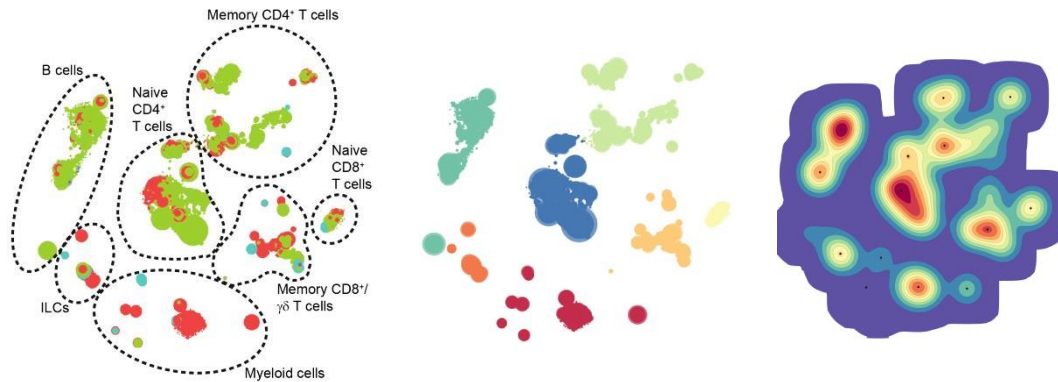


Mass Cytometry



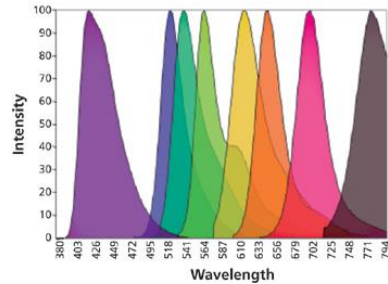
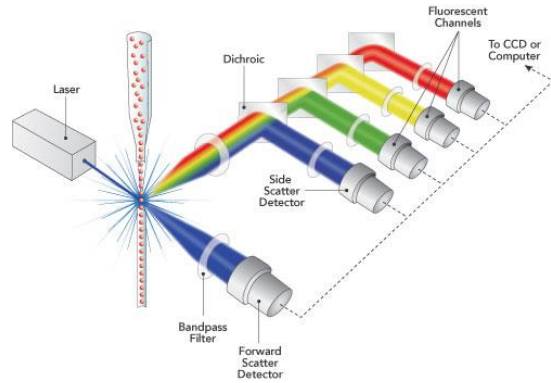
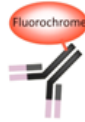
Natasja de Vries

PhD student

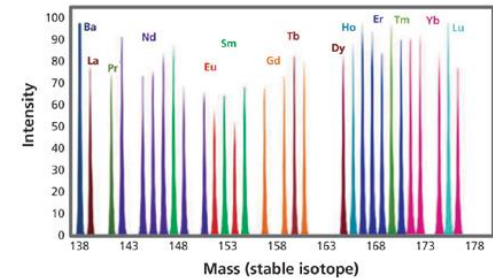
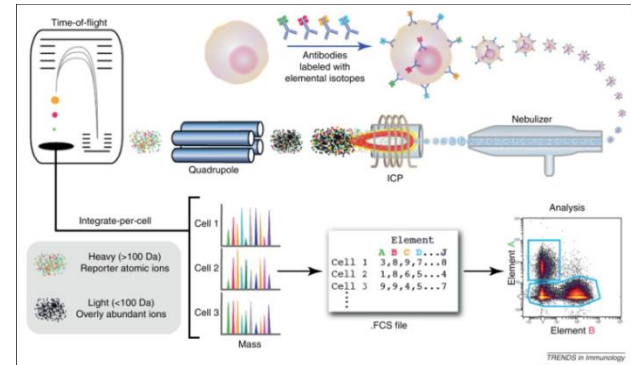
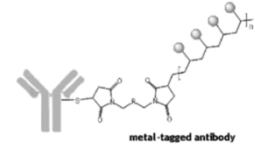
IHB & Pathology

Leiden University Medical Center

Flow cytometry



CyTOF



2009 CyTOF



2013 CyTOF2



2015 Helios



2017 Hyperion



[Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry.](#)

Bandura DR, Baranov VI, Ornatsky OI, Antonov A, Kinach R, Lou X, Pavlov S, Vorobiev S, Dick JE, Tanner SD.

Anal Chem. 2009 Aug 15;81(16):6813-22. doi: 10.1021/ac901049w.

PMID: 19601617

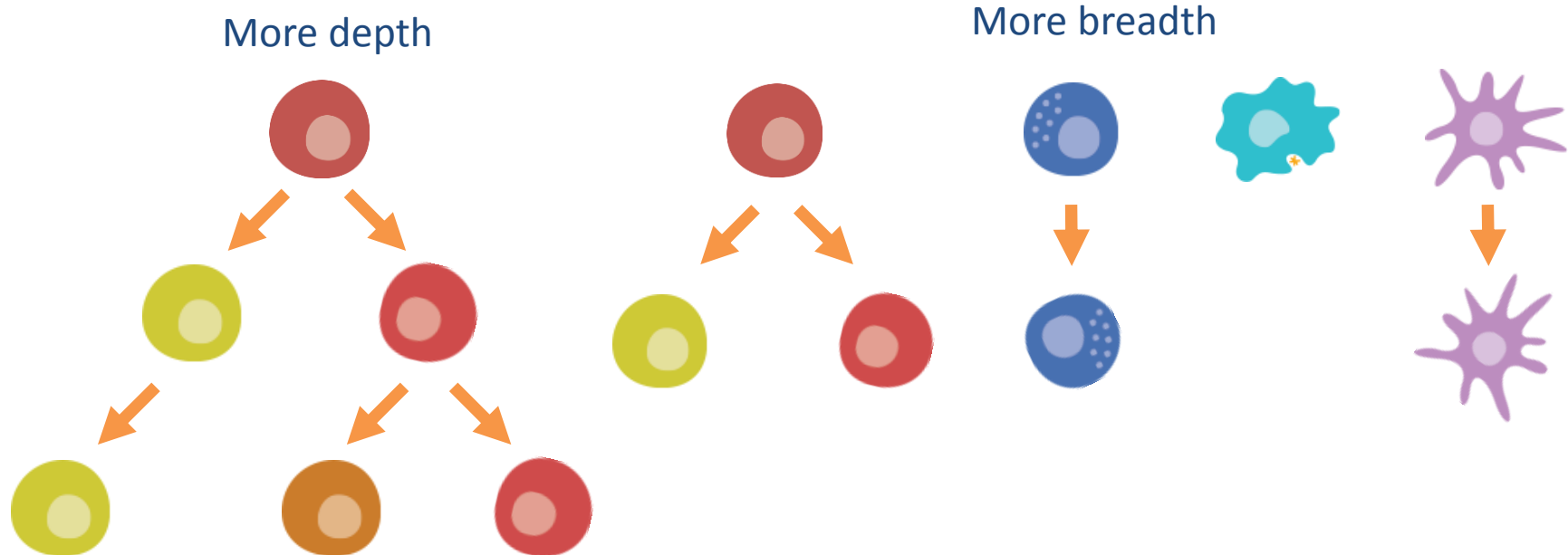
10/2019 on PubMed:

766 papers 'Mass cytometry' or 'CyTOF'

199 papers 'Imaging mass cytometry' or 'Hyperion'

Cytometry by Time-Of-Flight (CyTOF)

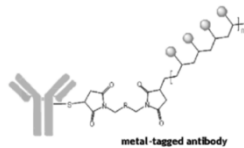
- Immune cells are extremely diverse, many markers are needed



Cytometry by Time-Of-Flight (CyTOF)

- Immune cells are extremely diverse, many markers are needed
- Heavy metal isotopes as antibody reporter

Periodic table



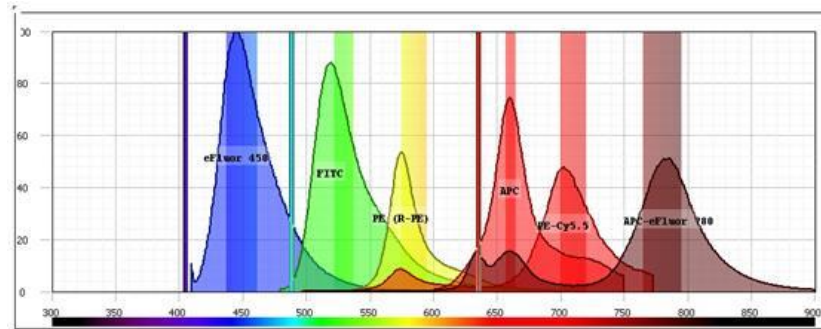
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Voor elementen zonder stabiele isotopen staat het massagetal van de isotoop met de hoogste halveringstijd tussen haakjes.

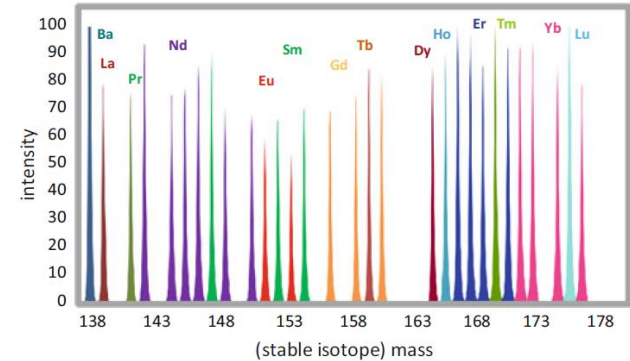
Periodiek Systeem Copyright voor het ontwerp en de interface © 1997 Michael Dayah. Ftable.com Laatst gewijzigd 16 jun. 2017

Cytometry by Time-Of-Flight (CyTOF)

- Immune cells are extremely diverse, many markers are needed
- Heavy metal isotopes as antibody reporter
- Ability to measure many (>40) antibodies



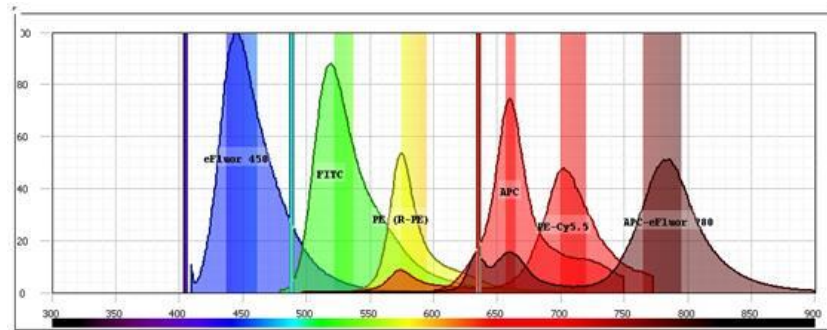
Flow Cytometry



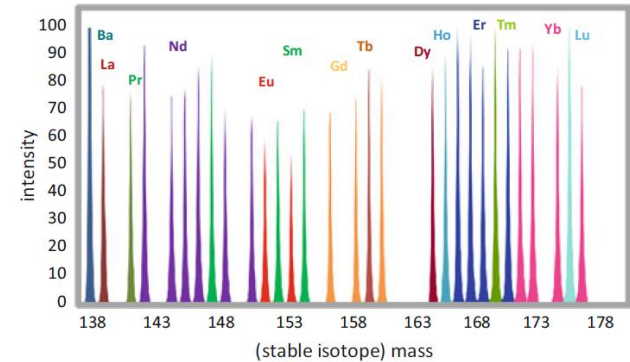
CyTOF

Cytometry by Time-Of-Flight (CyTOF)

- Immune cells are extremely diverse, many markers are needed
- Heavy metal isotopes as antibody reporter
- Ability to measure many (>40) antibodies
- Sharp peaks and less crosstalk between channels

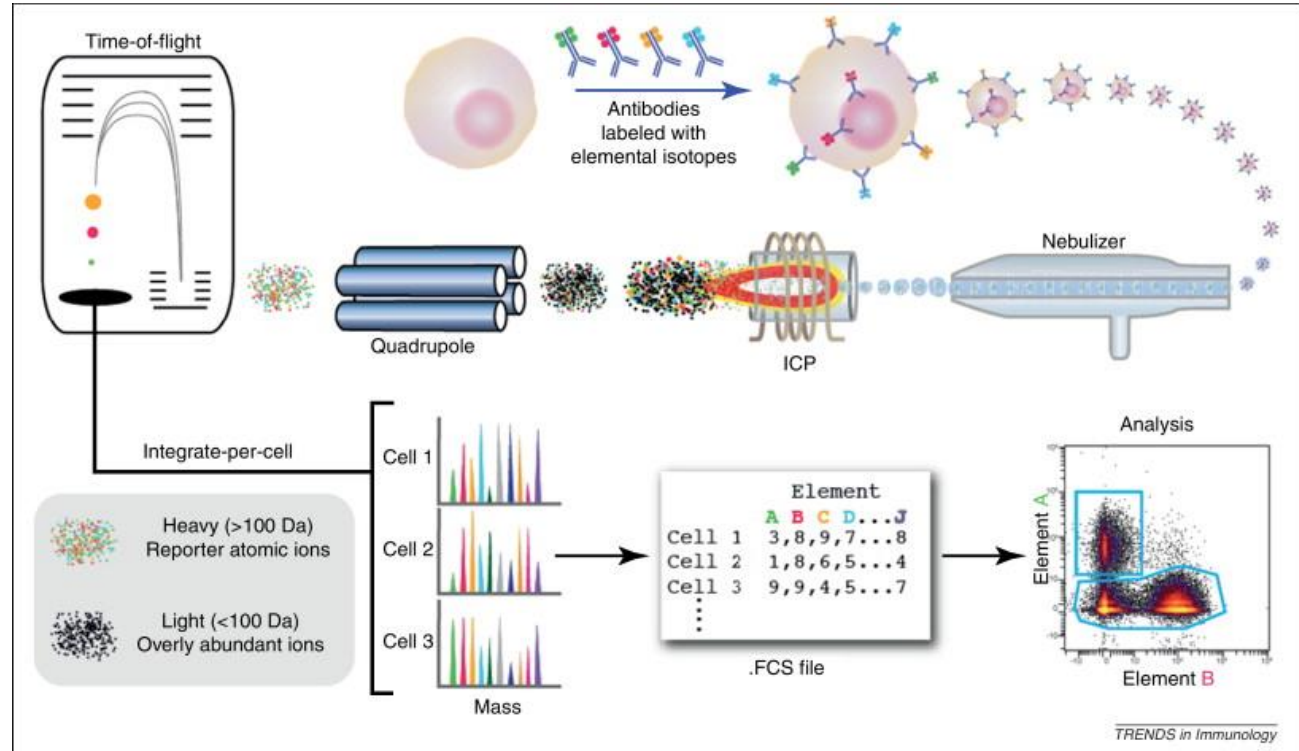


Flow Cytometry



CyTOF

CyTOF workflow



Lanthanides	57 La Lanthanum	58 Ce Cerium	59 Pr Praseodymium	60 Nd Neodymium	61 Pm Promethium	62 Sm Samarium	63 Eu Europium	64 Gd Gadolinium	65 Tb Terbium	66 Dy Dysprosium	67 Ho Holmium	68 Er Erbium	69 Tm Thulium	70 Yb Ytterbium	71 Lu Lutetium
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Advantages and disadvantages of CyTOF

Advantages:

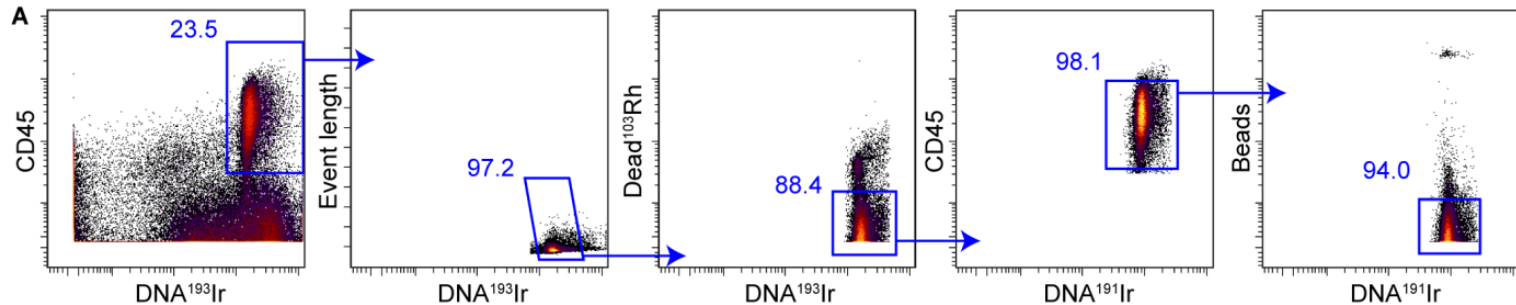
- Minimal overlap in metal signal
- In theory capable of detecting 100 parameters per cell
- Large amounts of data from each experiment

Limitations:

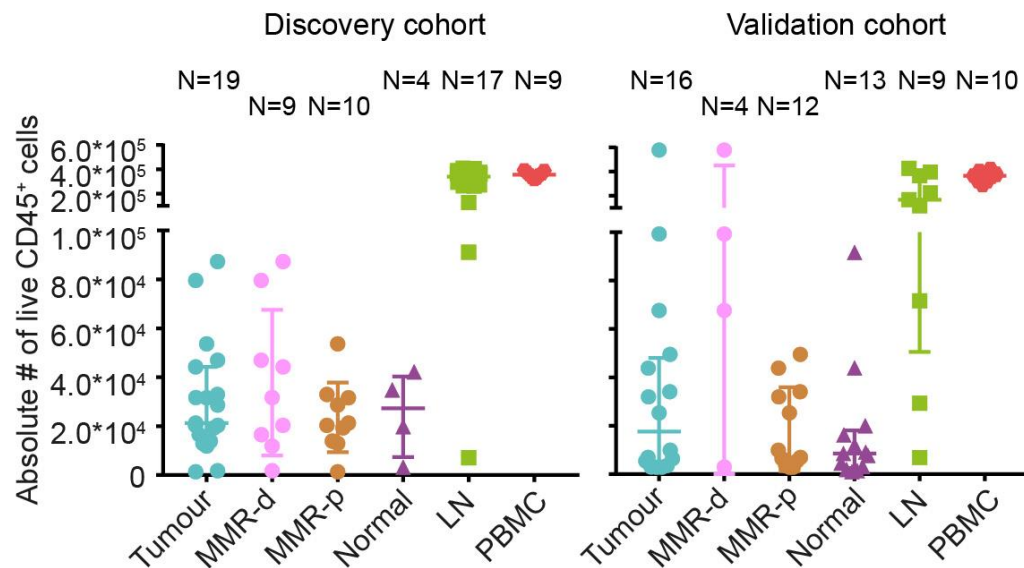
- Flow rate of around 500 cells per second
- Current chemical methods limits use to around 40 parameters per cell
- Expensive
- Cells cannot be sorted for further analysis

CyTOF gating strategy

- CD45: leukocyte common antigen
- DNA stains: 191 / 193 Ir
- Live/dead stain: 103 Rh
- Four element calibration beads: 140 Ce, 151 Eu, 165 Ho, 175 Lu



Absolute number of CD45⁺ cells



Antibody panel (N = 39) for the detection of immune cell markers

Major lineage markers	
CD45	Pan immune
CD3	T cells
CD4	CD4 T cells
CD8 α	CD8 T cells
TCR $\gamma\delta$	$\gamma\delta$ T cells
CD20	B cells
CD14	Monocytes
CD11c	Myeloid cells
CD7	ILCs

Fc/complement receptors	
CD16	Low affinity FC γ R3 α
CD11b	Complement R3

Apoptosis	
CD95	FASR/TNFRSF6

Differentiation/activation markers	
CD45RO	CD45 isoform
CD38	cADP ribose hydrolase
CD161	KLRB1
HLA-DR	Ag presentation
CD335	NKp46

Cytokine/chemokine receptors	
CD123	IL-3R α
CD127	IL-7R α
CD25	IL-2R α
CD122	IL-2R β
CCR6	Chemokine R6
CCR7	Chemokine R7
CXCR3	CXC chemokine R3
CD115	CSF1R

Immunomodulatory molecules	
PD-1	Co-inhibitory R
PD-L1	Co-inhibitory ligand
CD27	Co-stimulatory R
CD28	Co-stimulatory R
CD40	Co-stimulatory R
ICOS	Co-stimulatory R
CD86	Co-stimulatory ligand

Adhesion/homing markers	
CD44	Glycoprotein
CD54	ICAM-1
CD56	NCAM
CD69	Glycoprotein
CD103	Glycoprotein
CD163	High-affinity scavenger R
KLRG-1	Glycoprotein

CyTOF data analysis

- Large number of cells (8.9 million live CD45⁺ cells)
- High-dimensionality of the data

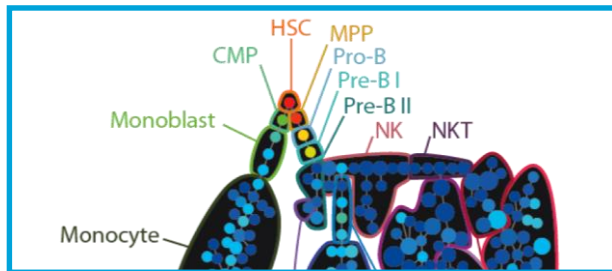
Colorectal tumor – Live CD45⁺ cells



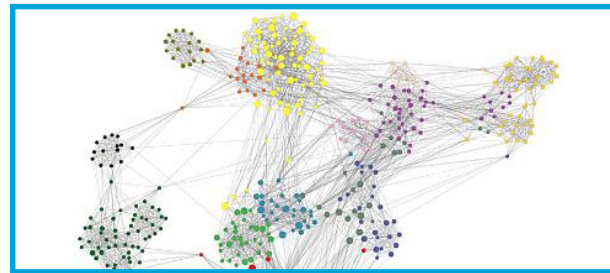
Current single-cell computational tools

Clustering-based

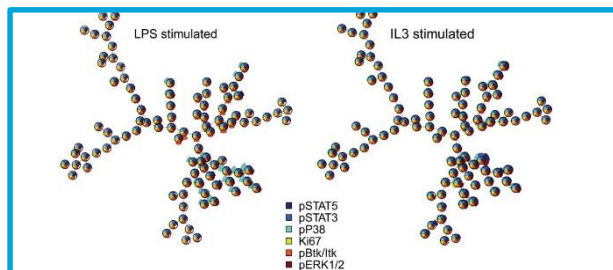
No single-cell resolution



[1] Qiu et al., *Extracting a Cellular Hierarchy from High-dimensional Cytometry Data with **SPADE***, Nature Biotechnology, 2011



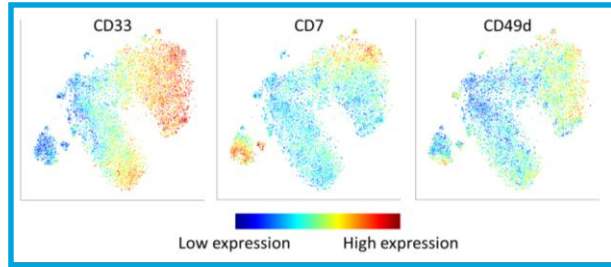
[2] Levine et al., *Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis (**Phenograph**)*, Cell 2015



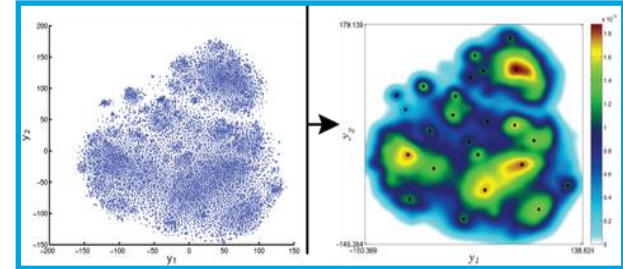
[3] Van Gassen et al., *FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data (**FlowSOM**)*, Cytometry A, 2015

Current single-cell computational tools

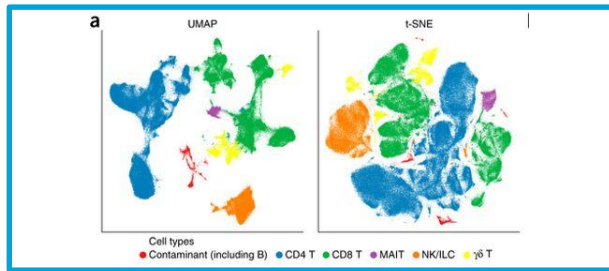
Dimensionality-Reduction-based



[1] Amir et al., **viSNE** enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia, Nature Biotech. 2013.



[2] Shekhar et al., Automatic classification of cellular expression by nonlinear stochastic embedding (**ACCENSE**), PNAS, 2014

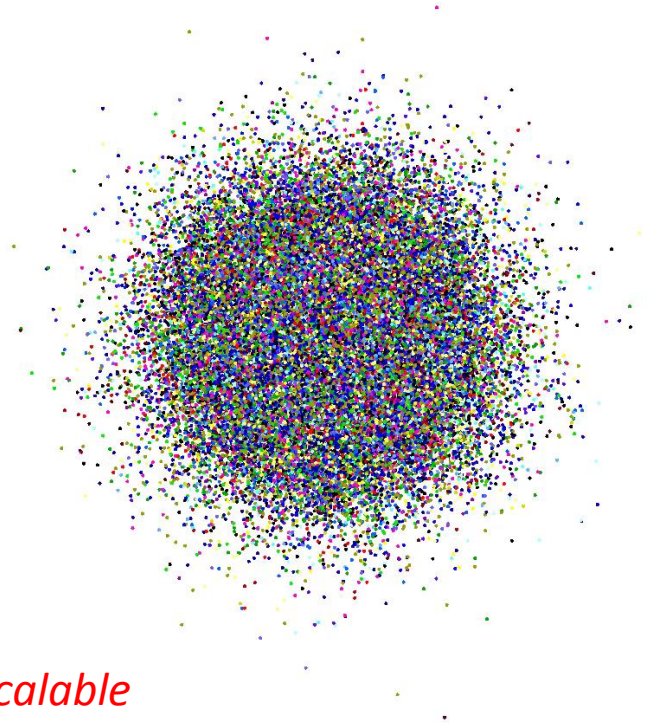


[3] Becht et al., Dimensionality reduction for visualizing single-cell data using **UMAP**, Nature Biotech. 2019

Current single-cell computational tools

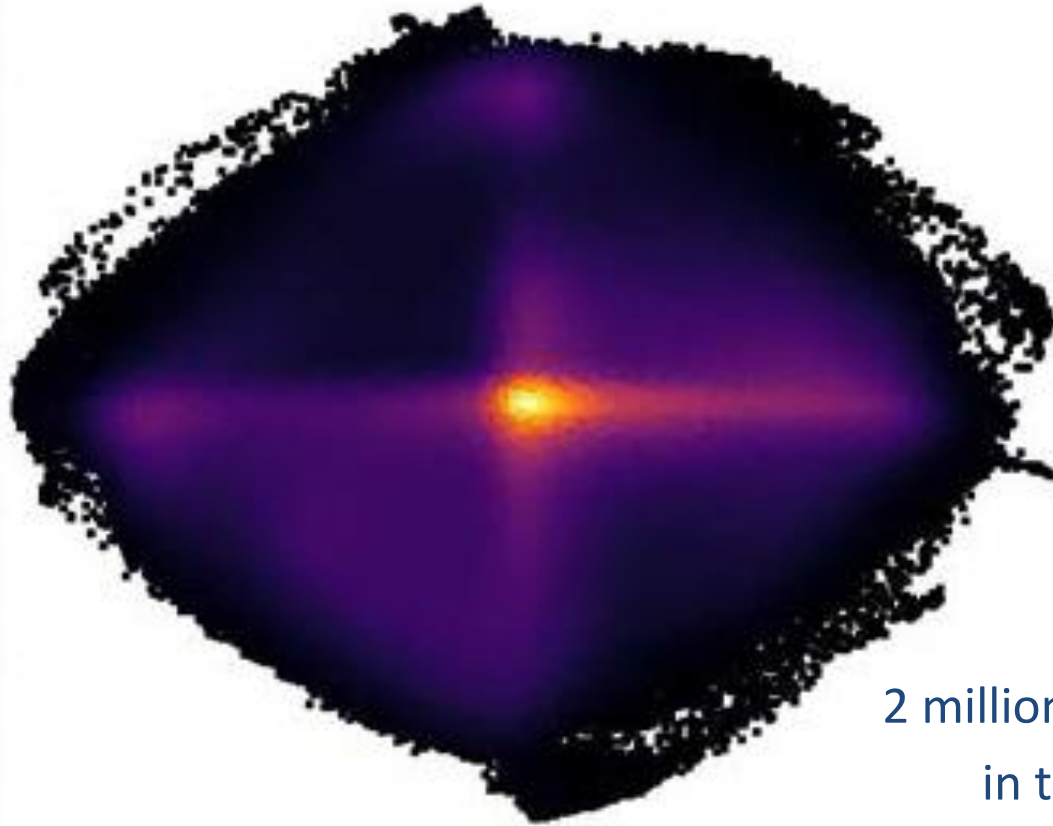
Dimensionality-Reduction-based

t-SNE analysis



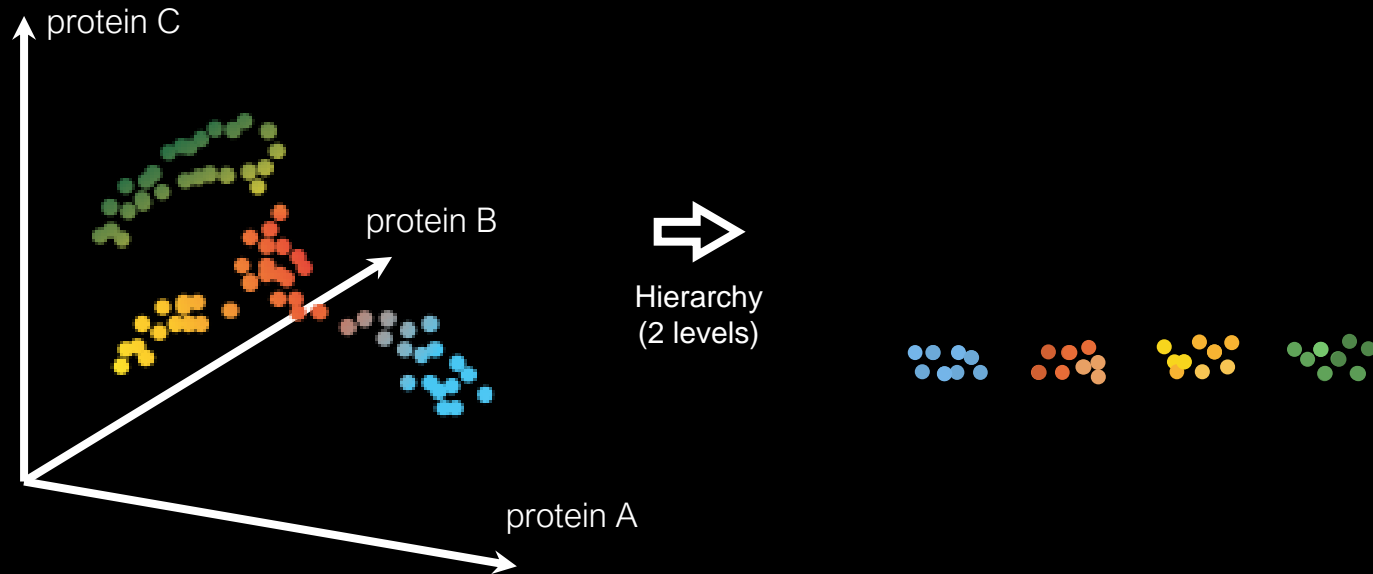
Not scalable

Problems with t-SNE analysis: data overcrowding

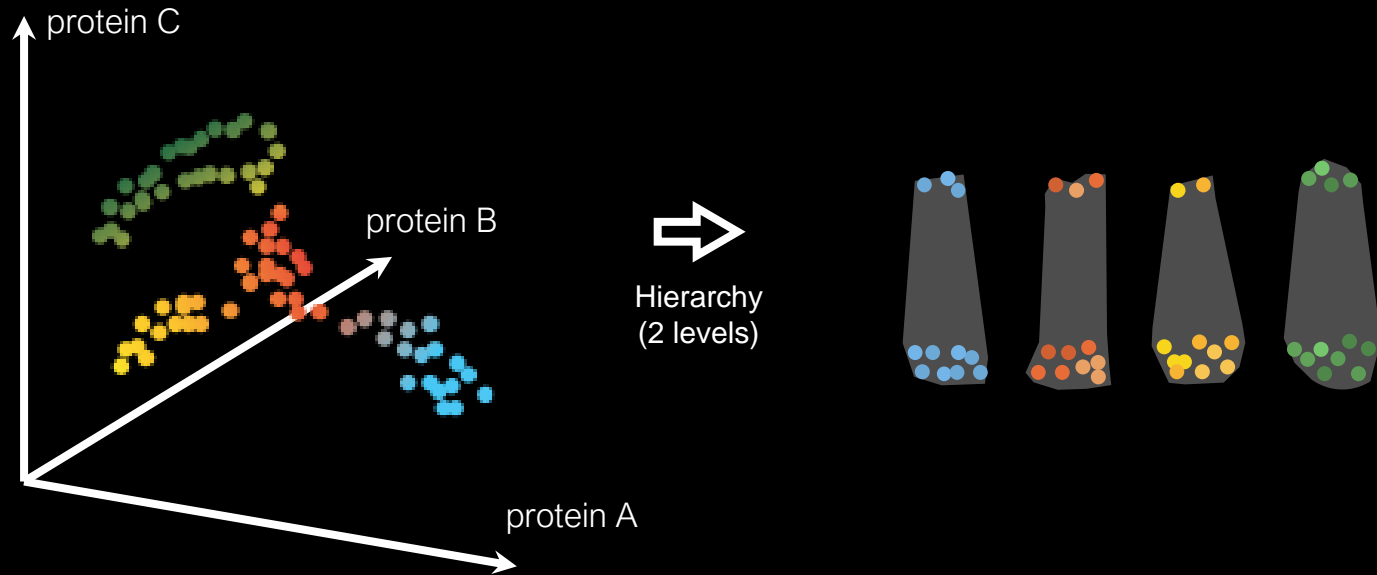


2 millions of cells
in t-SNE

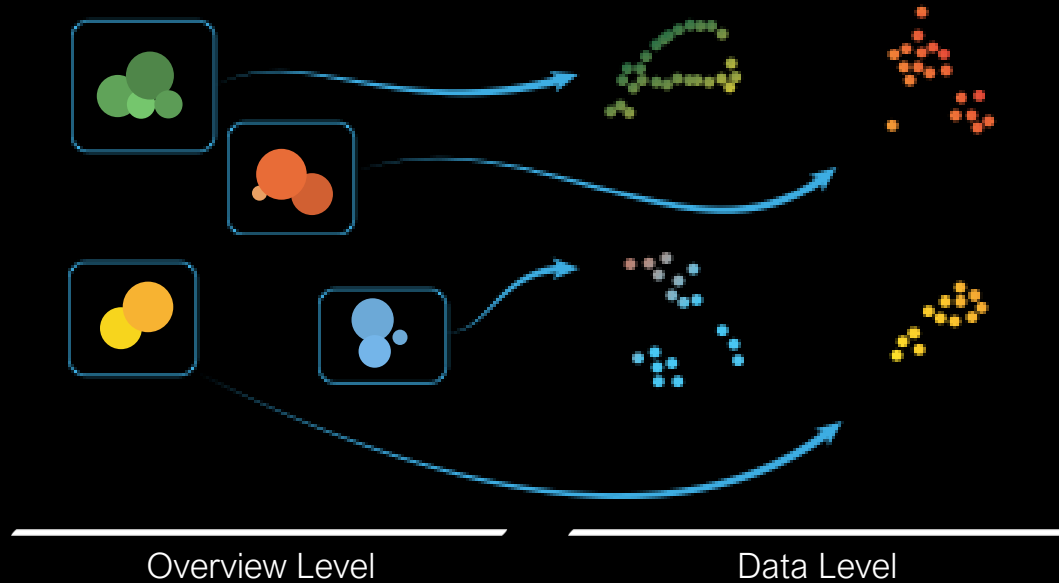
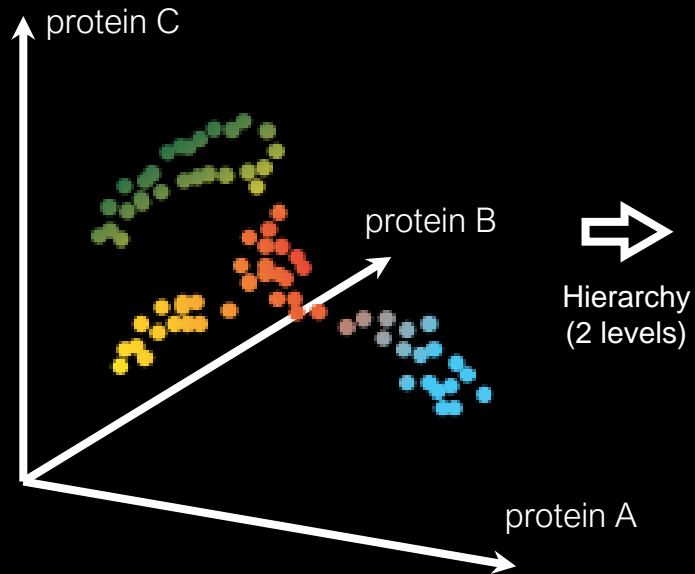
Hierarchical SNE



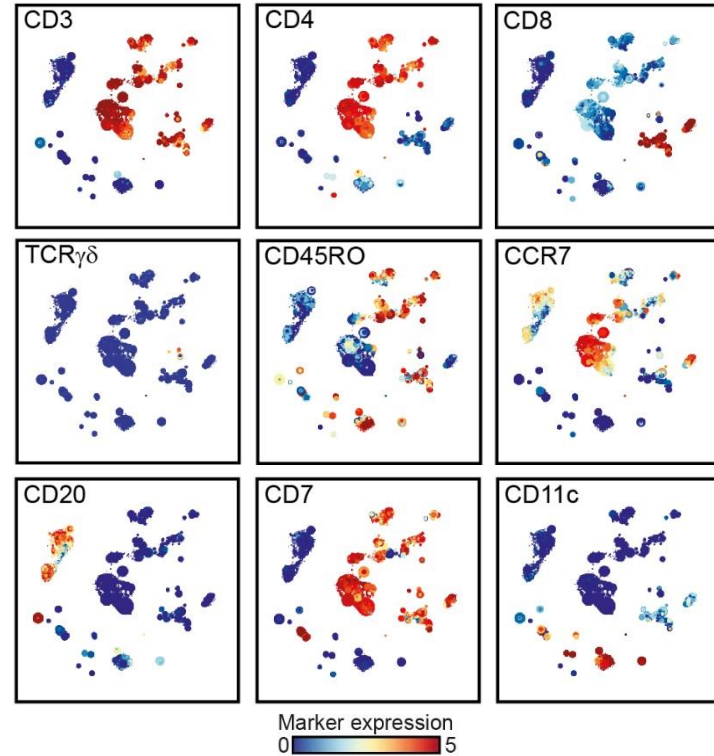
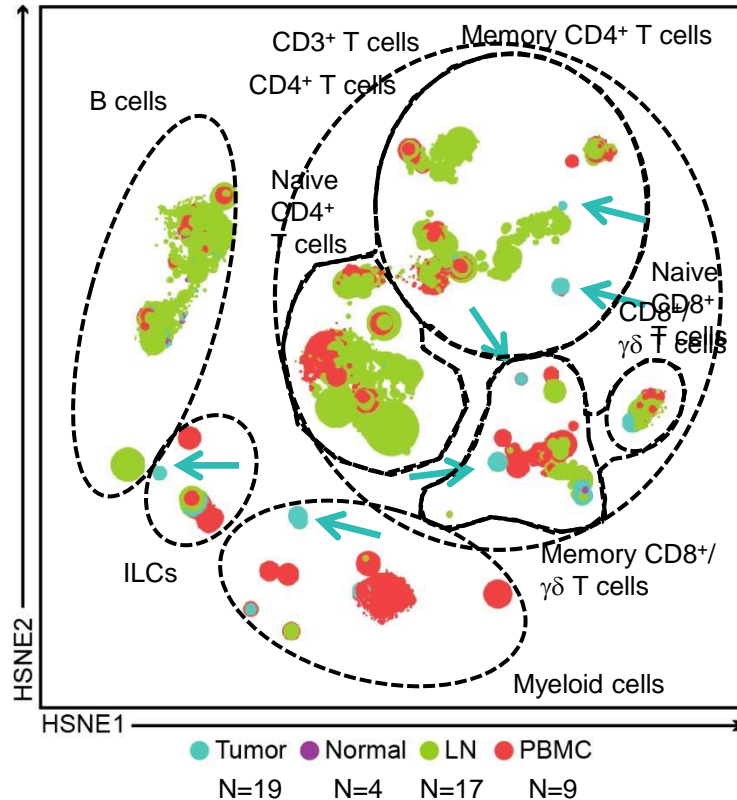
Hierarchical SNE



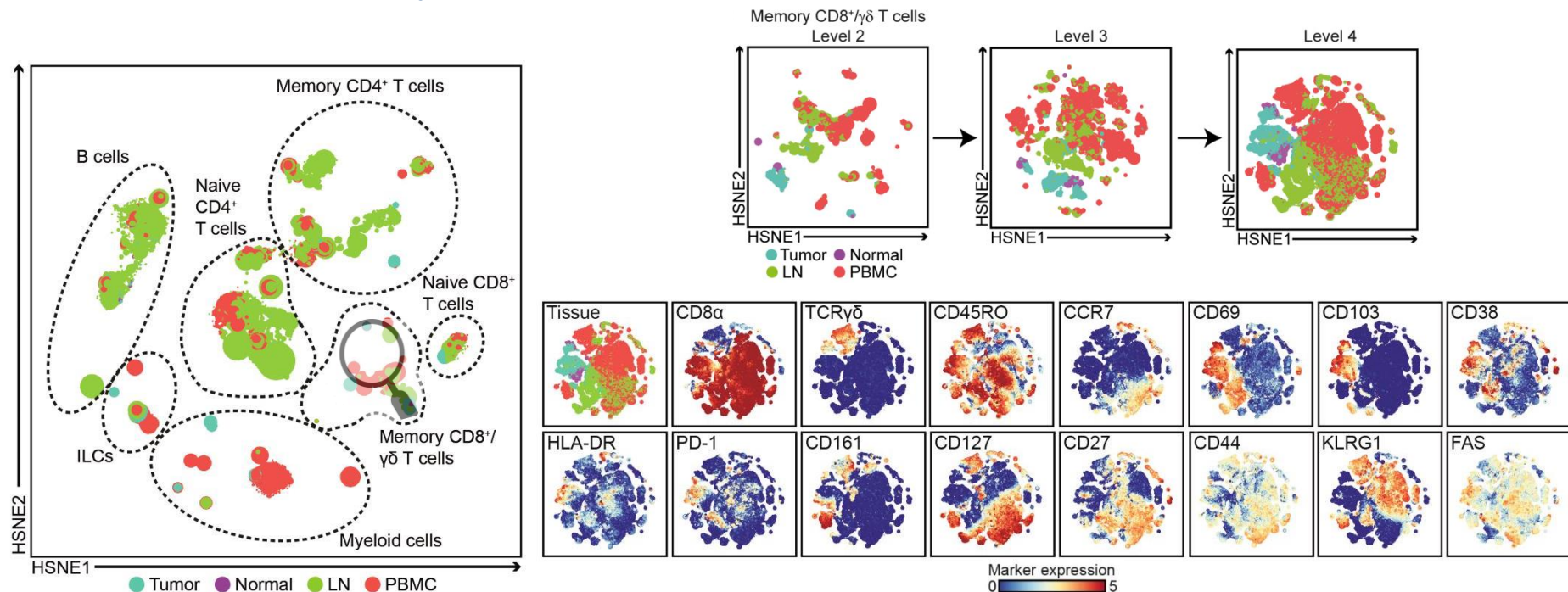
Hierarchical SNE



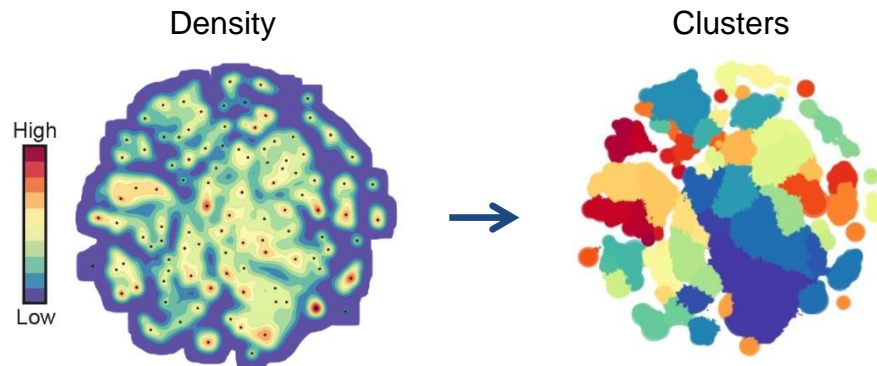
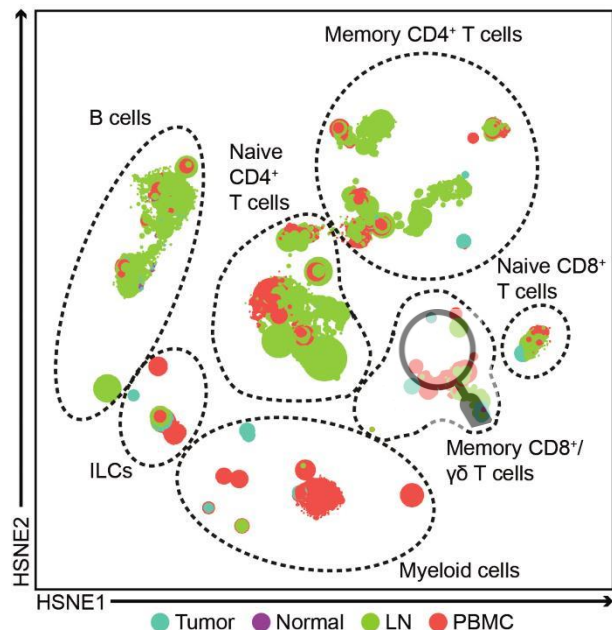
8.9 million immune cells derived from different samples of colorectal cancer (CRC) patients



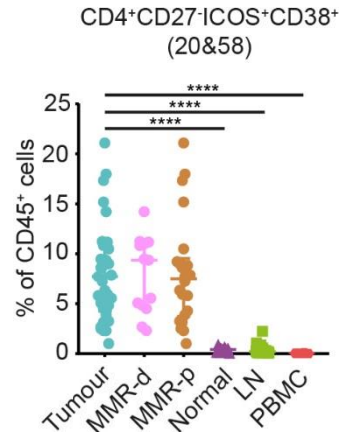
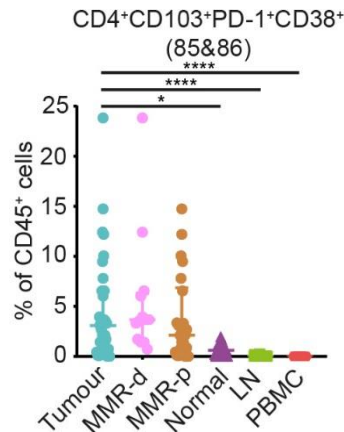
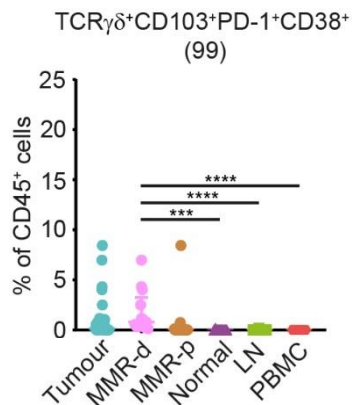
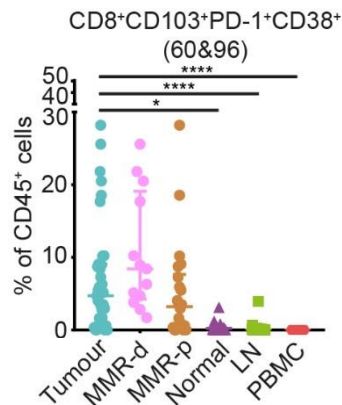
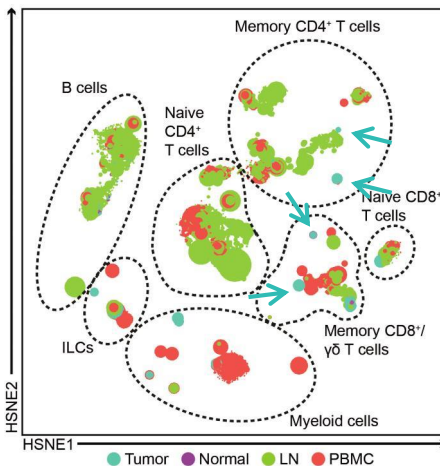
Hierarchical exploration of the memory CD8⁺/γδ T cell compartment by HSNE



Hierarchical exploration of the memory CD8⁺/γδ T cell compartment by HSNE



Identification of tumour tissue-specific T cell clusters

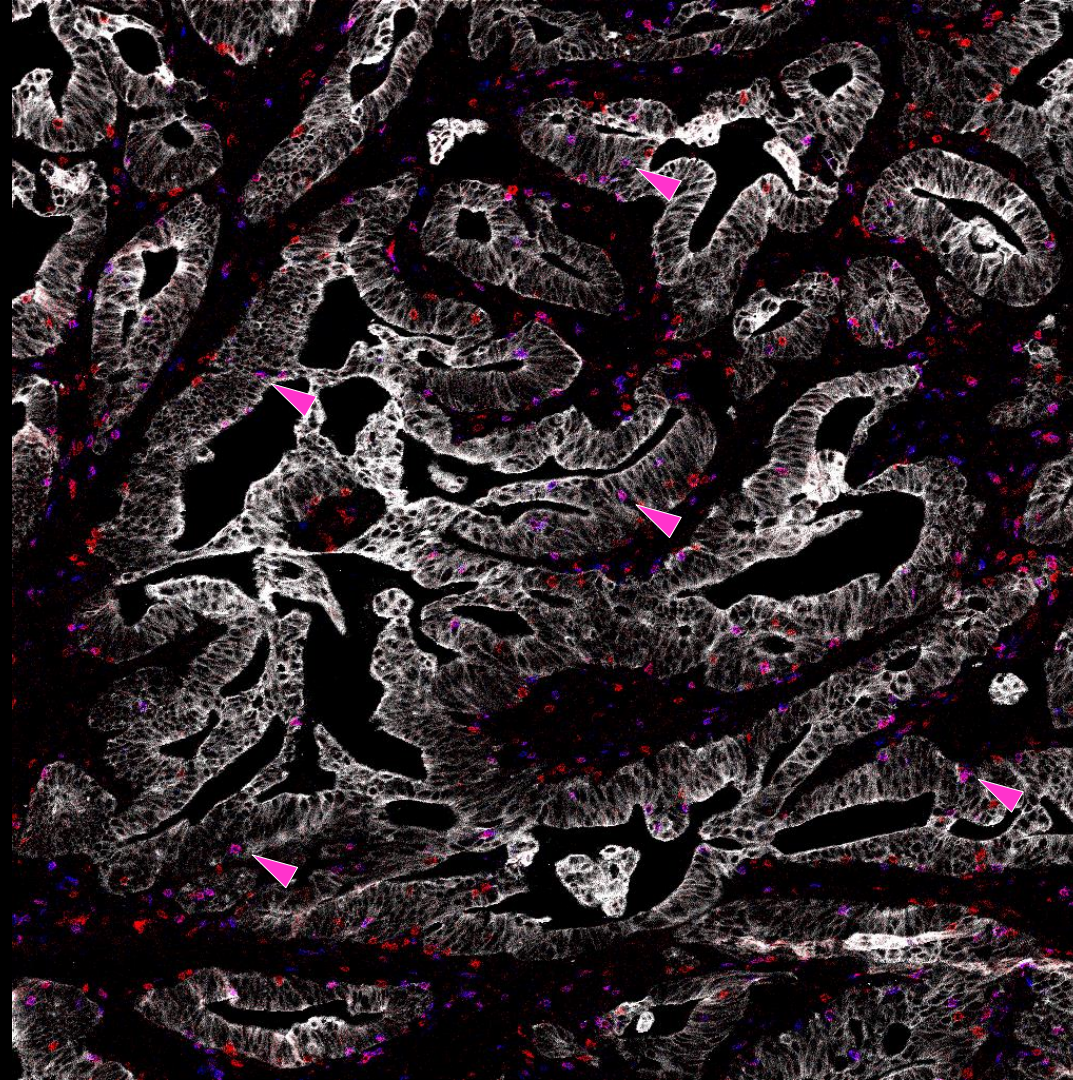


Keratin

CD8

CD103

CD8/CD103

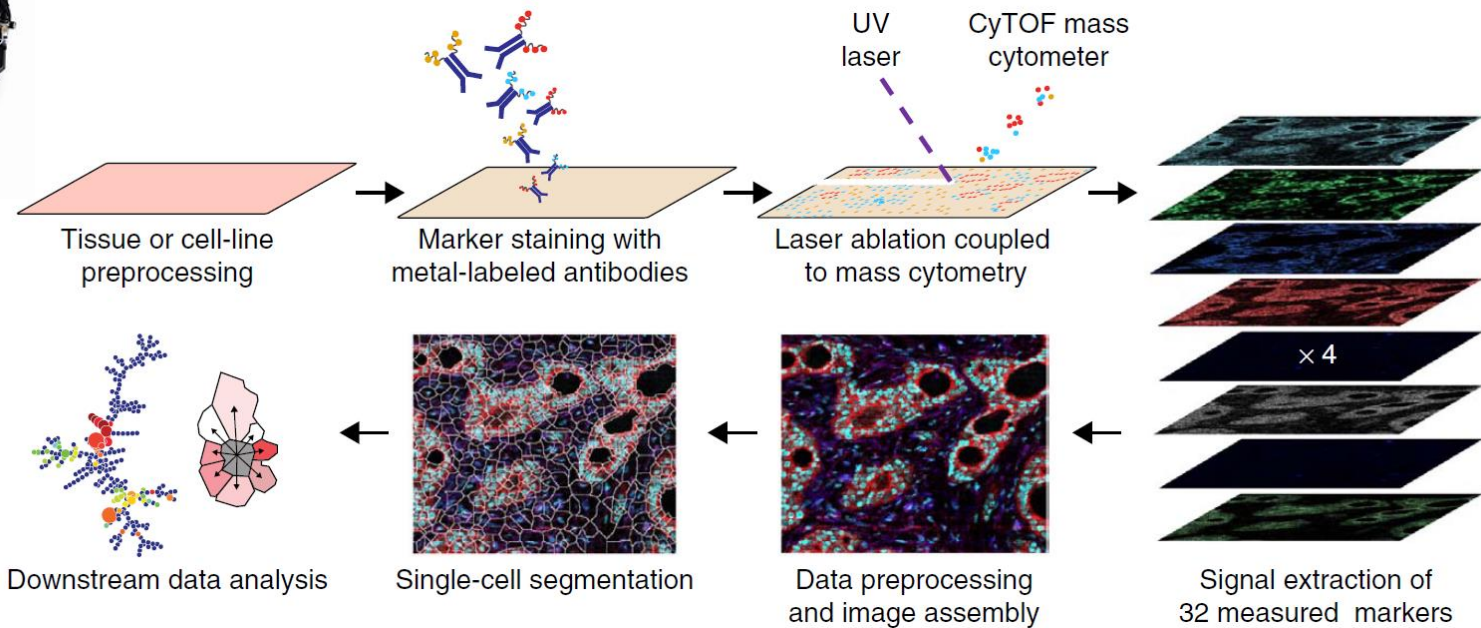


Colorectal tumour





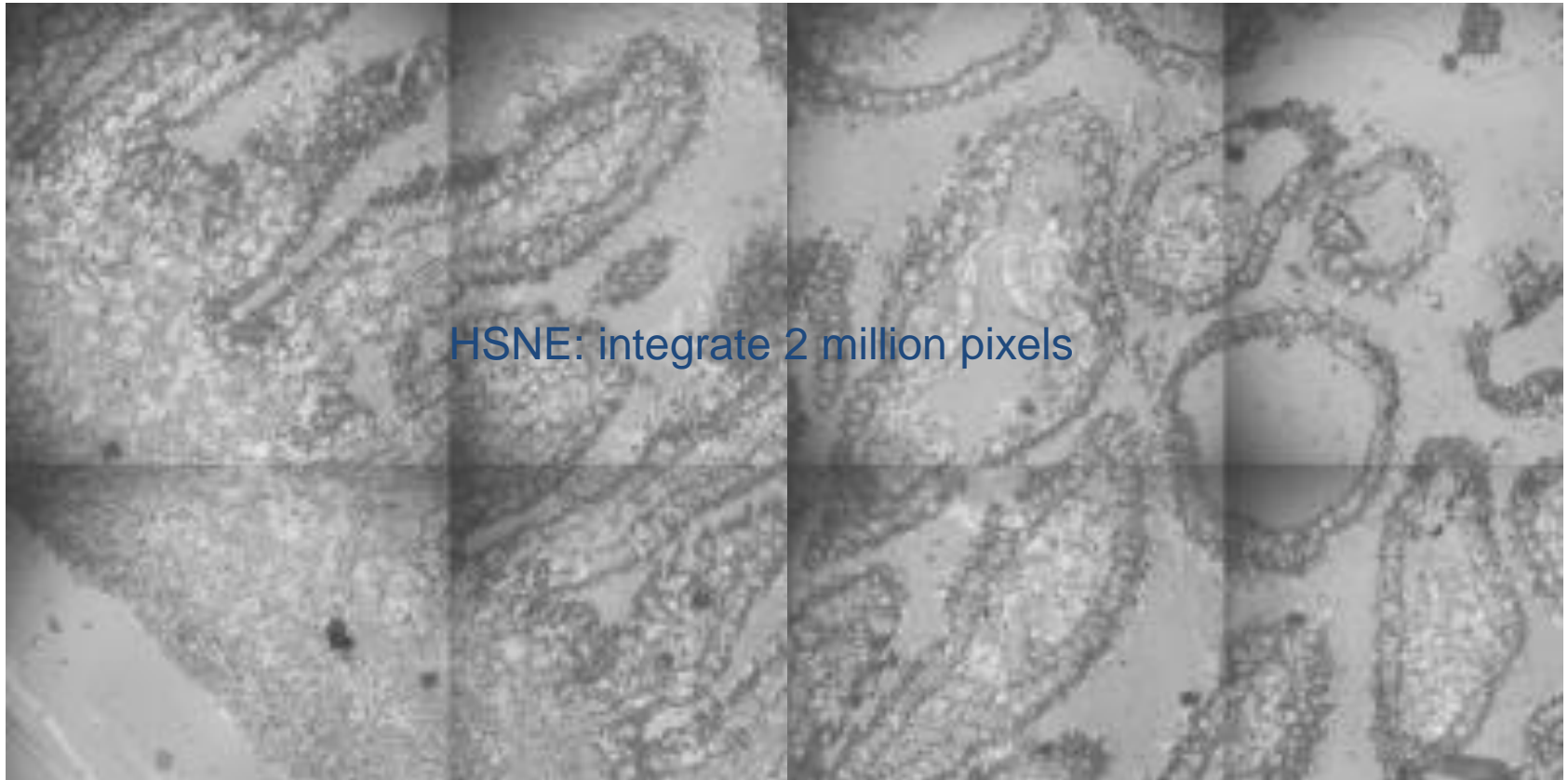
Imaging CyTOF (Hyperion)



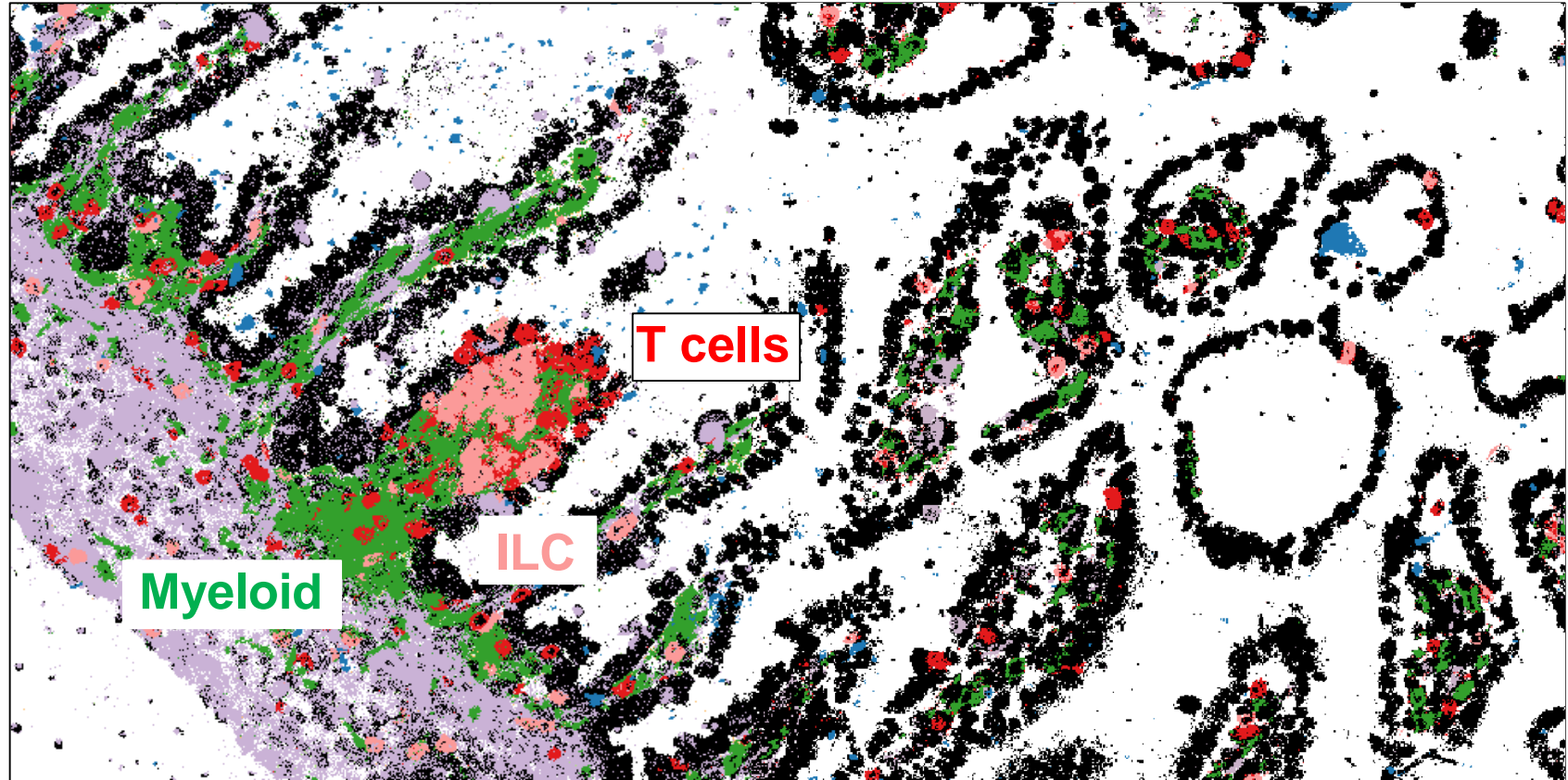
- FFPE and snapfrozen tissue sections
- 1 μM resolution
- 40+ markers
- No autofluorescence

Giesen *et al.* Nat Methods (2014)

Imaging CyTOF (Hyperion) – Fetal gut before ablation

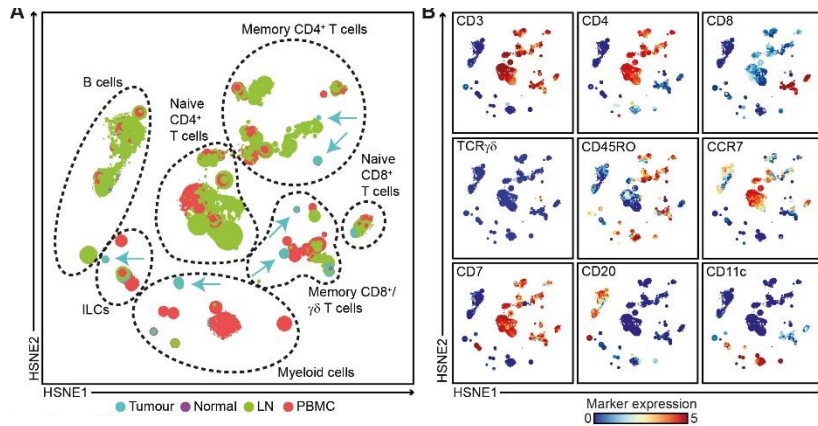


Imaging CyTOF (Hyperion) – HSNE analysis of the fetal gut

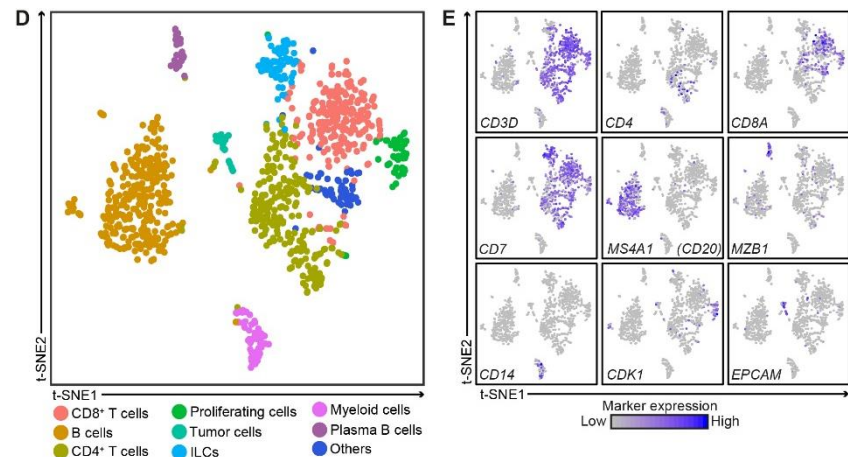


Single-cell RNA-sequencing of CRCs reveals the presence of similar immune cell clusters

Mass cytometry



Single-cell RNA-sequencing

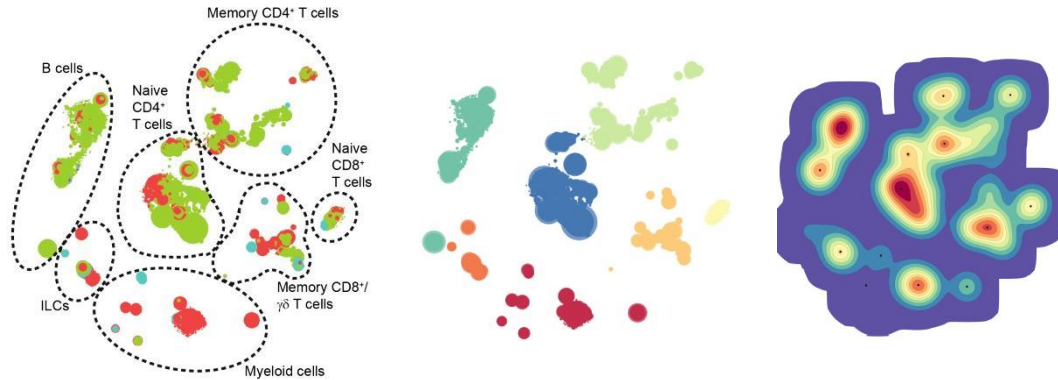


Take home messages

- Mass cytometry allows simultaneous analysis of 40+ markers on single-cells, resulting in highly complex datasets
- Conventional data analysis approaches are not suitable for such datasets
- Mass cytometry has broad range of research applications (e.g. immunology, hematology, oncology, etc.)
- It can be used for the detection of extra- and intracellular proteins, cytokines, signaling molecules
- “Data too big for t-SNE? Try HSNE!” (www.cytosplore.org)
 - Allows analysis of millions of cells, maintaining the non-linearity of the data
 - From global visualization to single-cell data resolution

N.L.de_Vries@lumc.nl

Mass Cytometry – Cytosplore practical



Introduction to Cytosplore practical

- Cytosplore + HSNE: <https://vimeo.com/205552113>

Cytosplore practical

- Colorectal cancer (CRC) patient S00184
 - Tumor (T)
 - Healthy mucosa (normal; N)
 - Tumor-associated lymph node (LN)
 - Peripheral blood mononuclear cells (PBMC)
- FCS files have been pre-gated on CD45⁺ immune cells
- Downsample to 5,000 cells / sample
- Cluster on immune cell markers
- t-SNE analysis versus 3-level HSNE analysis (without downsampling)
 - Marker expression profiles
 - Clustering based on density
 - Coloring of the samples
 - Heatmap
 - Data export