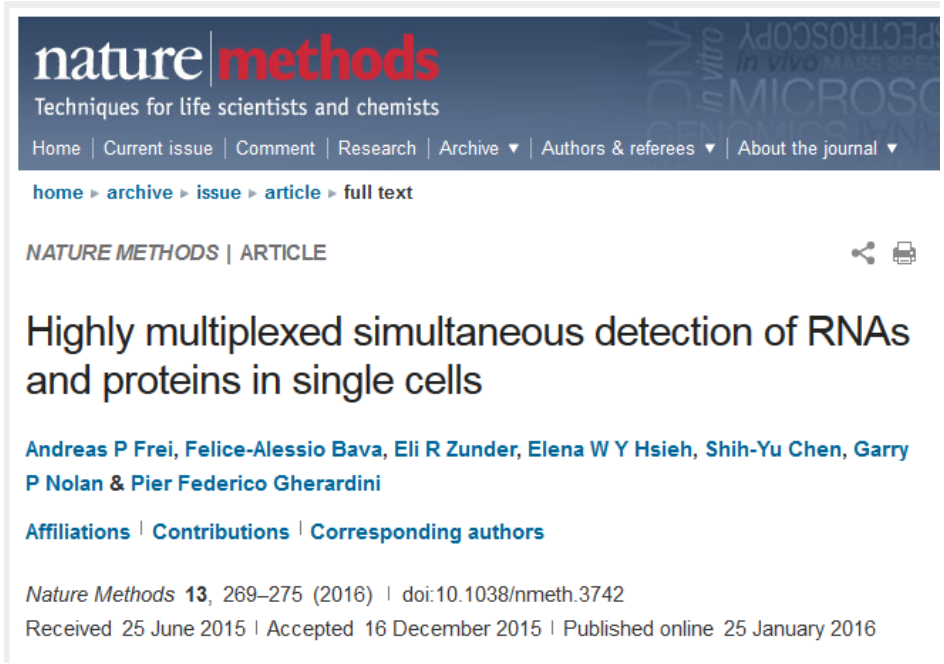
†To whom correspondence can be addressed. E-mail: gnolan@stanford.edu

* These authors contributed equally to this work.

Science 06 May 2011:
Vol. 332, Issue 6030, pp. 687-696
DOI: 10.1126/science.1198704

What is mass cytometry?

- Protein and RNA content
  - Antibody-based (proteins)
  - Nucleotide probe-based (RNA)



nature methods

Techniques for life scientists and chemists

Home | Current issue | Comment | Research | Archive ▾ | Authors & referees ▾ | About the journal ▾

home ▸ archive ▸ issue ▸ article ▸ full text

NATURE METHODS | ARTICLE

Highly multiplexed simultaneous detection of RNAs and proteins in single cells

Andreas P Frei, Felice-Alessio Bava, Eli R Zunder, Elena W Y Hsieh, Shih-Yu Chen, Garry P Nolan & Pier Federico Gherardini

Affiliations | Contributions | Corresponding authors

Nature Methods **13**, 269–275 (2016) | doi:10.1038/nmeth.3742

Received 25 June 2015 | Accepted 16 December 2015 | Published online 25 January 2016

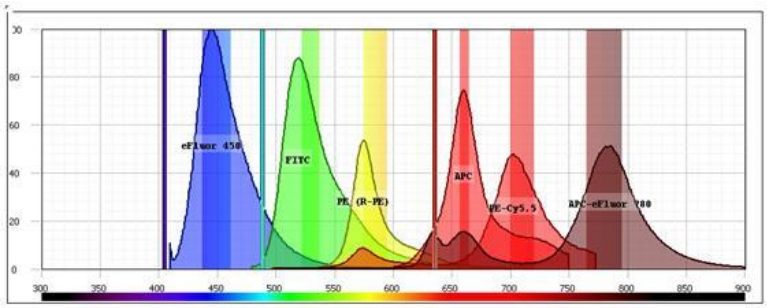
CyTOF = mass cytometry

- CyTOF - **Cy**tometry by **T**ime **o**f **F**light
- Currently, high end flow cytometers can measure up to 20 parameters simultaneously
  - FACSSymphony just came out with 30 detectors installed, but instrument performance and fluorochrome situation is unclear at the moment.
- CyTOF currently measures around 50 parameters!

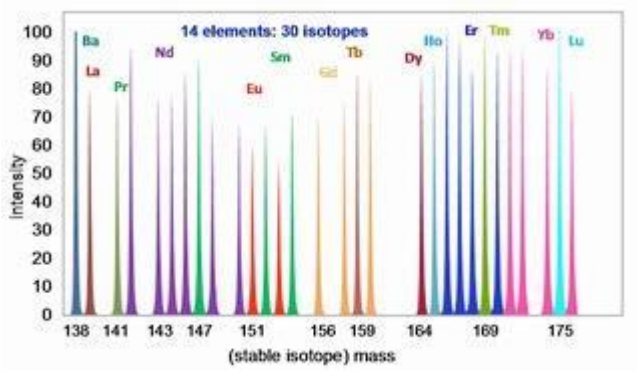
CyTOF

- How was this leap in dimensionality achieved?
  - Instead of fluorochromes, antibodies are tagged with stable metal isotopes (Lanthanides)

vs.



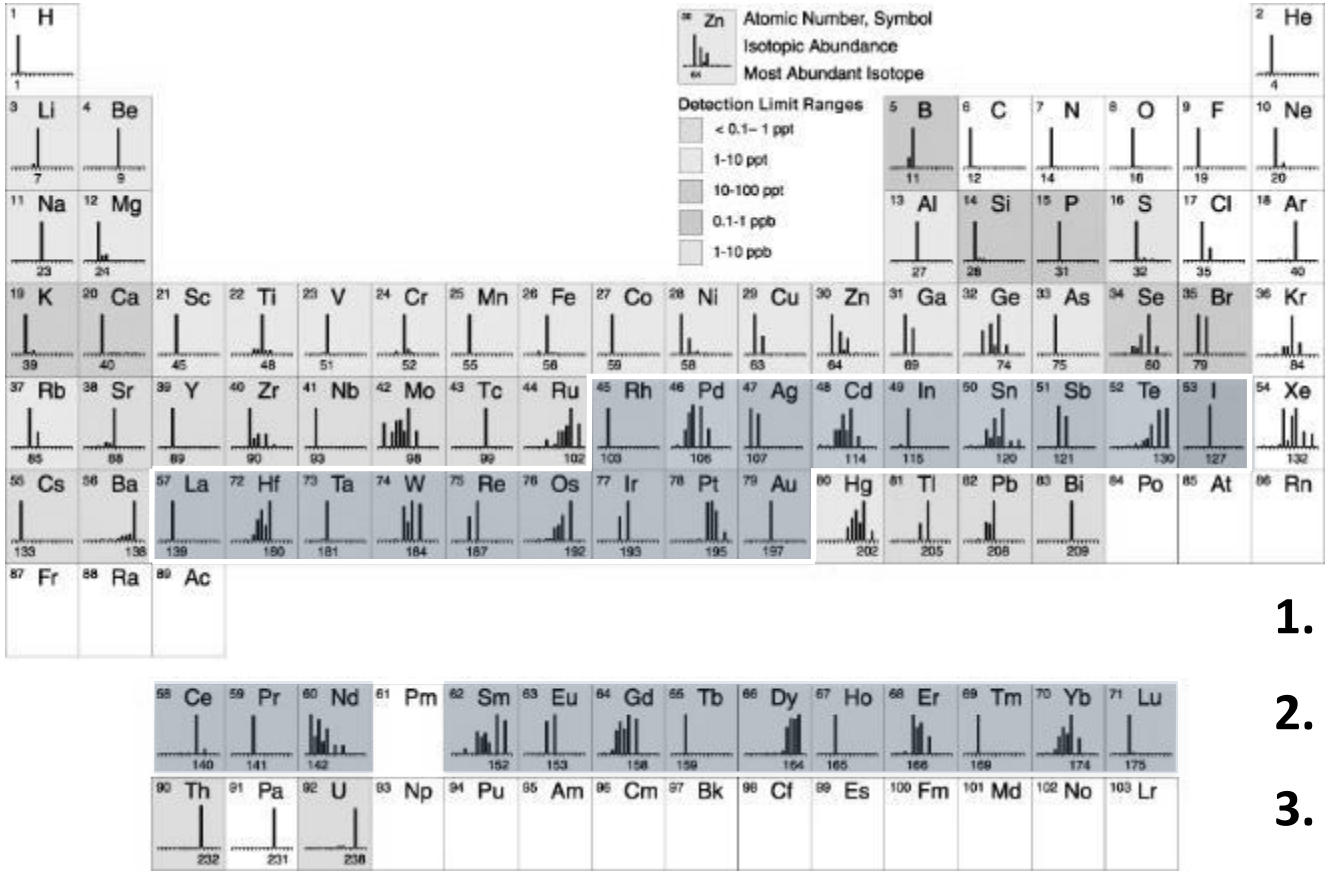
vs.



Source:BD, Fluidigm

Possibly useful elements

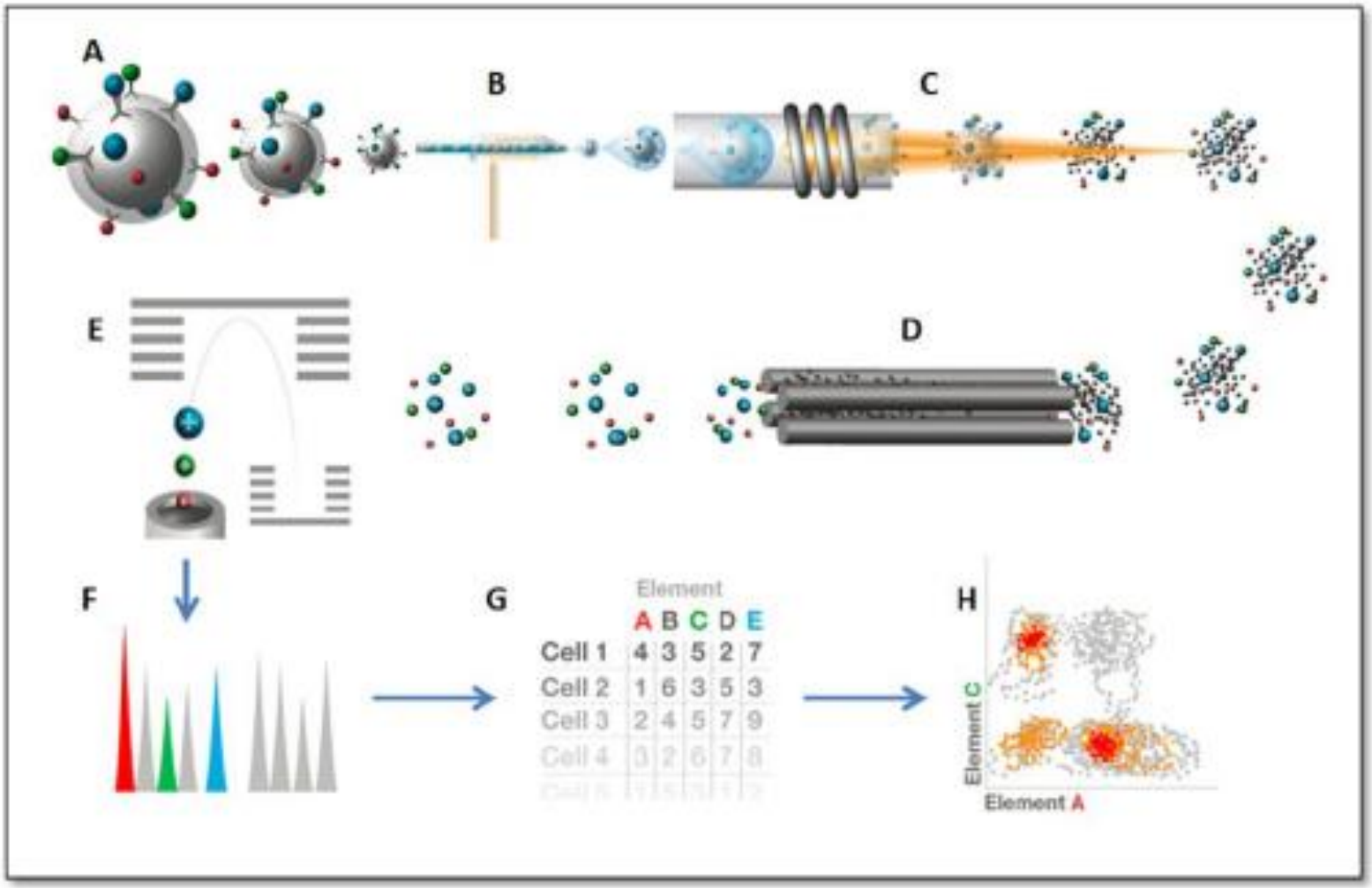
Source: Fluidigm



1. **Non-rare**
2. **Non-biological**
3. **Non-radioactive**

Overview

Source: Fluidigm



Pros and cons

- A lot of limitations stems from the fact that ions are measured the way they are
- Pros
  - Many channels
  - Lower «autofluorescence –like» background
- Cons
  - Slower (cells/sec)
  - Less sample transmission efficency
  - Less per-channel sensitivity (not really a problem)

Pros and cons

- Cons
  - …
  - Dependence on Abs (dominantly but not exclusively, i.e. cell cycle or proliferation analyses work beautifully with IdU directly).

Side-by-side comparison

Source: Bendall SC, Nolan GP, Roederer M, Chattopadhyay PK. A deep profiler's guide to cytometry. Trends in Immunology. 2012 Jul;33(7):323–32.

Table 1. Comparison of utility and performance of state of the art commercial fluorescence flow cytometry and mass cytometry single-cell analysis platforms.

Technology		Fluorescence flow cytometry	Mass cytometry
Measurement basis		Fluorescent probes	Stable mass isotope probes
Experimental design			
Max no. of measurements		20 (18 fluorescence)	37 (including DNA)
Theoretical no. of subsets[a]		2.6×10^5	1.4×10^{11}
Panel design complexity (no. of probes)	Easy	<8	37
	Moderate	8–12	
	Hard	12–18	
Sensitivity range for different probes[b]		0.1–10	1–2
Sample throughput			
Sampling efficiency		> 95%	< 30%
Measured cells/s		25 000	500–1000
Cells/h		25–60 million	2 million
Commercial reagent cost			
Per probe per test[c]		$2.00–$8.00	$1.50–$3.00

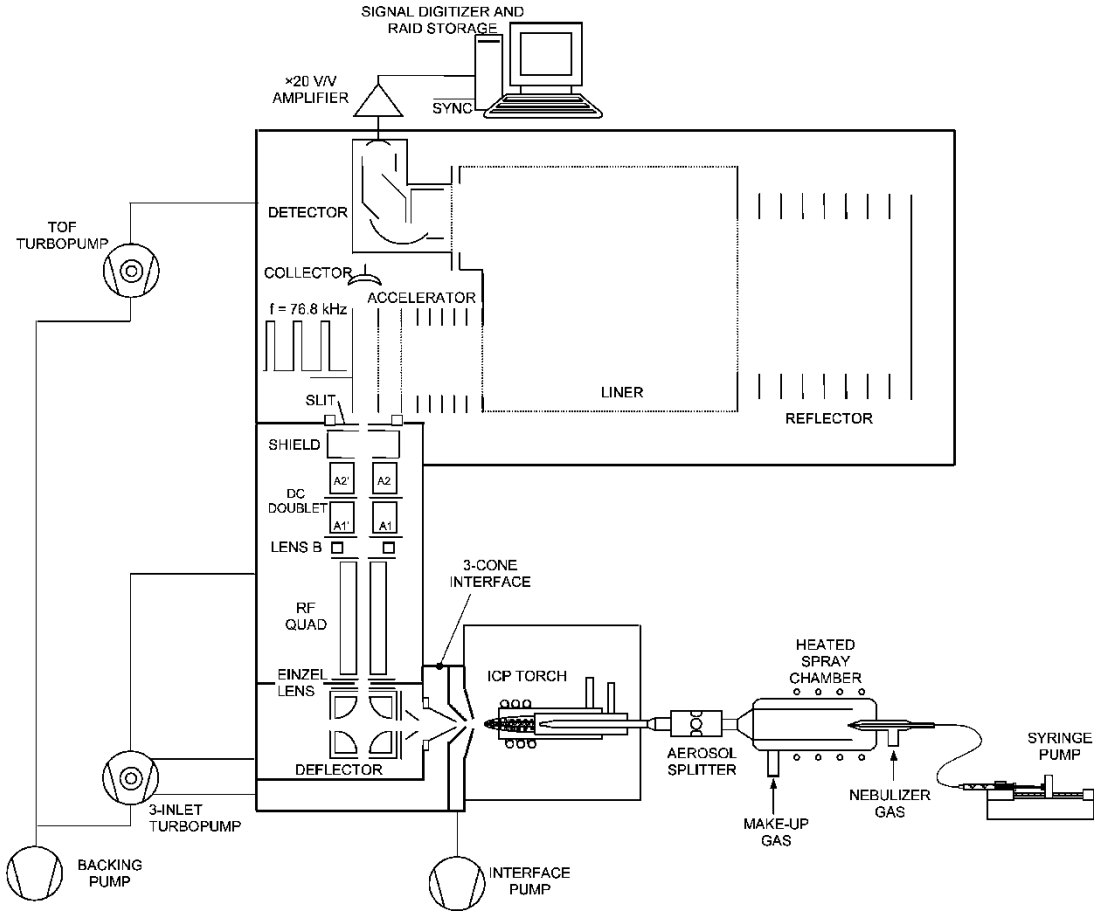
[a]Theoretical number of subsets is the number of distinct cell types determinable, assuming only on or off for each marker; that is, 2^{colors}.

[b]Sensitivity range is in arbitrary units, and compares the rough sensitivity for different probes (fluorescence or ICP-MS) to detect a given epitope on a cell by immunophenotyping.

[c]Estimated based on the price of commercially conjugated reagents or unconjugated antibodies and commercial conjugation kits.

CyTOF

Source: Source: http://pubs.acs.org/action/showImage?doi=10.1021%2Fac901049w&iName=master.img-000.png&type=master

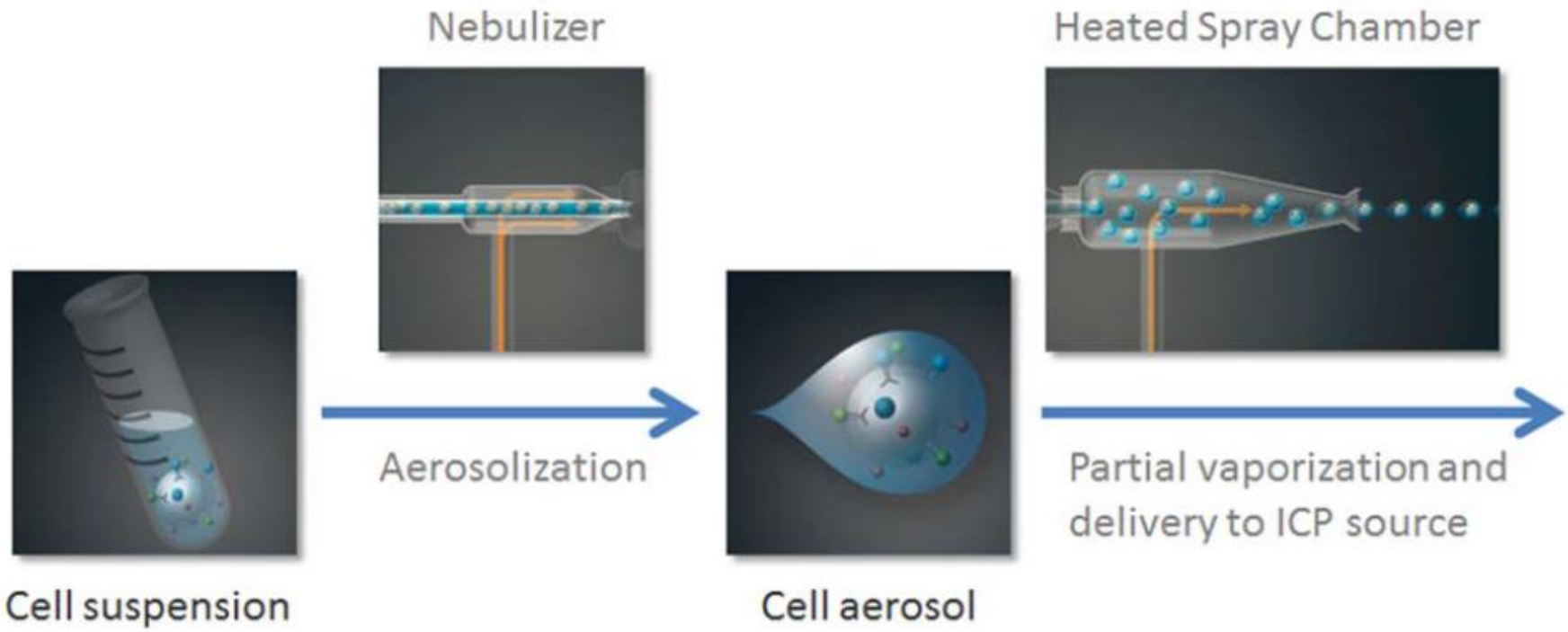


Sample introduction and ionization

- A lot of cells get lost on the way to the detector in the tubing and the glassware.
- CyTOF2 had 30% to 40% cell transmission efficiency (i.e. you have 100K cells in the tube and end up with 30K to 40K cells on the FCS file).
- Helios (3rd generation CyTOF instrument) has 50% to 70%
- Caveat! 75% on beads translates into 50% on hPBMCs (in our hands)

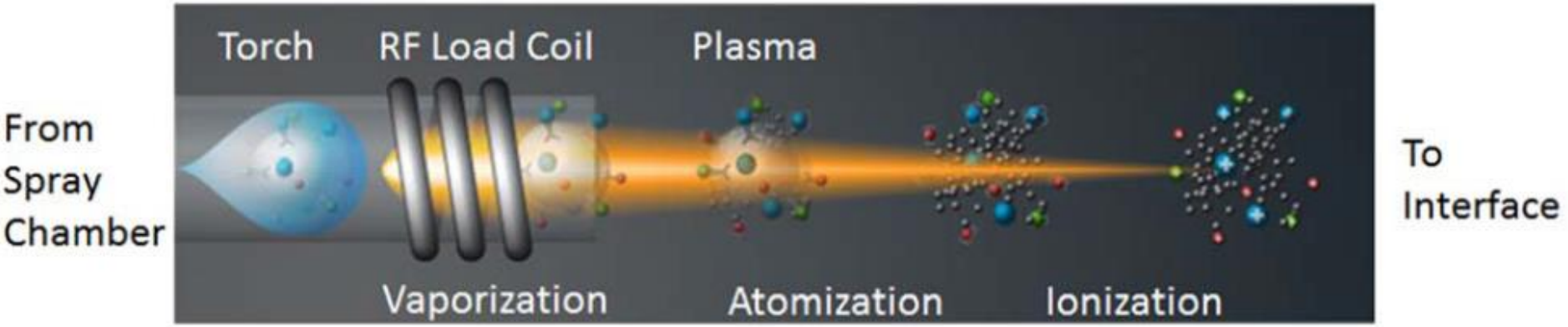
Sample introduction

Source: Fluidigm



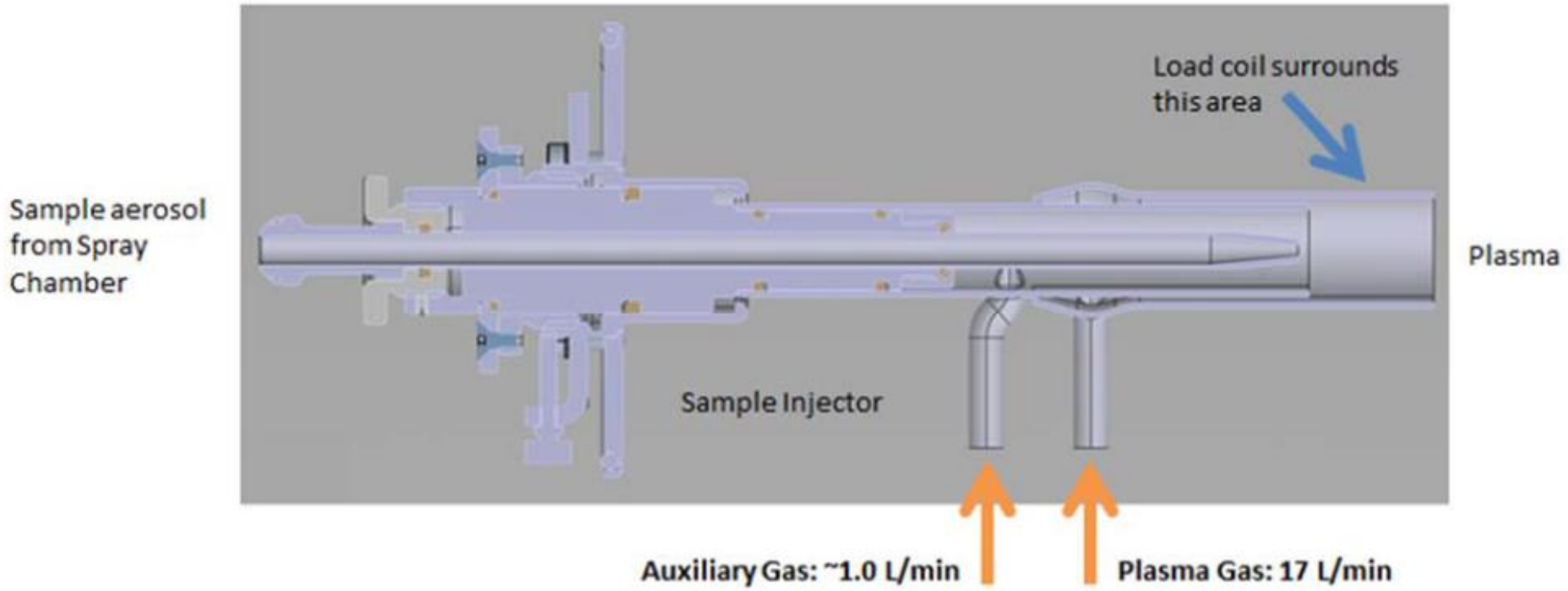
Sample introduction and ionization

Source: Fluidigm



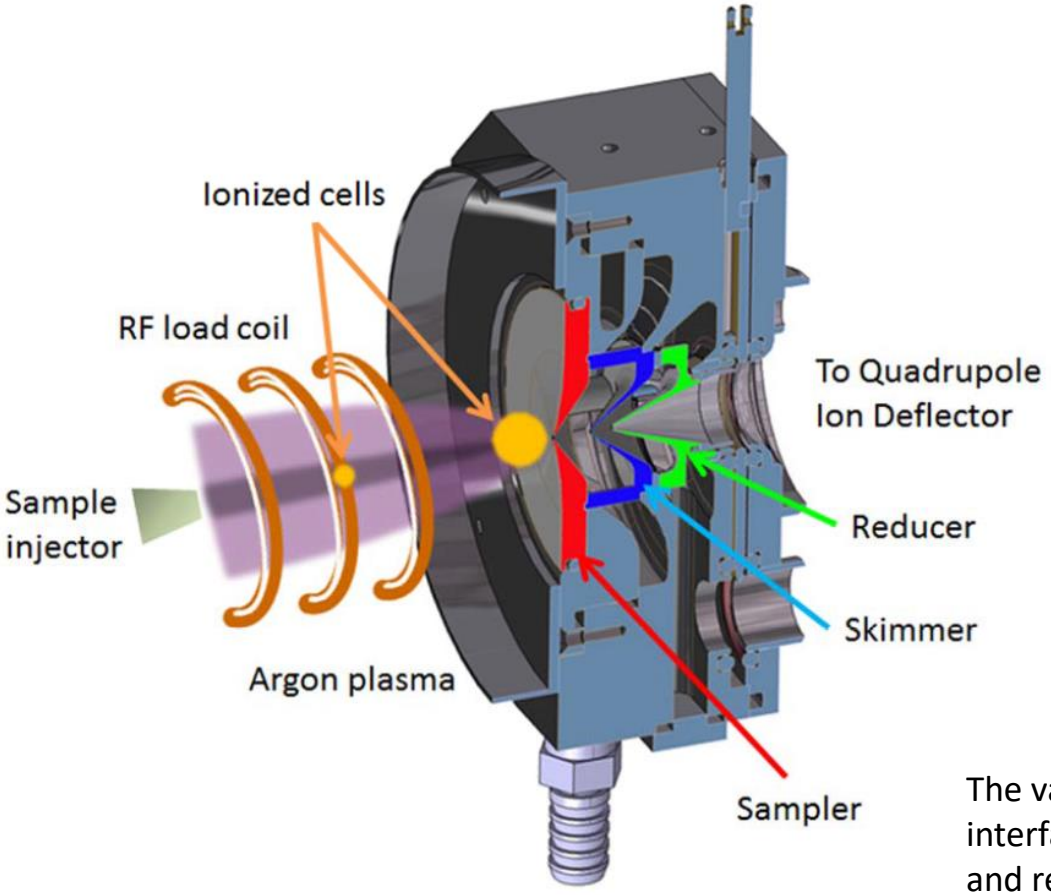
Sample introduction and ionization

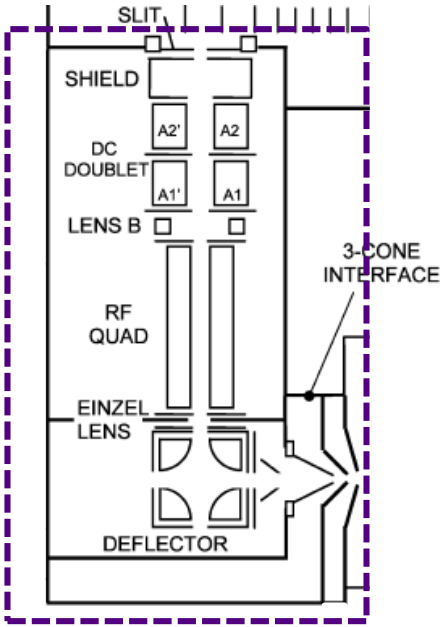
Source: Fluidigm



Processing of the ion cloud

Source: Fluidigm





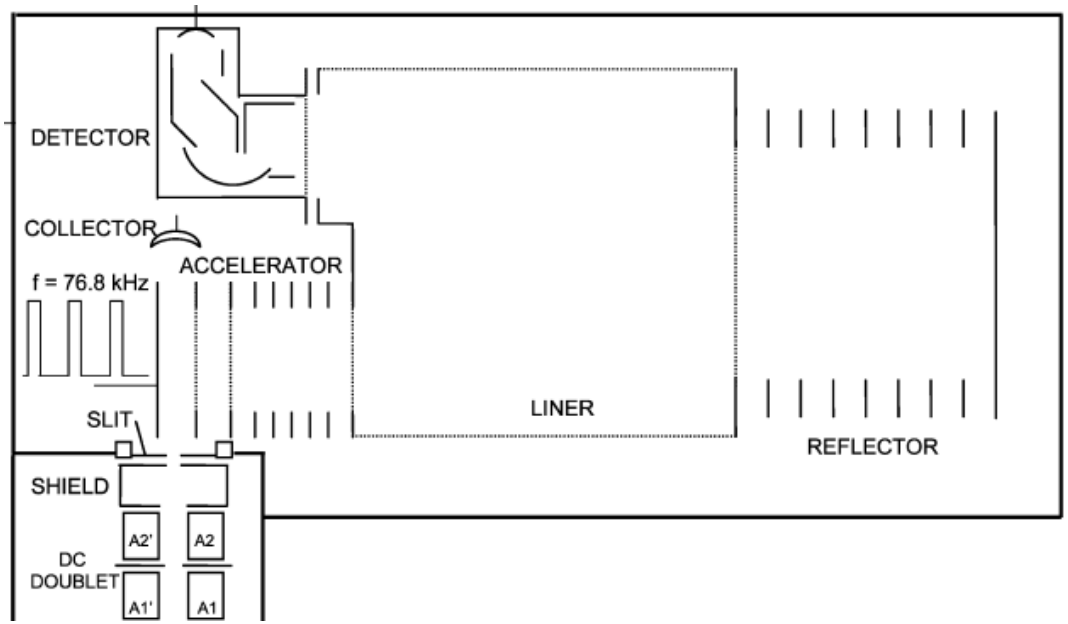
The vacuum interface includes the three nickel interface cones: sampler (red), skimmer (blue) and reducer (green).

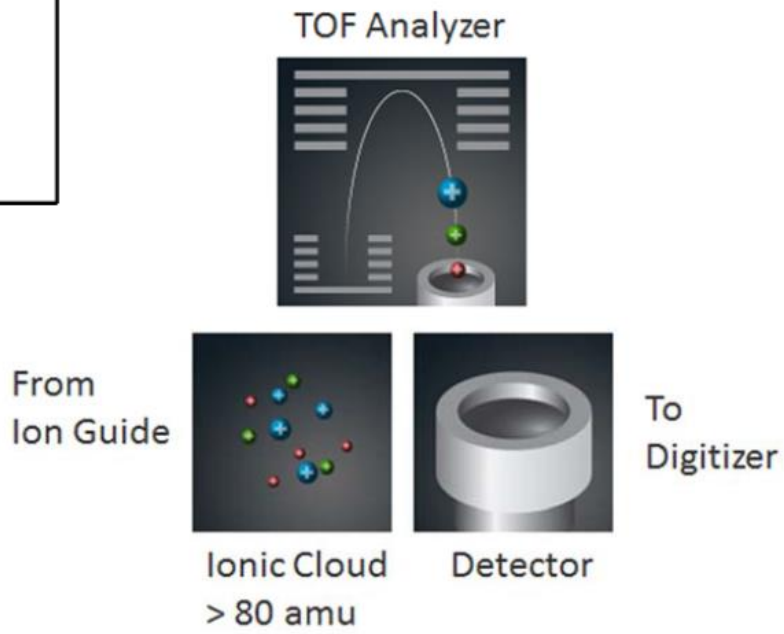
Processing of the ion cloud

Source: Fluidigm

Ion separation and detection

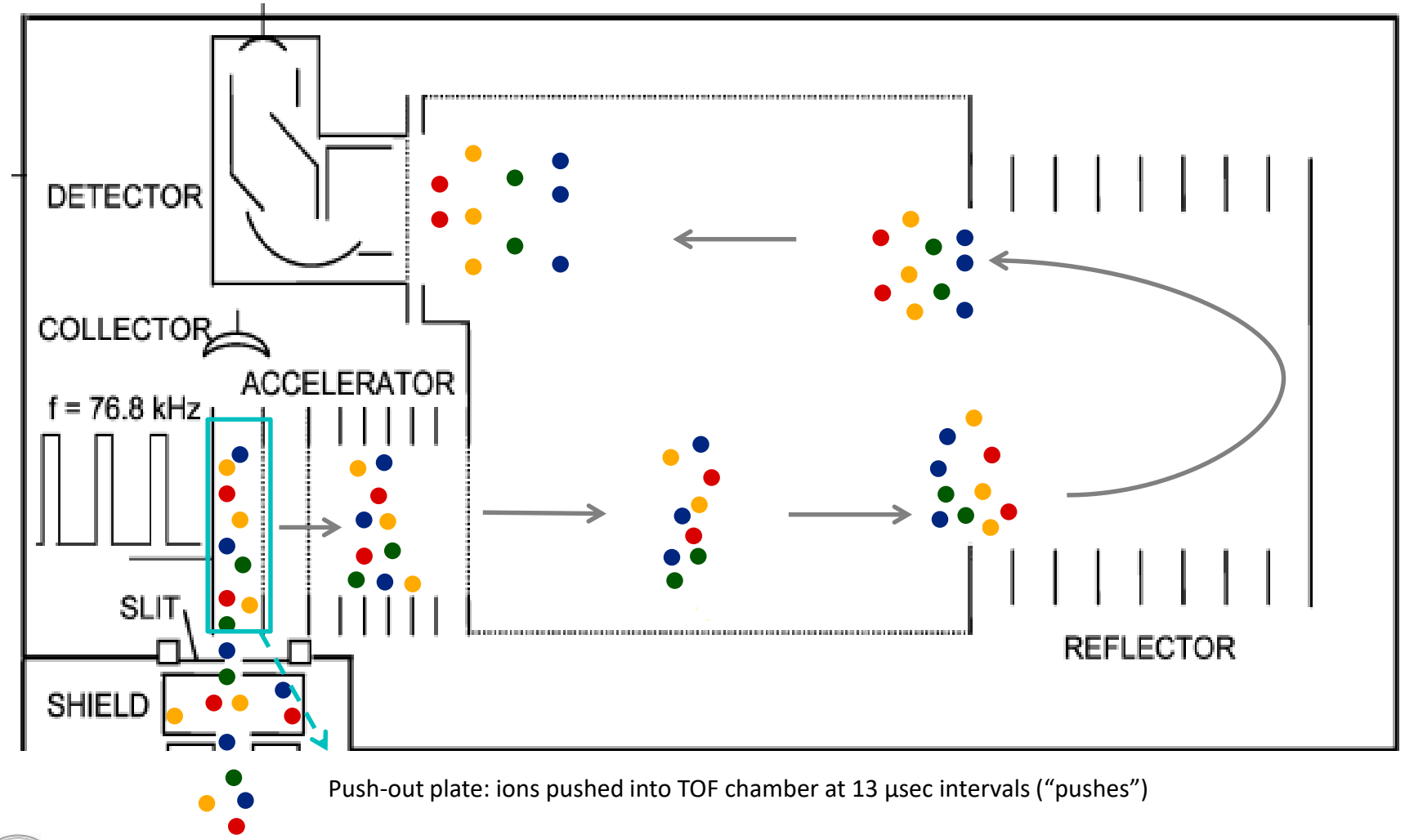
Source: Fluidigm





Ion separation and detection

Source: Fluidigm

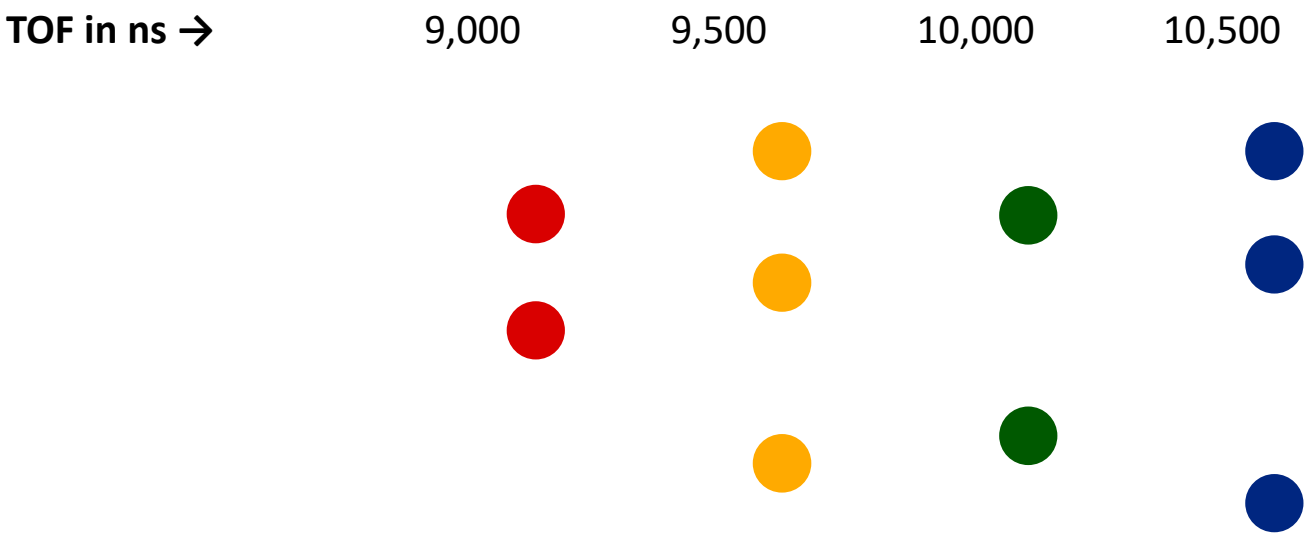


Push-out plate: ions pushed into TOF chamber at 13 µsec intervals (“pushes”)

Push

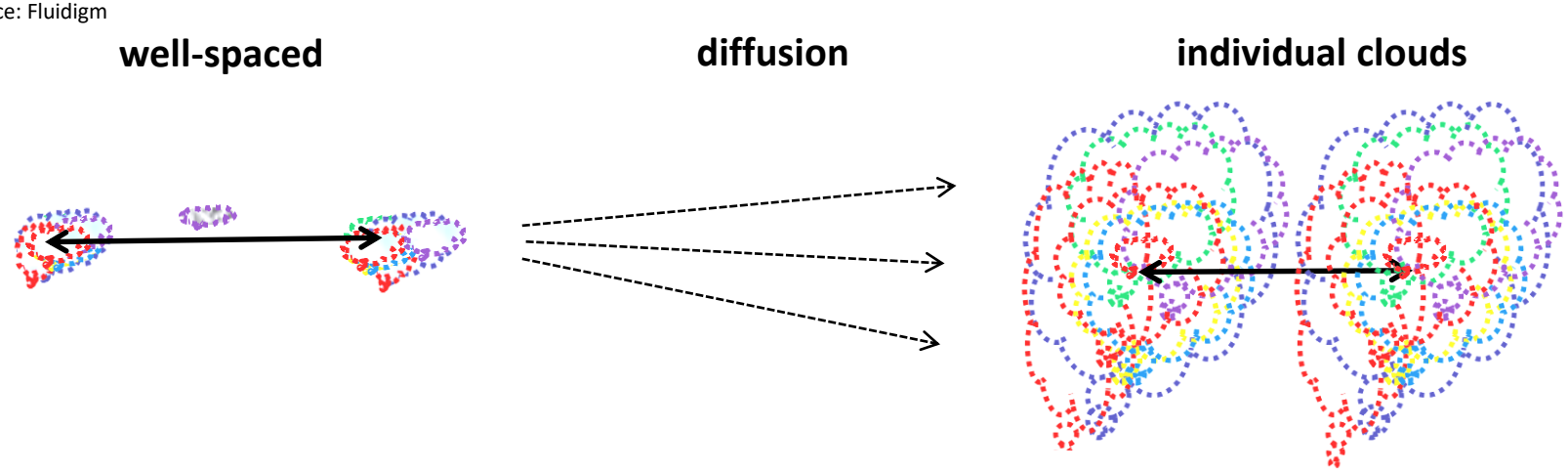
Source: Fluidigm

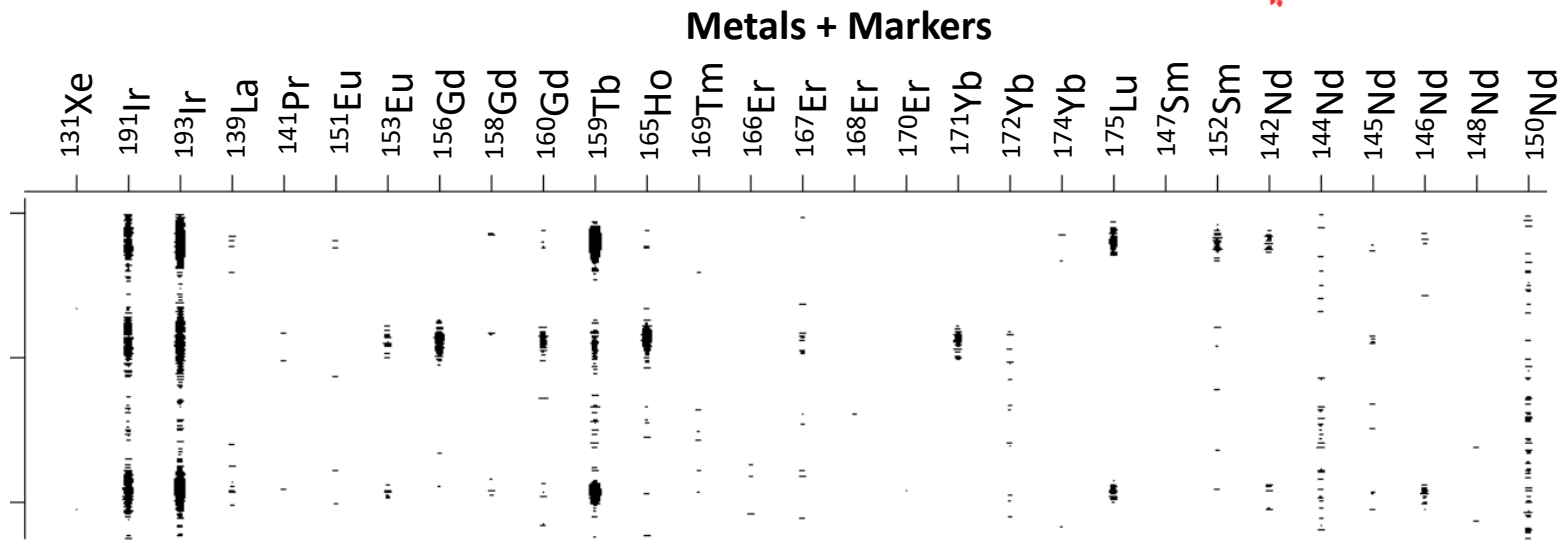
- **Pushes:** 13 µsec slice of the ion stream entering the ToF chamber.
- **TOF:** Separation of ions **within a push** by mass.



From cell to ion cloud

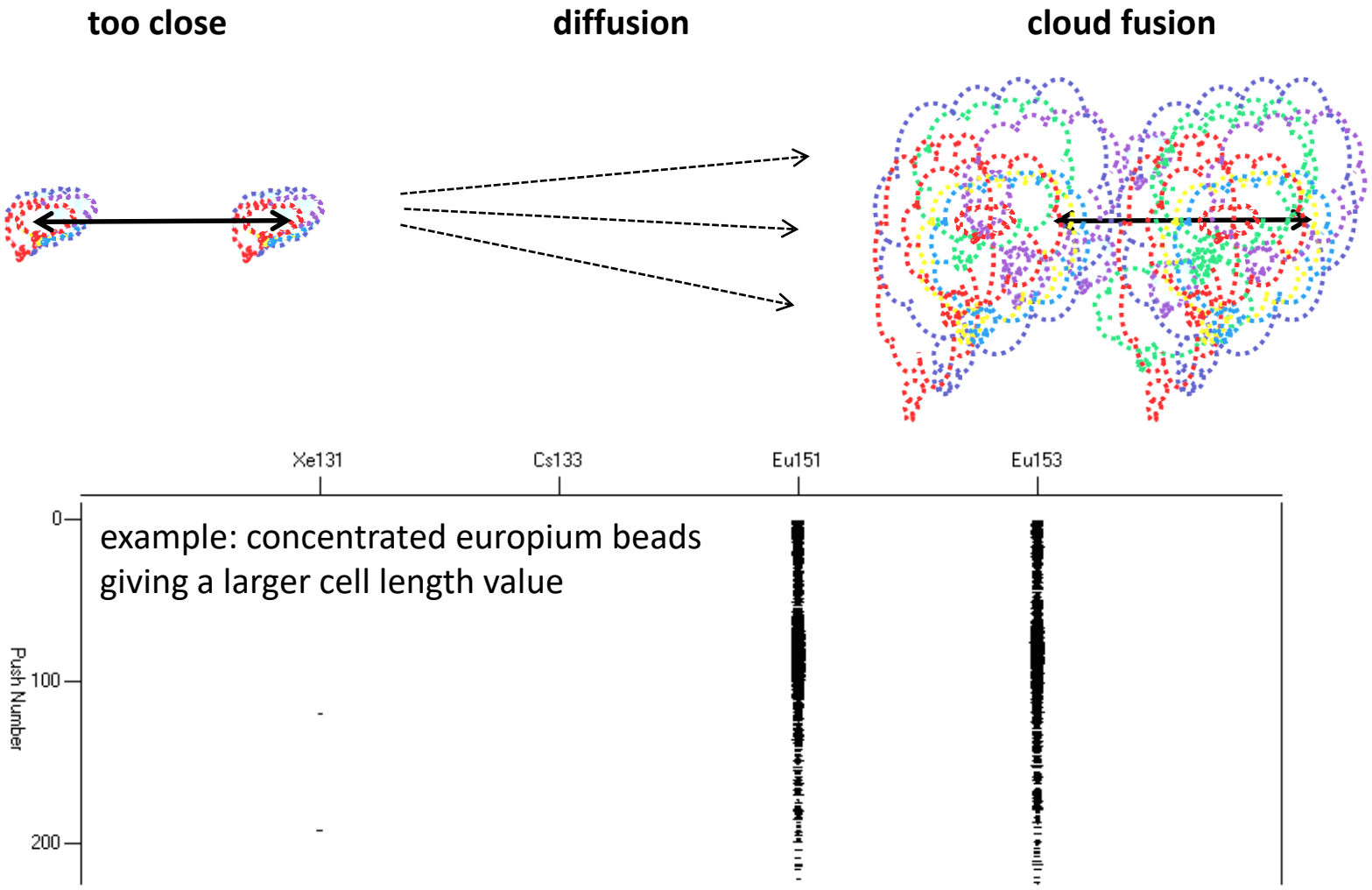
Source: Fluidigm





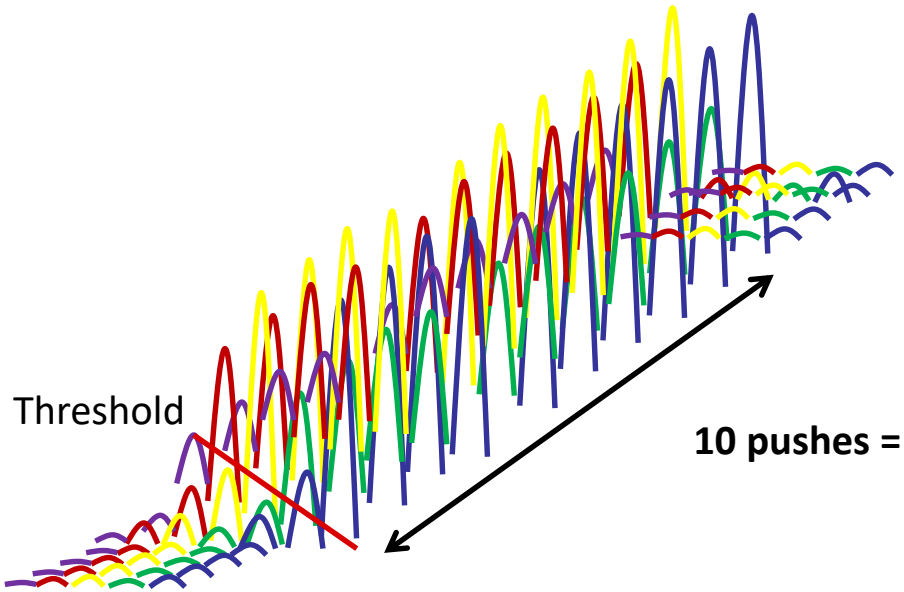
From cell to ion cloud

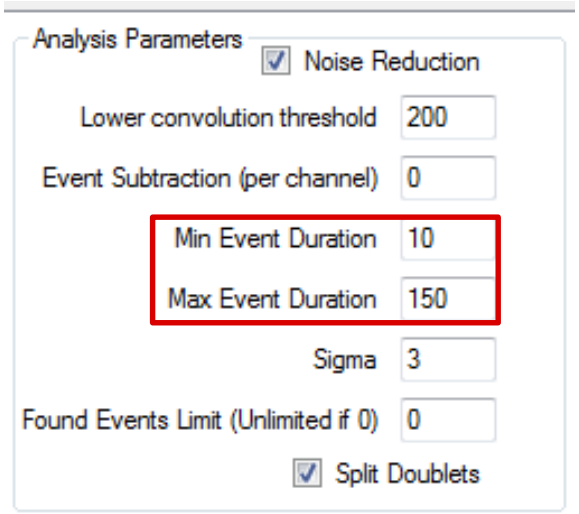
Source: Fluidigm



From cell to ion cloud

Source: Fluidigm





- Event duration is the period of time, measured in pushes, that the ion signal intensity is above the threshold.
- Any events outside the range of 10 to 150 pushes are excluded from conversion to the FCS file data set.

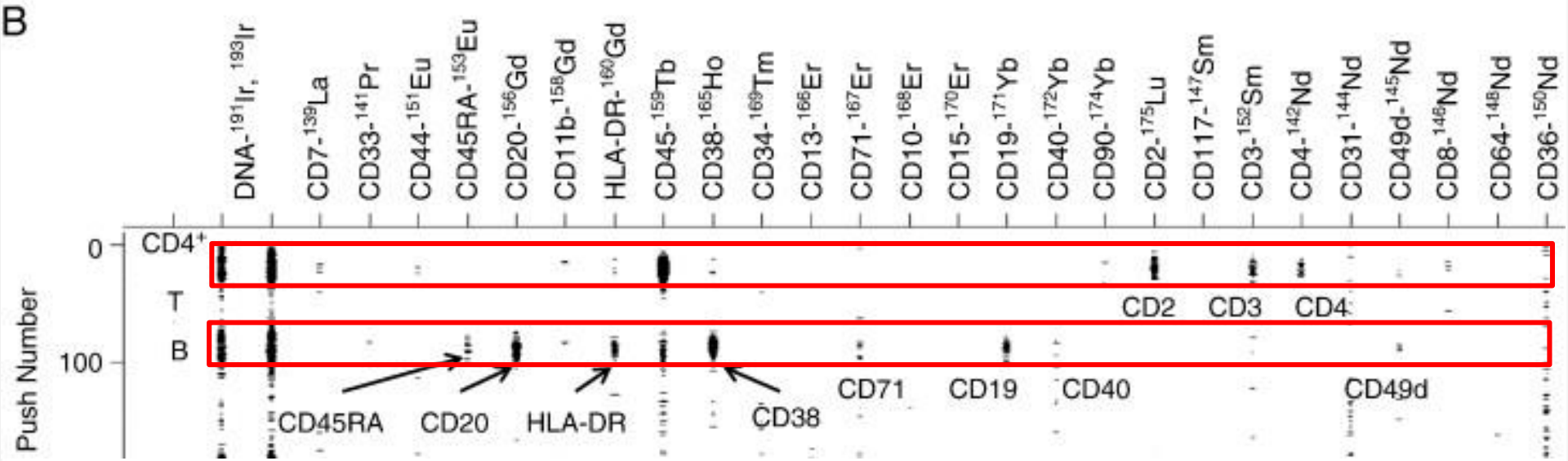
Raw data – rain plot

Source: Ornatsky et al., J Immunol Methods 2010

A

CD2-^{175}Lu	CD11b-^{158}Gd	CD33-^{141}Pr	CD45-^{159}Tb	CD90-^{174}Yb
CD3-^{152}Sm	CD13-^{166}Er	CD34-^{169}Tm	CD45RA-^{153}Eu	CD117-^{147}Sm
CD4-^{142}Nd	CD15-^{170}Er	CD36-^{150}Nd	CD49d-^{145}Nd	HLA-DR-^{160}Gd
CD7-^{139}La	CD19-^{171}Yb	CD38-^{165}Ho	CD56-^{176}Yb	DNA -^{191}Ir, ^{193}Ir
CD8-^{146}Nd	CD20-^{156}Gd	CD40-^{172}Yb	CD64-^{148}Nd	
CD10-^{168}Er	CD31-^{144}Nd	CD44-^{151}Eu	CD71-^{167}Er	

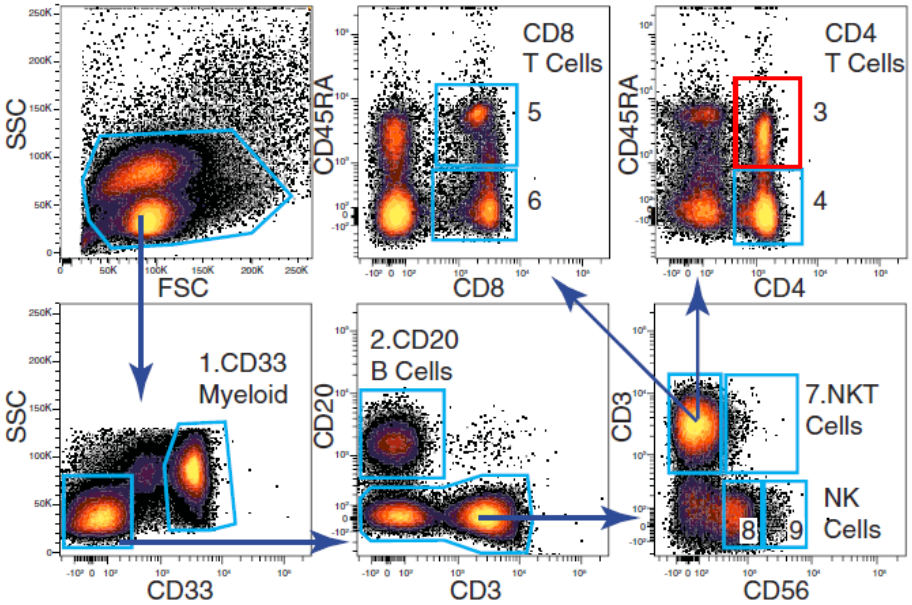
B



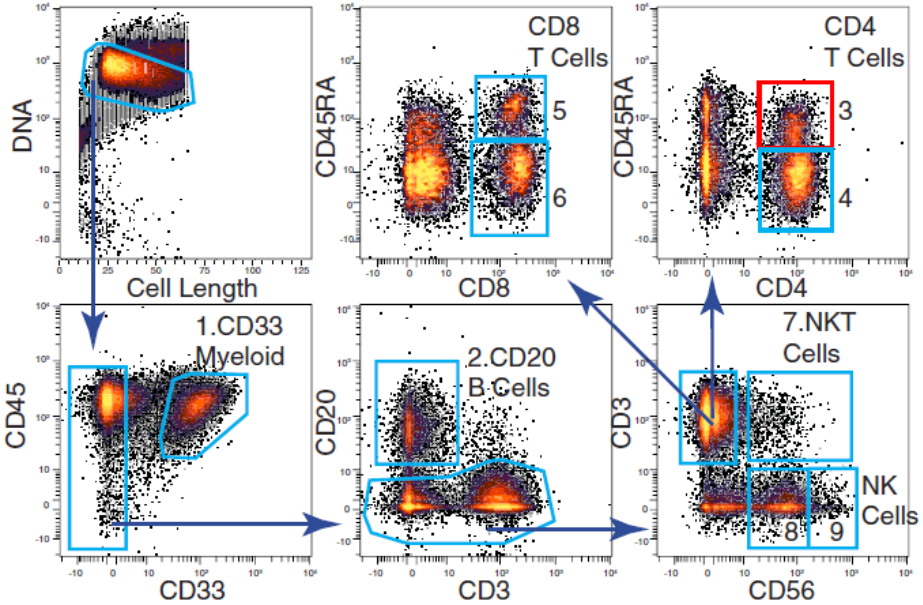
FCS file in the end

Source: Bendall, S. C. *et al.* Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum. *Science* **332,** 687–696 (2011).

Conventional flow cytometry



Mass cytometry



Application highlights

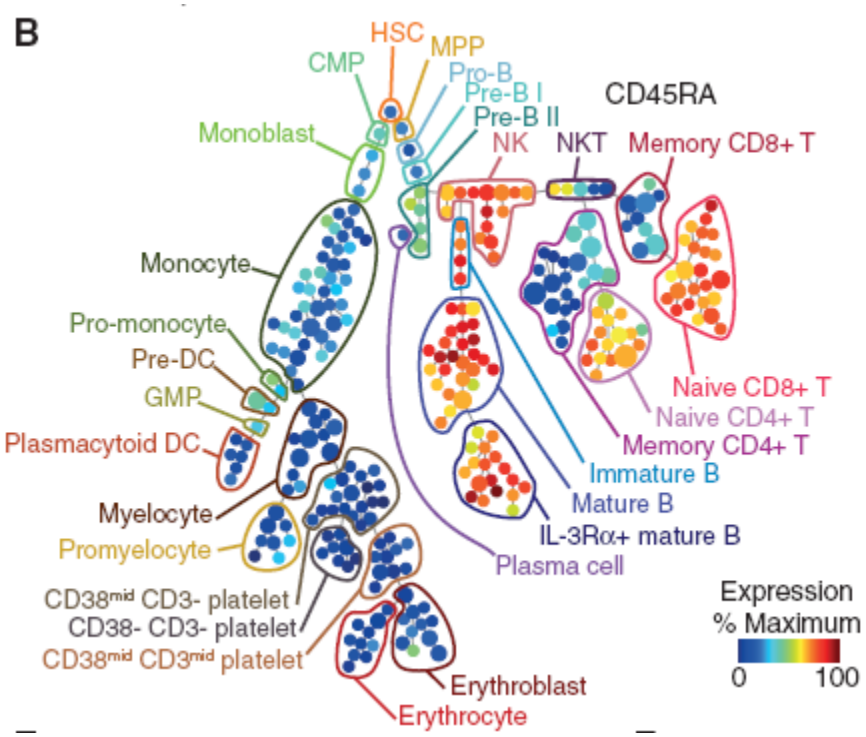
Aplications

- No sorting or calcium influx ☺
- Extensive phenotyping and functional profiling
  - Immunophenotype
  - Signaling state
  - Cytokine/chemokine expression
  - Health & Viability
  - Proliferation
  - Apoptosis
  - …

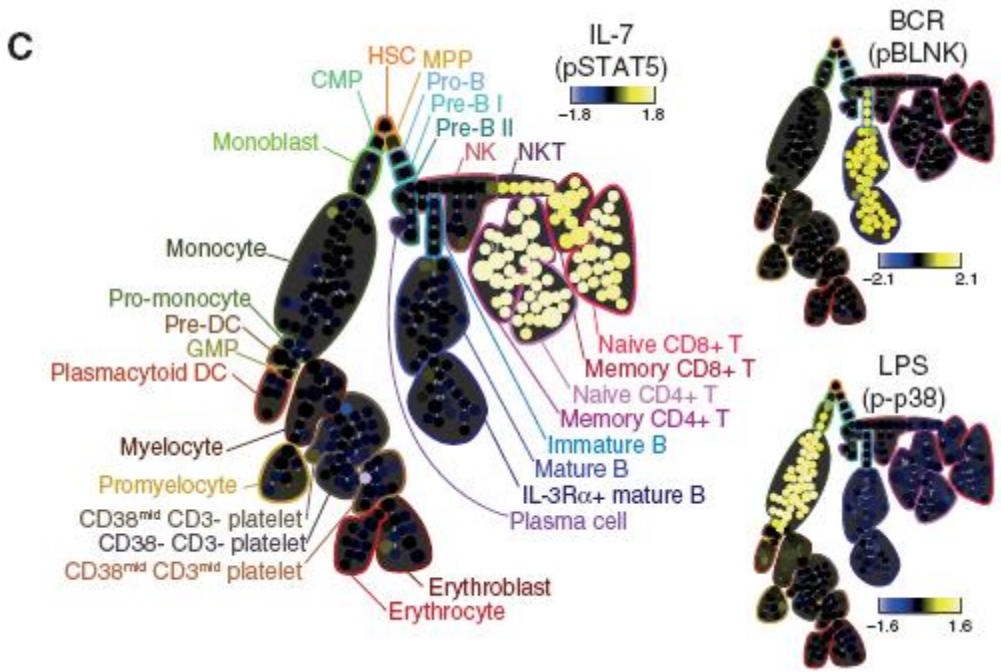
Seminal work

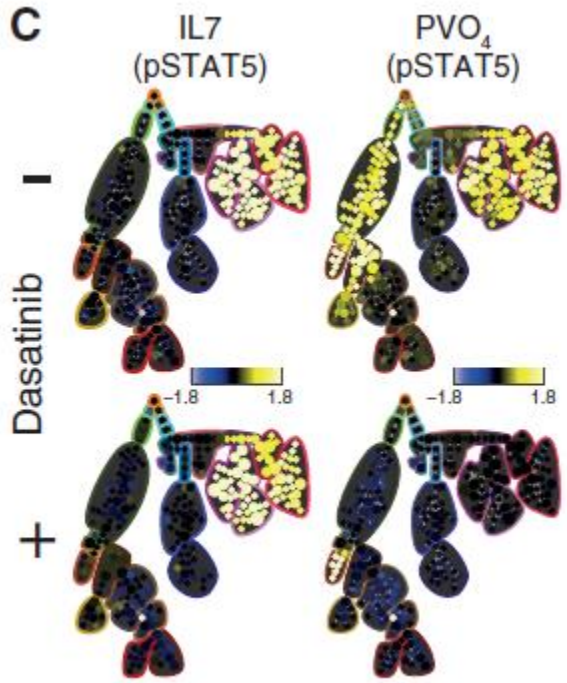
- Bendall SC, Simonds EF, Qiu P, Amir ED, Krutzik PO, Finck R, et al. Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum. Science. 2011 May 6;332(6030):687–96.
- 13 „core“ lineage markers, 18 subset-specific surface markers, 18 intracellular epitopes, 13 ex-vivo stimulation conditions.
- Huge scope of the results (929 pages of SOM)

Bendall et al.



Bendall et al.

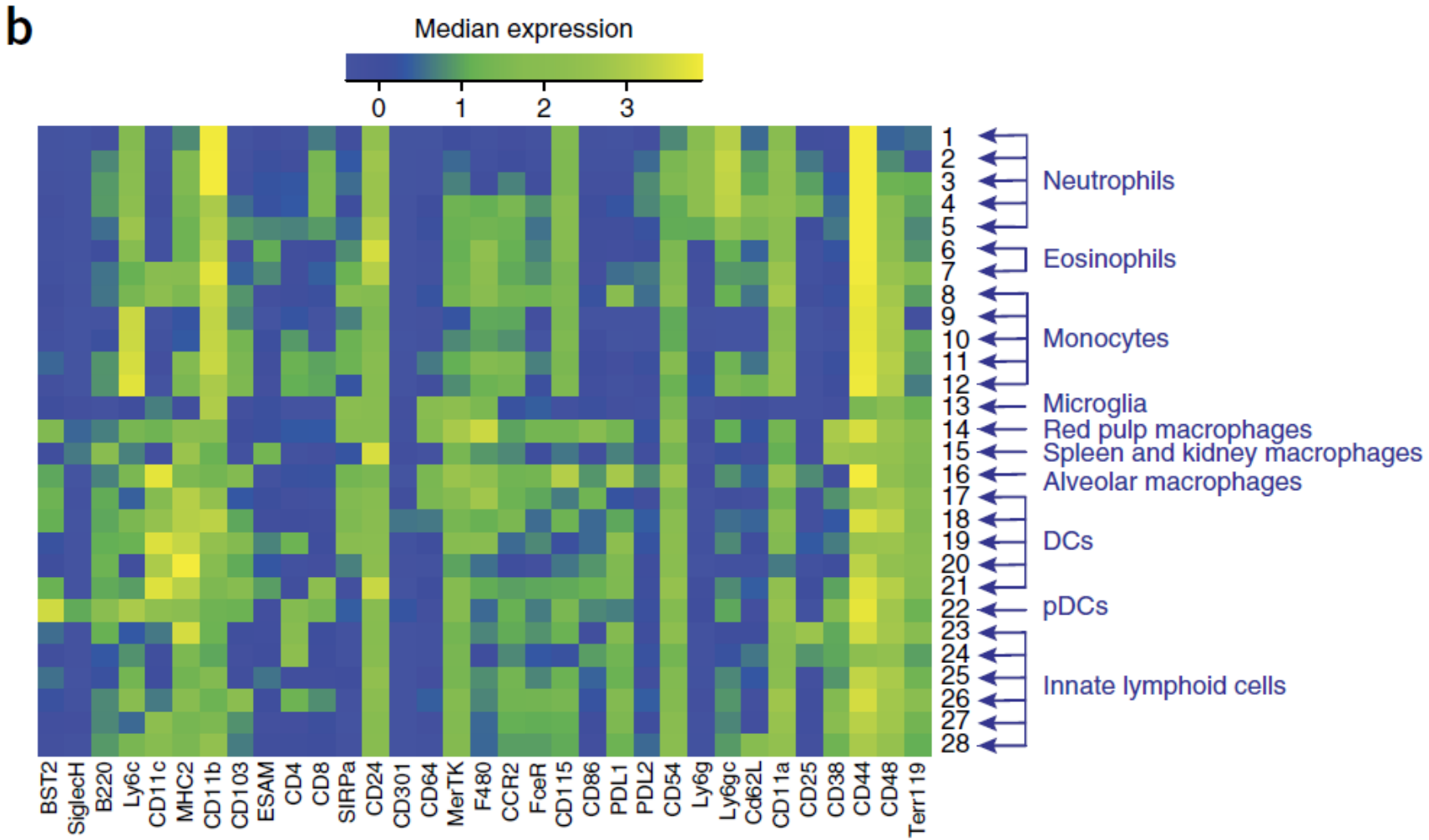




Closer to home

- Becher B, Schlitzer A, Chen J, Mair F, Sumatoh HR, Teng KWW, et al. High-dimensional analysis of the murine myeloid cell system. Nat Immunol. 2014 Dezember;15(12):1181–9.

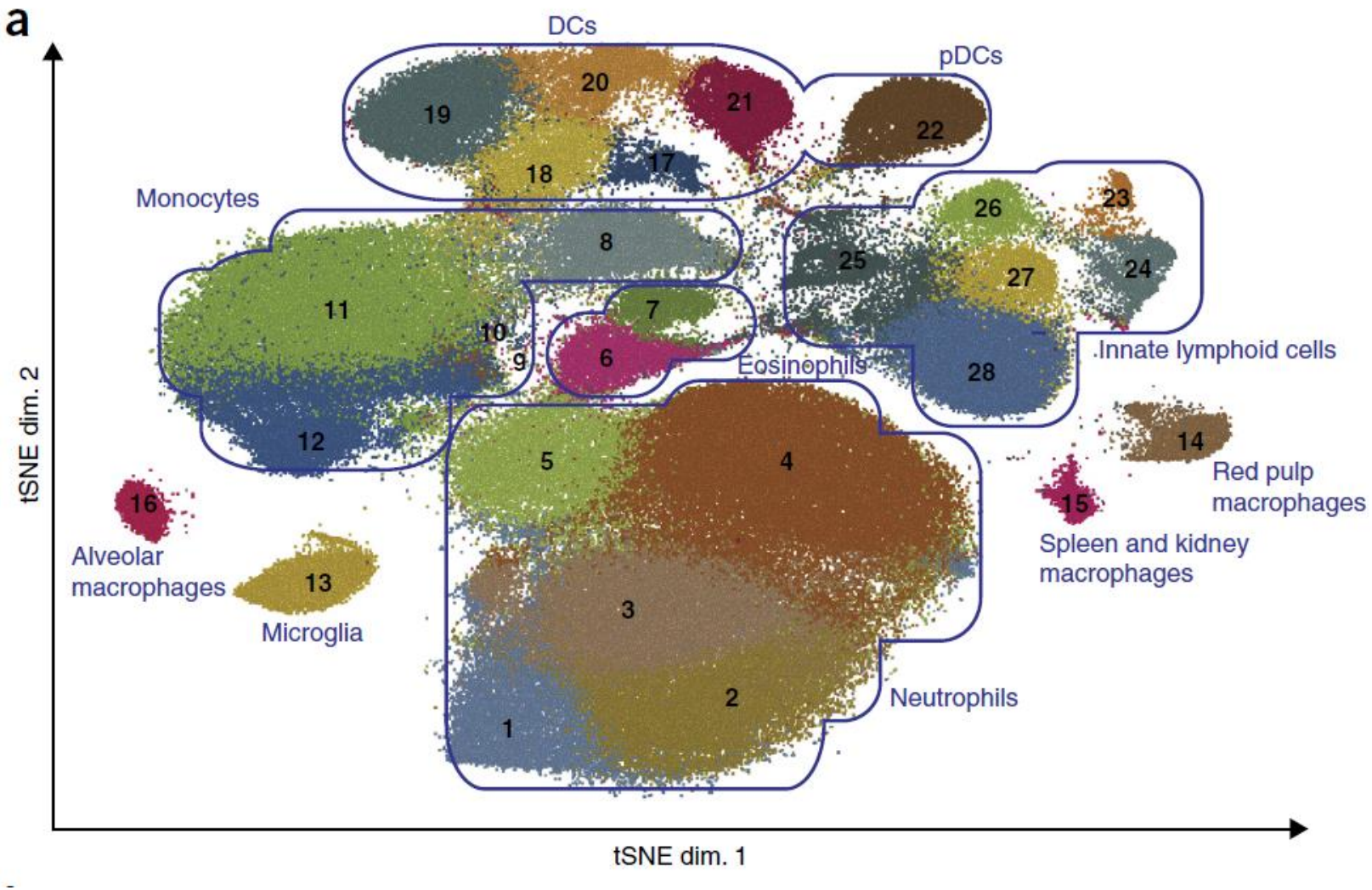
Becher et al.



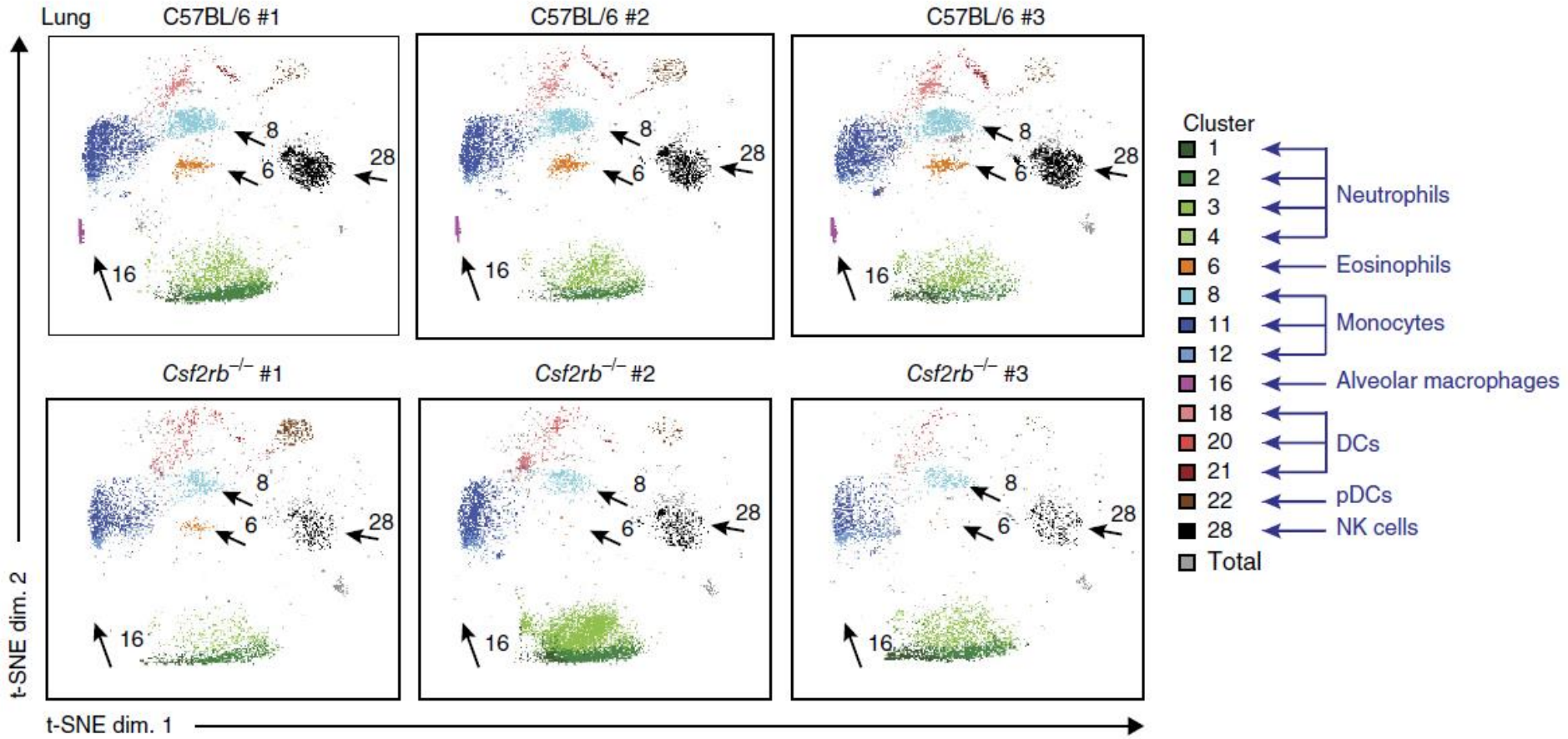
University of Zurich[UZH]

Mass Cytometry Facility

Becher et al.


a
DCs
pDCs
Monocytes
Eosinophils
Innate lymphoid cells
Red pulp macrophages
Spleen and kidney macrophages
Alveolar macrophages
Microglia
Neutrophils
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
tSNE dim. 2
tSNE dim. 1

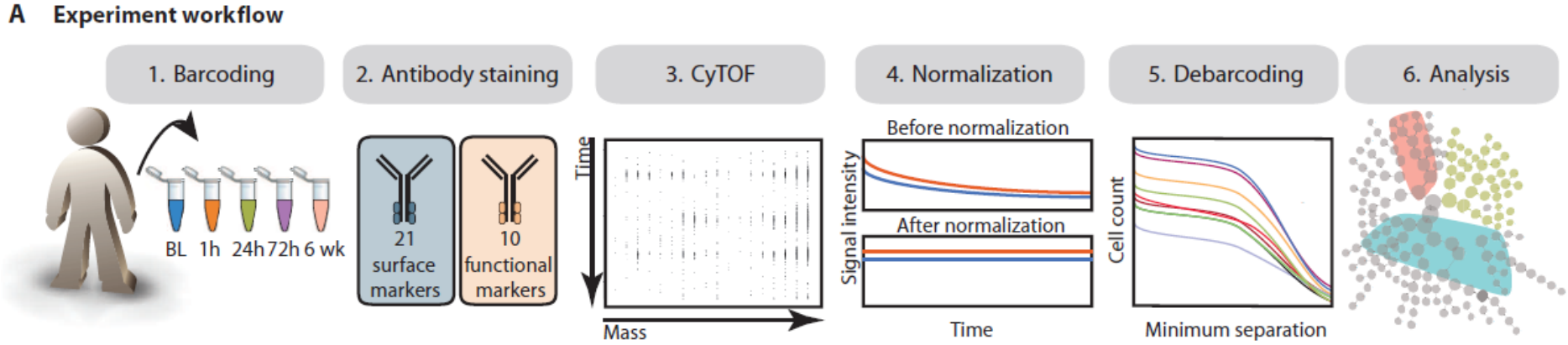
Becher et al.


Lung
C57BL/6 #1
C57BL/6 #2
C57BL/6 #3
Csf2rb−/− #1
Csf2rb−/− #2
Csf2rb−/− #3
8
6
28
16
t-SNE dim. 2
t-SNE dim. 1
Cluster
1
2
3
4
6
8
11
12
16
18
20
21
22
28
Total
Neutrophils
Eosinophils
Monocytes
Alveolar macrophages
DCs
pDCs
NK cells

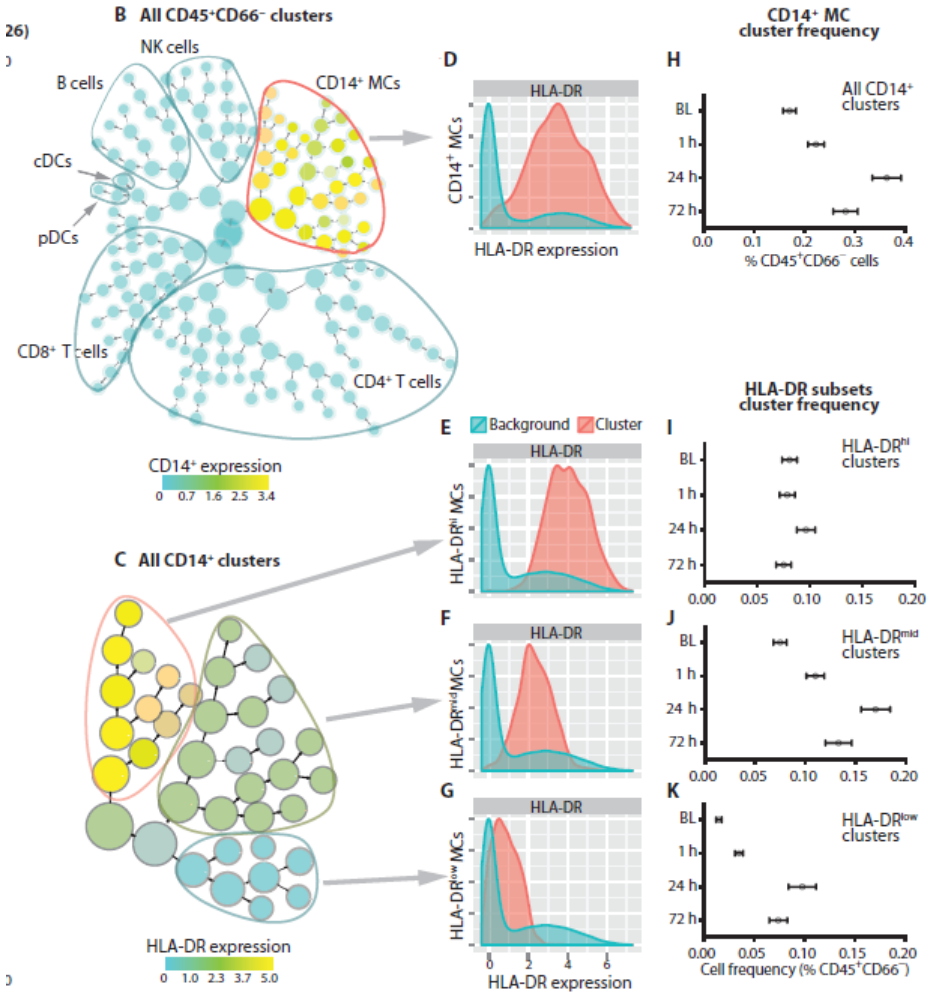
Around the same time

- Gaudillière B, Fragiadakis GK, Bruggner RV, Nicolau M, Finck R, Tingle M, et al. Clinical recovery from surgery correlates with single-cell immune signatures. Science Translational Medicine. 2014 Sep 24;6(255)

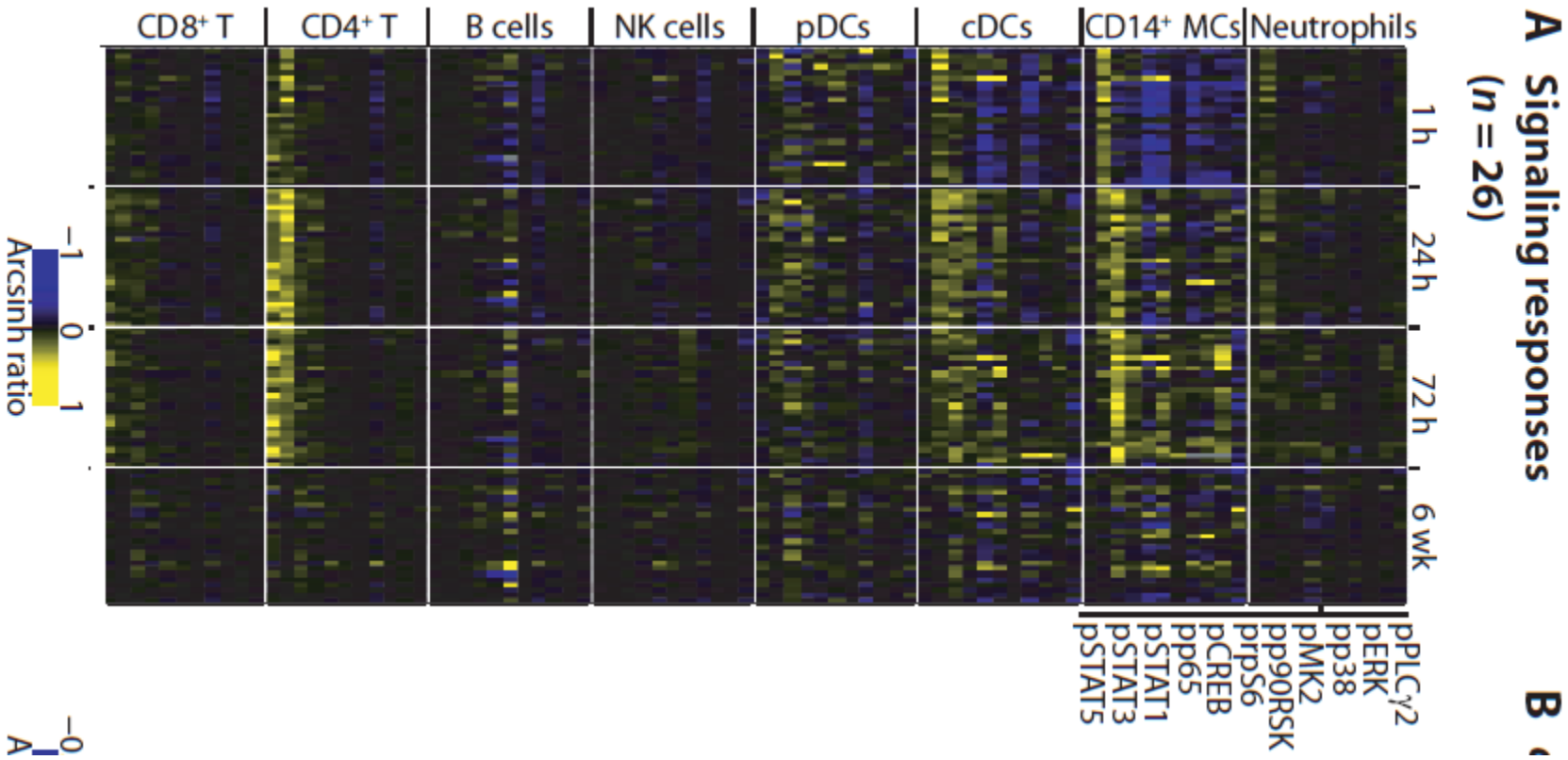
Gaudillière et al.



Gaudillière et al.



Gaudillière et al.



The mass cytometry toolbox

The challenge?

- Let’s CyTOF!
- Most common concerns
  - Panel assembly (know-how and price)
  - Barcoding yes/no?
  - Data analysis

Price of a mass cytometry pilot experiment

- No stocks of metal-labeled antibodies around
- Off-the-shelf cemicals/buffers often weren't good enough for elemental analysis
- Whole labs not suited for elemental analysis!
- Cyto 2014 – CyTOF UGM
  Jared K. Burks
  MD Anderson, TX, USA
- Reagent repository!

Reagent repository

- 100+ metal-conjugated antibodies (Ms, Hu)
- All (!) metal conjugation kits
- All the buffers required to perform the standard surface, ICS and nuclear staining.
- Additonal reagents
  - Intercalator(s), Dead cell reagent(s)
    - Iridium, Rhodium, cisplatin
  - normalization beads
    - EQ four element beads

- The aim – provide the means for «the quick test» if mass cytometry works for you without breaking the budget.

Reagent repository

Ly6G
CD11c
CD115
CD69
CD25
CD3e
CD335, NKp46
CD62L
CCR7
CD8a
TCRβ
NK1.1
B220
CD45
CD11b (MAC1)
CD19
Ly6C
CD44
CD4
I-A/I-E

\+  for 4 samples ≈ 150 CHF

Know-how

- By now, quite some support information out there (Fluidigm, peer-reviewed journals, UGMs,...)
- MaxPar reagents are a good start
- On average, conceiving and validating the panel not as hard as generally perceived (depends on the application!)
- Panel building takes from 0 to ∞
- Having deep experience with FC helps!

Instrumentation know-how

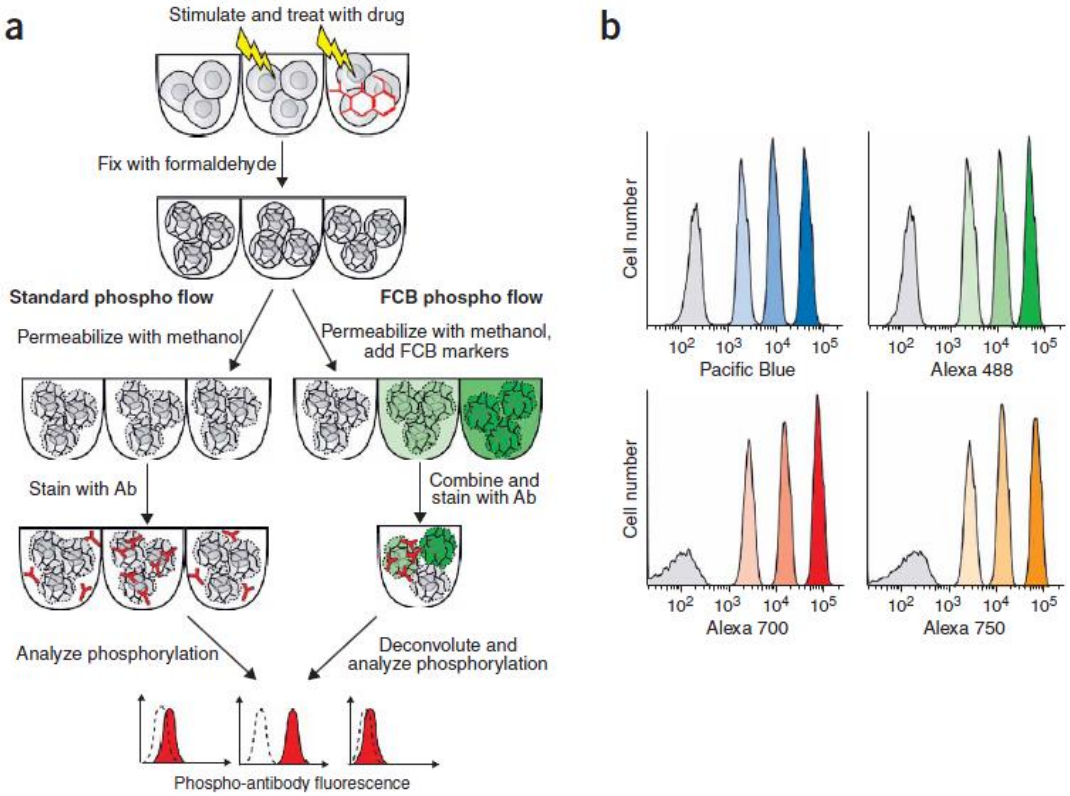
- Howard Shaprio, Cerberus

Barcoding

- Barcoding really found it’s place in mass cytometry – should be considered as well as means of to reduce the batch effects and improve throughput.

Barcoding

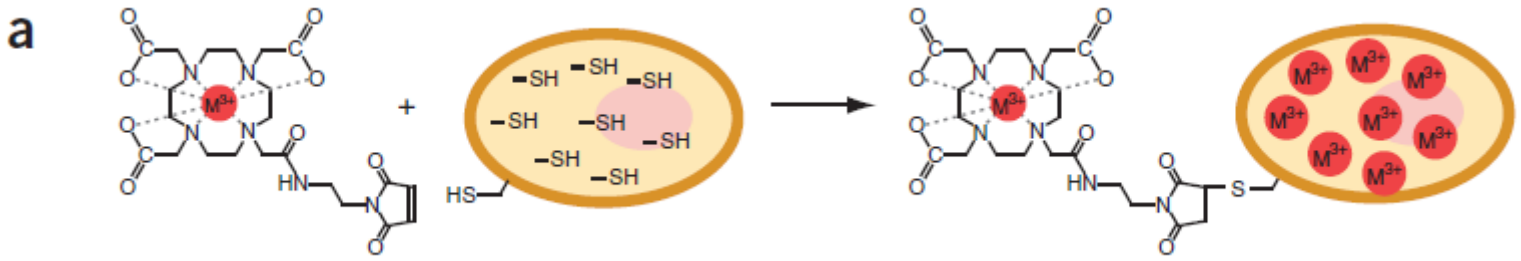
Source: KrutzikPO, Nolan GP. Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. Nat Meth. 2006 May;3(5):361–8.



Barcoding

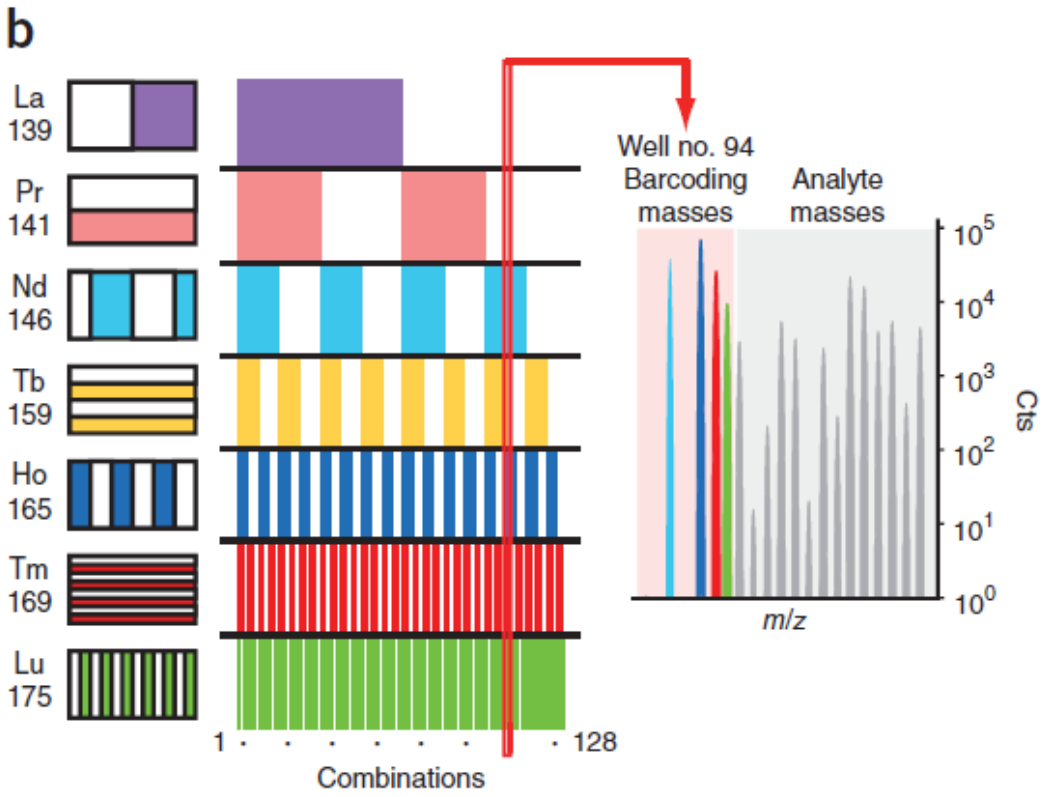
Source: Bodenmiller B, Zunder ER, Finck R, Chen TJ, Savig ES, Bruggner RV, et al. Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. Nat Biotech. 2012 Sep;30(9):858–67.

- Mass cytometry barcoding (MCB).
- Instead of NHS-functionalized fluorochromes (FCB), MCB used mDOTA (maleimido-mono-amide-DOTA).



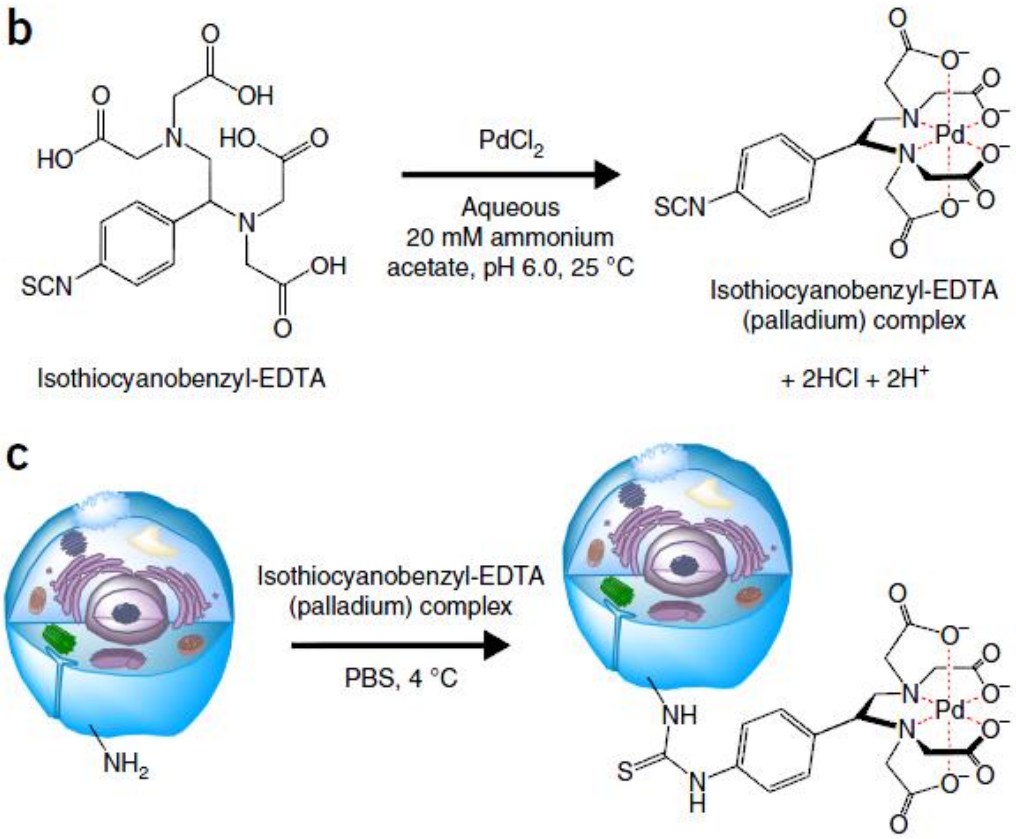
Barcoding

Source: Bodenmiller B, Zunder ER, Finck R, Chen TJ, Savig ES, Bruggner RV, et al. Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. Nat Biotech. 2012 Sep;30(9):858–67.

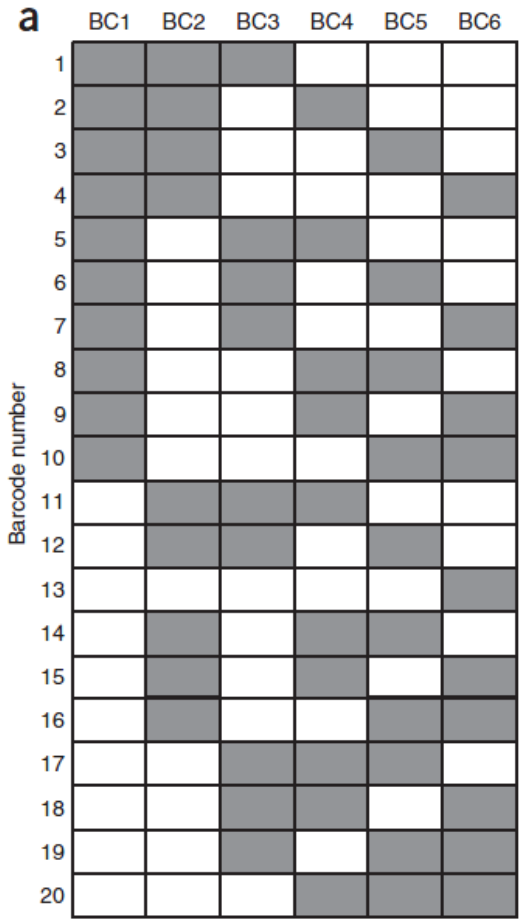


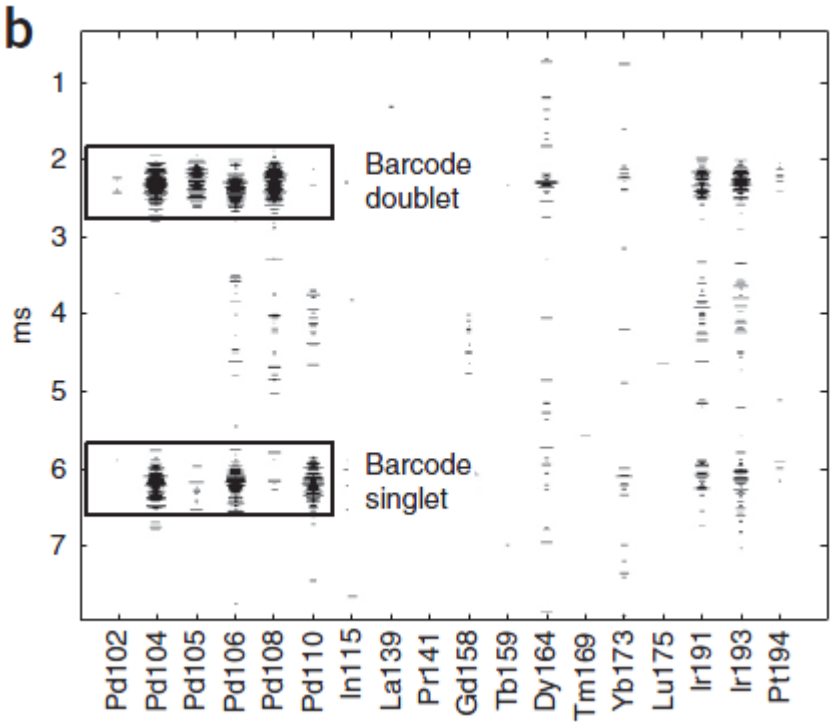
Barcoding

Source: Zunder ER, Finck R, Behbehani GK, Amir ED, Krishnaswamy S, Gonzalez VD, et al. Palladium-based mass tag cell barcoding with a doublet-filtering scheme and single-cell deconvolution algorithm. Nat Protocols. 2015 Feb;10(2):316–33.



Barcoding


a
BC1
BC2
BC3
BC4
BC5
BC6
Barcode number
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20


b
ms
1
2
3
4
5
6
7
Barcode doublet
Barcode singlet
Pd102
Pd104
Pd105
Pd106
Pd108
Pd110
In115
La139
Pr141
Gd158
Tb159
Dy164
Tm169
Yb173
Lu175
Ir191
Ir193
Pt194

Data analysis

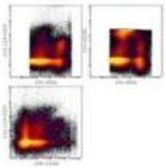
- The biggest perceived challenge of all!

2 parameters

1 plot

3 parameters

3 plots

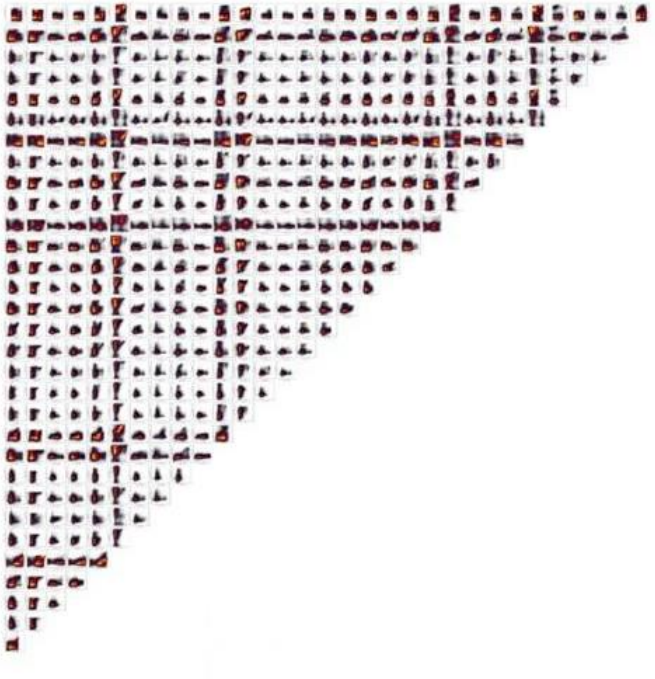


9 parameters

36 plots

32 parameters

496 plots



Data analysis

- “Unfortunately, the use of three or more independent fluorescent parameters complicates the analysis of the resulting data significantly.” *Murphy, Cytometry (1985)*
- Does one have to become a bioinformatician to analyze CyTOF data?
- «Hypothesis-driven analysis» vs. «Exploratory data analysis»

Tools for automated data analysis

- Bioconductor
- FlowJo
- Cytobank
- GemStone
- Cyt

Bioconductor

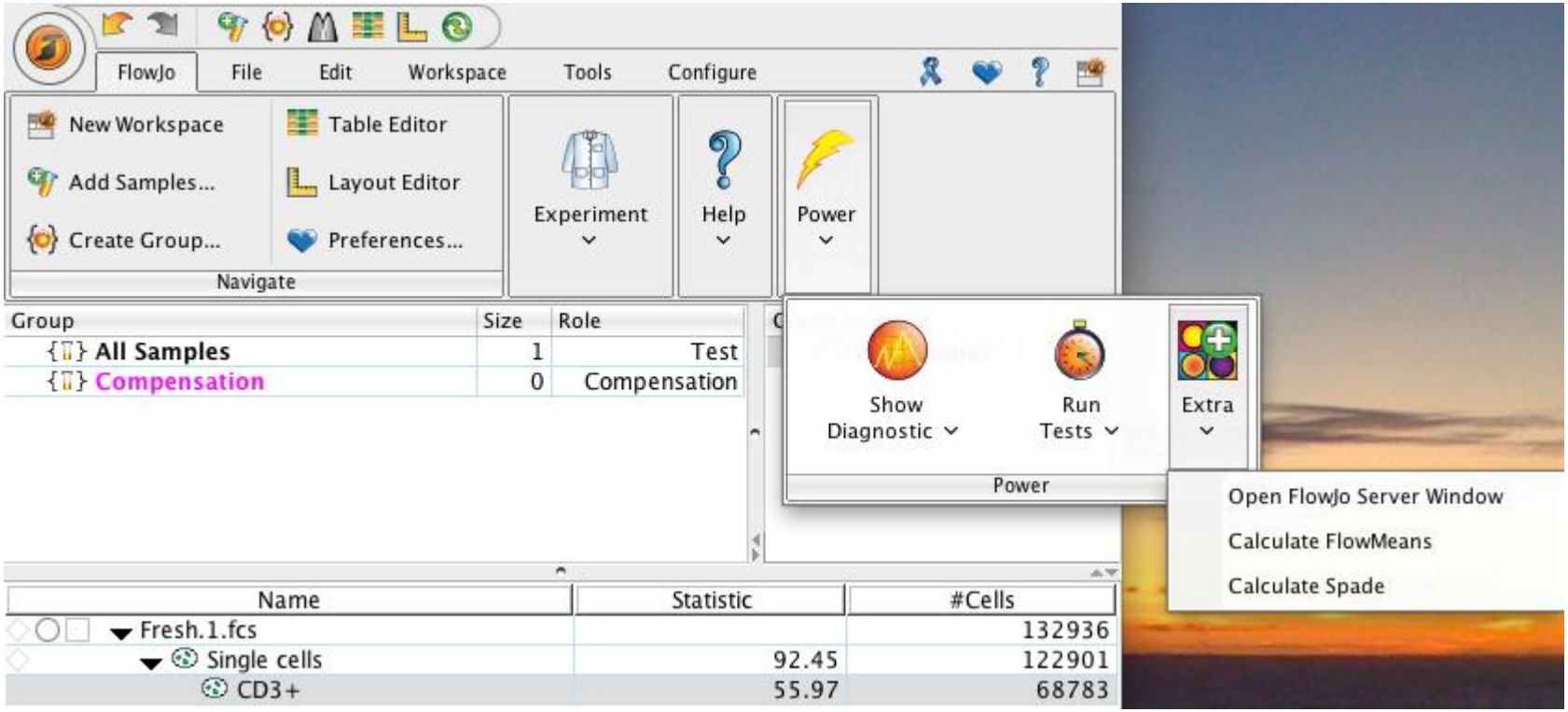
- http://bioconductor.org
- BioConductor provides R software modules for biological and clinical data analysis
- A scripted approach to high throughput data analysis
  - Non-interactive, reproducible
  - Breaks problem into smaller pieces (packages)
  - Modules can plug-in & swap-out

Why R?

- Big community
- A lot of available packages
- Well suited for developing new workflows
  - Interactive mode vs. batch mode
- Designed with data analysis in mind
- New algorithms will often be made available in R by the authors *right away*

FlowJo

- Historically, FlowJo offered some basic integration with few popular R packages

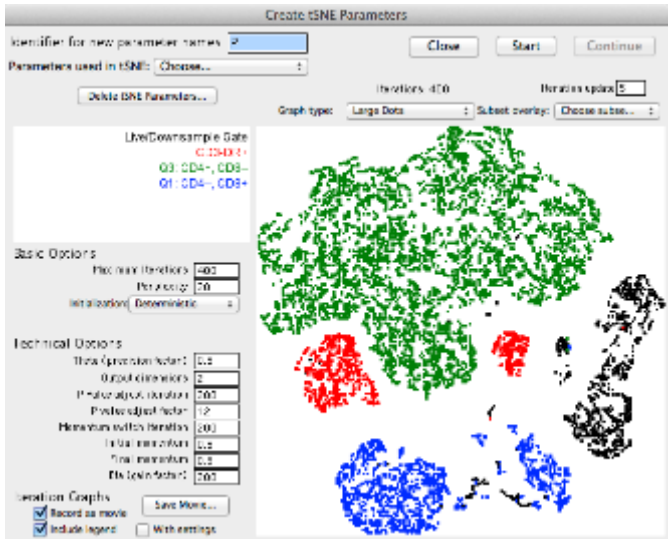


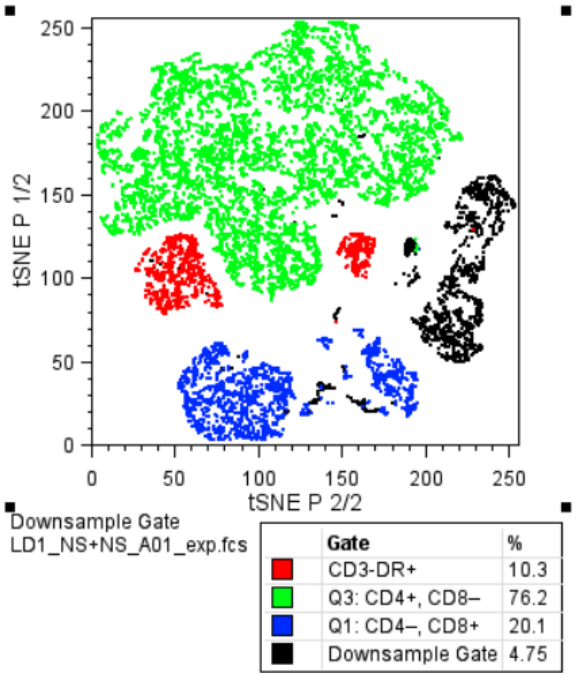
FlowJo Exchange

- https://github.com/FlowJo
- FlowJo Exchange houses scripts and plugins for FlowJo that anyone can contribute to or download from.
- New version 10.1 promised “Multiple improvements which allow any algorithm to be plugged into FlowJo.”
- At the beginning 5 scripts on the exchange - Indexed Sorting, Basic Looping, Collection Order Sort, Control Based Inclusion Gating, and a Relative Gate.

FlowJo v9

- http://docs.flowjo.com/v9/flowjo-v9-documentation-home/platforms/t-sne/



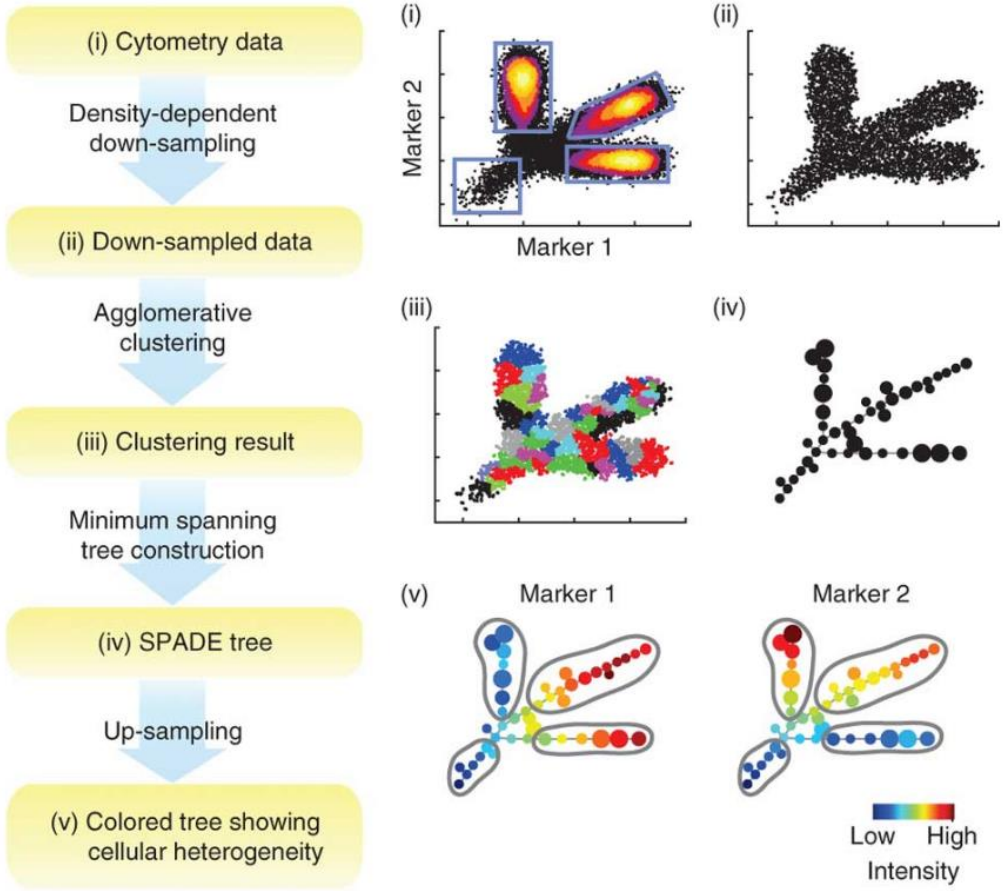


Cytobank with SPADE, viSNE, CITRUS

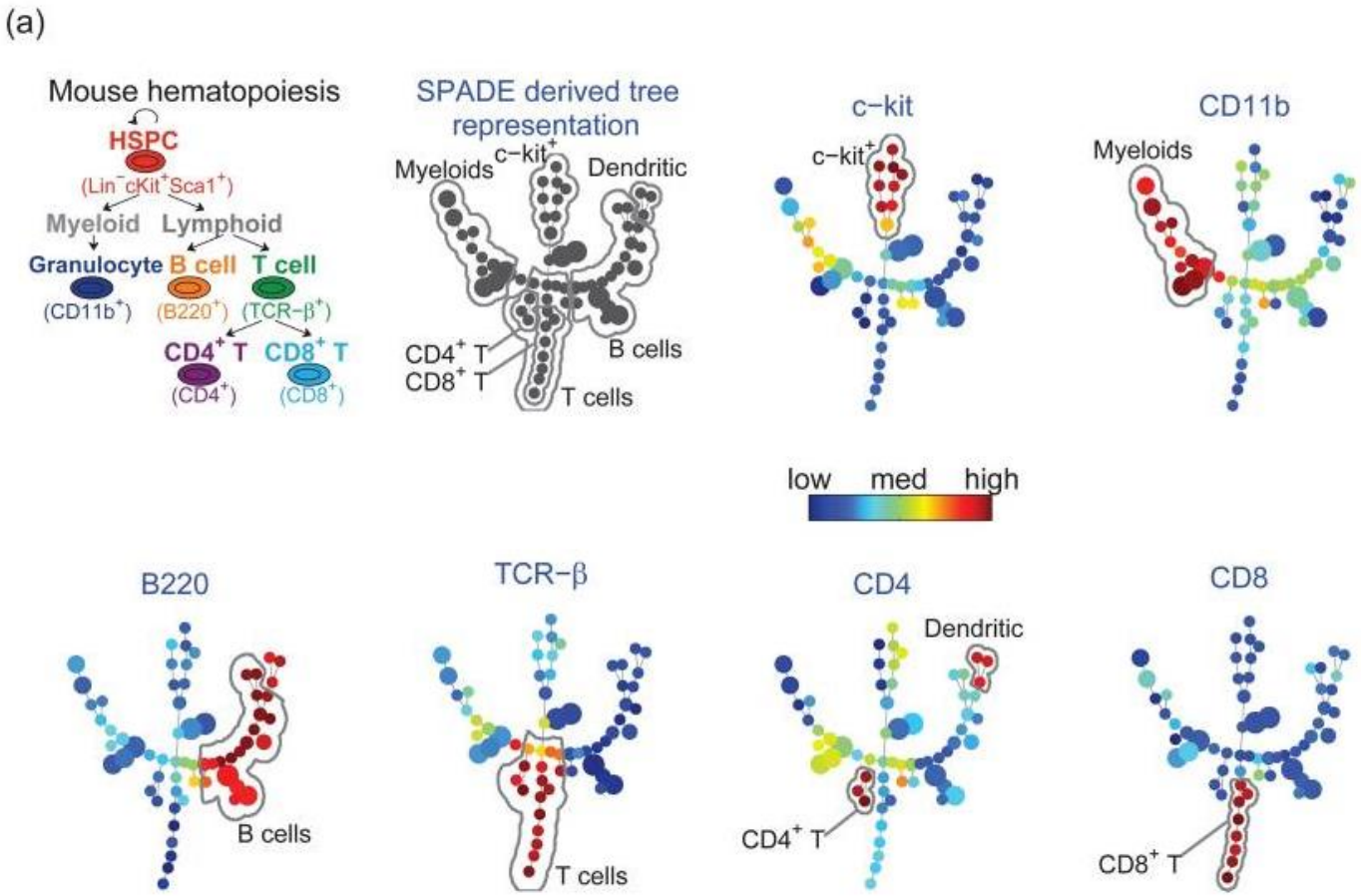
- Kotecha N, Krutzik PO, Irish JM. Web-based analysis and publication of flow cytometry experiments. Curr Protoc Cytom. 2010 Jul;Chapter 10:Unit10.17.
- Qiu P, Simonds EF, Bendall SC, Gibbs KD Jr, Bruggner RV, Linderman MD, et al. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. Nat. Biotechnol. 2011 Oct;29(10):886–91.
- Amir ED, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nat Biotech. 2013 Jun;31(6):545–52.
- Bruggner RV, Bodenmiller B, Dill DL, Tibshirani RJ, Nolan GP. Automated identification of stratifying signatures in cellular subpopulations. PNAS. 2014 Jul 1;111(26):E2770–7.

SPADE

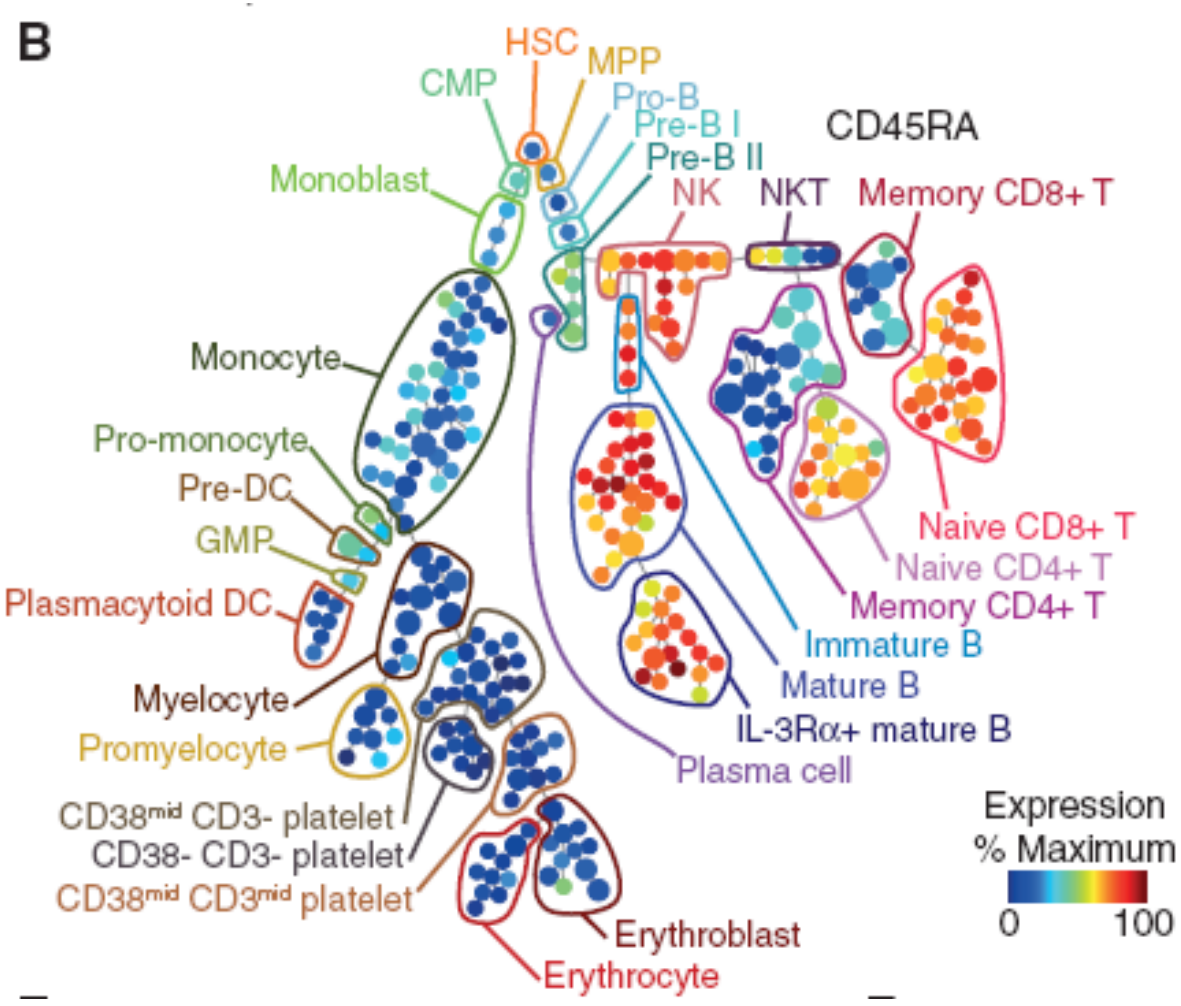
- Clustering algorithm



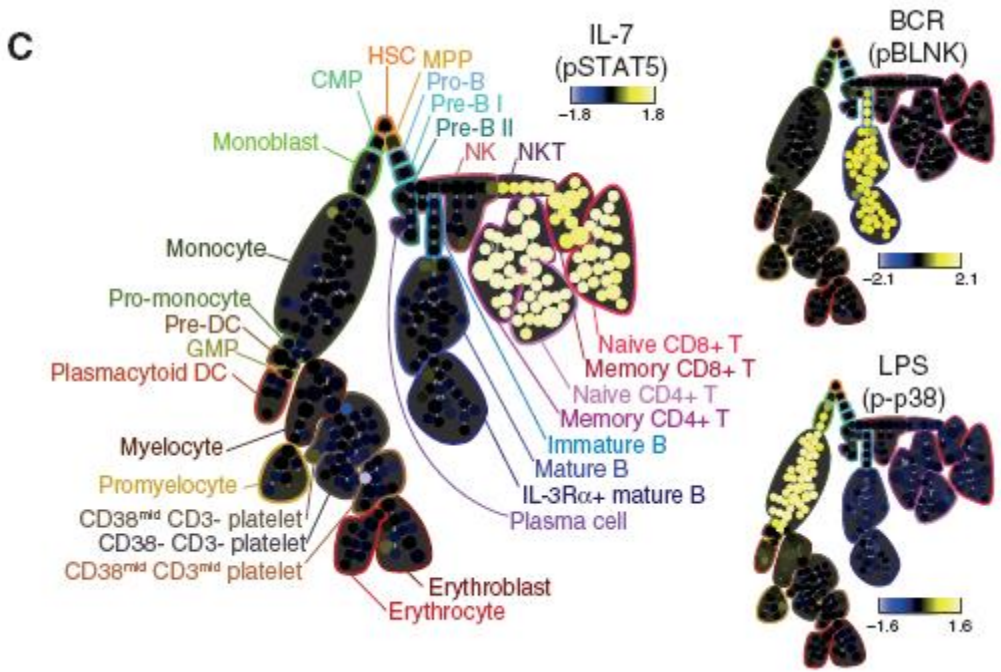
SPADE

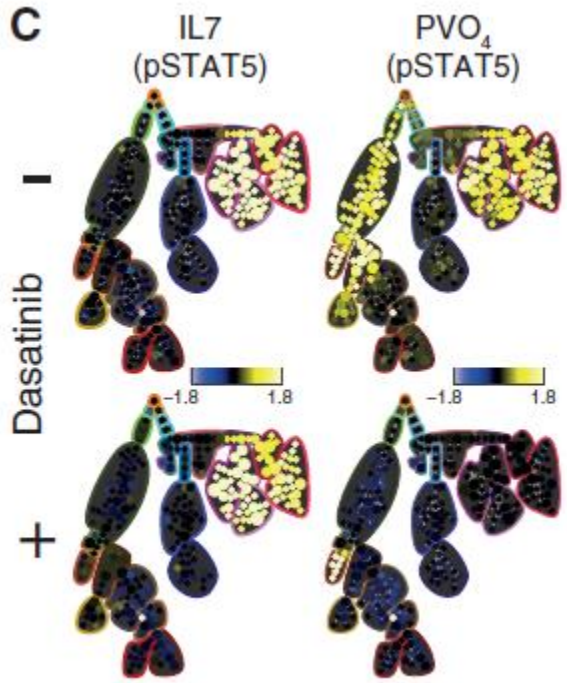

(a)
Mouse hematopoiesis
HSPC
(Lin⁻cKit⁺Sca1⁺)
Myeloid
Lymphoid
Granulocyte
B cell
T cell
(CD11b⁺)
(B220⁺)
(TCR-β⁺)
CD4⁺ T
CD8⁺ T
(CD4⁺)
(CD8⁺)
SPADE derived tree representation
Myeloids
c-kit⁺
Dendritic
CD4⁺ T
CD8⁺ T
B cells
T cells
c-kit
c-kit⁺
CD11b
Myeloids
low
med
high
B220
B cells
TCR-β
T cells
CD4
Dendritic
CD4⁺ T
CD8
CD8⁺ T

Bendall et al.



Bendall et al.





viSNE (t-SNE, bh SNE)

- Dimensionality reduction algorithm

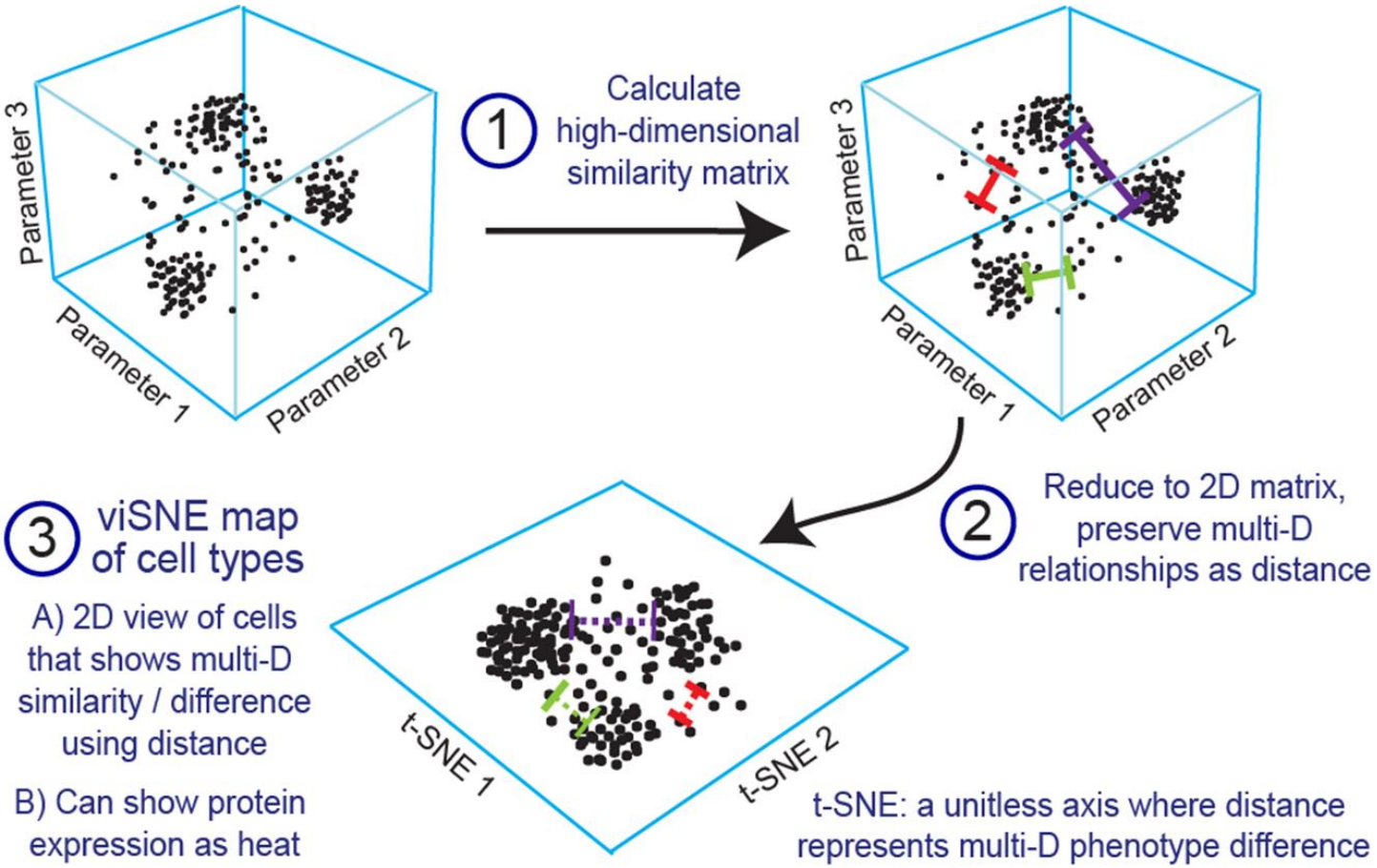
ARTICLES

nature biotechnology

viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia

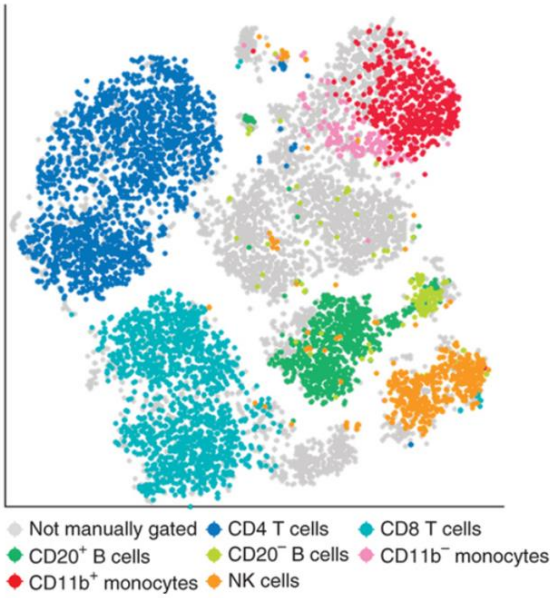
El-ad David Amir[1], Kara L Davis[2,3], Michelle D Tadmor[1,3], Erin F Simonds[2,3], Jacob H Levine[1,3], Sean C Bendall[2,3], Daniel K Shenfeld[1,3], Smita Krishnaswamy[1], Garry P Nolan[2,4] & Dana Pe'er[1,4]

viSNE (t-SNE, bh SNE)



viSNE (t-SNE, bh SNE)

- Visualization of high-dimensional single-cell data in 2D
- The resulting map provides a visual representation of the single-cell data where the positions of cells reflects their proximity in high-dimensional space.
- Color can be utilized as a third dimension to interactively visualize features of these cells.



CITRUS

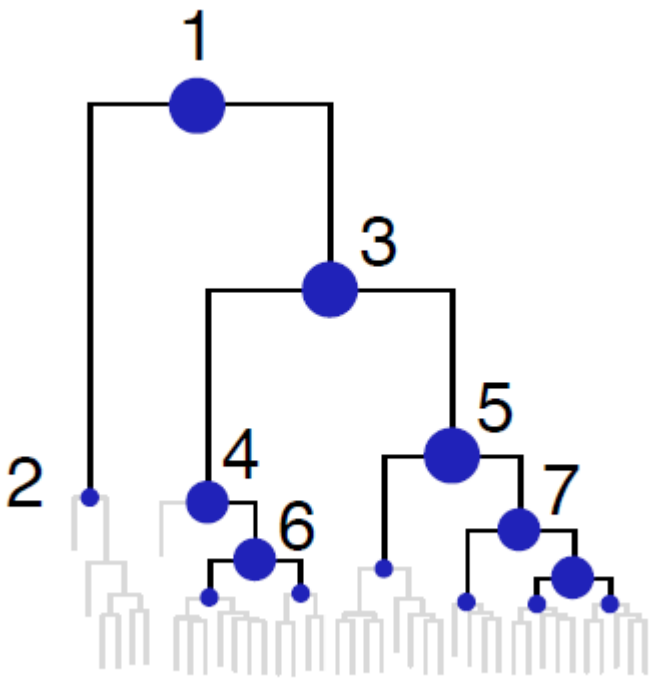
- Bruggner RV, Bodenmiller B, Dill DL, Tibshirani RJ, Nolan GP. Automated identification of stratifying signatures in cellular subpopulations. PNAS. 2014 Jul 1;111(26):E2770–7.
- “cluster identification, characterization, and regression”

CITRUS

- Cells from N samples are combined and clustered in a semi(un)supervised manner to automatically identify C clusters of related cells.
- Descriptive statistics characterizing various properties of each cluster (cluster features) are extracted on a per-sample basis.
- Extracted cluster features are used in conjunction with a user-specified endpoint of interest to train a supervised model.
- Internal cross-validation is used to evaluate model fit and select the appropriate regularization threshold for a final model.
- Model features are plotted as a function of endpoint of interest and cluster phenotypes are determined by density plots of markers used for clustering.

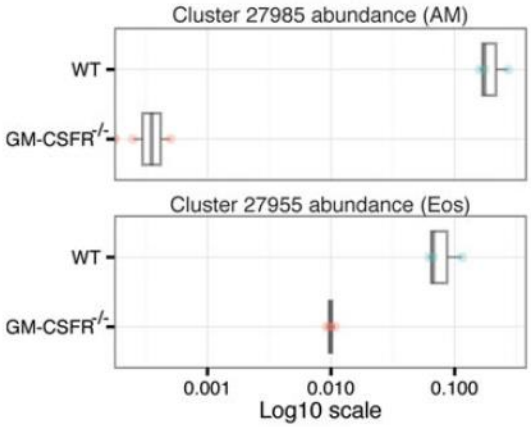
CITRUS

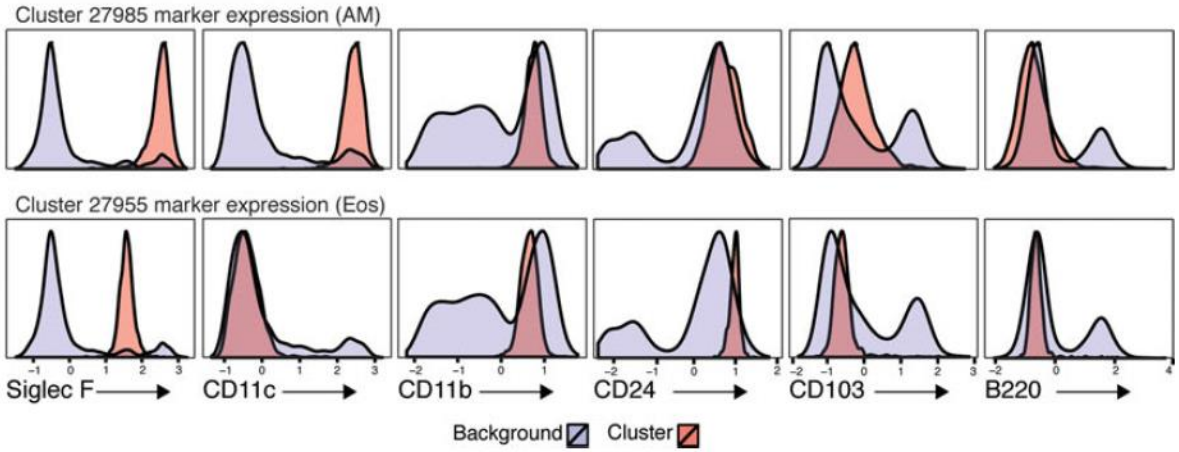
- Rather than cutting the dendrogram at a fixed height to identify clusters, all clusters C in the hierarchy of merged clusters larger than a user-specified size are retained for subsequent analysis.



CITRUS

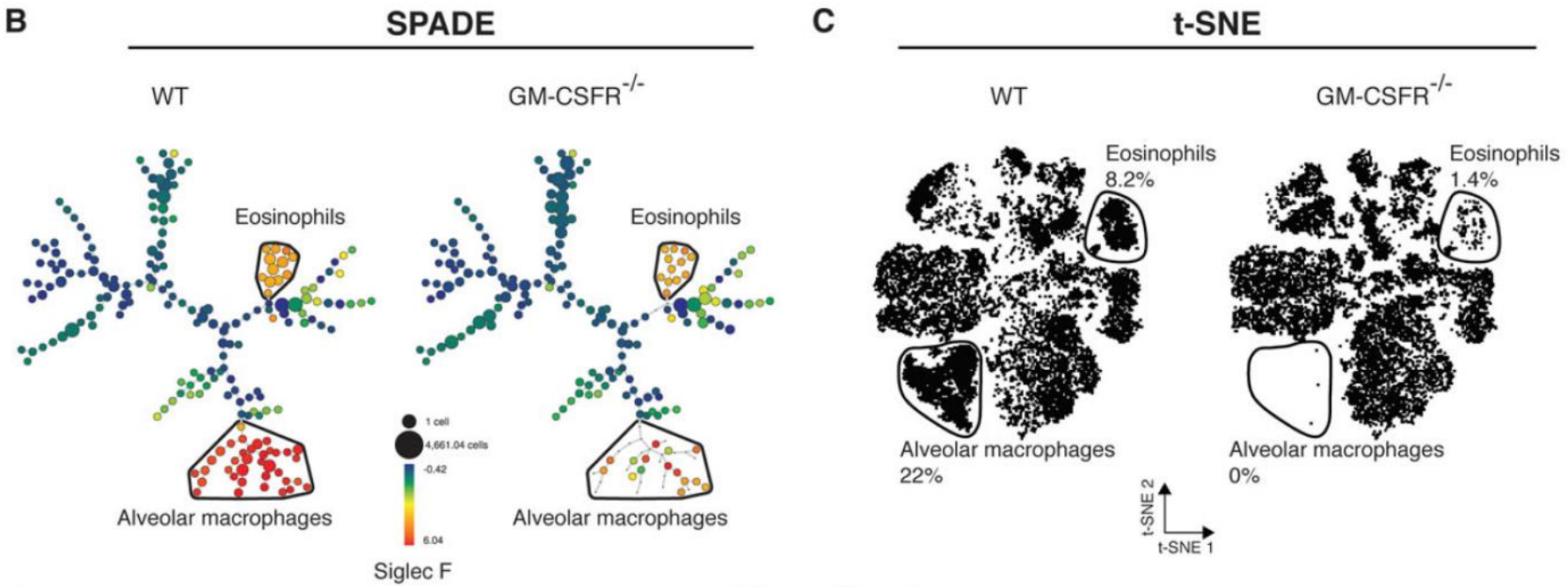
Mair F, Hartmann FJ, Mrdjen D, Tosevski V, Krieg C, Becher B. The end of gating? An introduction to automated analysis of high dimensional cytometry data. Eur J Immunol. 2015 Nov 1;n/a-n/a.





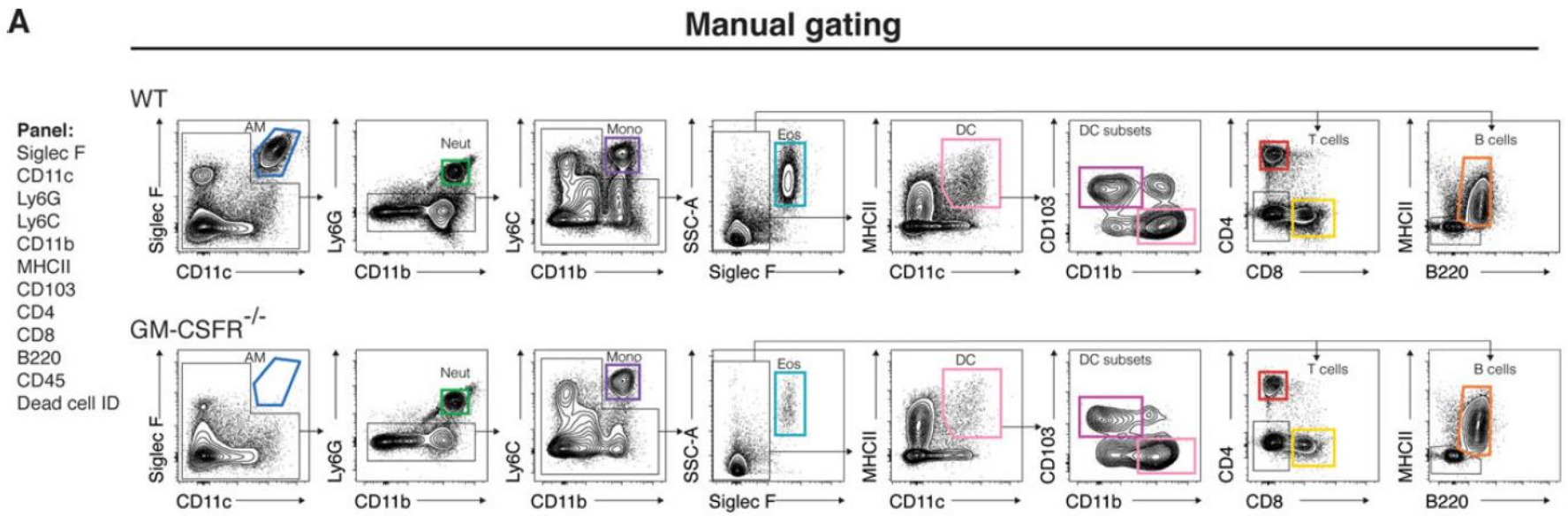
CITRUS

Mair F, Hartmann FJ, Mrdjen D, Tosevski V, Krieg C, Becher B. The end of gating? An introduction to automated analysis of high dimensional cytometry data. Eur J Immunol. 2015 Nov 1;n/a-n/a.



CITRUS

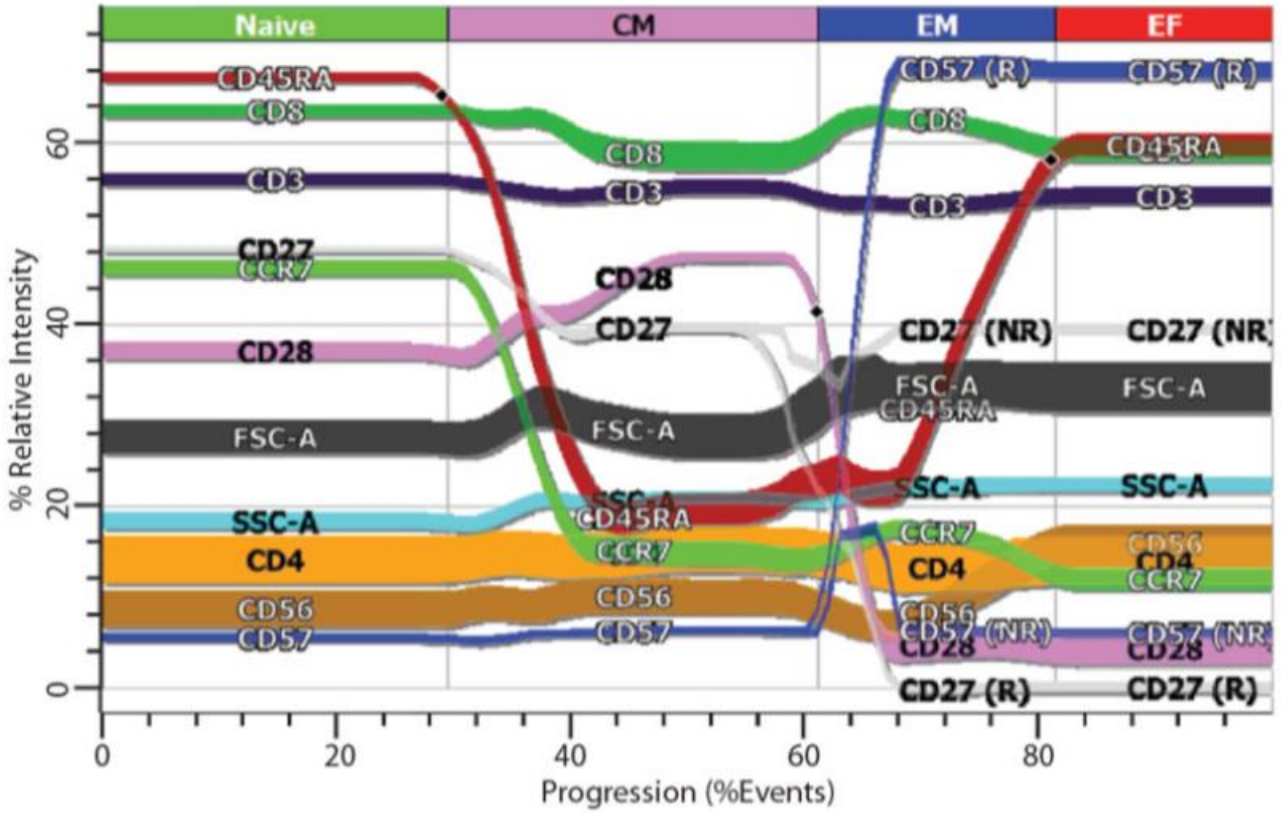
Mair F, Hartmann FJ, Mrdjen D, Tosevski V, Krieg C, Becher B. The end of gating? An introduction to automated analysis of high dimensional cytometry data. Eur J Immunol. 2015 Nov 1;n/a-n/a.



GemStone

- Bagwell CB. Breaking the dimensionality barrier. Methods Mol. Biol. 2011;699:31–51.
- Uses continuous expression patterns of various parameters and employs probability state modeling to organize and visualize cell populations relative to one another.
- While it still requires a priori knowledge of the relationship between at least some of the markers measured, it still visually summarizes all cells in a given sample and can reveal cell subsets and relationships that other tools may not.

GemStone



Analysis of CD8 T cells showing progression of phenotypic markers including branching expression of markers like CD57

cyt

- CYT is an interactive visualization tool designed for the analysis of high-dimensional mass or flow cytometry data. The tool encompasses multiple computational features (viSNE, Wanderlust, PhenoGraph and more).

cyt

- viSNE: see before
- PhenoGraph: computing PhenoGraph clusters. The clusters can be visualized by a marker expression heatmap or the cluster centroids can be used to generate a tSNE map to be gated on or overlaid with other markers.
- Wanderlust: computing a wanderlust trajectory (a nonlinear pricipal component or ordering of the data). Cyt can visualize the average expression of markers as a function of the wanderlust trajectory (or any desired marker).
- Wishbone: to align single cells from differentiation systems with bifurcating branches.
- cyt also implements some basic data analysis techniques such as PCA, kMeans, EMGM, and more.

Mass Cytometry Facility, University of Zurich

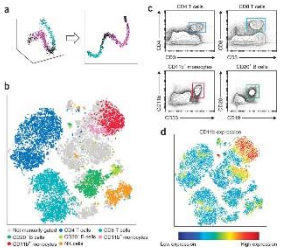
Current organization and scope of service

- We are able to support the entire hypothetical workflow based on mass cytometry!

- Project
  - Panel design
  - Sample preparation
  - Troubleshooting/optimization

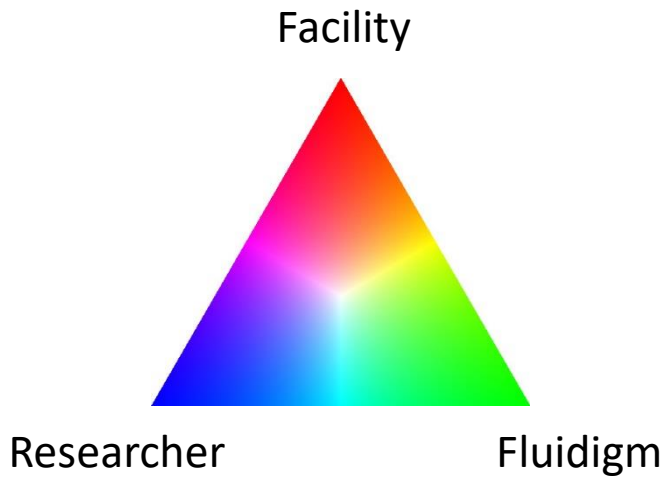
- Reagents
  - Reagent repository (Abs, buffers, conjugation kits)
- Instrument
  - Sample acquisiton
  - Maintenance
  - Repairs
  - QC

- Data analysis
  - Preprocessing
  - Exploratory data analysis



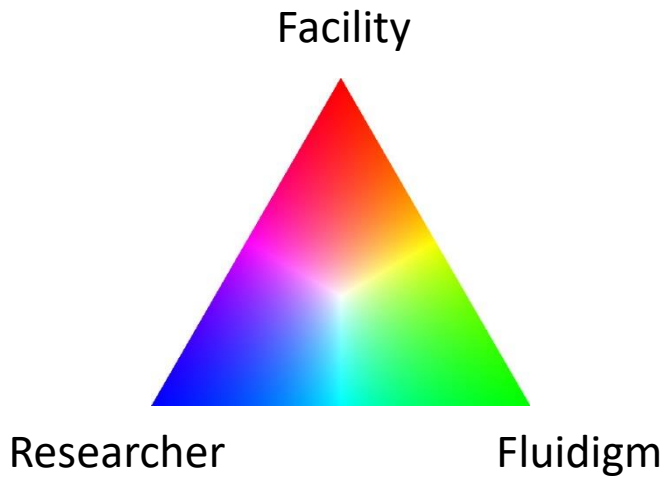
Current organization and scope of service

- Project
  - Panel design
  - Sample preparation
  - Troubleshooting/optimization
- Reagents
  - Reagent repository (Abs, buffers, conjugation kits)
- Instrument
  - Sample acquisition
  - Maintenance
  - Repairs
  - QC
- Data analysis
  - Preprocessing
  - Exploratory data analysis



Current organization and scope of service

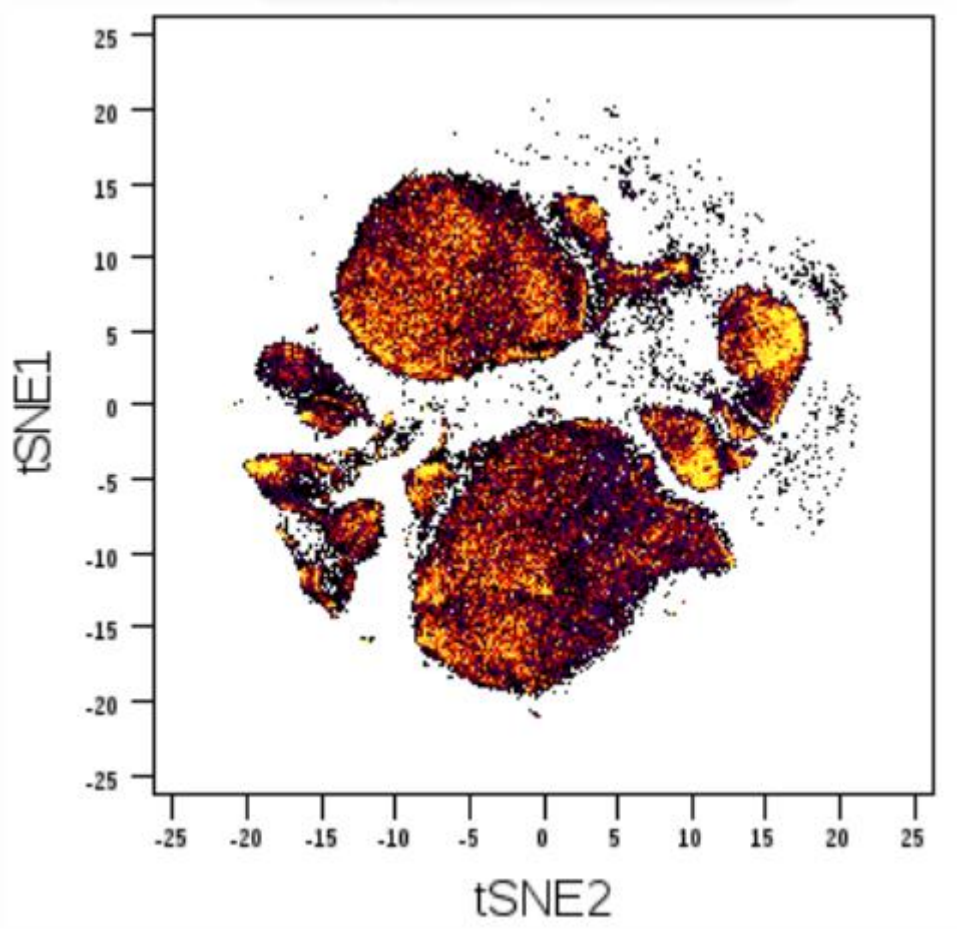
- Project
  - Panel design
  - Sample preparation
  - Troubleshooting/optimization
- Reagents
  - Reagent repository (Abs, buffers, conjugation kits)
- Instrument
  - Sample acquisition
  - Maintenance
  - Repairs
  - QC
- Data analysis
  - Preprocessing
  - Exploratory data analysis



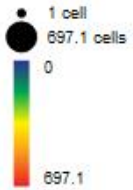
Cytobank

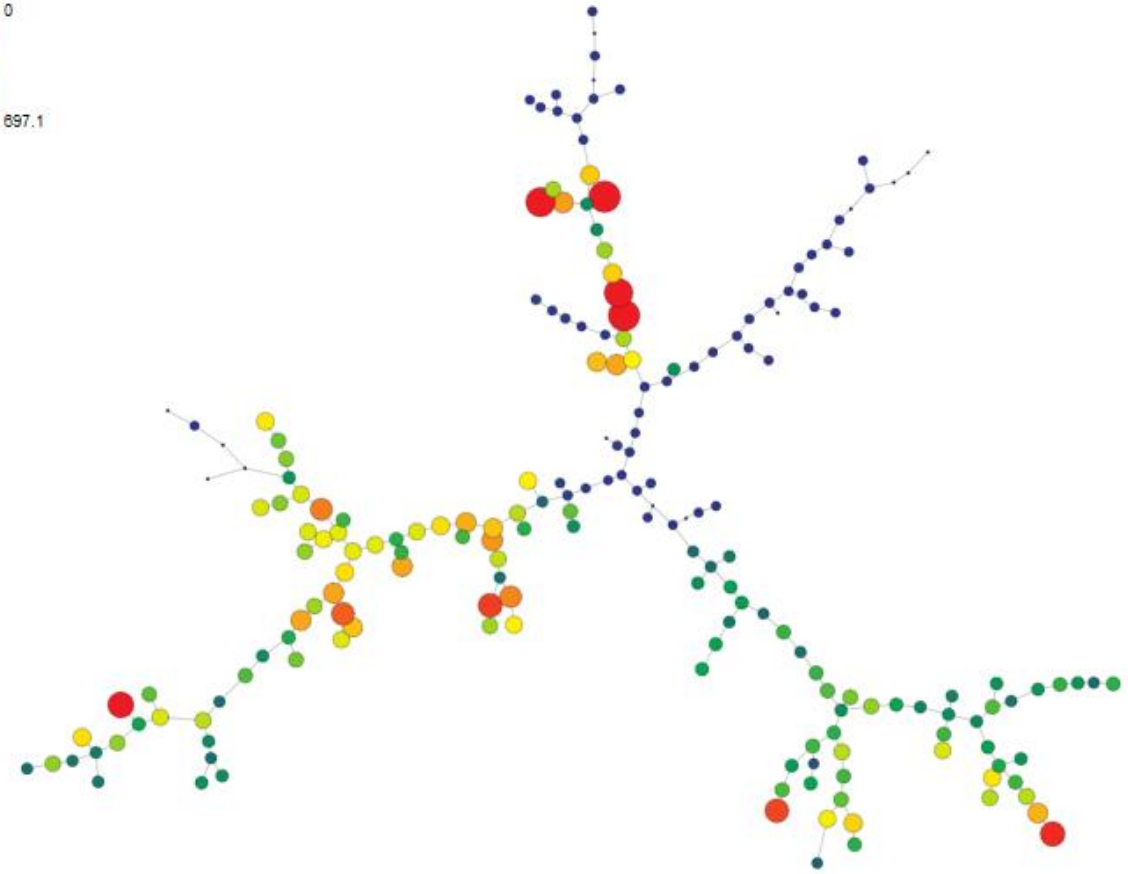
- uzh.cytobank.org
- Great tool for data storage, sharing and analysis
- Well suited for core facilities as it simplifies support
- Has SPADE, viSNE, CITRUS
- Limitations?

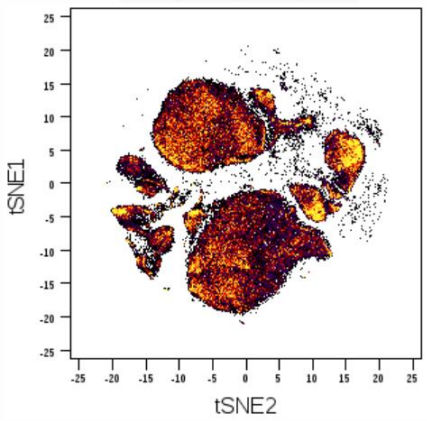
Cytobank



Cytobank







University of Zurich[UZH]

Mass Cytometry Facility

Hybrid workflow

- Cytobank – Bioconductor – Cytobank
- Cytobank – Bioconductor
- (Cytobank ↔ custom code through API)

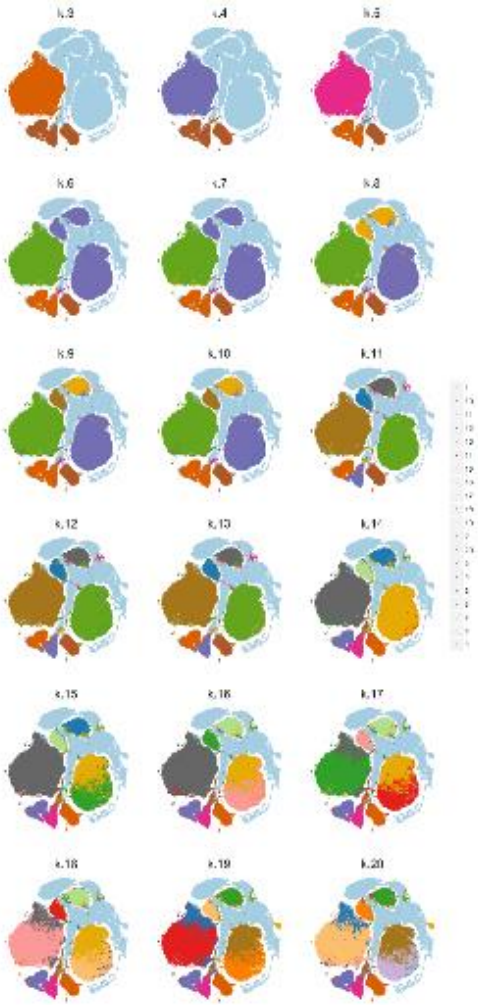
Hybrid workflow

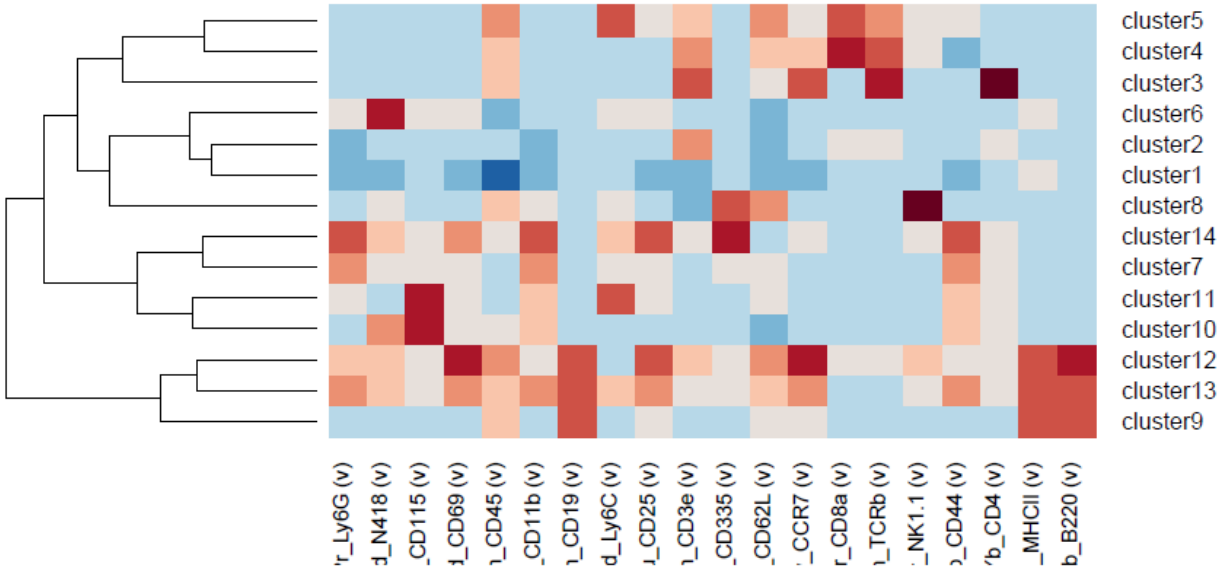
F. Hartmann

- We run clustering on the dataset and visualize the resulting clusters on the viSNE map
- Currently we use FlowSOM
  - SAMSpectral, Phenograph, densityCut...
- Good reference to check:
  - Weber LM, Robinson MD. Comparison of Clustering Methods for High-Dimensional Single-Cell Flow and Mass Cytometry Data. bioRxiv. 2016 Sep 8;47613.
- Metaclustering step
  - Existing knowledge of the number of populations
  - «elbow» method
  - Guided by the tSNE map?

Hybrid workflow

F. Hartmann





University of Zurich[UZH]

Mass Cytometry Facility

Conclusion

- Since few years now there has been a massive excitement about mass cytometry
- Not every site that purchased the instrument ended up happy, though…
- Be well prepared
- Well designed shared resource labs can minimize the entry barriers

We’re open for collaboration

- Discussion never harms…
- http://www.cytometry.uzh.ch/mcf
- vinko.tosevski@uzh.ch

Thank you for your attention!