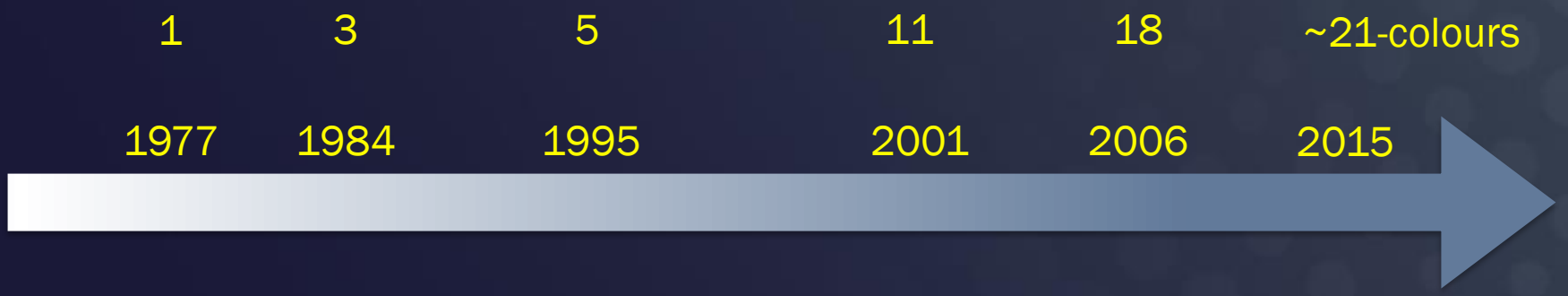
Mass Cytometry
(Cytometry by Time-Of-Flight)

How many markers do you need to define a T cell population?



T cells lineages

CD3
CD4
CD8
TCRγδ-Vd1
TCRγδ-Vd2
MAIT (Va7.2,CD161)
NKT (CD1d-GalCer)

CD4 T cell subsets

Tregs CTLA4
Th1 IFNg, GZB, TNF
Th2 IL4, IL13
Tfh IL21
Th17 IL17, TNF
Th22 IL22
Th9 IL9

Phenotypic markers

CD25
FOXP3
CD127
CCR4
CXCR3
CCR6
CCR10
CXCR5

Memory

CD45RA
CD45RO
CCR7
CD27

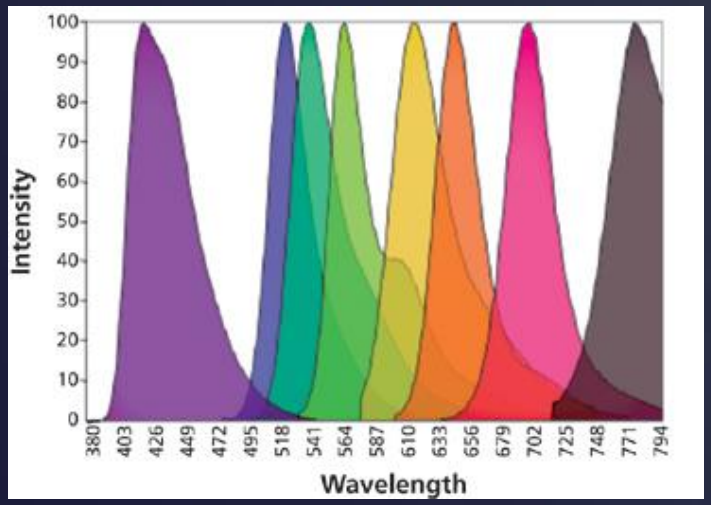
Activation

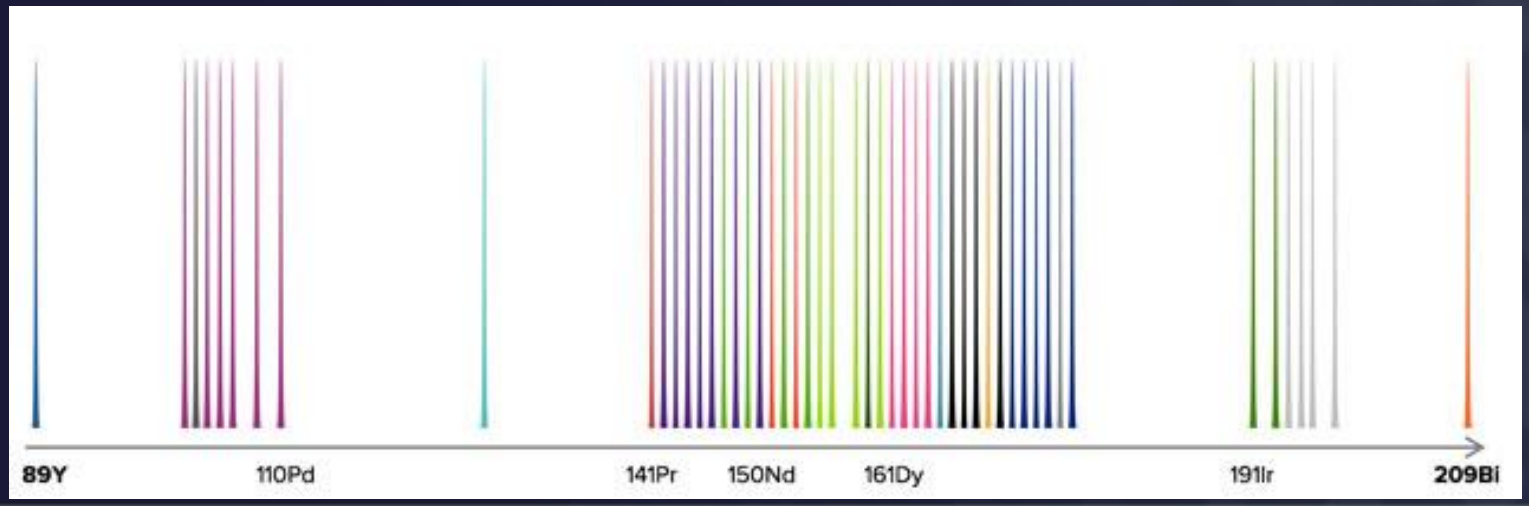
ICOS
Ki67
Bcl2
PD1
HLADR
CD38

Homing

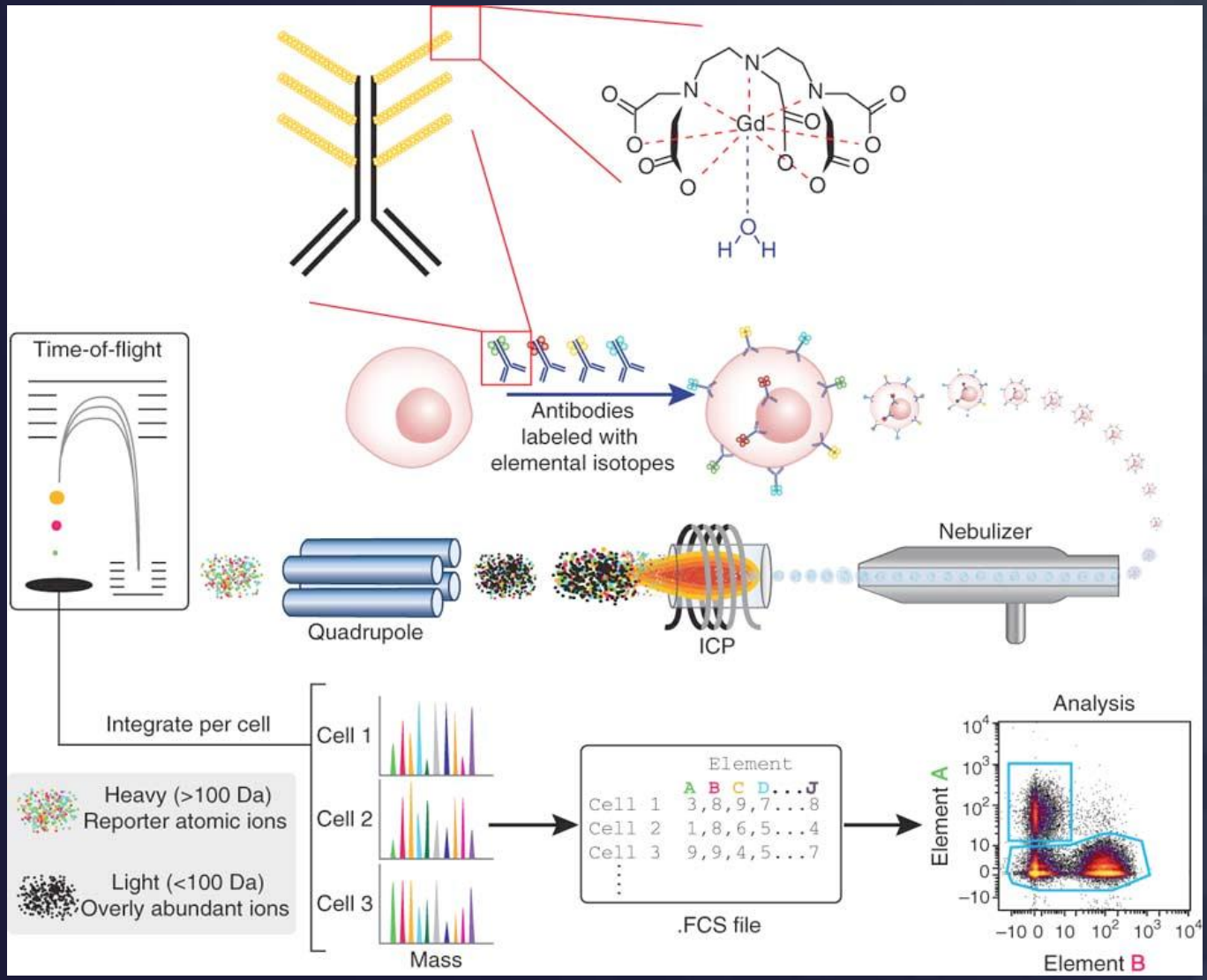
Intb7
CD49a
CD49d
CLA
CCR2
CX3CR1
CXCR5
CD103
CCR9

Spectral overlap hinders the expansion of multicolour flow cytometry





Cytometry by Time-Of-Flight - CyTOF



Bendall et al, 2012

CyTOF measures only metals

Cells identification:	Iridium as DNA intercalator
Live Dead:	Cisplatin
Markers:	Heavy metals isotopes
S-phase:	Iodine (IdU)
In vivo tracking:	Gold nanoparticles
Barcoding:	Palladium
Size:	Osmium

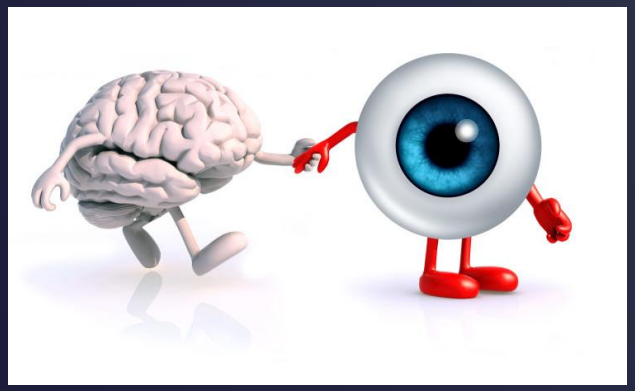
Before you start your project

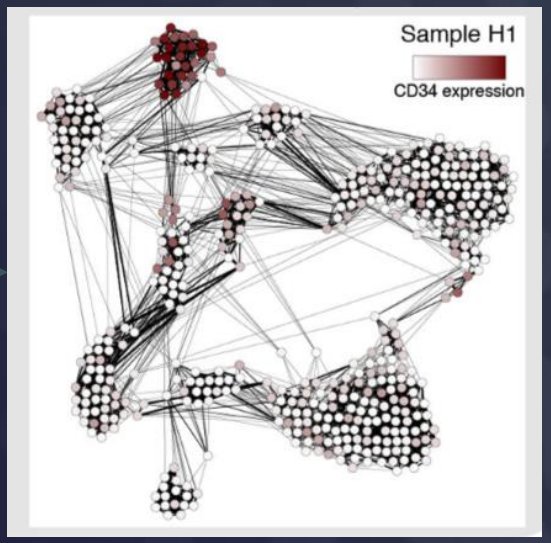
- Does Mass Cytometry help to answer my question?
- Well defined cohort/dataset
- Spend time in designing your panel (spillover, don’t forget any marker)
- Need bioinformatics support?

- Expensive (?)
- Low Throughput
- Takes time

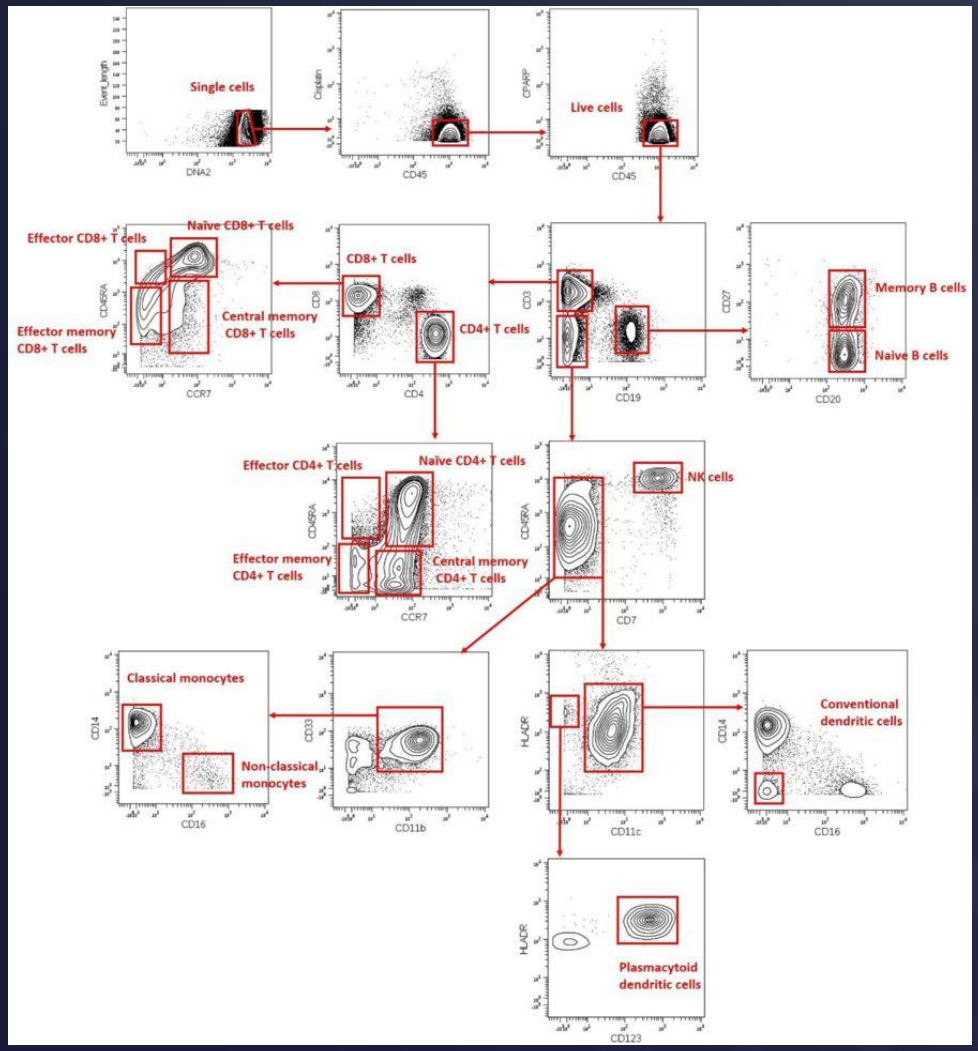
Your Mass Cytometry project

Hope
Reality



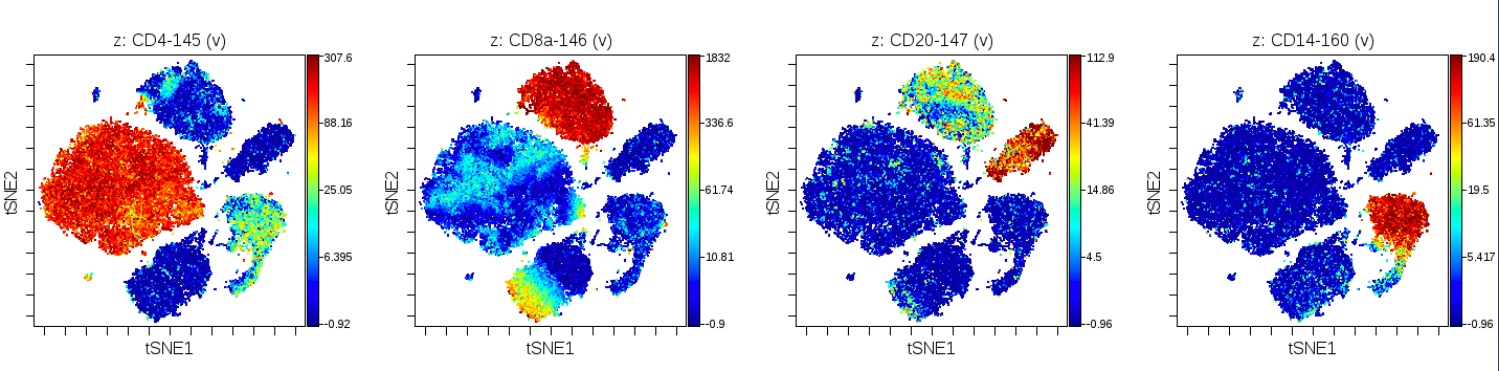


Standard manual gating using bivariate plots



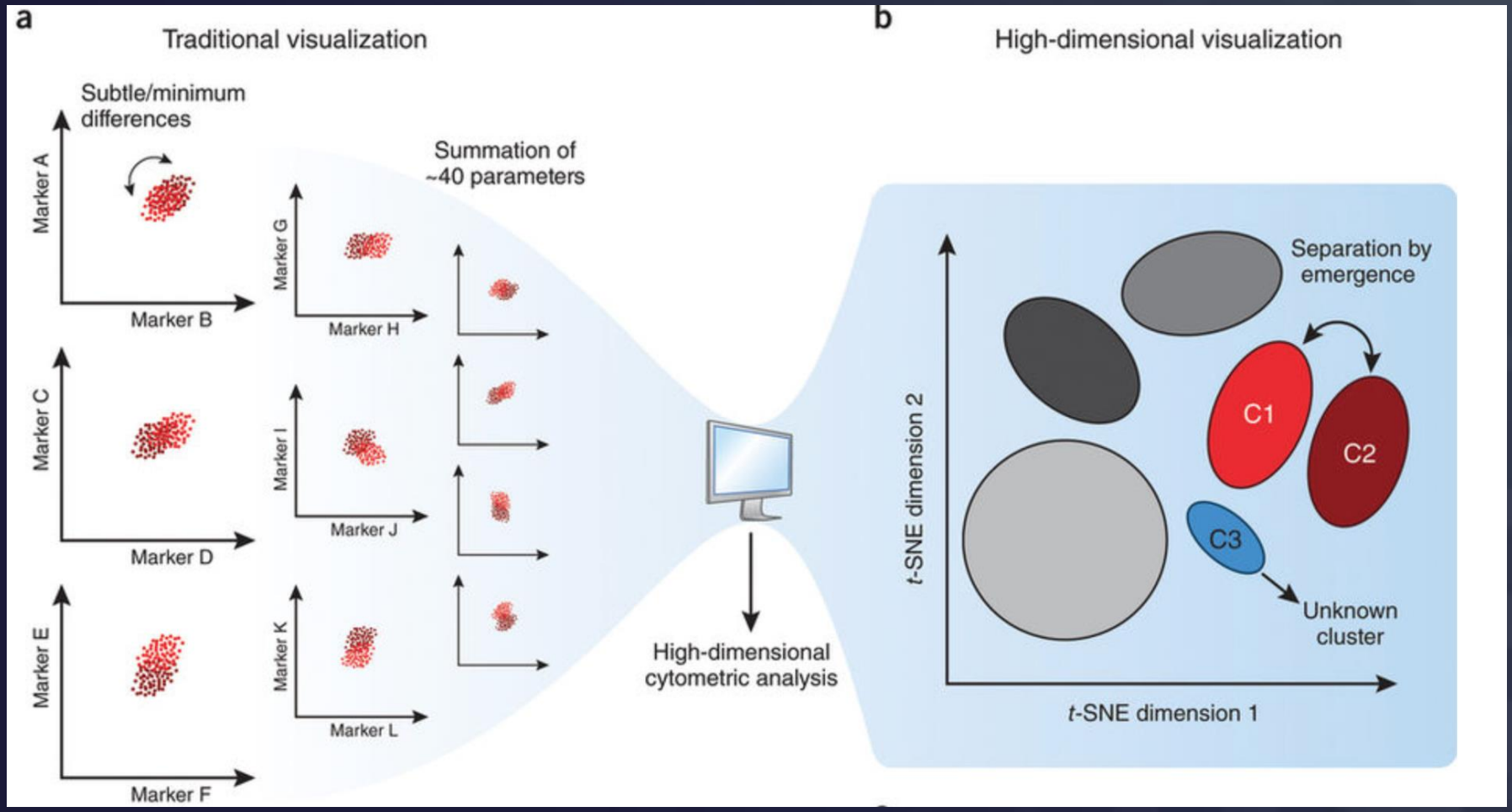
N Markers= NxN/2 bivariate plots

Multidimensionality reduction



Nature Biotechnology **volume 31**, pages 545–552 (2013)

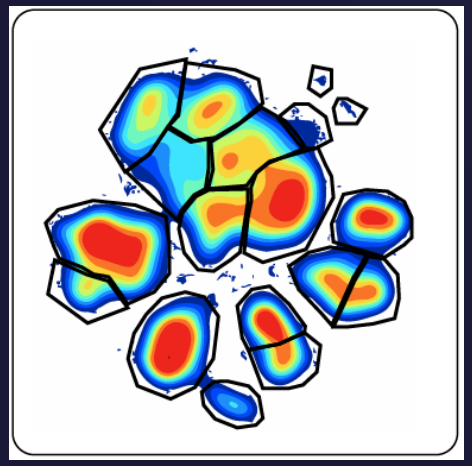
The power of high dimensional analysis



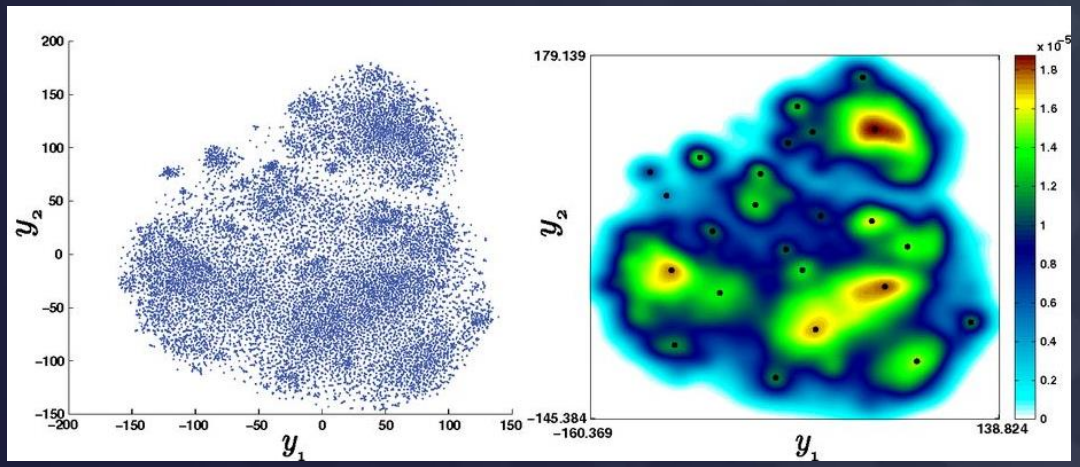
Nature Immunology volume 17, pages 890–895 (2016)

Defining distinct populations on viSNE plots

Manual-gating on a bi-dimensional map

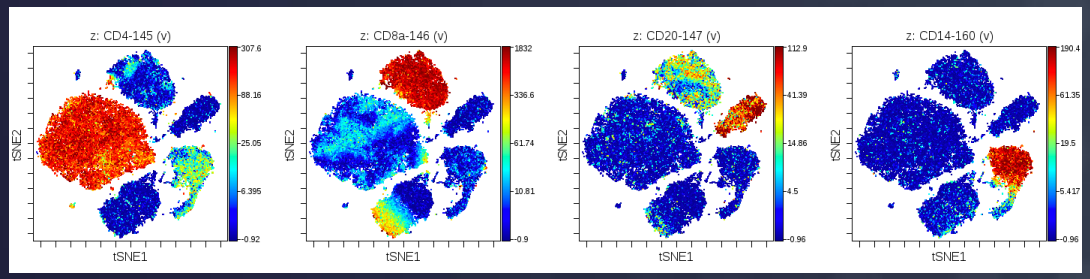


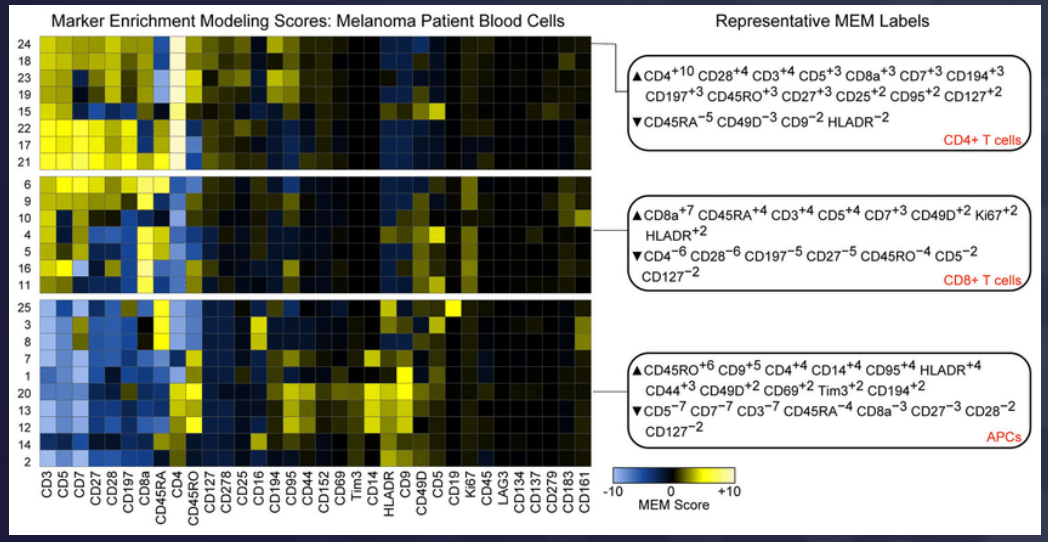
Unsupervised clustering (ACCENSE, DensVM, ClusterX)



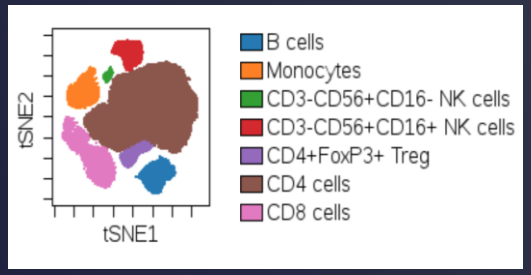
Define the features of the different clusters

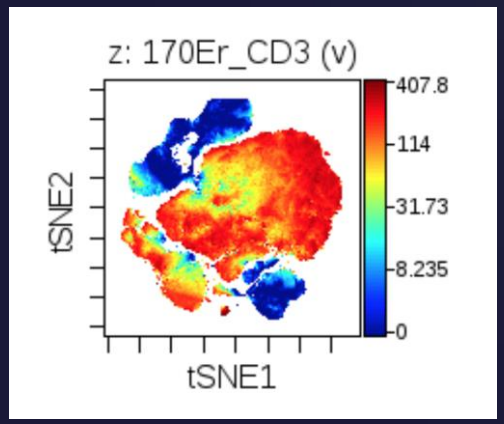
- Visualization of marker expression (viSNE)
- Heatmaps (Cytofkit, Cytobank)
- MEM (marker enrichment model)

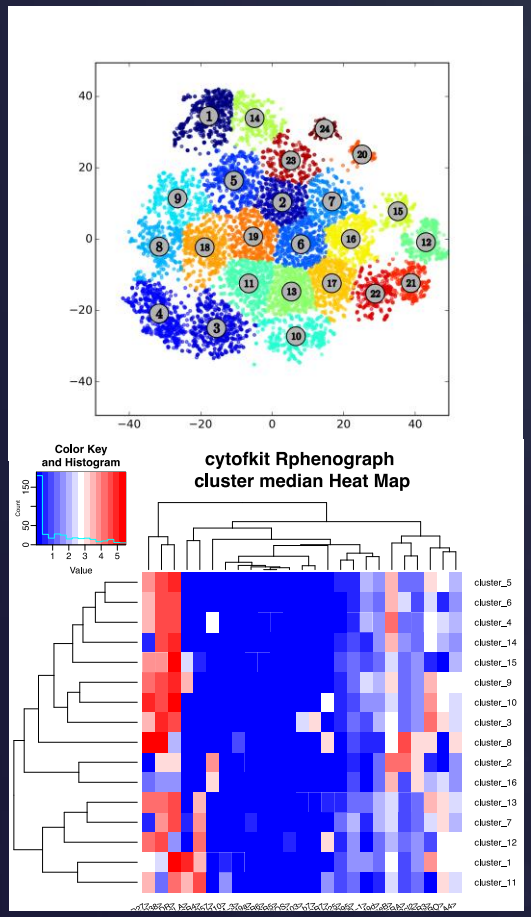


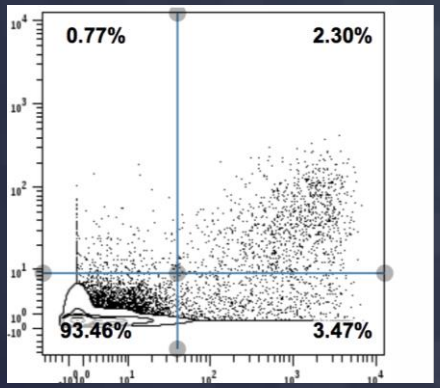


Data analysis workflow









Software

Cytobank

- heavily down-sampled
- SPADE
- viSNE
- Citrus
- flowSOM

Cytofkit

- Phenograph
- UMAP
- Cluster X
- DensVM
- t-SNE
- Isomap
- PCA

- Phenograph
- UMAP
- t-SNE
- PCA
- SPADE
- FlowSOM
- downsampler

Issues in data analysis

Batch effects

Computationally heavy

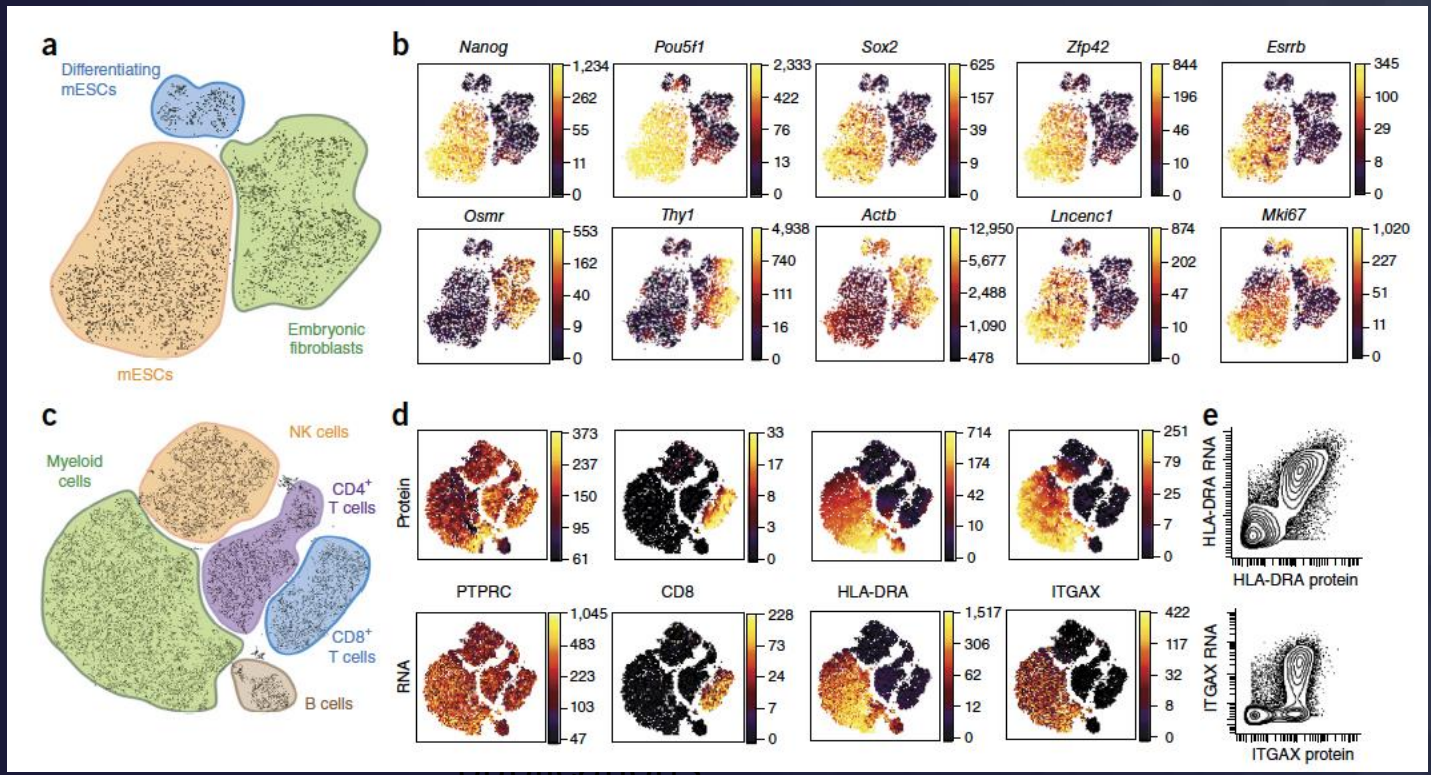
Kinetics, longitudinal studies, biological models, etc.

Need for bioinformatics support

nature methods

Highly multiplexed simultaneous detection of RNAs and proteins in single cells

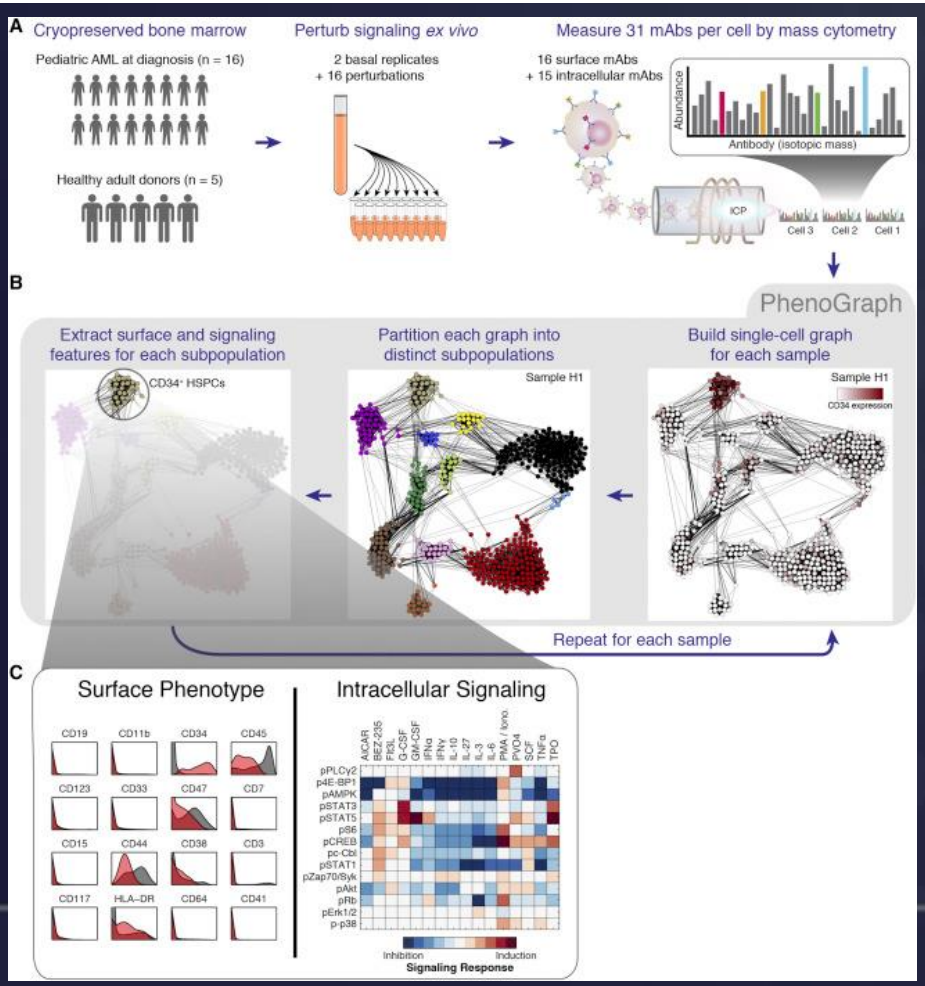
Andreas P Frei[1,5], Felice-Alessio Bava[1,5], Eli R Zunder[1,2], Elena W Y Hsieh[1,3,4], Shih-Yu Chen[1], Garry P Nolan[1] & Pier Federico Gherardini[1]



publications

Cell

Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis



16-phenotypic markers
15-phospho-proteins

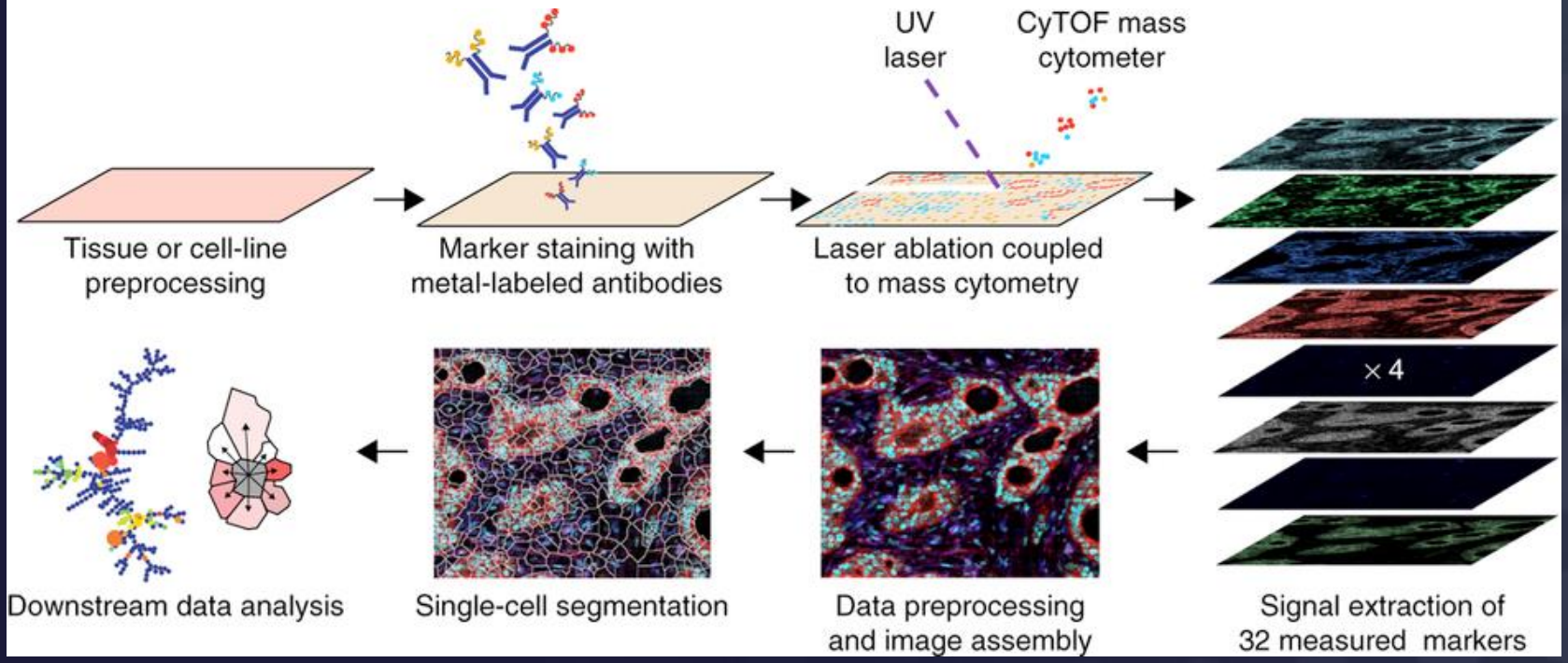
Levine JH et al, 2015

Hyperion™
Imaging System

nature methods

Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry

Charlotte Giesen[1,8], Hao A O Wang[2,3,8], Denis Schapiro[1,4], Nevena Zivanovic[1,5], Andrea Jacobs[1], Bodo Hattendorf[2], Peter J Schüffler[6], Daniel Grolimund[3], Joachim M Buhmann[6], Simone Brandt[7], Zsuzsanna Varga[7], Peter J Wild[7], Detlef Günther[2] & Bernd Bodenmiller[1]



FACS vs CyTOF

Parameters	4-10 easy 10-20 hard 20-30 very hard	<35 easy 42-48 hard (Limit 135)
Auto-fluorescence	Yes	No
Sorting	Yes	No
Acquisition speed	10000-20000/s	150-500/s

Competing technologies

Mass Cytometry	Multi Colour Flow Cytometry	Cite-Seq (DNA Tags)
45-60 (Limit 135)	30-50 Fluorochromes	No limits in number of antibodies
	Difficult to build panel Custom made conjugations Limited Flexibility	Low Throughput Low number of cells Sensitivity? $$$$$$
	➢ High Throughput ➢ Cell isolation ➢ High sensitivity	➢ Protein detection+ scRNA sequencing

WIMM Mass Cytometry Facility

https://www.imm.ox.ac.uk/research/facilities/mass-cytometry-facility

Panels available:

Human:
- T cell phenotyping
- T cell functional
- Myeloid
- Phospho-proteins, fixed cells

Mouse:
- General phenotyping