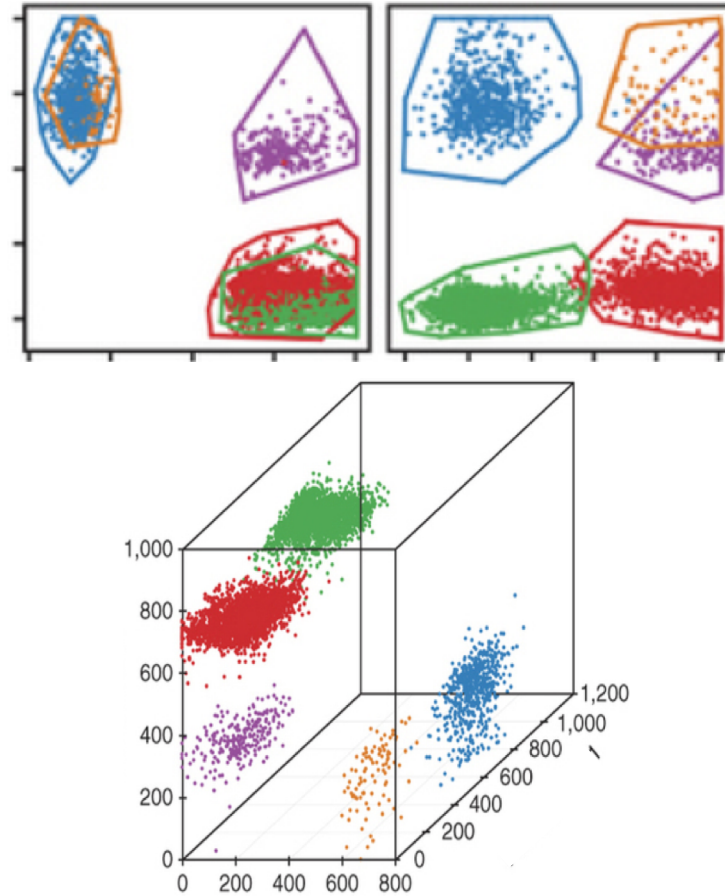


Power of Algorithmic Analysis



Lisa Borghesi
Professor of Immunology
Director, Unified Flow Core

Power of Algorithmic Analysis

High-dimensional analysis – Human brain is not so hot at >3 dimensions. What does 18 dimensional data (18 color) even look like in high dimensional space?

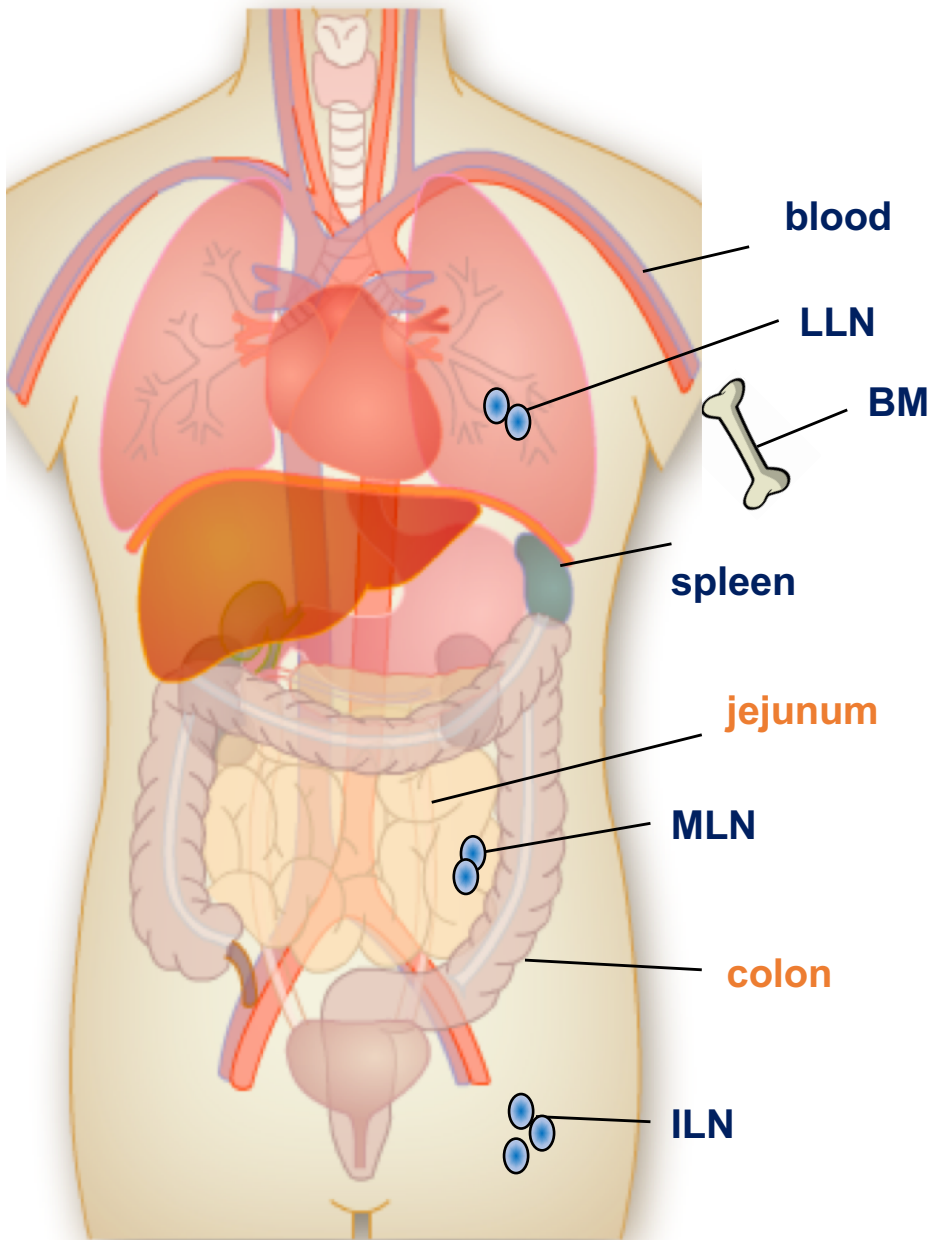
Highlight patterns in the data – Manual analysis with a plethora of iterative bivariate plots is highly inefficient.

Facilitates population discovery – Meaningful populations can be overlooked b/c biases and a priori knowledge dictate analysis.

Automated/more unbiased – Manual analysis is subjective and biased.

Fun!

Creating a Human B cell Atlas



30+ donors

CD19
CD27
CD38
CD138
IgM
IgD
IgG
CD10
CD95

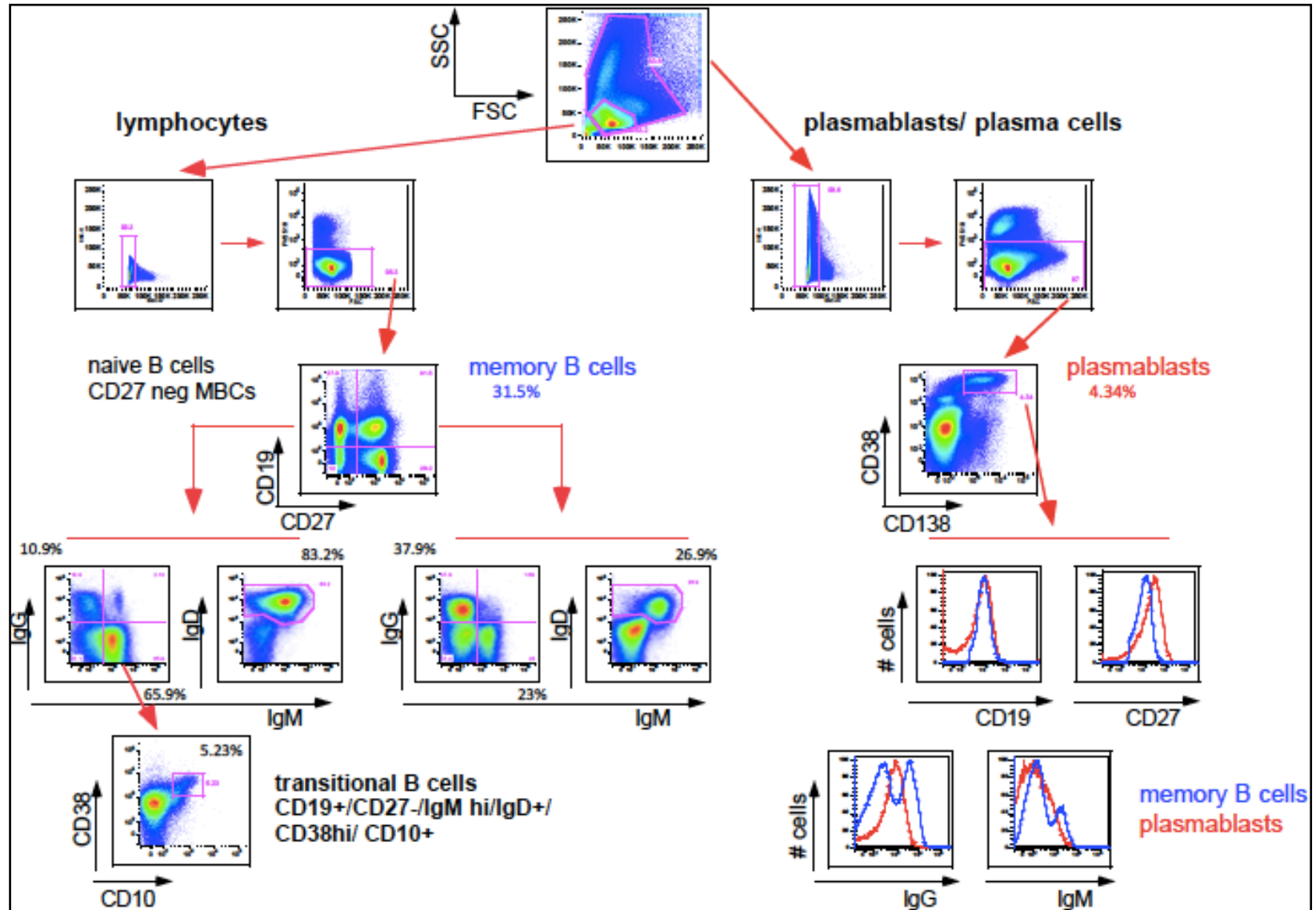
CD45RB
CD69
CD21
CD200
CD218a
CD370
etc.

ERK/MAPK
S6
AKT
mTOR
stat3
zbtb32
bach2



A day in the life of a FlowJo analyst

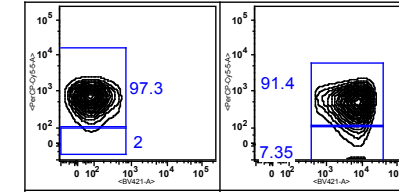
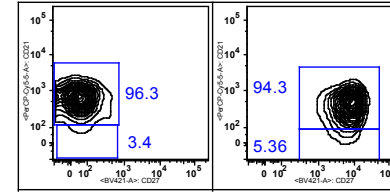
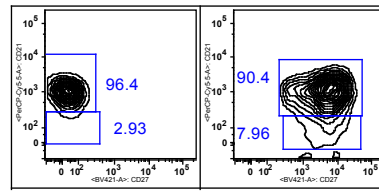
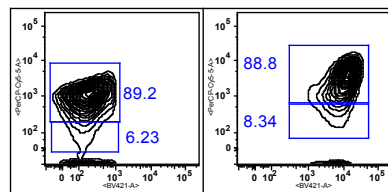
Donor 1, Tissue 1



Courtesy: Florian Weisel

D256 various samples – subsets CD45RB/ CD69

CD21
↑
CD27



CD45RB
↑
CD69

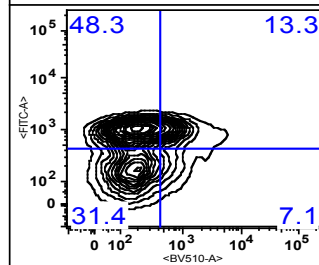
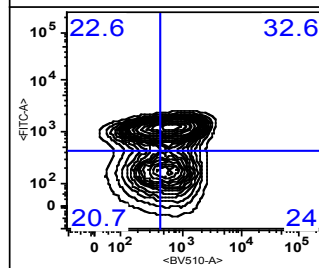
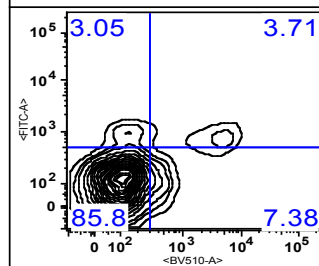
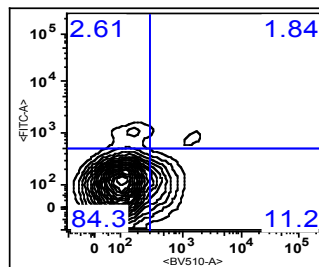
Naive B cells
CD21+ CD27-

atypical B cells
CD21- CD27-

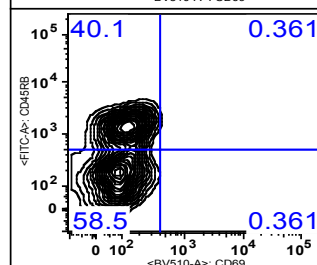
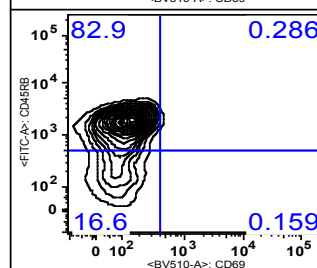
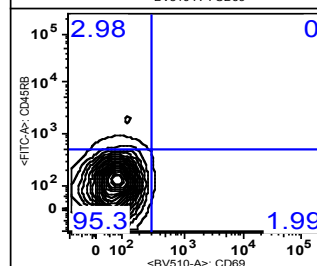
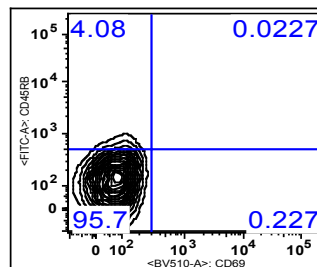
classical MBC
CD21+ CD27+

activated MBC
CD21- CD27+

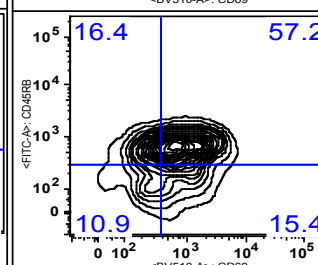
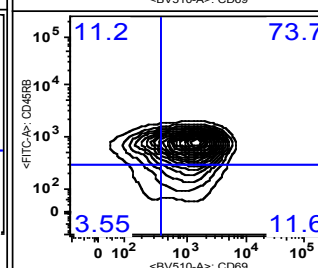
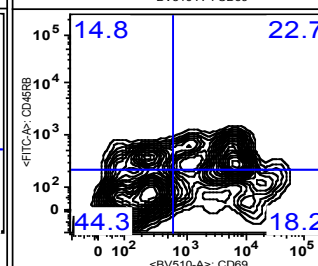
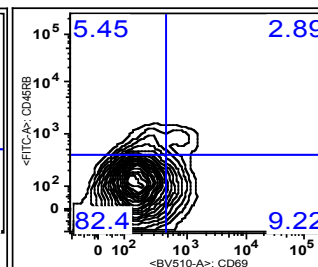
D256_SP



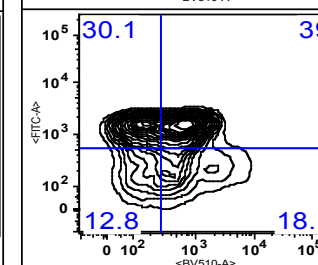
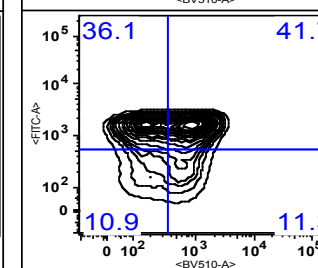
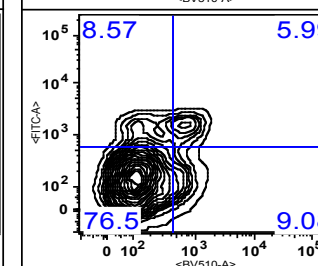
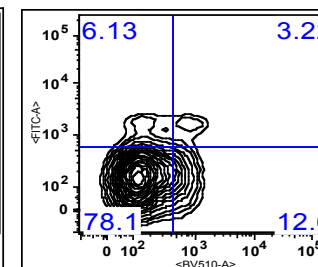
D256_B



D256_Col

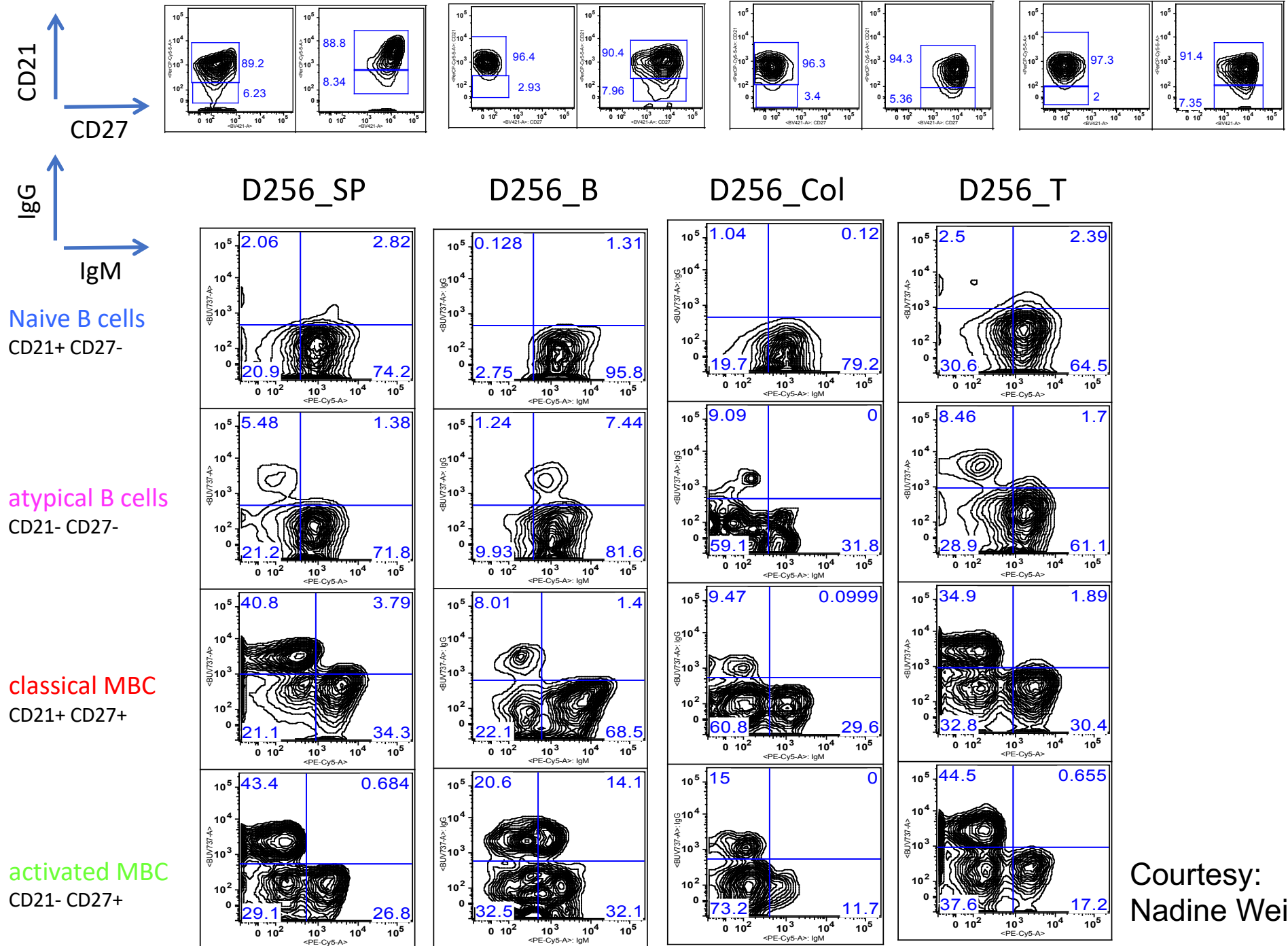


D256_T



Courtesy:
Nadine Weisel

D256 various samples – IgG/ IgM distribution in B cell populations



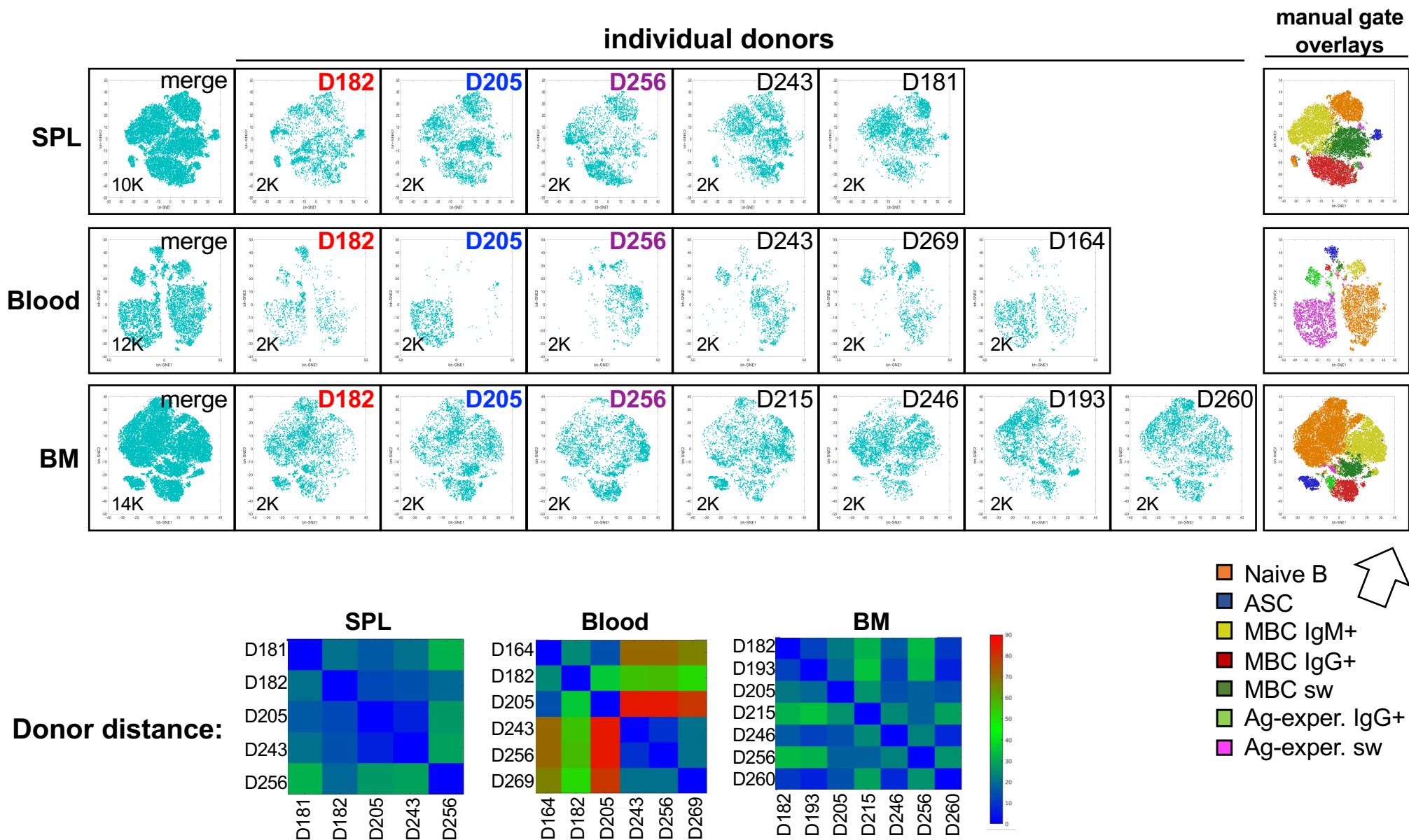
Courtesy:
Nadine Weisel

Problem: how do you visualize **millions of cells** marked by a **rainbow of colors** in order to establish the underlying structure or **principles of organization**?

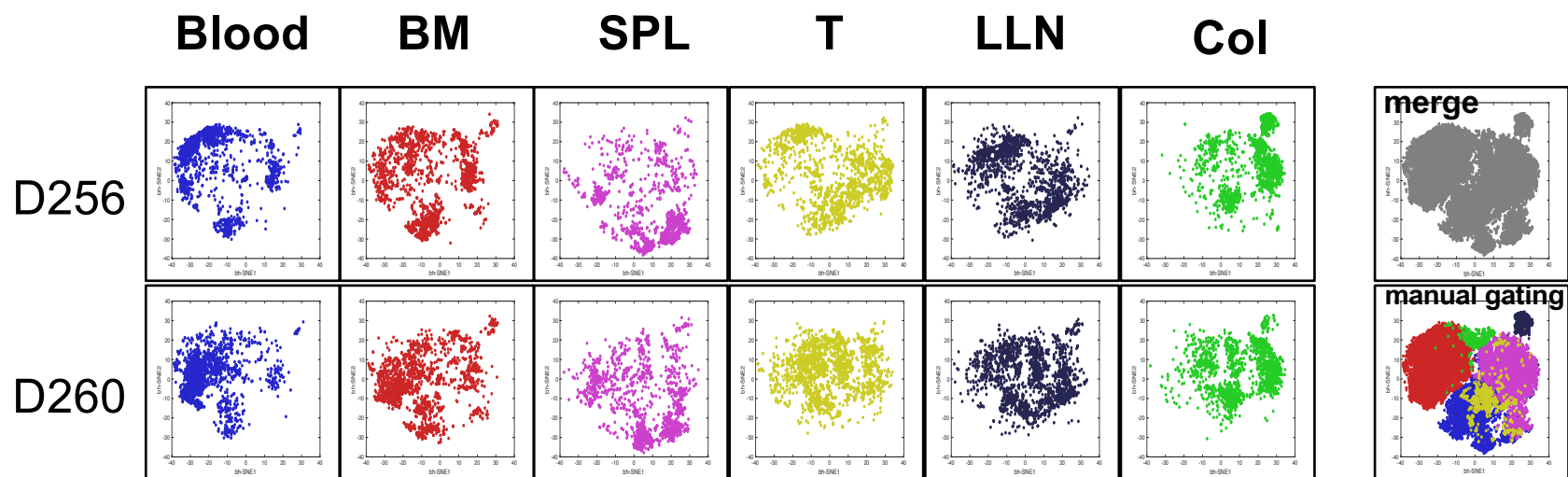
Solution: take high-D spaces and embed this information into low-D spaces that our brains can understand

→ algorithms identify **local patterns** and **global patterns** that our brains may miss when performing manual analysis via iterative pairwise plots

Unbiased analysis of across-donor heterogeneity



Tissue-specific B cell signatures

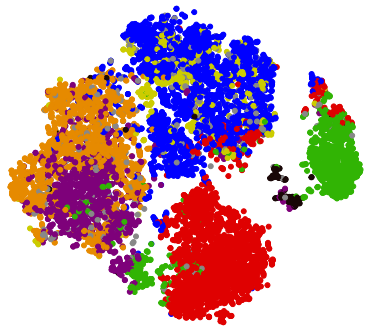


Naive B
 ASC
 MBC IgM+IgD-
 MBC IgD+
 MBC sw
 Ag-exper. sw

Next Presentations: How the algorithms work and how you can install on your personal computer

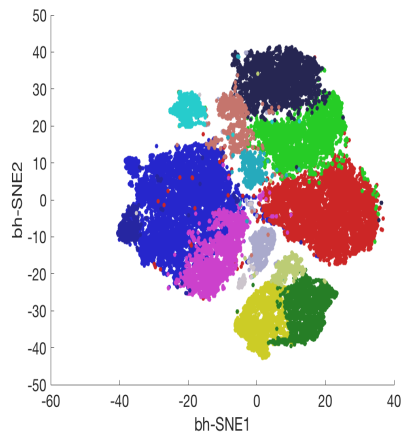
t-SNE

Single cell resolution



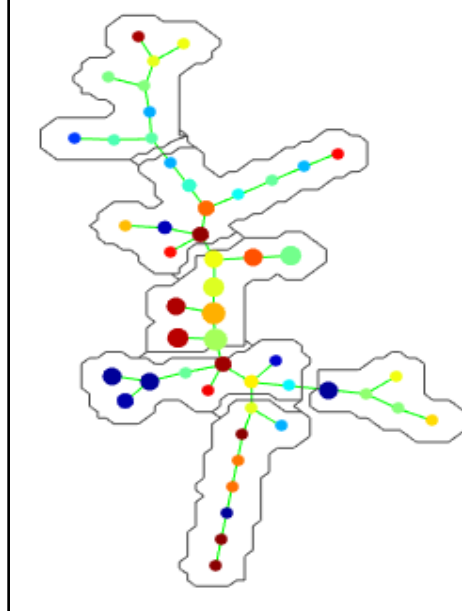
PhenoGraph

Automated population identification



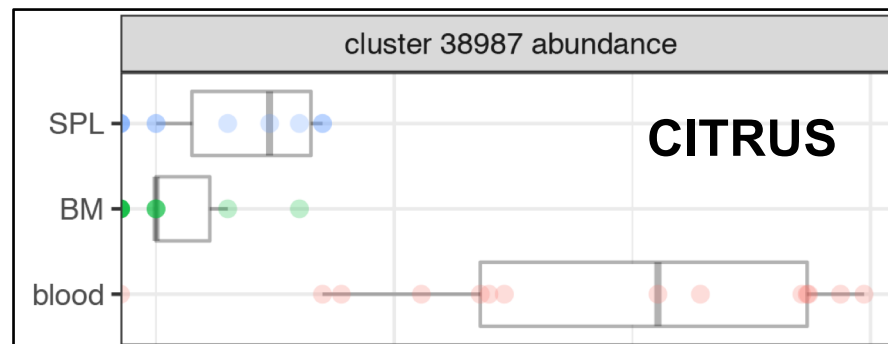
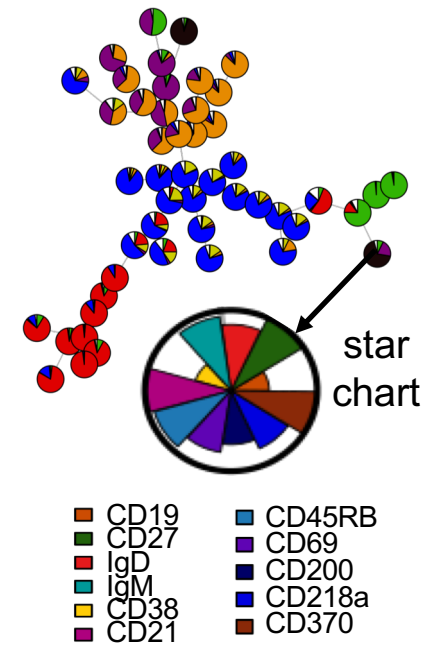
SPADE

Tree of relationships



FlowSOM

Tree of relationships



Resources

Useful starting places - reviews

1. Kimball AK, Oko LM, Bullock BL, Nemenoff RA, van Dyk LF, Clambey ET. A Beginner's Guide to Analyzing and Visualizing Mass Cytometry Data. J Immunol. 2018 Jan 1;200(1):3-22.
2. Saeys Y, Gassen SV, Lambrecht BN. Computational flow cytometry: helping to make sense of high-dimensional immunology data. Nat Rev Immunol. 2016 Jul;16(7):449-62.
3. 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. 2016 Jan;46(1):34-43.
4. Chester C & Maecker HT. J Immunol. 2015 Aug 1;195(3):773-9. doi: 10.4049/jimmunol.1500633. Algorithmic Tools for Mining High-Dimensional Cytometry Data. J Immunol. 2015 Aug 1;195(3):773-9.

Original application of algorithms

1. Qiu P, Simonds EF, Bendall SC, Gibbs KD Jr, Bruggner RV, Linderman MD, Sachs K, Nolan GP, Plevritis SK. Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. Nat Biotechnol. 2011 Oct 2;29(10):886-91. **SPADE**
2. Amir el-AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, Shenfeld DK, Krishnaswamy S, Nolan GP, Pe'er D. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Amir el-AD, Davis KL, 3. Tadmor MD, Simonds EF, Levine JH, Bendall SC, Shenfeld DK, Krishnaswamy S, Nolan GP, Pe'er D. Nat Biotechnol. 2013 Jun;31(6):545-52. **viSNE**
3. Levine JH, Simonds EF, Bendall SC, Davis KL, Amir el-AD, Tadmor MD, Litvin O, Fienberg HG, Jager A, Zunder ER, Finck R, Gedman AL, Radtke I, Downing JR, Pe'er D, Nolan GP. Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. Cell. 2015 Jul 2;162(1):184-97. **PhenoGraph**
4. Bruggner RV, Bodenmiller B, Dill DL, Tibshirani RJ, Nolan GP. Automated identification of stratifying signatures in cellular subpopulations. Proc Natl Acad Sci U S A. 2014 Jul 1;111(26):E2770-7. **CITRUS**
5. Setty M, Tadmor MD, Reich-Zeliger S, Angel O, Salame TM, Kathail P, Choi K, Bendall S, Friedman N, Pe'er D. Wishbone identifies bifurcating developmental trajectories from single-cell data. Nat Biotechnol. 2016 Jun;34(6):637-45. **Wishbone**
6. Bendall SC, Davis KL, Amir el-AD, Tadmor MD, Simonds EF, Chen TJ, Shenfeld DK, Nolan GP, Pe'er D. Single-cell trajectory detection uncovers progression and regulatory coordination in human B cell development. Cell. 2014 Apr 24;157(3):714-25. **Wanderlust**
7. McInnes L, Healy J, Melville J. UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction. <https://arxiv.org/abs/1802.03426> **UMAP (for advanced Matlab Users, no GUI interface)**