A visual interrogation of dimension reduction tools for single-cell analysis

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AG Mei, DRFZ Berlin

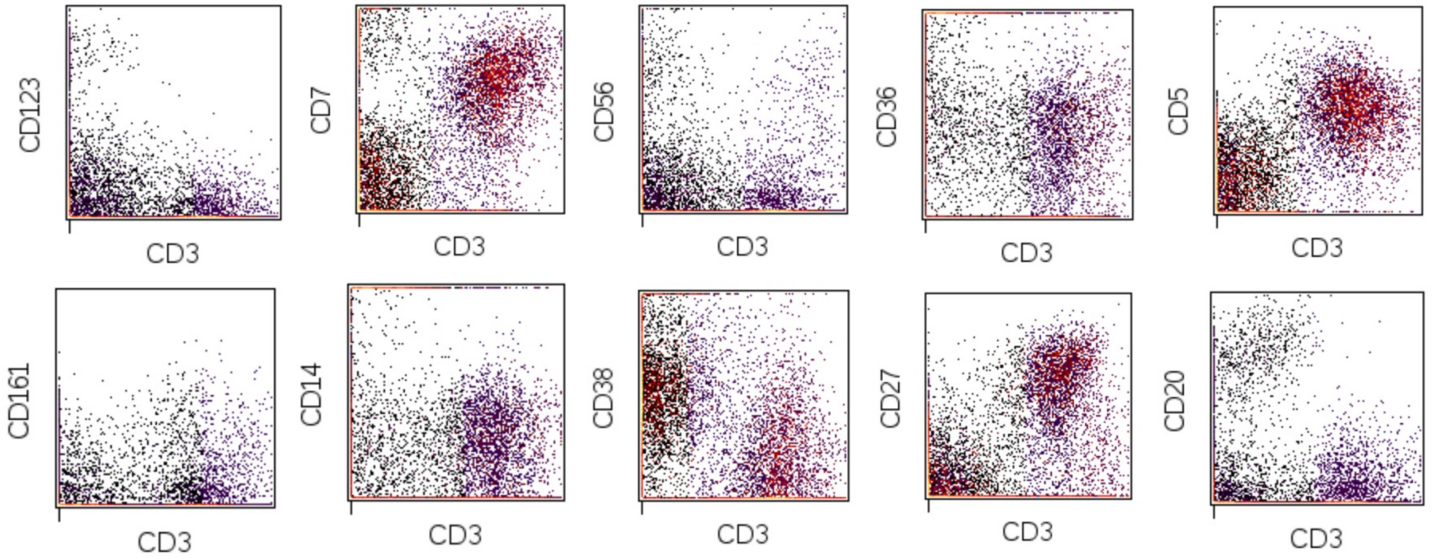
Outline

- Part 1: Introduction
- Part 2: Preservation of local structure
- Part 3: Preservation of global structure

Outline

- **Part 1: Introduction**
- Part 2: Preservation of local structure
- Part 3: Preservation of global structure

Dimension reduction makes large amounts of information human-readable without too much human work




```
# A tibble: 10,000 x 30
   CD235_61   CD45     CD7     CD19    CD11b     CD4      CD8    CD127     CCR7    CD123   CD45RA    NKp44     CD33    CD11c     CD14     CD69
      <dbl>  <dbl>   <dbl>    <dbl>    <dbl>   <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>
 1  0.109   0.965  0.149    0.110    0.251    0.122   4.20     0.0795   0.100    0.0660   0.0226   0.0246   0.117    0.102    0.0859   1.28
 2  0.0235  1.34   2.39     0.164    0.371    0.355   0.00938  0.0435   0.0368   0.0725   2.37     0.0527   0.0280   0.0478   0.0522   0.396
 3  0.00146 0.0749 0.0681   0.1000   1.31     0.186   0.321    0.0664   0.0239   0.0965   0.114    0.141    0.127    0.378    0.0597   0.0377
 4  0.0320  2.14   0.0684   0.101    0.565    0.145   0.184    0.151    0.102    0.0396   0.637    0.0602   1.90     2.96     2.30     0.179
 5  0.0837  1.44   0.0496   0.0144   0.102    0.846   0.0531   0.0406   0.0141   1.23     3.17     0.265    0.252    2.54     0.0802   0.0284
 6  0.0989  0.939  0.929    0.0595   0.0305   0.0283  2.70     0.0303   0.0236   0.0646   0.0293   0.0701   0.103    0.0413   0.0782   0.613
 7  0.123   0.167  0.00865  0.00632  1.26     0.127   0.315    0.0410   0.184    0.0140   0.00240  0.0855   0.196    0.727    0.150    0.0864
 8  0.0512  0.385  0.0642   0.116    1.54     0.713   0.0576   0.0625   0.00486  0.0715   0.146    0.134    0.155    0.0125   0.166    0.284
 9  0.0826  0.262  0.181    0.0847   2.49     0.135   0.168    0.0706   0.109    0.0492   0.0467   0.141    0.175    0.0554   0.274    0.142
10  0.123   0.0829 0.0339   0.127    1.21     0.0545  0.0907   0.119    0.0835   0.129    0.0768   0.134    0.0329   0.0990   0.0405   0.151
# ... with 9,990 more rows, and 14 more variables: CD16 <dbl>, CD25 <dbl>, CD3 <dbl>, CD66 <dbl>, CD56 <dbl>,
#   HLADR <dbl>, V1 <dbl>, V2 <dbl>, BC1 <dbl>, BC2 <dbl>, BC3 <dbl>, BC4 <dbl>, BC5 <dbl>, BC6 <dbl>
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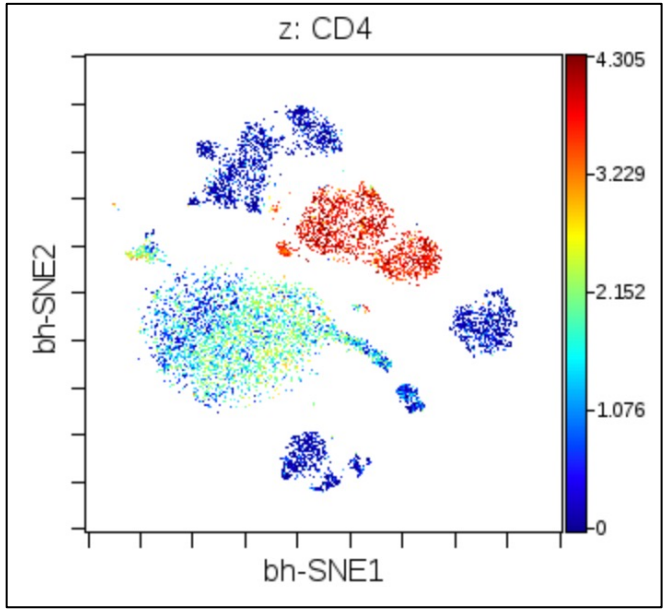
t-SNE(cells)



Amir et al, *Nat Biotechnology* 2013

Early dimension reduction tools: Principal Component Analysis (PCA)

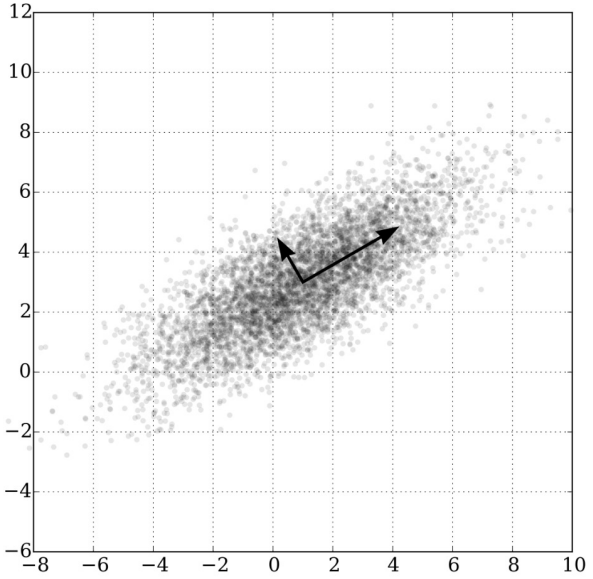
LIII. *On Lines and Planes of Closest Fit to Systems of Points in Space.* *By* KARL PEARSON, *F.R.S., University College, London* *. (1901)

(1) IN many physical, statistical, and biological investigations it is desirable to represent a system of points in plane, three, or higher dimensioned space by the "best-fitting" straight line or plane. Analytically this consists in taking

$$y = a_0 + a_1 x, \quad \text{or} \quad z = a_0 + a_1 x + b_1 y,$$
$$\text{or} \quad z = a_0 + a_1 x_1 + a_2 x_2 + a_3 x_3 + \ldots + a_n x_n,$$

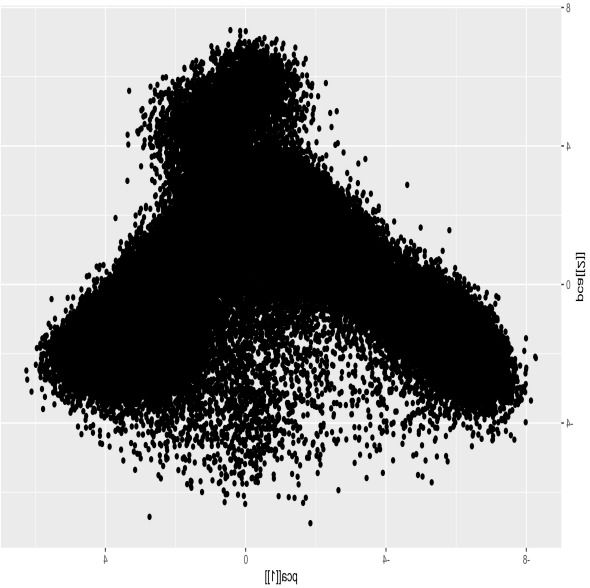
where y, x, z, x_1, x_2, $\ldots x_n$ are variables, and determining the "best" values for the constants a_0, a_1, b_1, a_0, a_1, a_2, a_3, $\ldots a_n$ in relation to the observed corresponding values of the variables. In nearly all the cases dealt with in the text-books of least squares, the variables on the right of our equations are treated as the independent, those on the left as the dependent variables. The result of this treatment is that we get one straight line or plane if we treat some one variable as independent, and a quite different one if we treat another variable as the independent variable. There is no paradox about this; it is, in fact, an easily understood and most im-

Axes span the direction with highest variance



https://en.wikipedia.org/wiki/Principal_component_analysis

Samusik_01 bone marrow CyTOF dataset



t-SNE preserves local information, produces more well clustered maps

Visualizing Data using t-SNE

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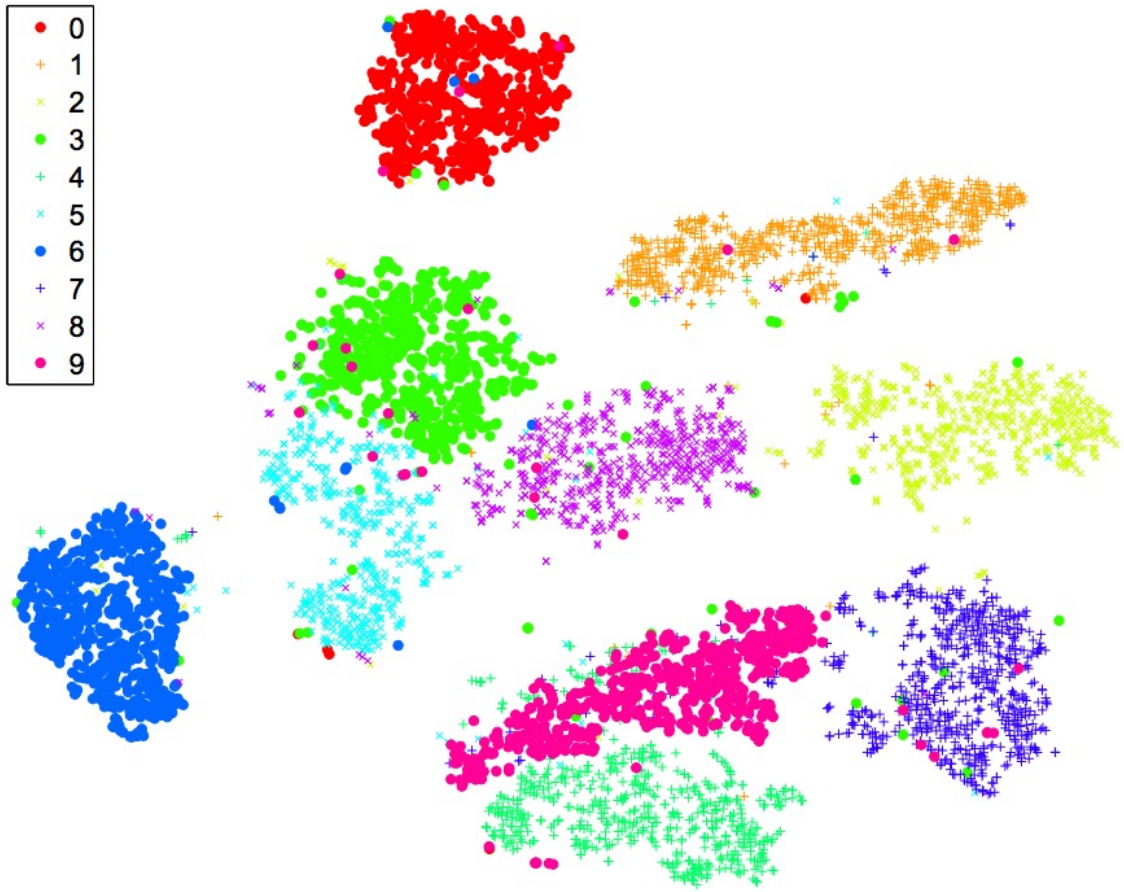
Editor: Yoshua Bengio

Abstract

We present a new technique called "t-SNE" that visualizes high-dimensional data by giving each datapoint a location in a two or three-dimensional map. The technique is a variation of Stochastic Neighbor Embedding (Hinton and Roweis, 2002) that is much easier to optimize, and produces significantly better visualizations by reducing the tendency to crowd points together in the center of the map. t-SNE is better than existing techniques at creating a single map that reveals structure at many different scales. This is particularly important for high-dimensional data that lie on several different, but related, low-dimensional manifolds, such as images of objects from multiple classes seen from multiple viewpoints. For visualizing the structure of very large data sets, we show how t-SNE can use random walks on neighborhood graphs to allow the implicit structure of all of the data to influence the way in which a subset of the data is displayed. We illustrate the performance of t-SNE on a wide variety of data sets and compare it with many other non-parametric visualization techniques, including Sammon mapping, Isomap, and Locally Linear Embedding. The visualizations produced by t-SNE are significantly better than those produced by the other techniques on almost all of the data sets.

Keywords: visualization, dimensionality reduction, manifold learning, embedding algorithms, multidimensional scaling



(a) Visualization by t-SNE.

viSNE: the adaptation of t-SNE to CyTOF

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PMCID: PMC4076922
NIHMSID: NIHMS586764
PMID: 23685480

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

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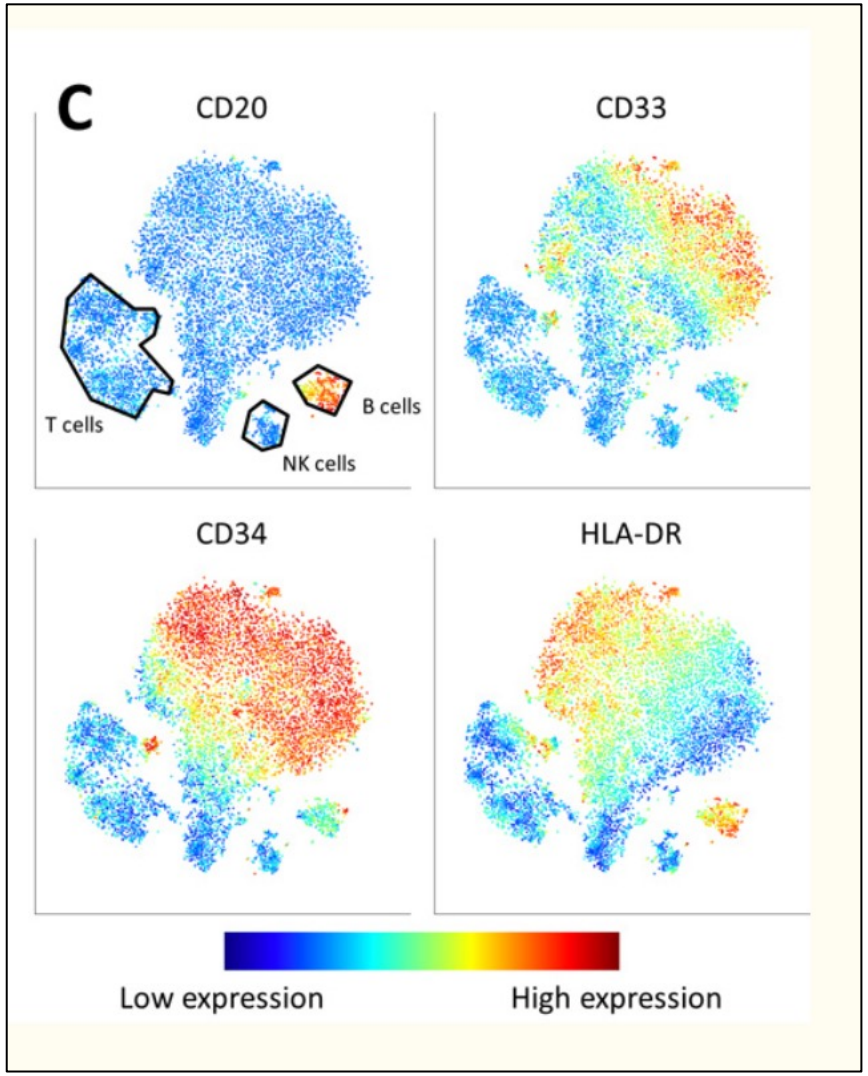
Abstract

Go to:

High-dimensional single-cell technologies are revolutionizing the way we understand biological systems. Technologies such as mass cytometry measure dozens of parameters simultaneously in individual cells, making interpretation daunting. We developed viSNE, a tool to map high-dimensional cytometry data onto 2D while conserving high-dimensional structure. We integrated mass cytometry with viSNE to map healthy and cancerous bone marrow samples. Healthy bone marrow maps into a canonical shape that separates between immune subtypes. In leukemia, however, the shape is malformed: the maps of cancer samples are distinct from the healthy map and from each other. viSNE highlights structure in the heterogeneity of surface phenotype expression in cancer, traverses the progression from diagnosis to relapse, and identifies a rare leukemia population in minimal residual disease settings. As several new technologies raise the number of simultaneously measured parameters in each cell to the hundreds, viSNE will become a mainstay in analyzing and interpreting such experiments.



Emergence of UMAP as an alternative to t-SNE for single-cell analysis

UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction

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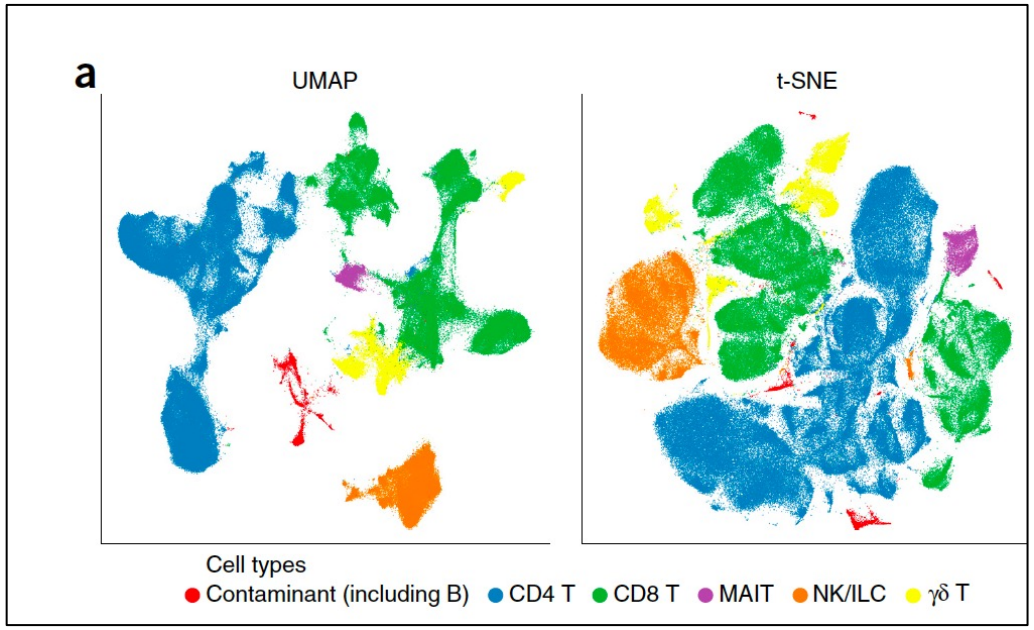
ANALYSIS

nature biotechnology

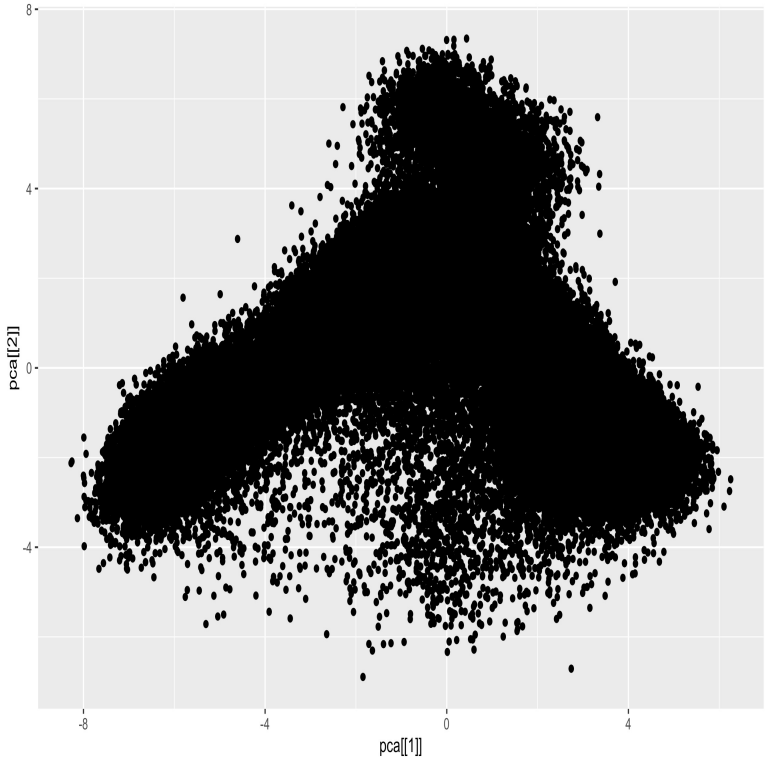
Dimensionality reduction for visualizing single-cell data using UMAP

Etienne Becht[1], Leland McInnes[2], John Healy[2], Charles-Antoine Dutertre[1], Immanuel W H Kwok[1], Lai Guan Ng[1], Florent Ginhoux[1] & Evan W Newell[1,3]

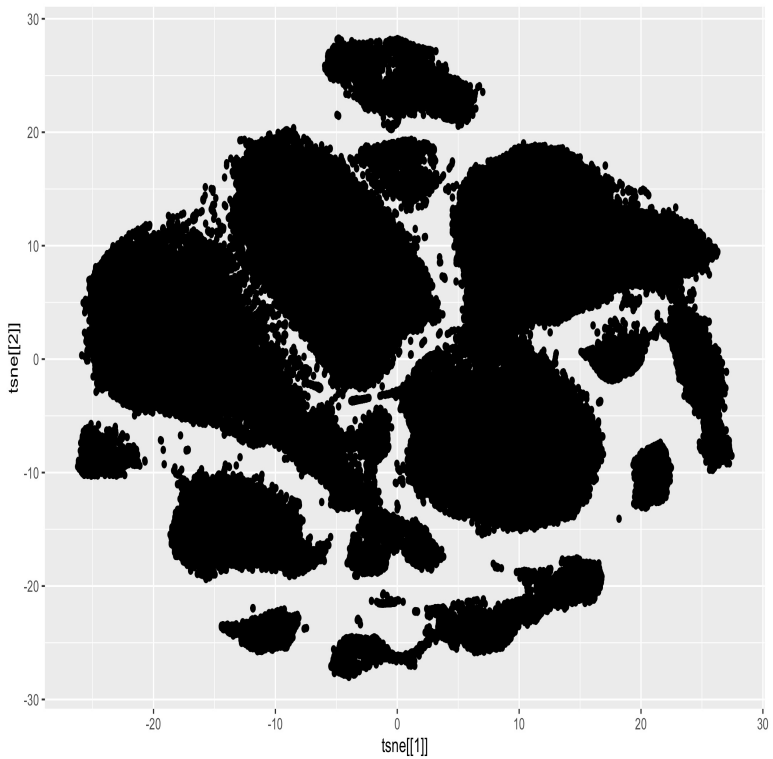


What PCA, t-SNE and UMAP look like on a bone marrow CyTOF dataset

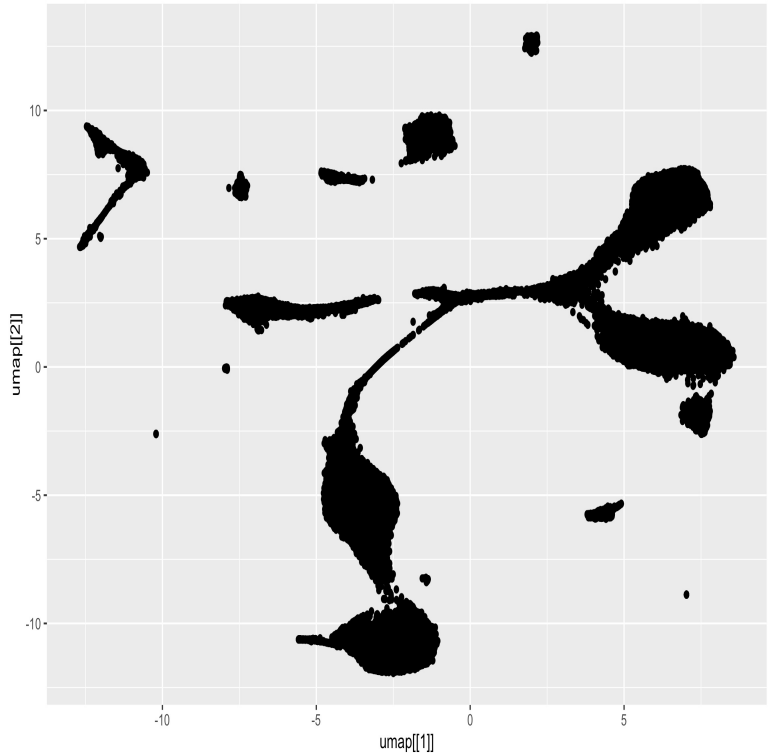
PCA



t-SNE



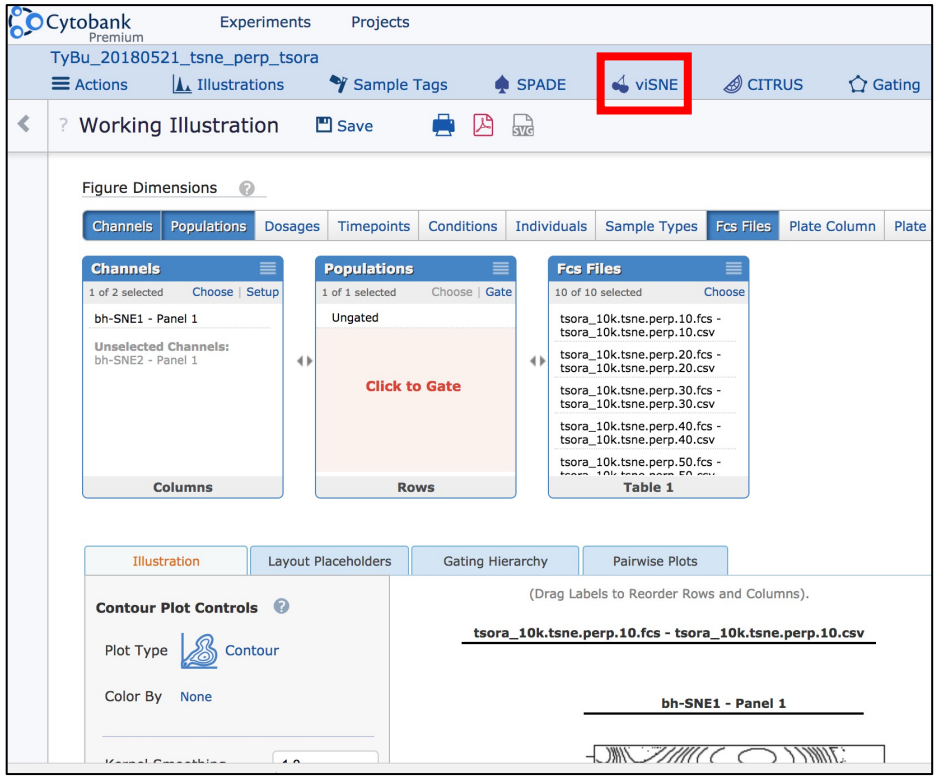
UMAP



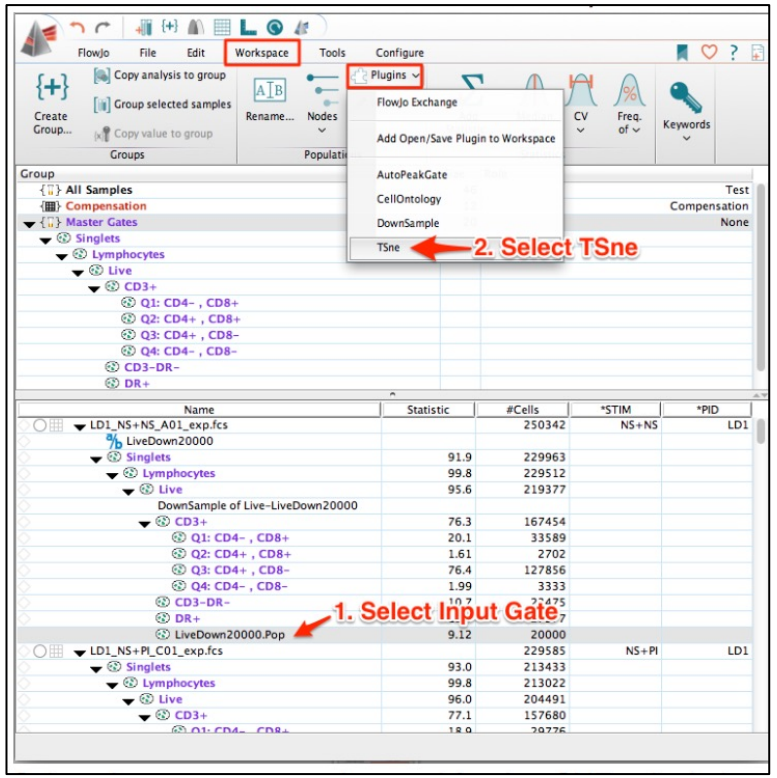
Dataset: Samusik *et al, Nature Methods* 2016

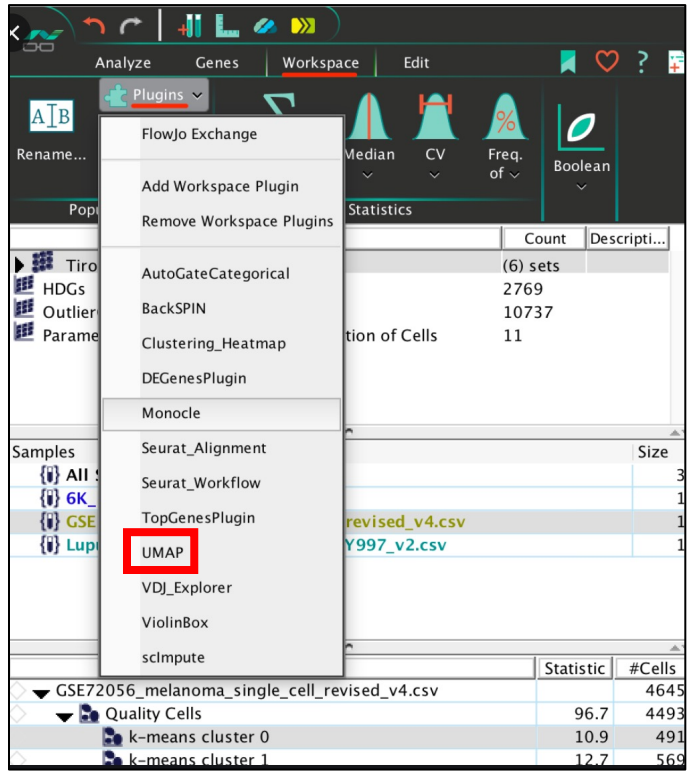
t-SNE and UMAP are accessible from single-cell analysis user interfaces

Cytobank



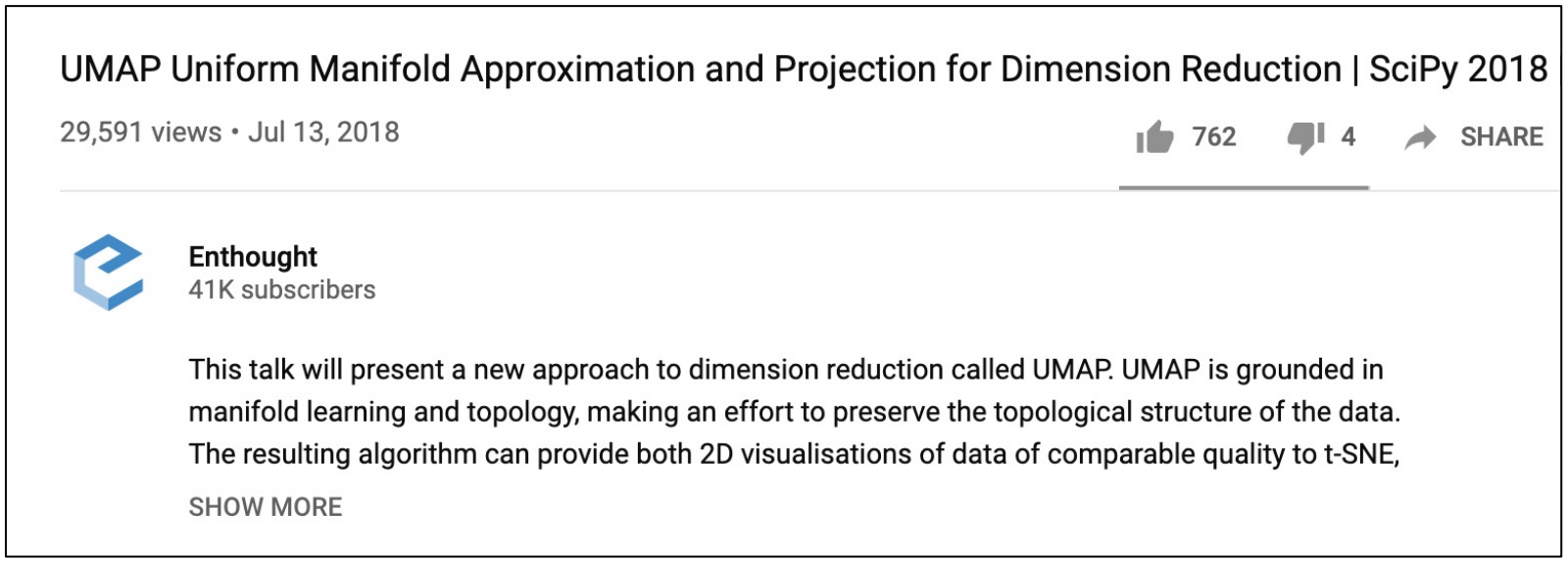
FlowJo

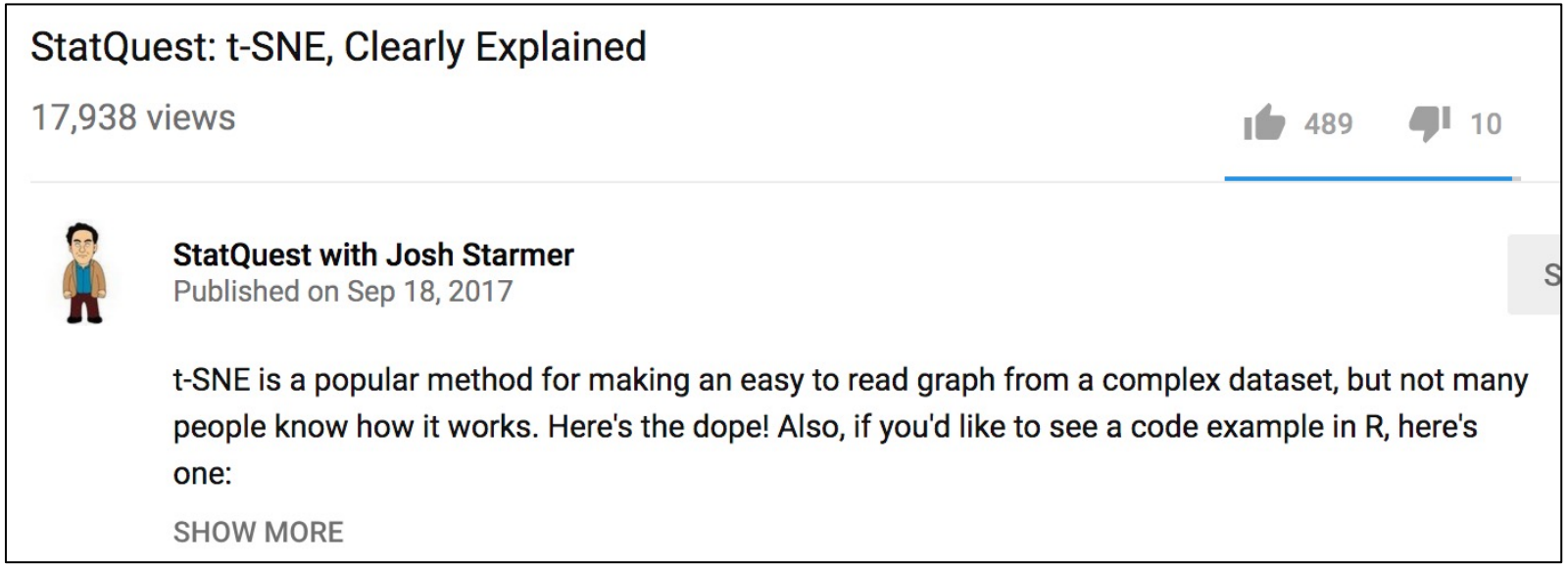




What additional information about dimension reduction maps should we know for their proper use?

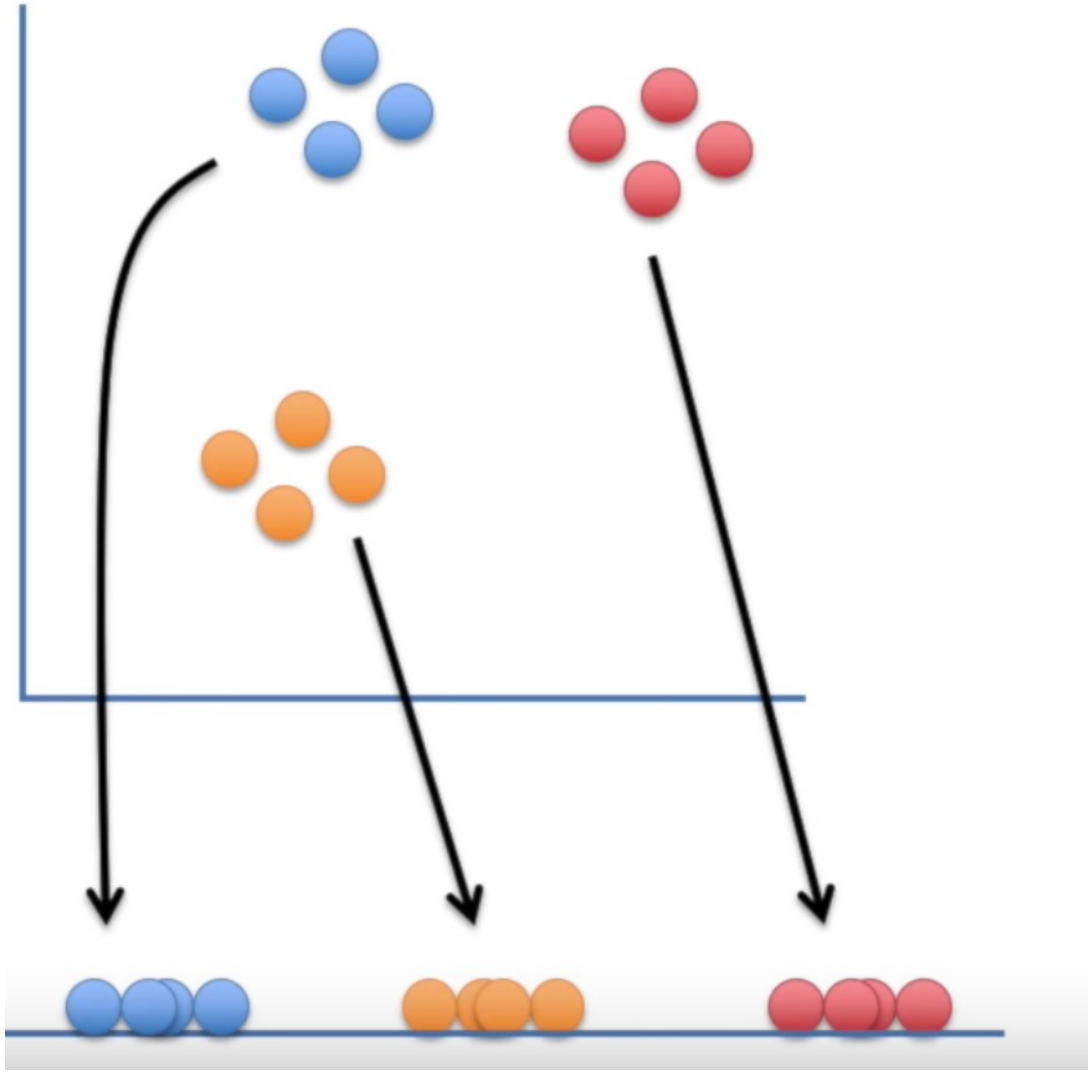
Credit for the following t-SNE and UMAP explanations





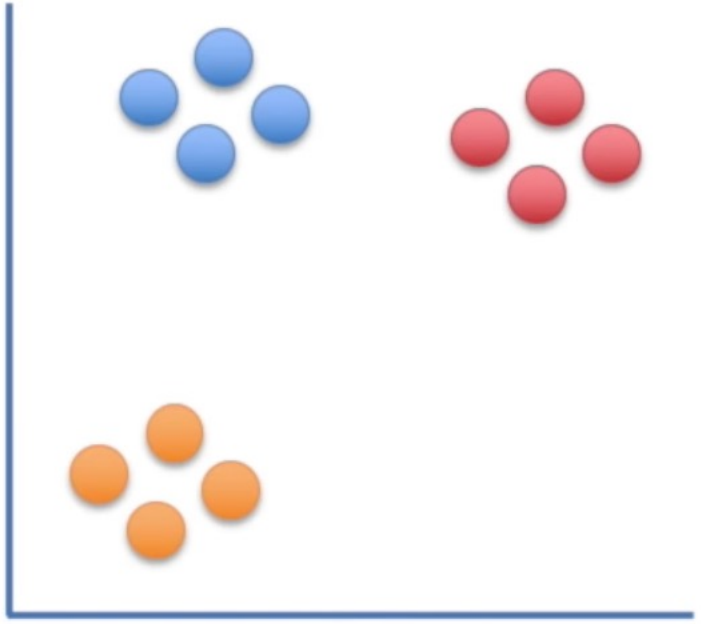
The goal of t-SNE and UMAP is to reduce dimensions while preserving specific information about each cell's neighbors

Higher dimensional space



Low dimensional embedding

t-SNE and UMAP start with a low-dimensional embedding of randomly placed points



t-SNE weights its neighbors based on distance fitted to a distribution

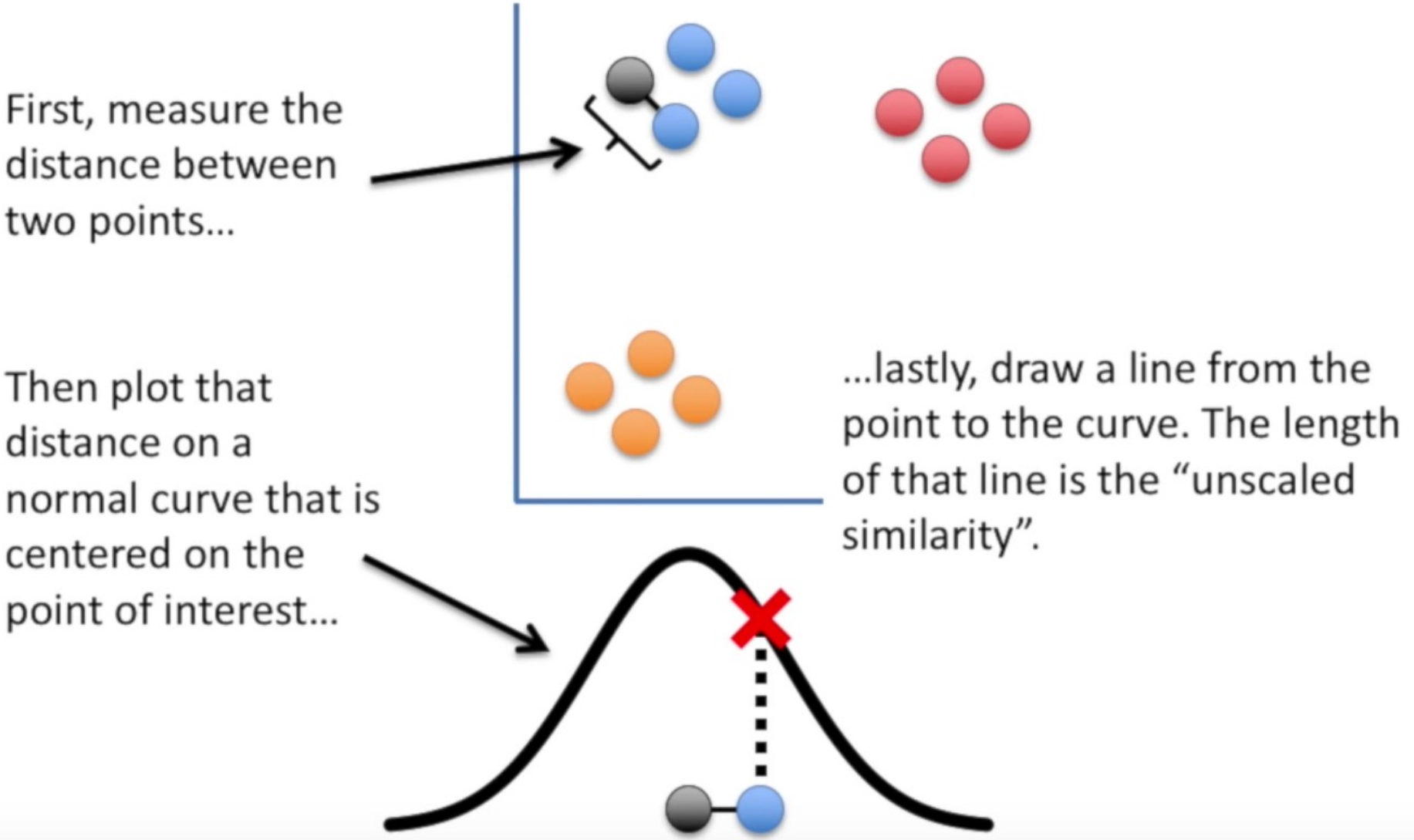
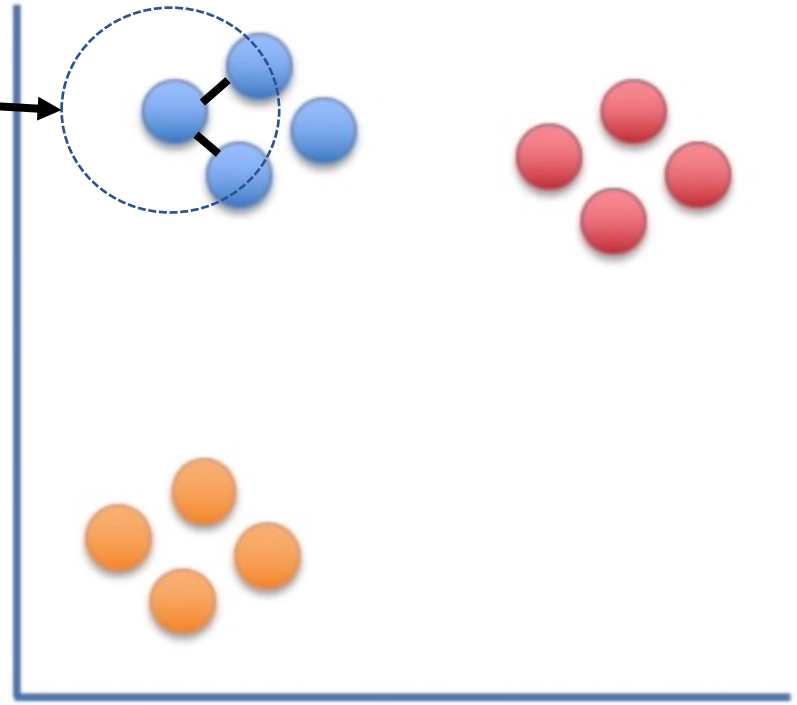


Image source: YouTube: StatQuest with Josh Starmer: *t-SNE, clearly explained*

UMAP weights its neighbors based on pure topology

Find the diameter of a neighborhood. Think of it like a ball.



Find the probability that a 1-simplex exists between two points in a neighborhood

Image source: https://umap-learn.readthedocs.io/en/latest/how_umap_works.html

Resulting neighbor graph is a bunch of simplexes glued together (simplicial complex). Simpler structure but preserves topological information.

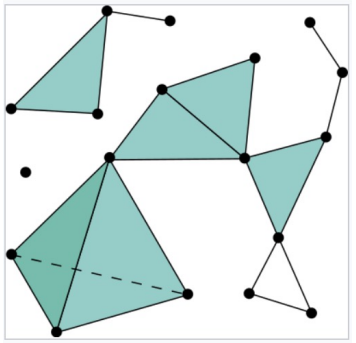
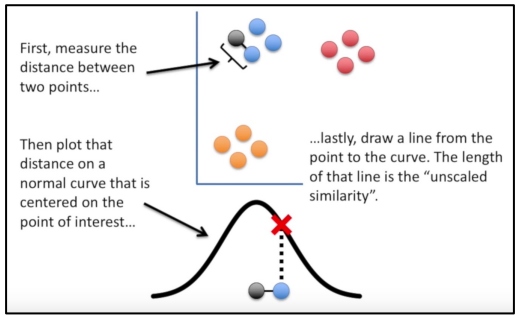
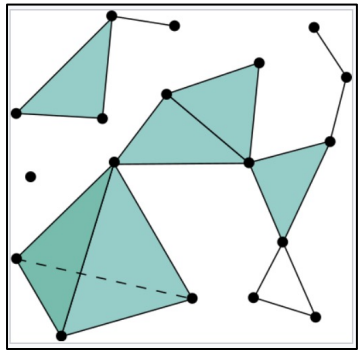


Image source: https://en.wikipedia.org/wiki/Simplicial_complex

The weighted neighborhood graphs can be represented as similarity matrices





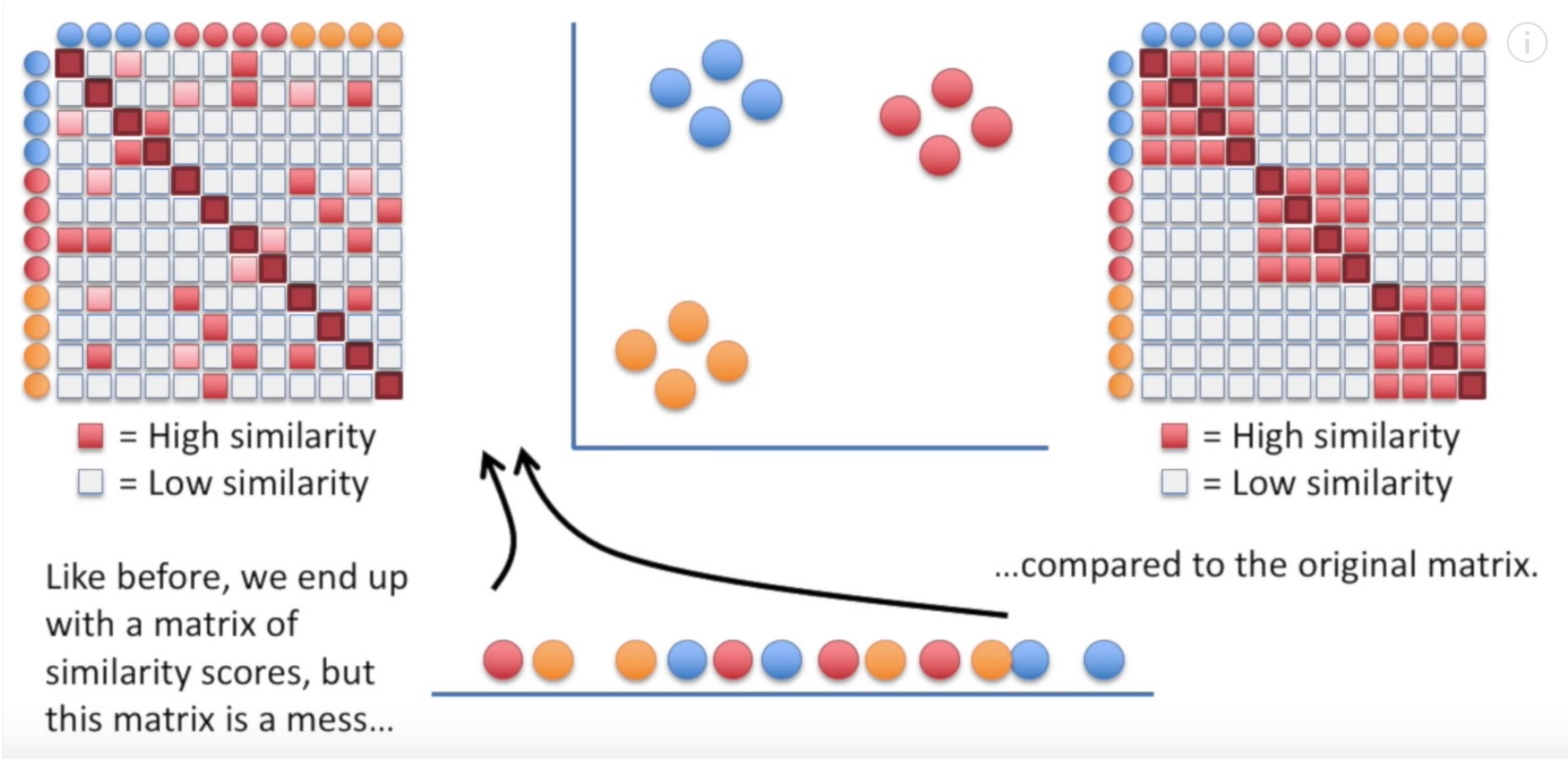
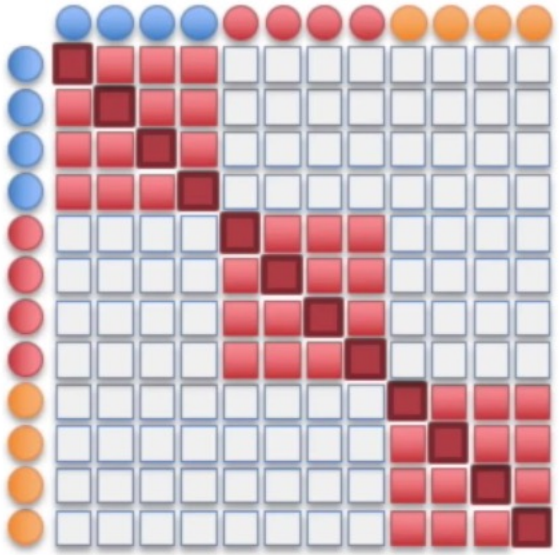
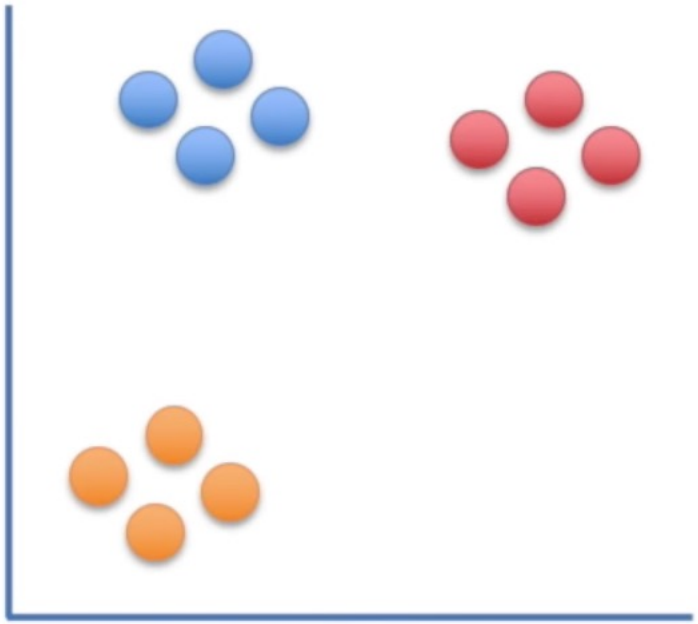
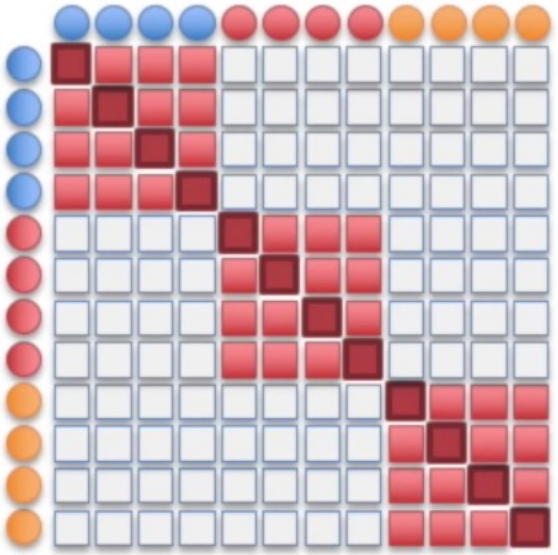


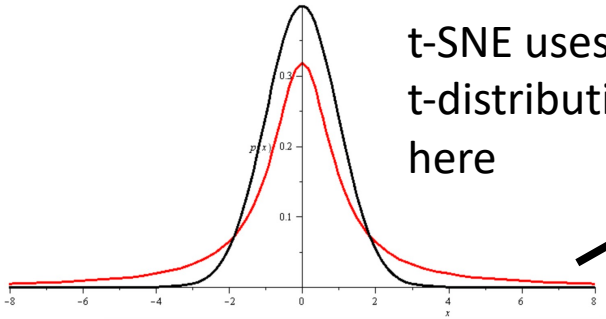
Image source: YouTube: StatQuest with Josh Starmer: *t-SNE, clearly explained*

Make these similarity matrices as similar to each other as possible, and then you're done







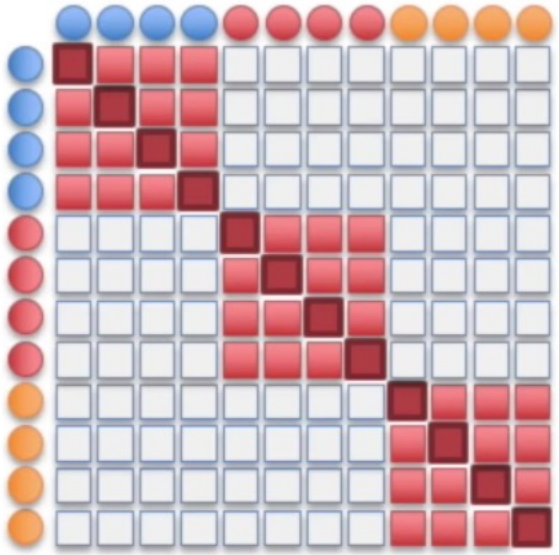


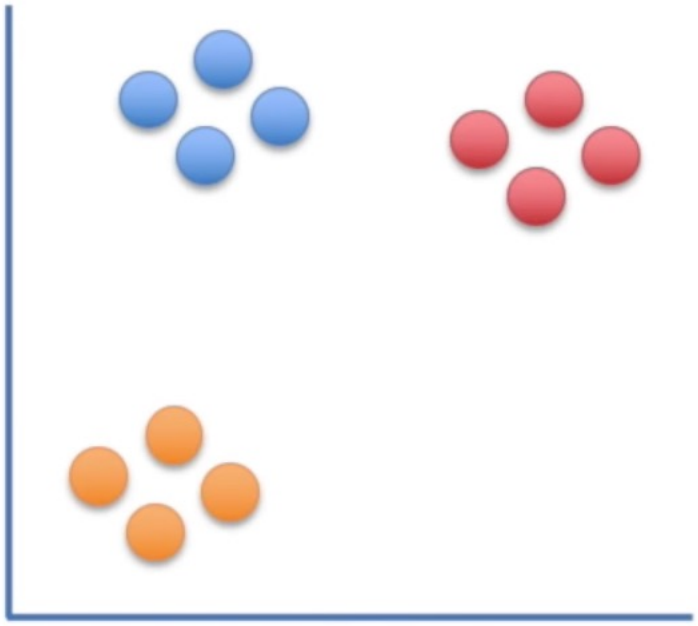
t-SNE uses a t-distribution here

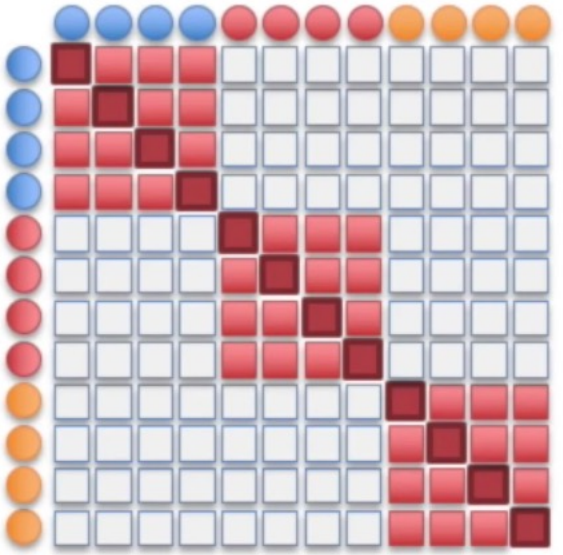
…without it the clusters would all clump up in the middle and be harder to see.

Image source: YouTube: StatQuest with Josh Starmer: *t-SNE, clearly explained*

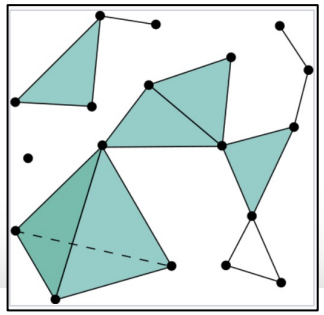
Make these similarity matrices as similar to each other as possible, and then you're done



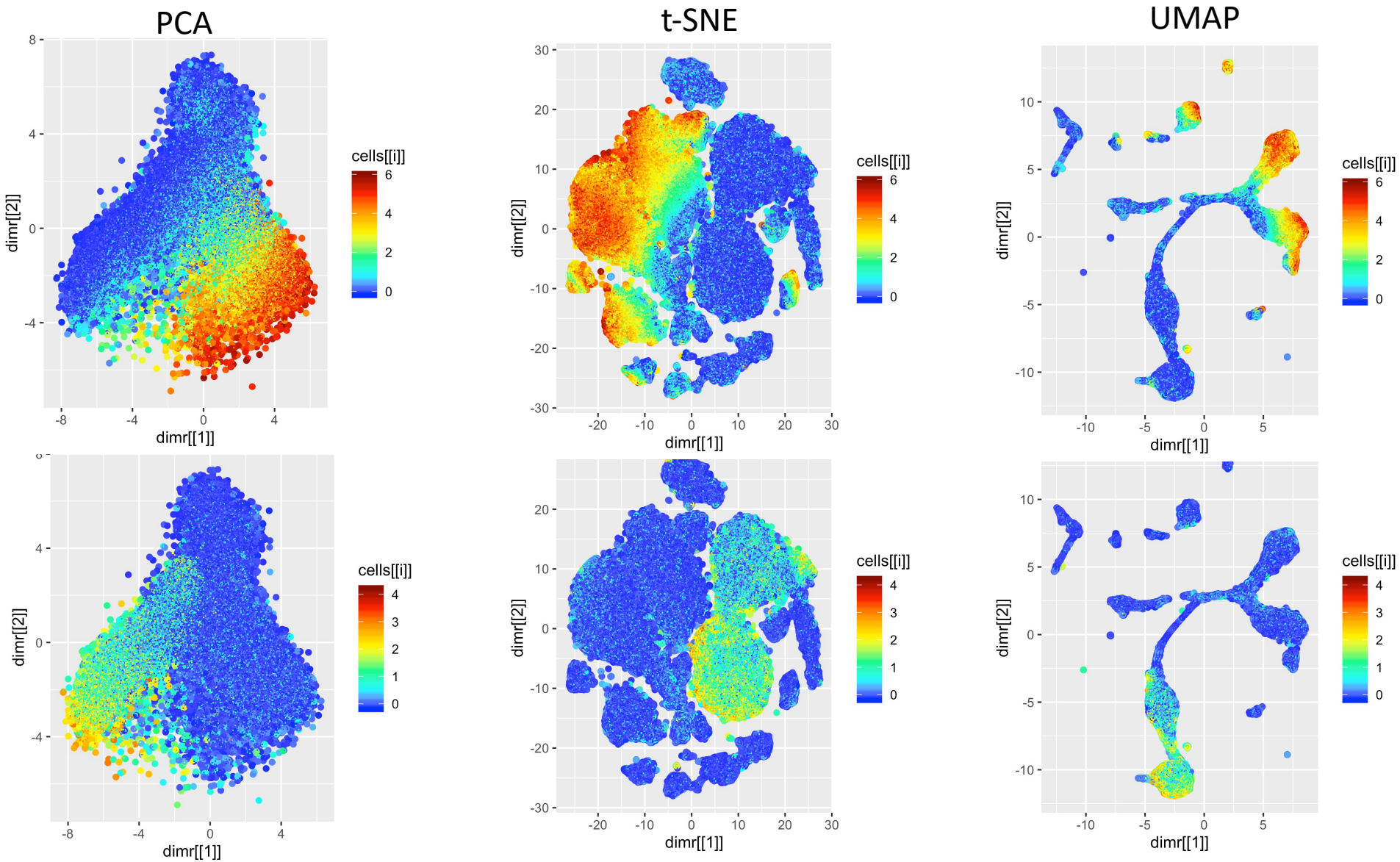




UMAP makes a
2-D simplicial complex



Dimension reduction maps group similar cells near each other

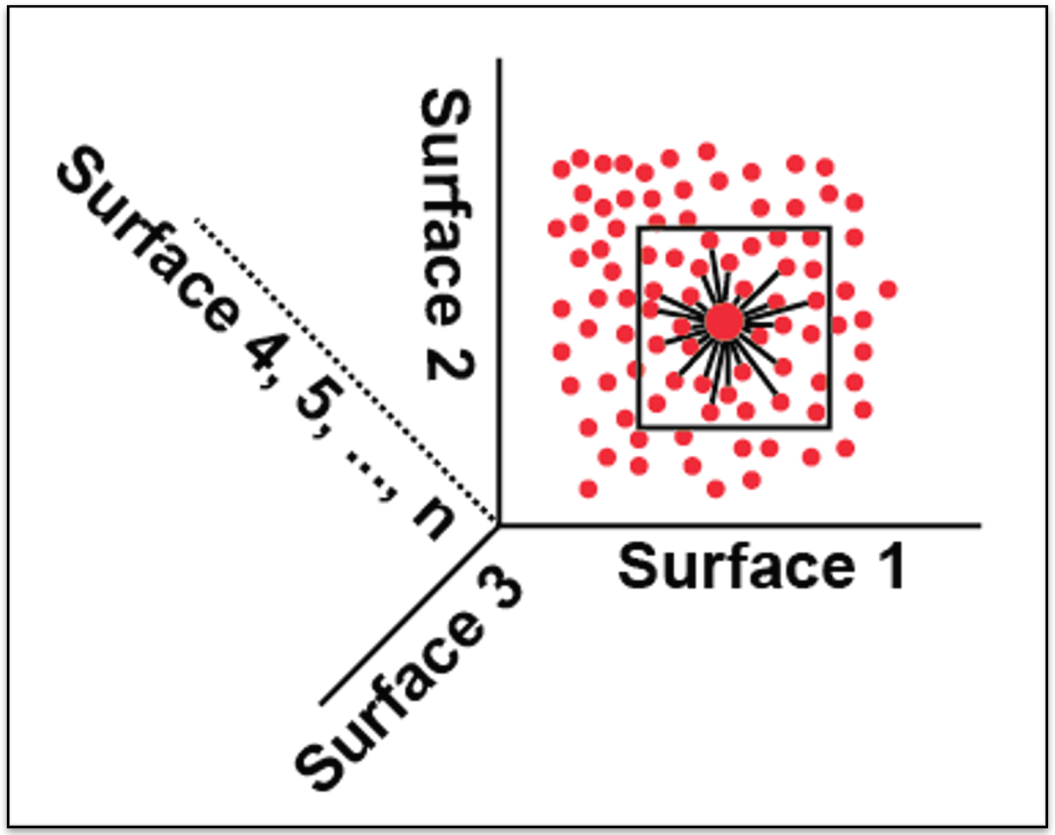


Samusik Bone marrow

Outline

- Part 1: Introduction
- **Part 2: Preservation of local structure**
- Part 3: Preservation of global structure

KNN without manual gating to determine preservation of lower dimensional embeddings



Find KNN for each cell from high-dim space

Find KNN for each cell from a 2-D embedding (eg tSNE)

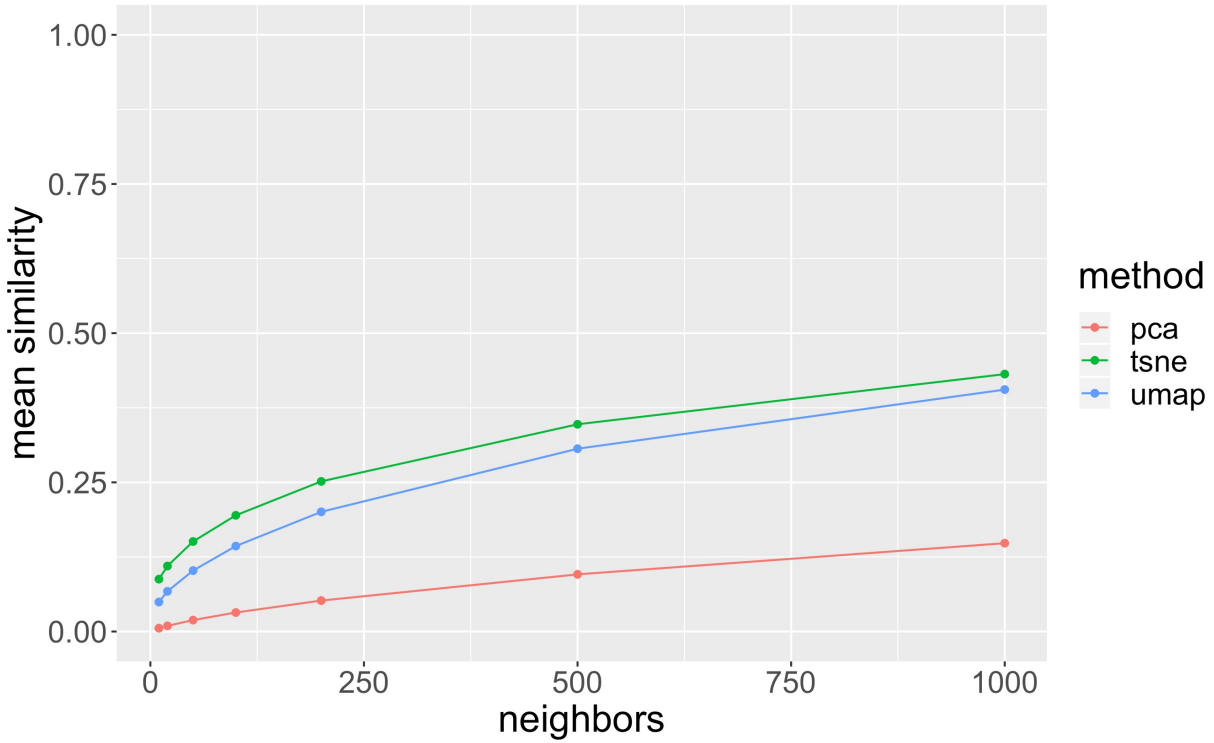
Compare KNN identities from the 2-D embedding and high-dim space

Repeat across a wide range of values for K

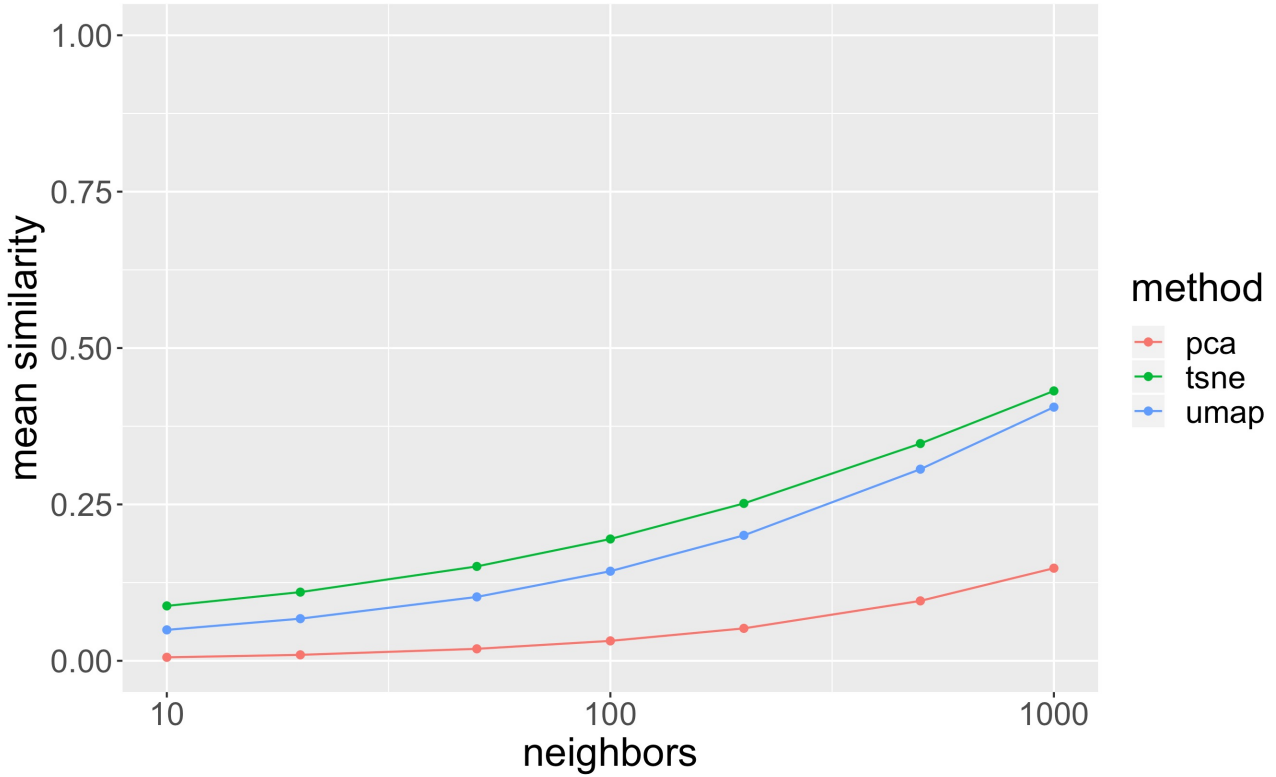
Bioconductor package: Sconify

Global KNN comparison between t-SNE, UMAP, and PCA

Dataset: Samusik Bone marrow (public)
Num. cells: 100k



X axis is on a log scale



t-SNE outperforms UMAP in KNN preservation, has been observed in scRNA seq data

ARTICLE

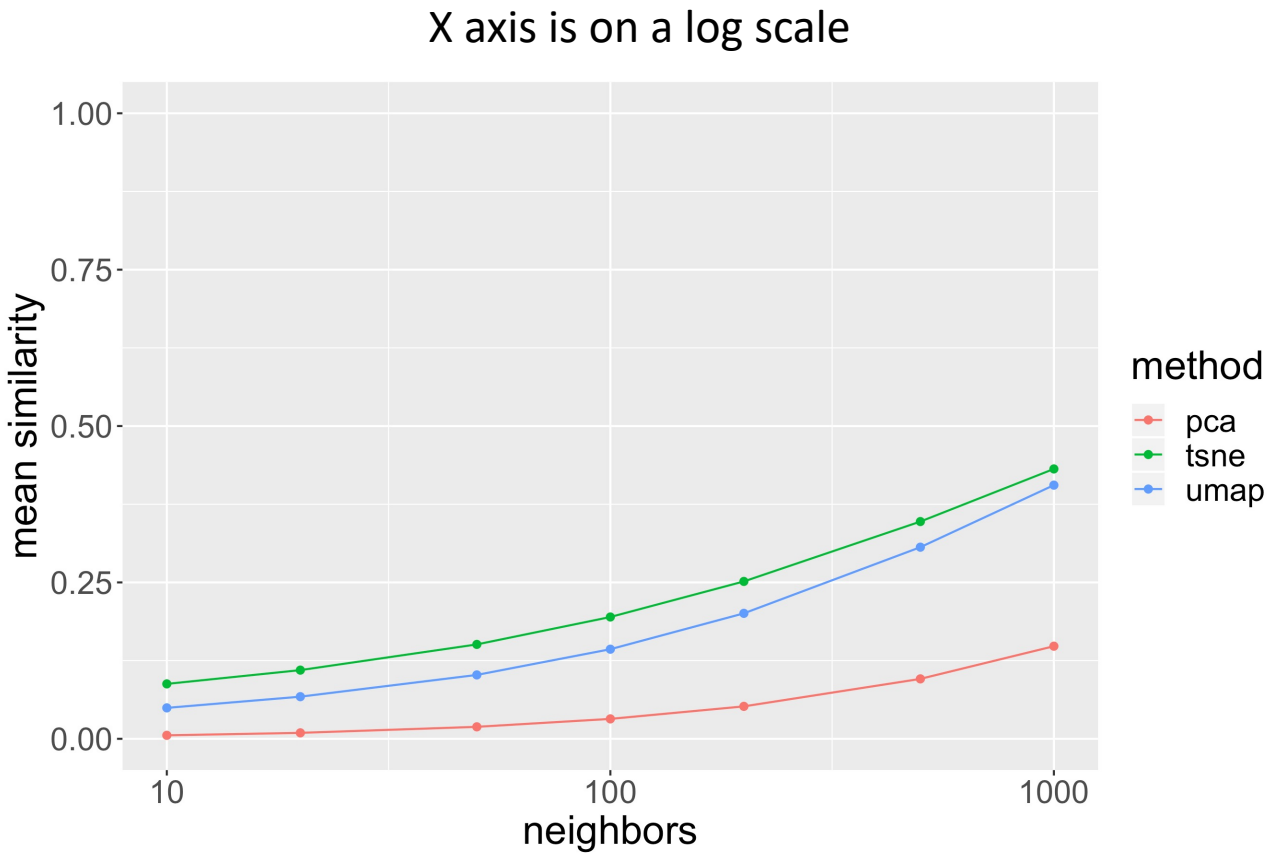
https://doi.org/10.1038/s41467-019-13056-x OPEN

The art of using t-SNE for single-cell transcriptomics (2019)

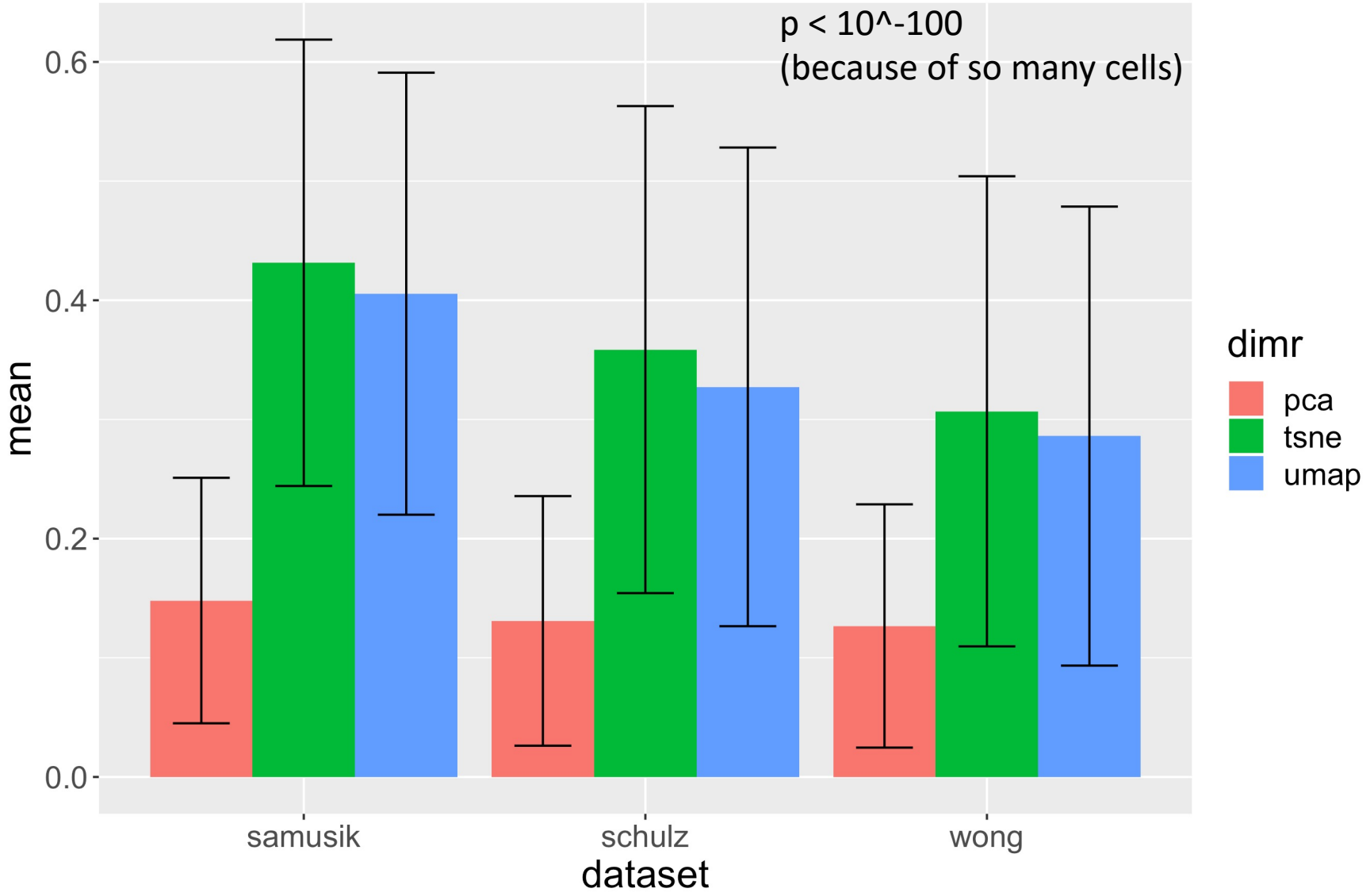
Dmitry Kobak [1*] & Philipp Berens [1,2,3,4*]

(Also did KNN preservation, K = 10 only)

To compare UMAP with our t-SNE approach in terms of preservation of global structure, we first ran UMAP on the synthetic and on the Tasic et al.[3] data sets (Supplementary Fig. 2). We used the default UMAP parameters, and also modified the two key parameters (number of neighbours and tightness of the embedding) to produce a more t-SNE-like embedding. In both cases and for both data sets, all three metrics (KNN, KNC, and CPD) were considerably lower than with our t-SNE approach.

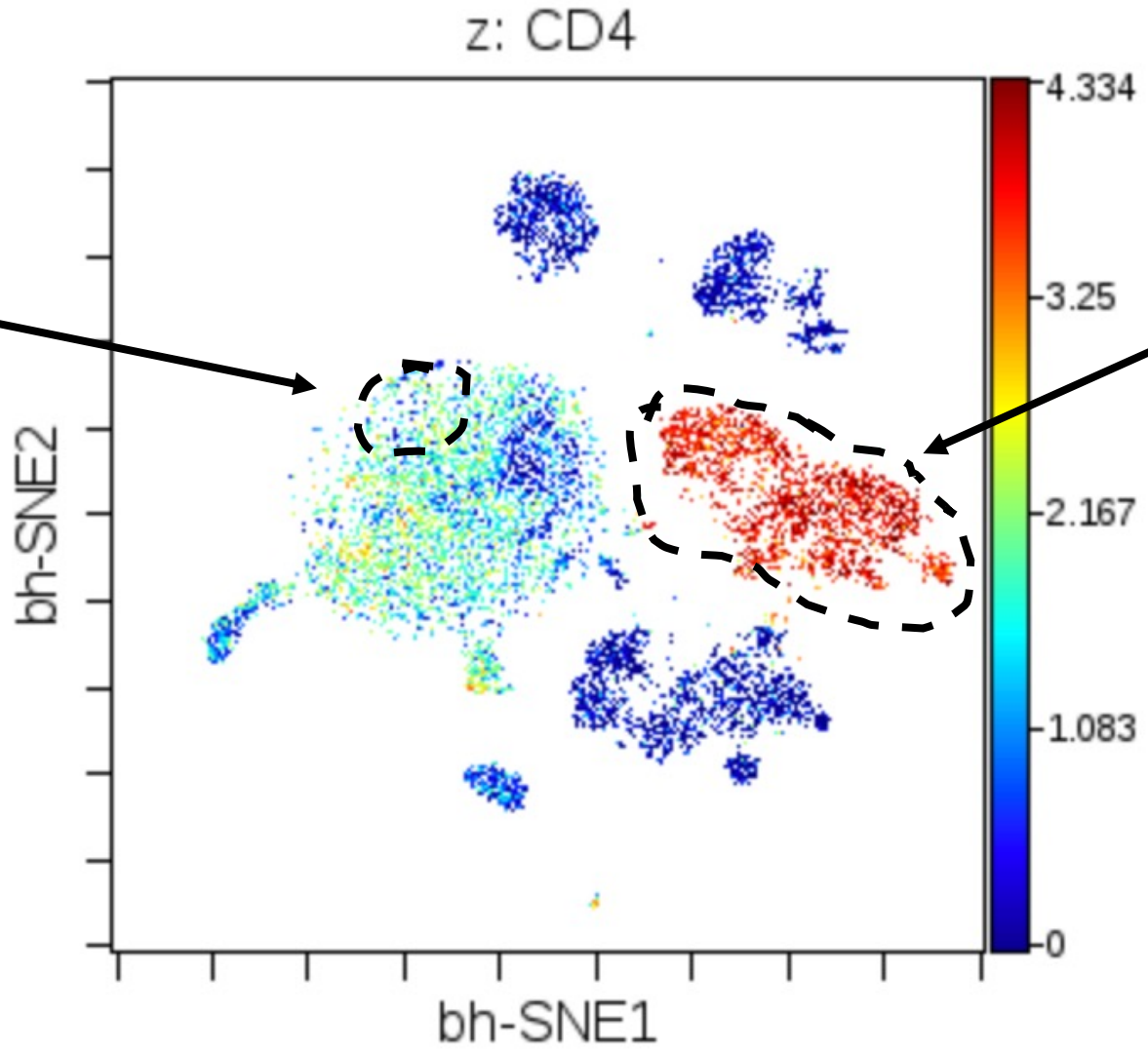


Results confirmed across 3 datasets, but with very large standard deviation



Cells: 100k
k = 1000

Does dimension reduction maps preserve some regions better than others (should and/or how should we gate the map?)



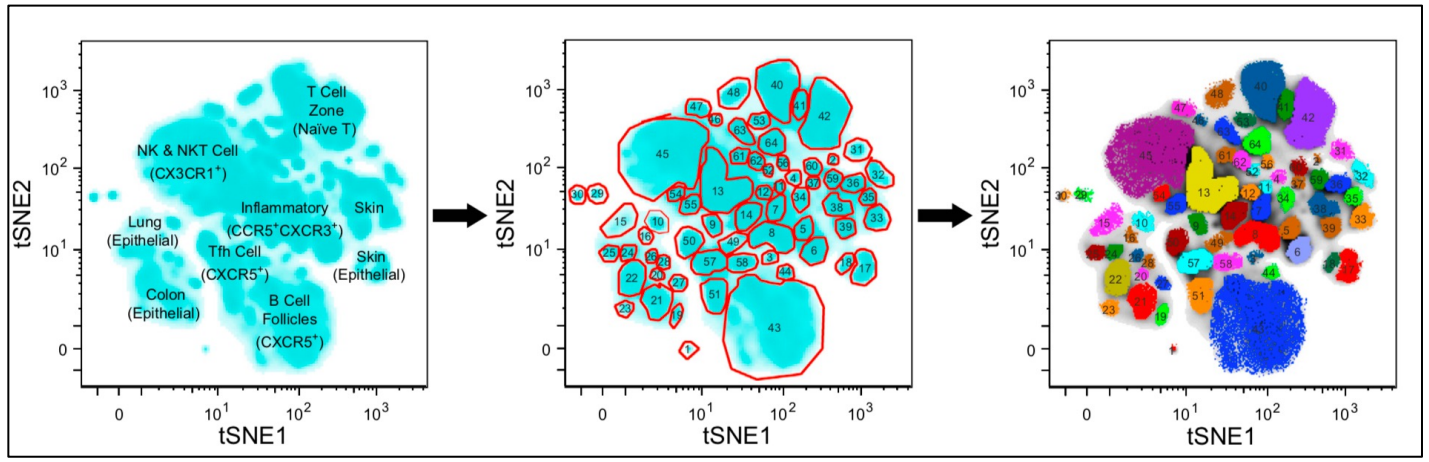
Is this a valid gate?

Is this a valid gate?

Data: Axel Schulz, PhD

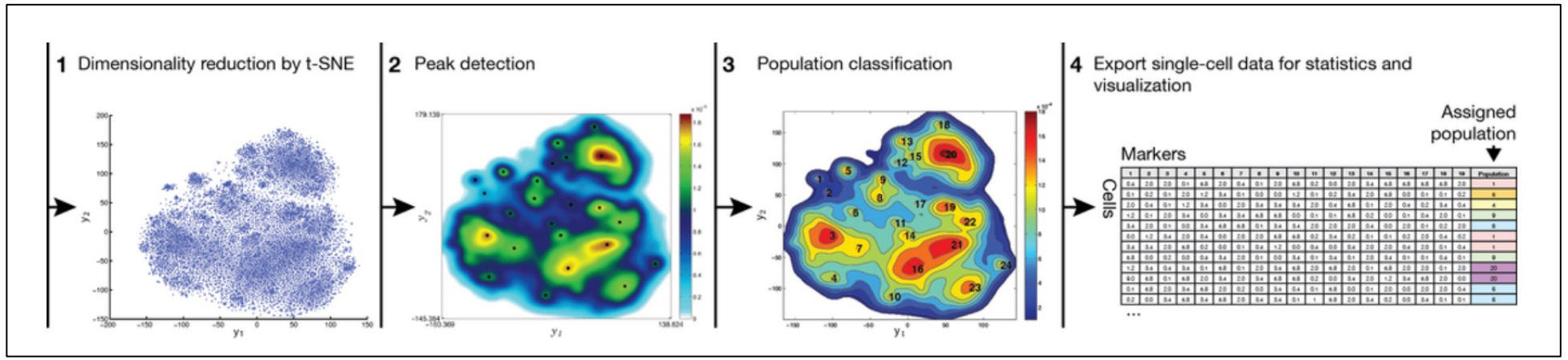
People are already gating and clustering dimension reduction maps. Guidelines are needed!

Michael Wong and Evan Newell: Manually gating a t-SNE map



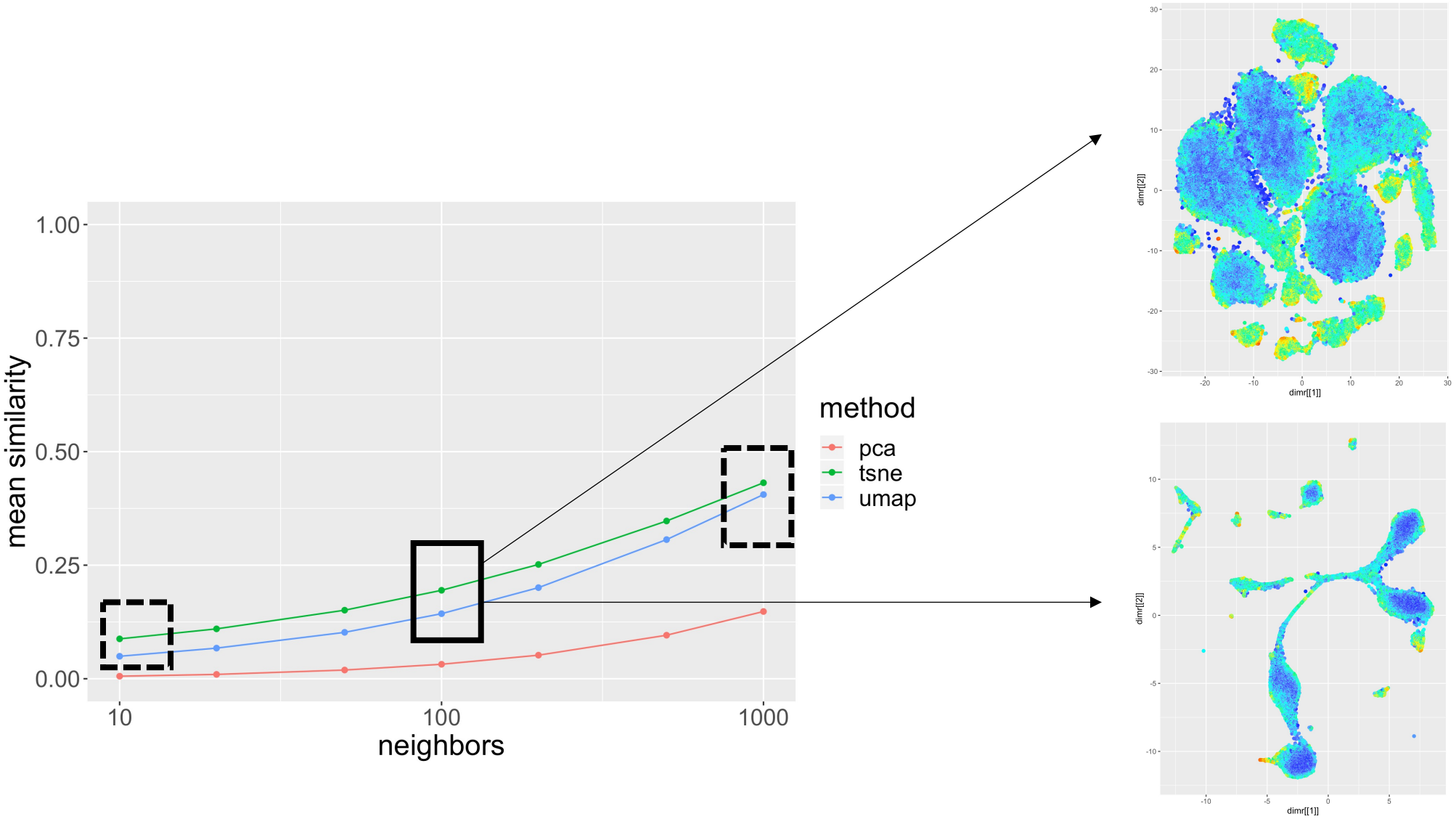
Wong *et al*, *Cell* 2016

Accense (Petter Brodin): Clustering a t-SNE map



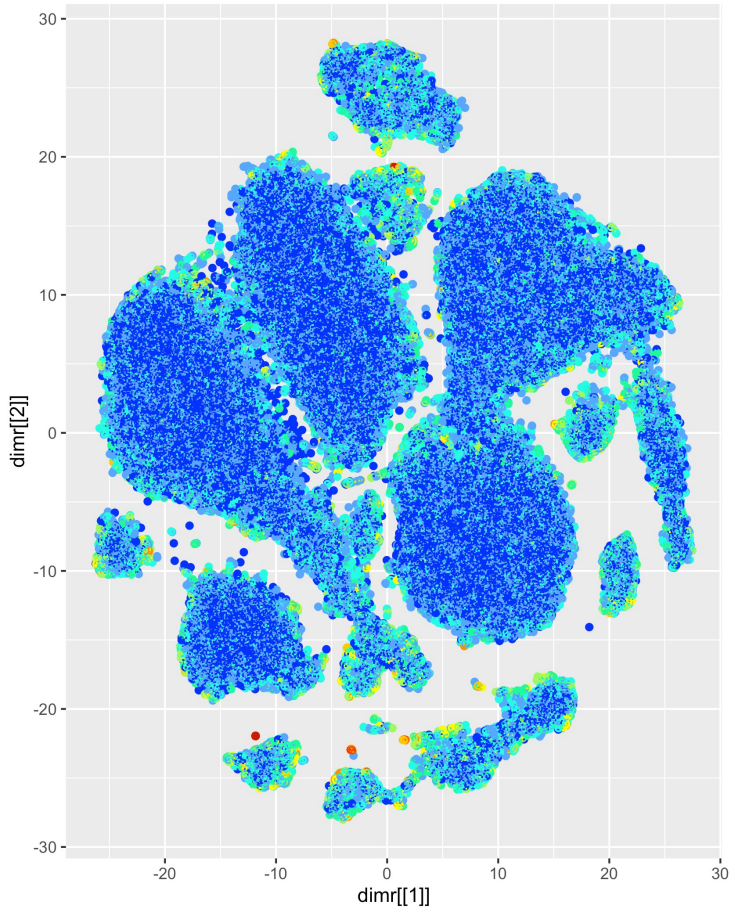
Shekar *et al*, *PNAS* 2014

Color a dimension reduction map by it's own neighborhood preservation, given k

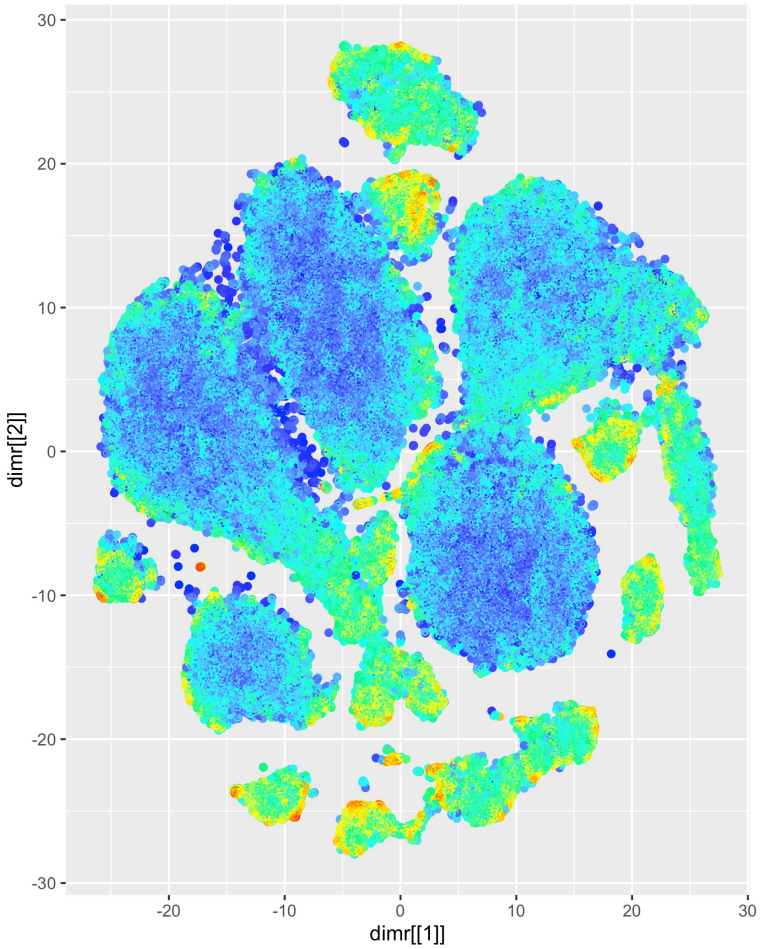


Local comparison for t-SNE

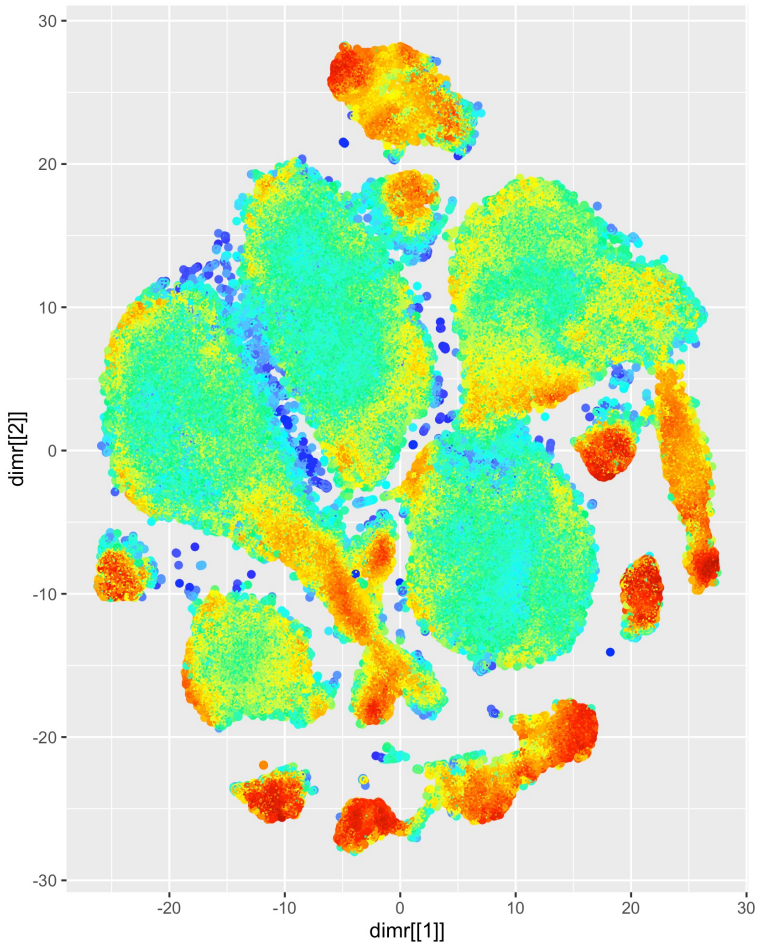
K = 10



K = 100

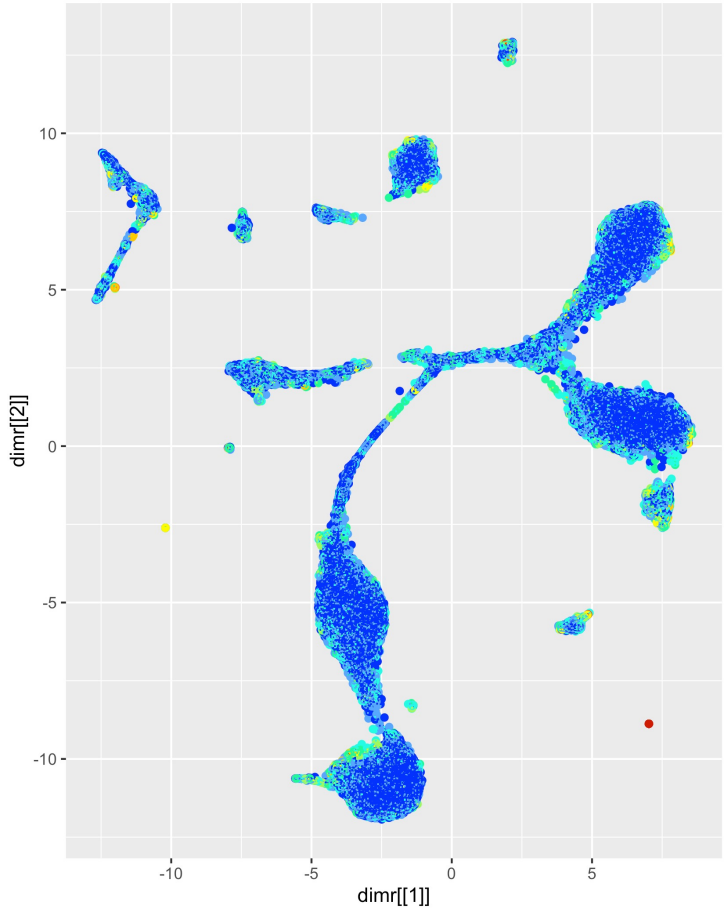


K = 1000

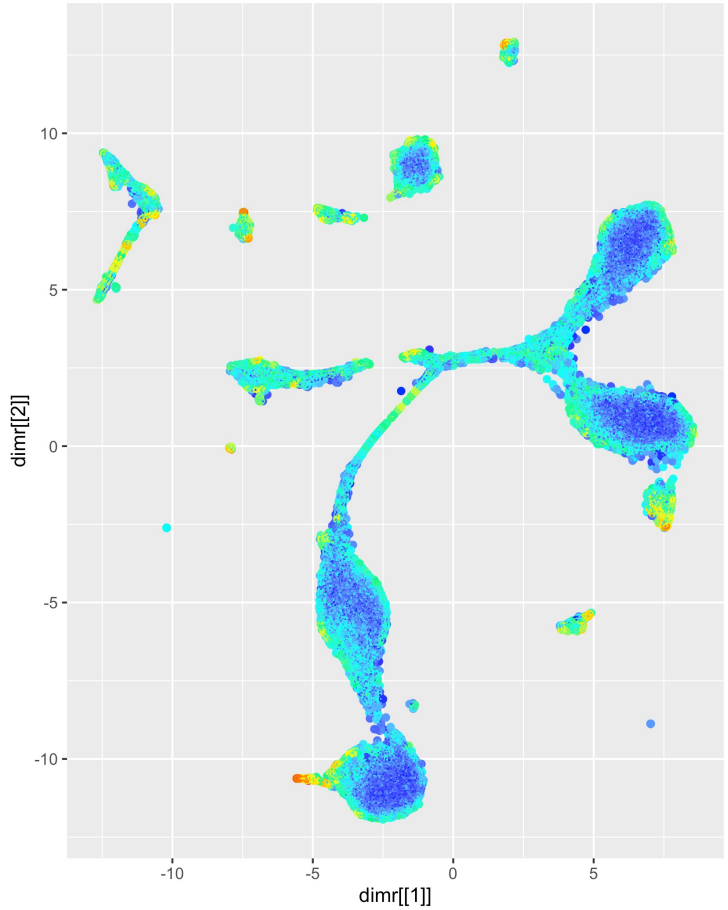


Local comparison for t-SNE

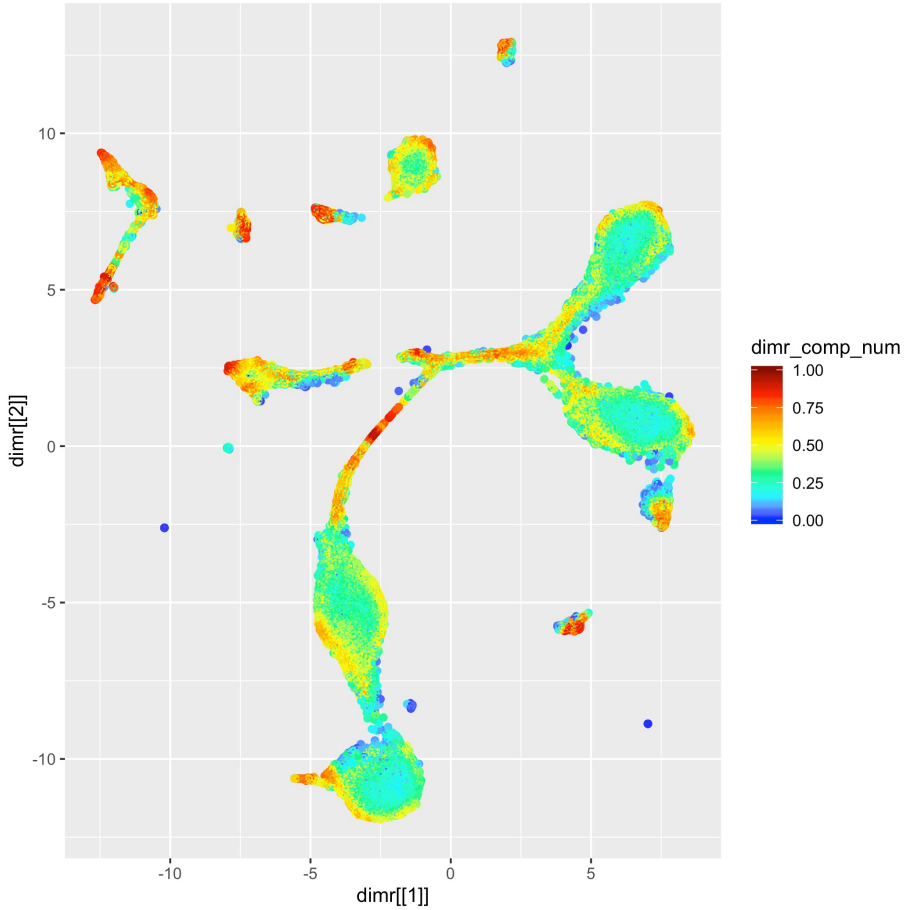
K = 10



K = 100

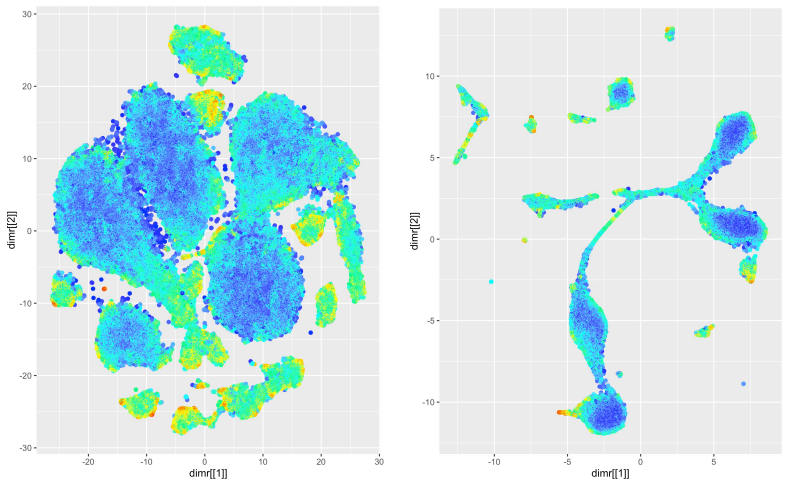


K = 1000

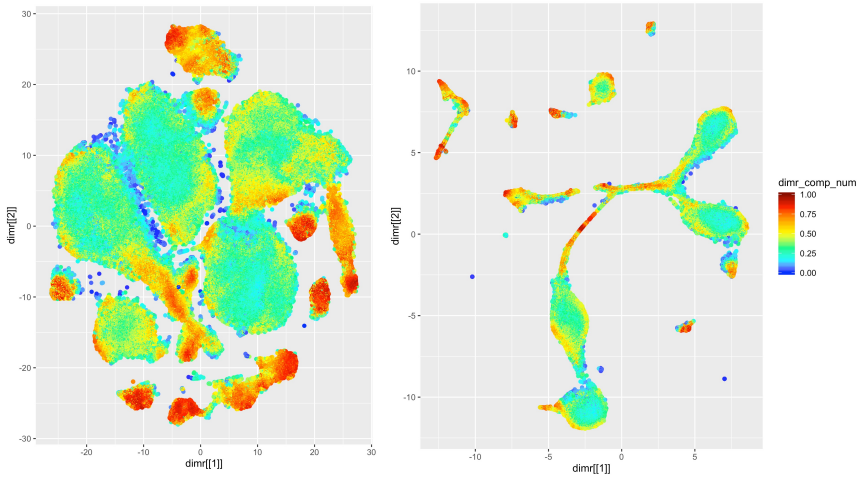


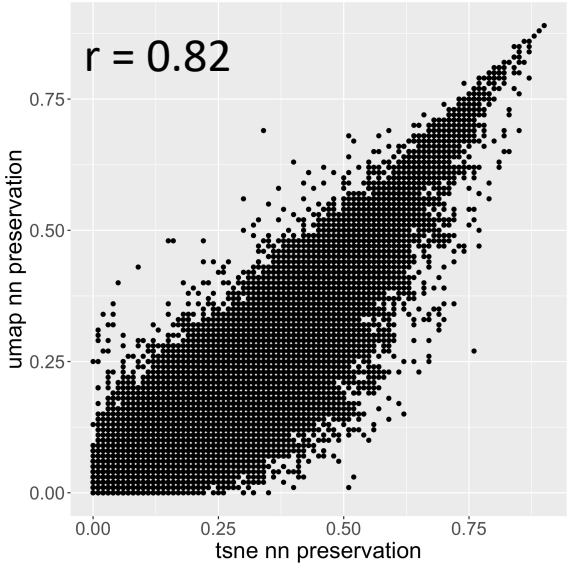
t-SNE and UMAP are preserving the data in a similar manner

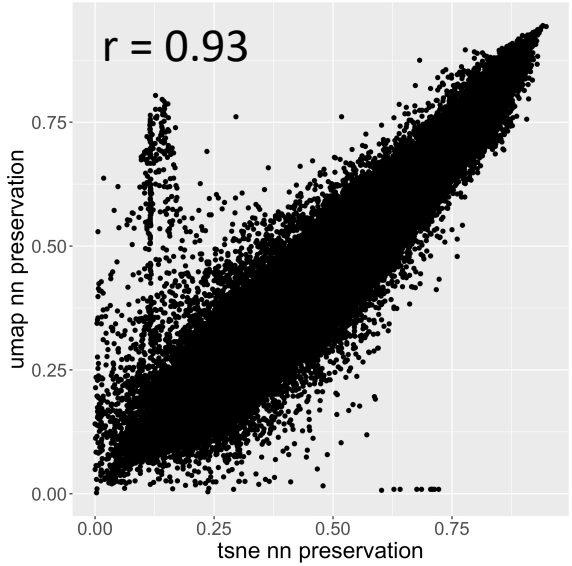
K = 100



K = 1000







Part 1 conclusions

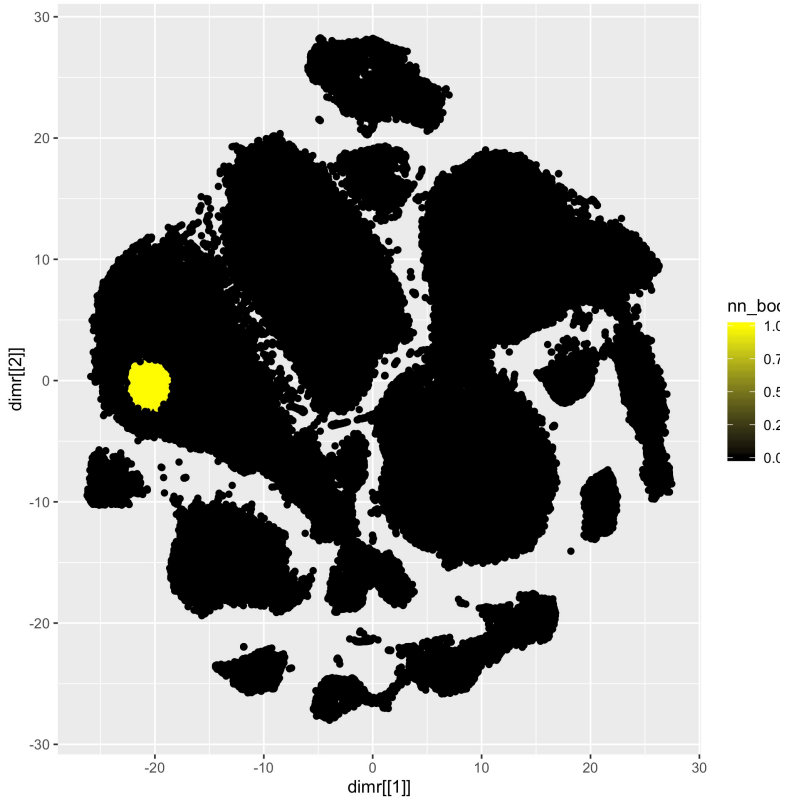
- t-SNE outperforms UMAP (though only slightly) in KNN preservation
- Both t-SNE and UMAP outperform PCA in KNN preservation
- KNN preservation performance varies in specific patterns across both t-SNE and UMAP
- t-SNE and UMAP have better KNN preservation in smaller islands/corridors in the data. Implications on how to gate the maps

Outline

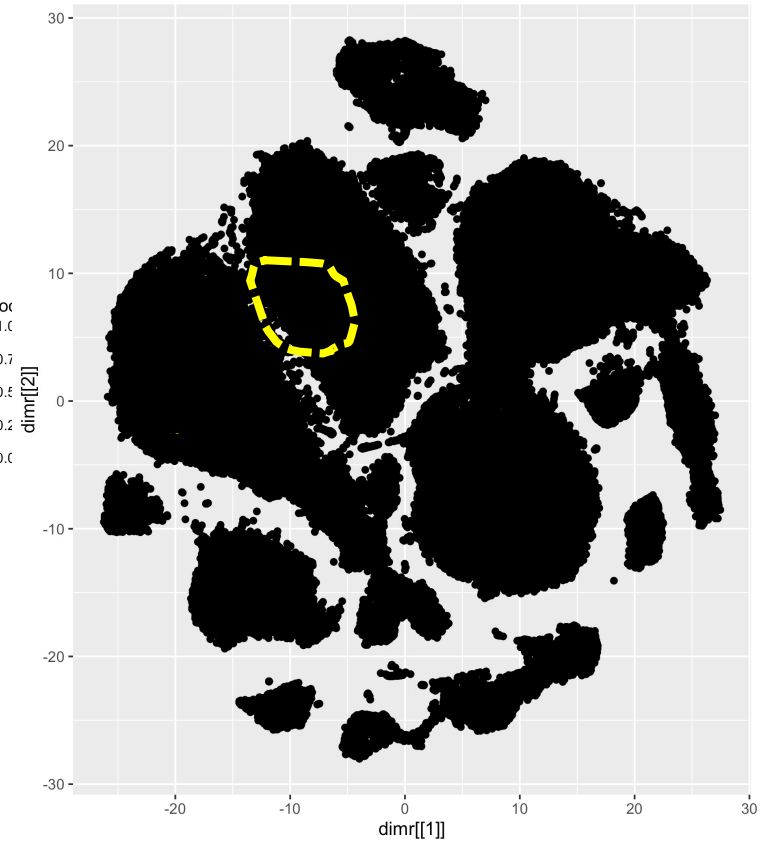
- Part 1: Introduction
- Part 2: Preservation of local structure
- **Part 3: Preservation of global structure**

If a “gate” on the map has 30% KNN preservation, where are the other cells?

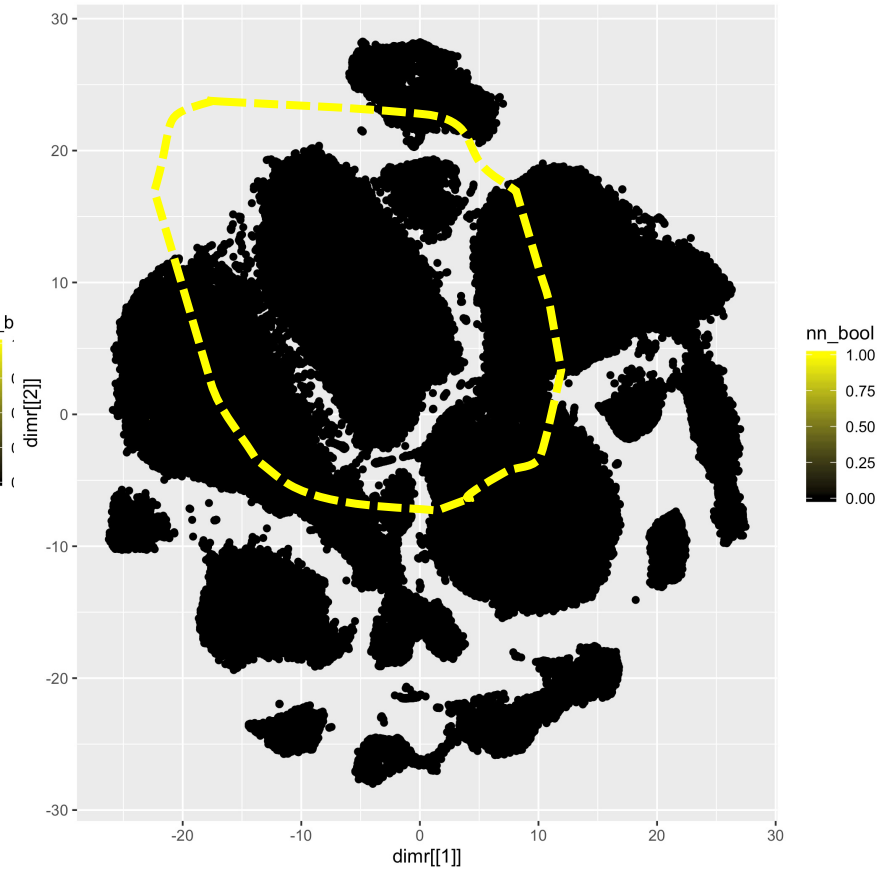
KNN ID on t-SNE map



KNN from hi-D, locations
Hypothesis 1

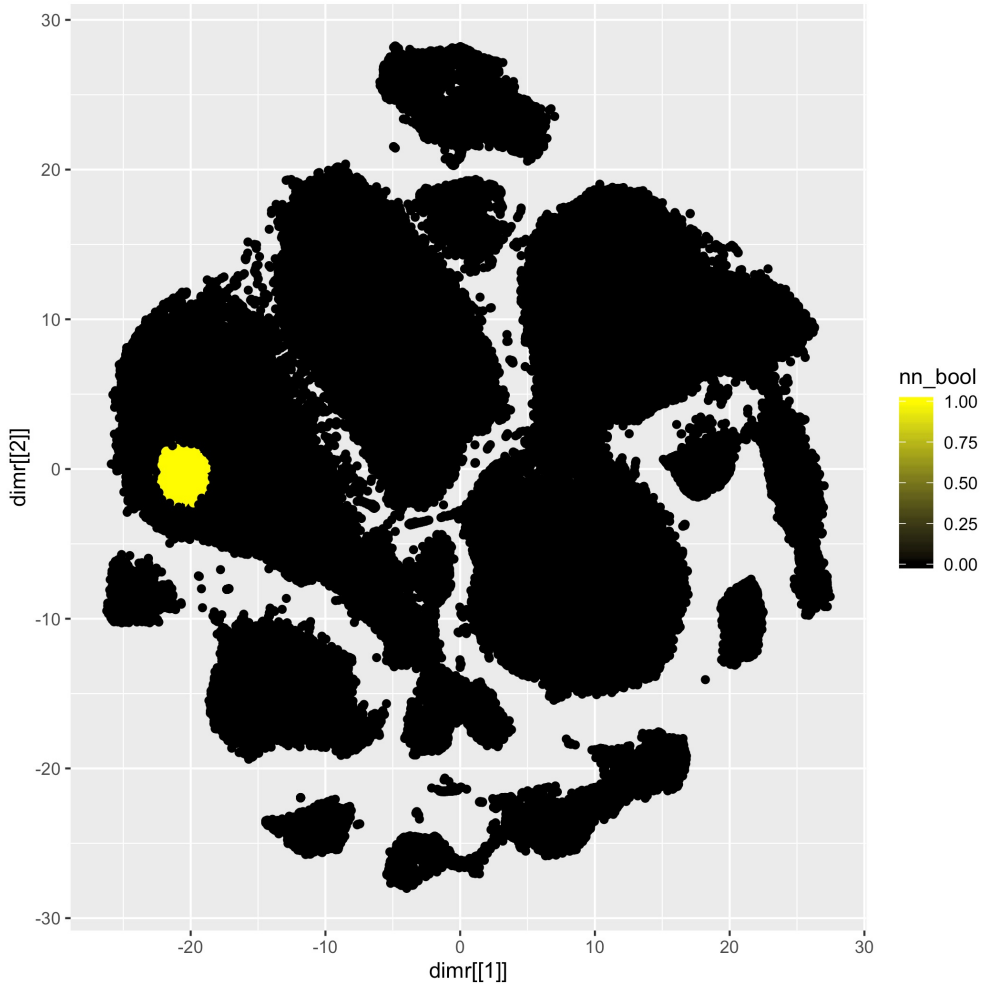


KNN from hi-D, locations
Hypothesis 2

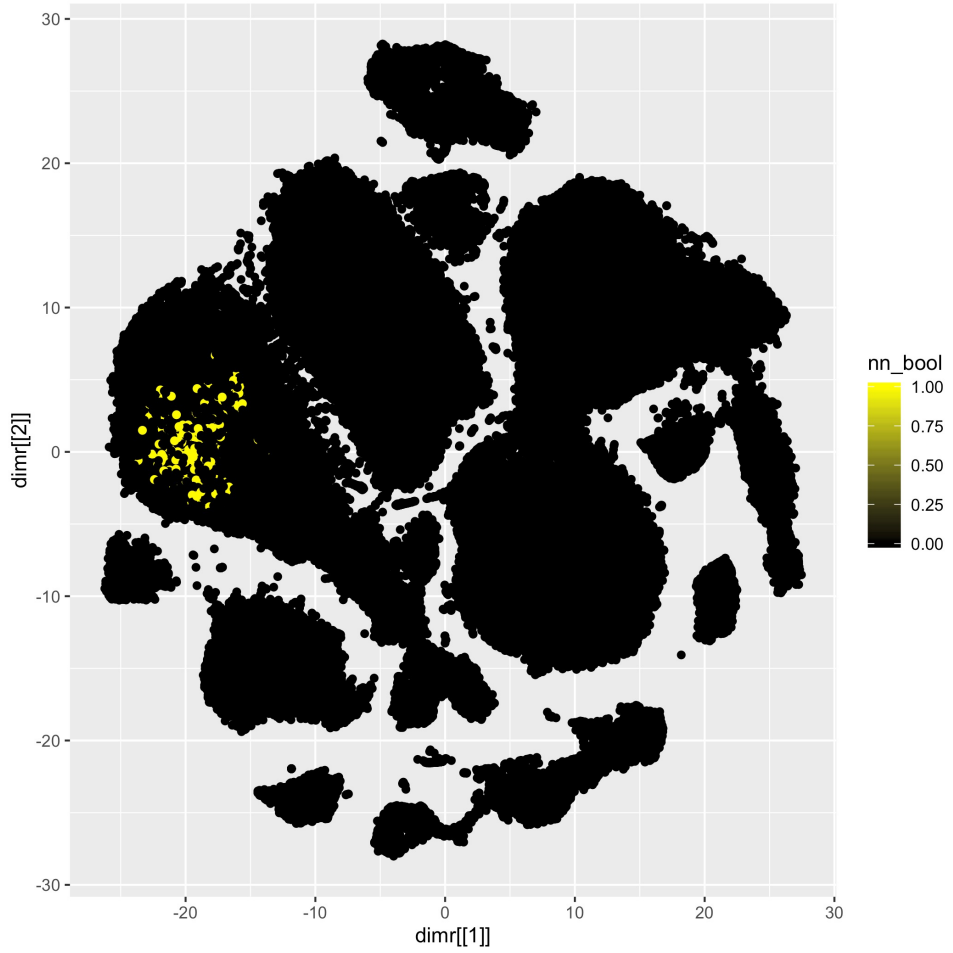


KNN identity for t-SNE, k = 1000, cell 1

Dimr neighbors

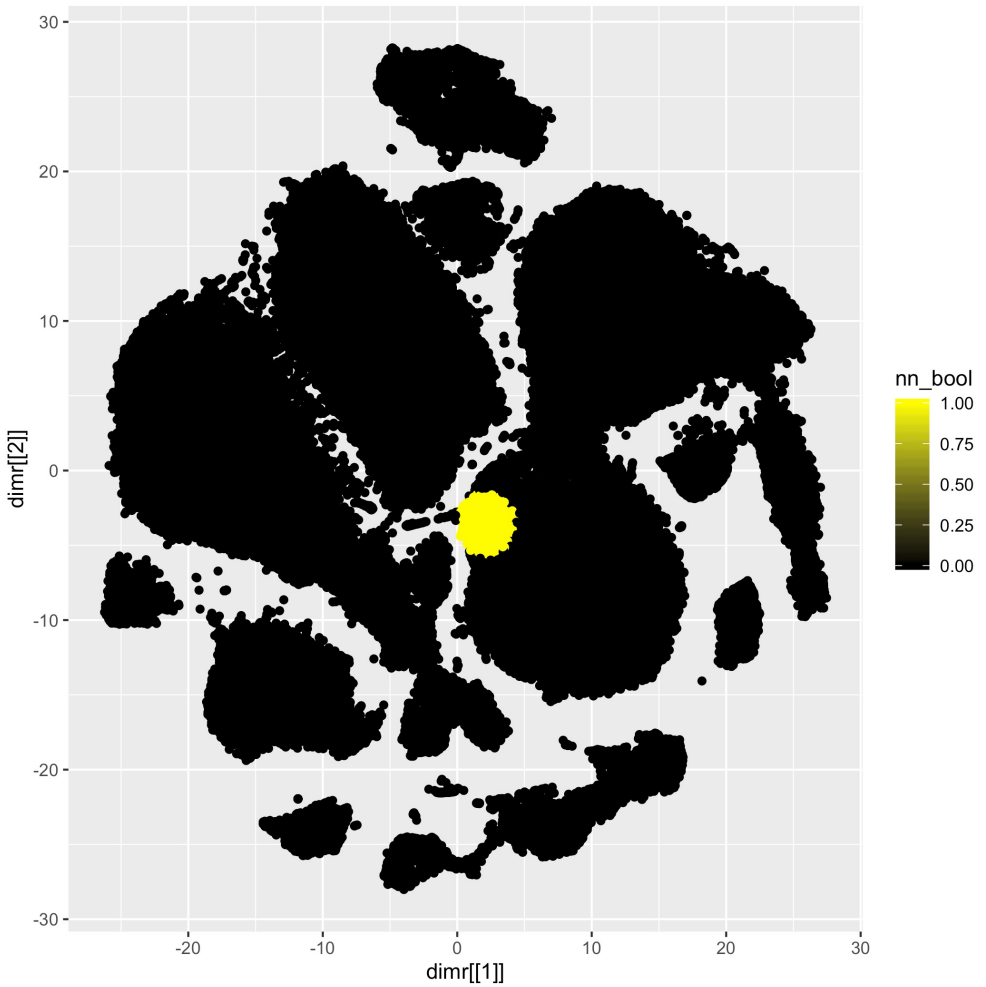


High-D neighbors

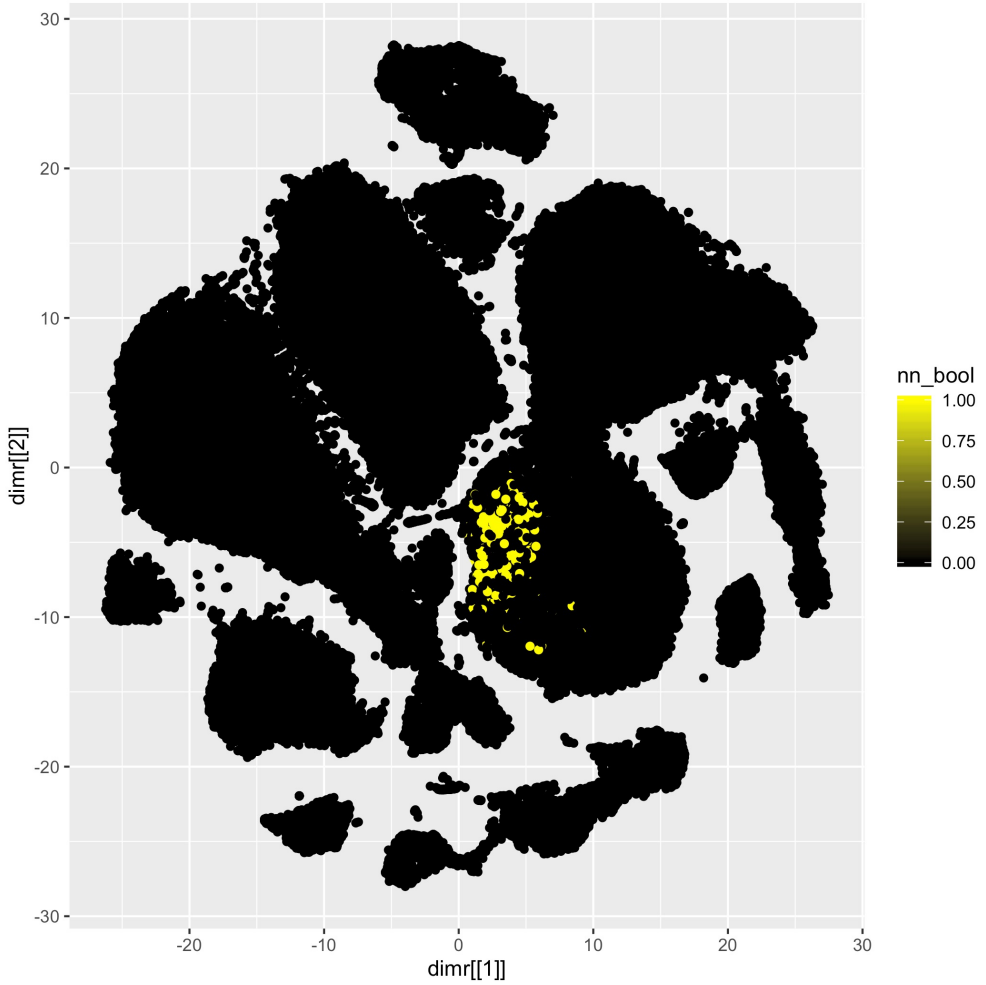


KNN identity for t-SNE, k = 1000, cell 4

Dimr neighbors

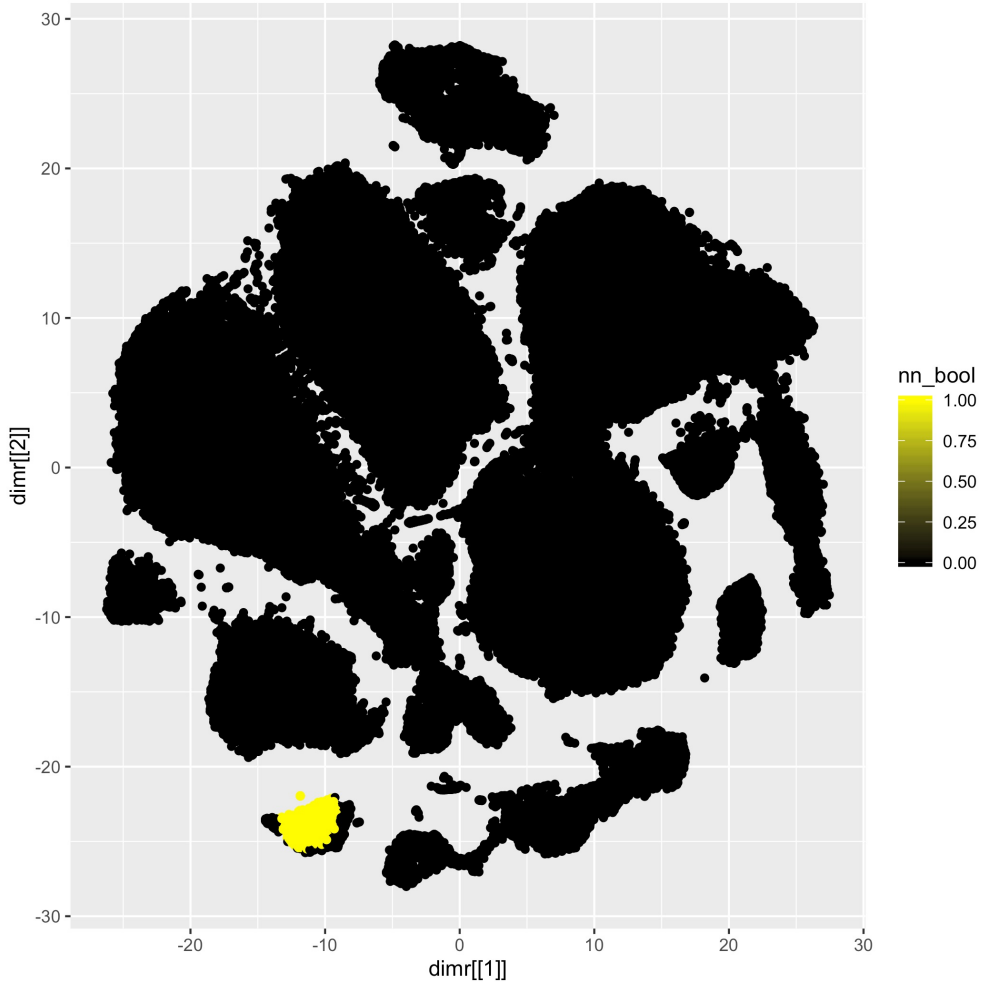


High-D neighbors

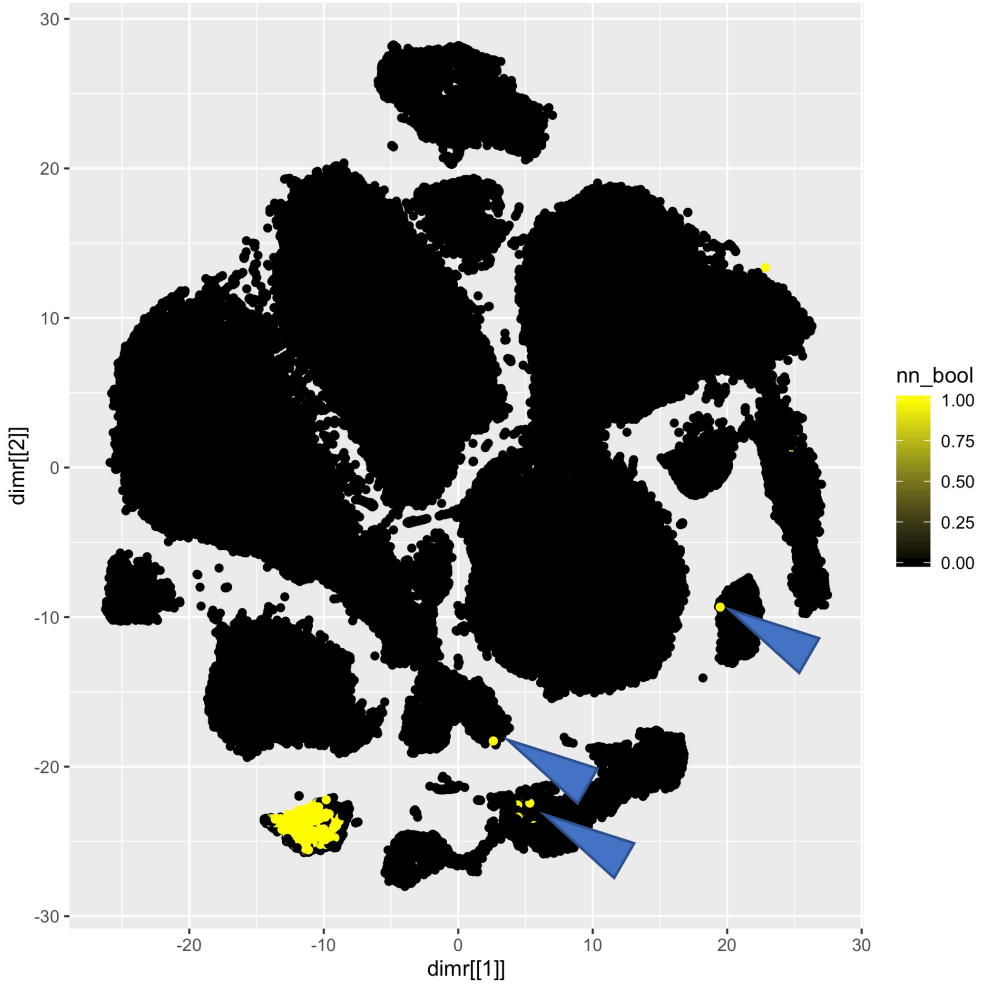


KNN identity for t-SNE, k = 1000, cell 6

Dimr neighbors

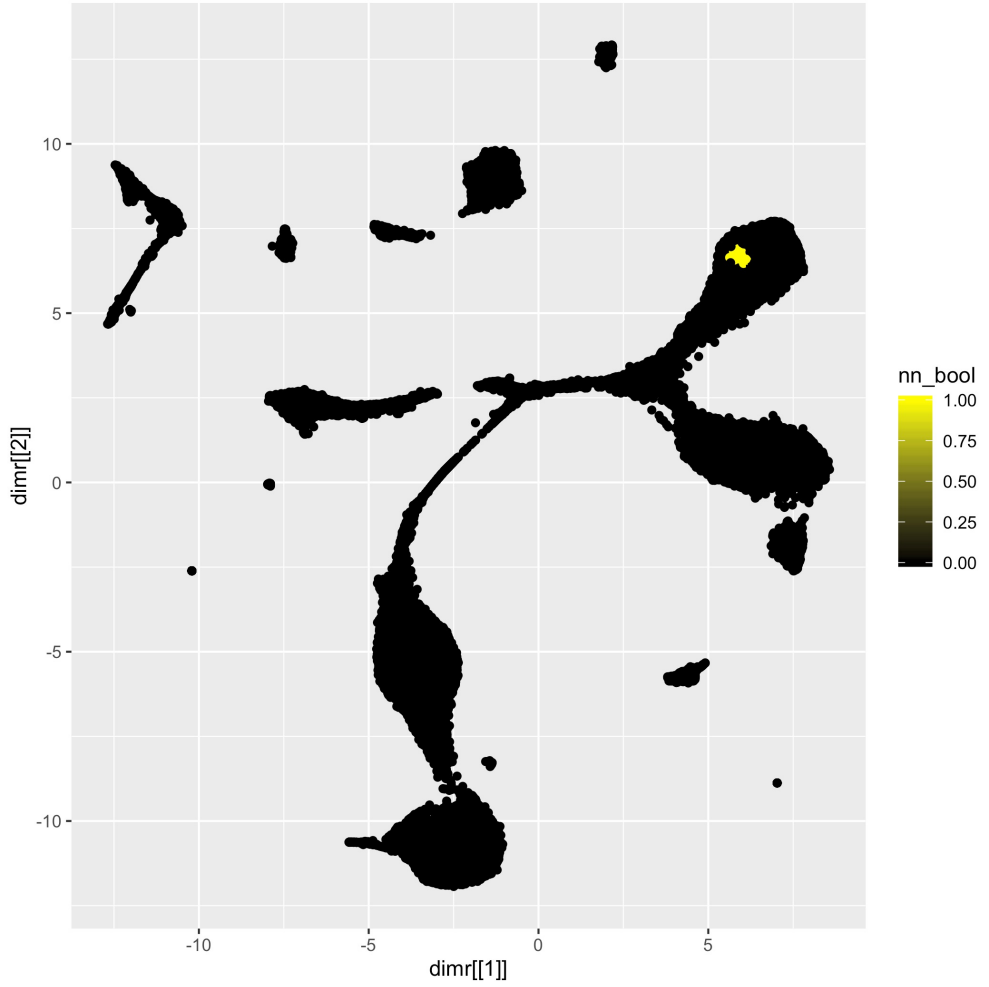


High-D neighbors

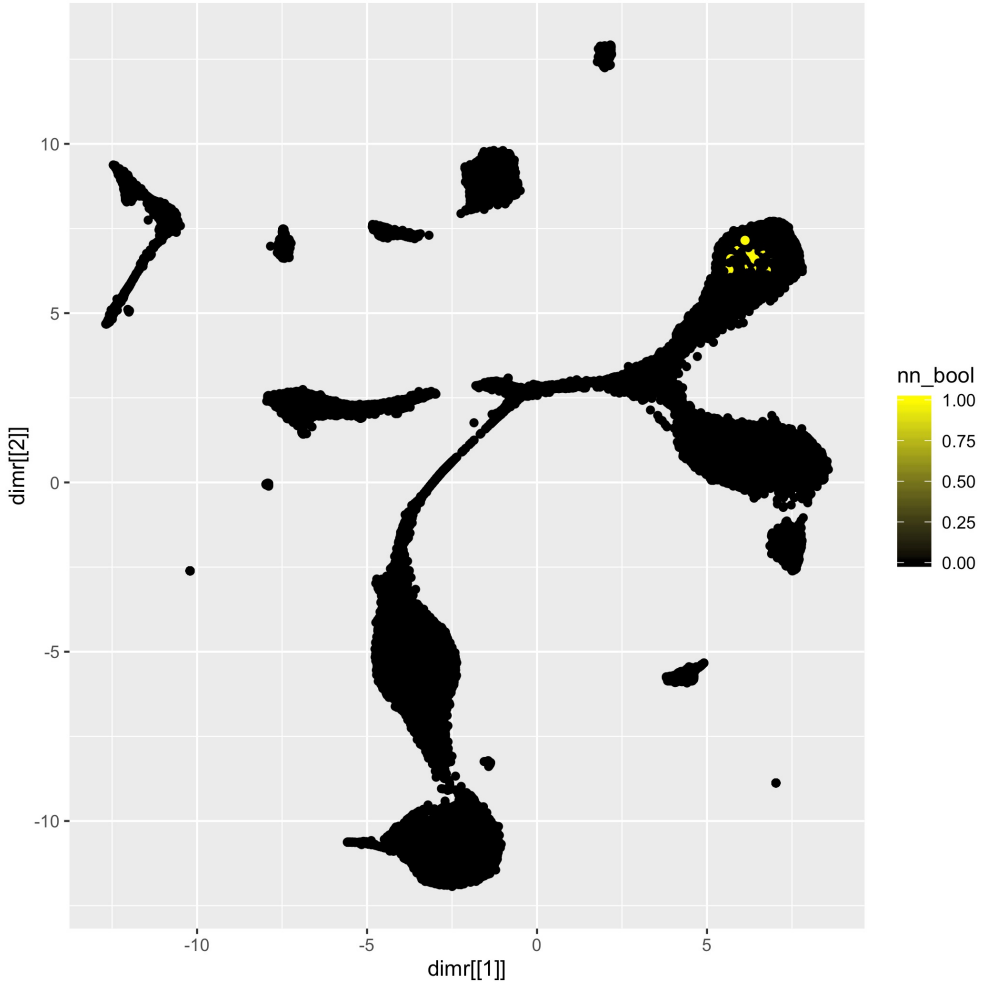


KNN identity for UMAP, k = 1000, cell 1

Dimr neighbors

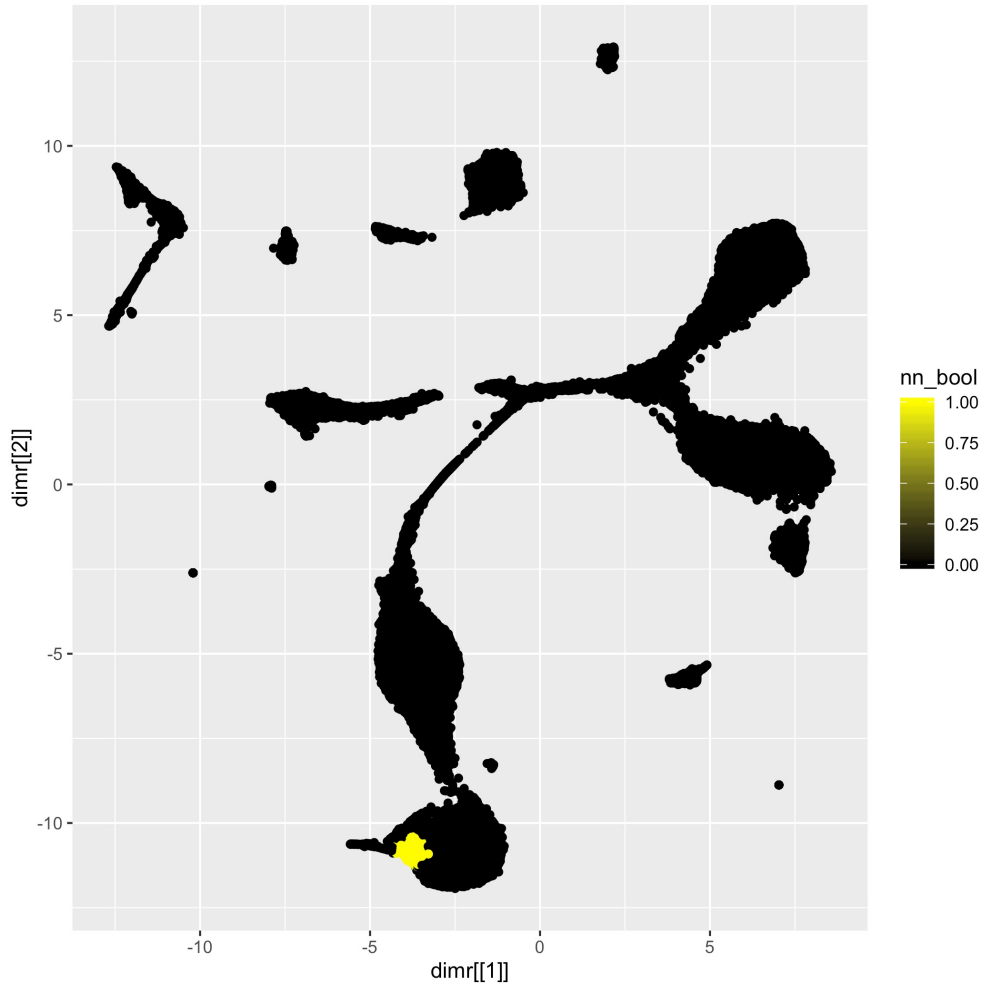


High-D neighbors

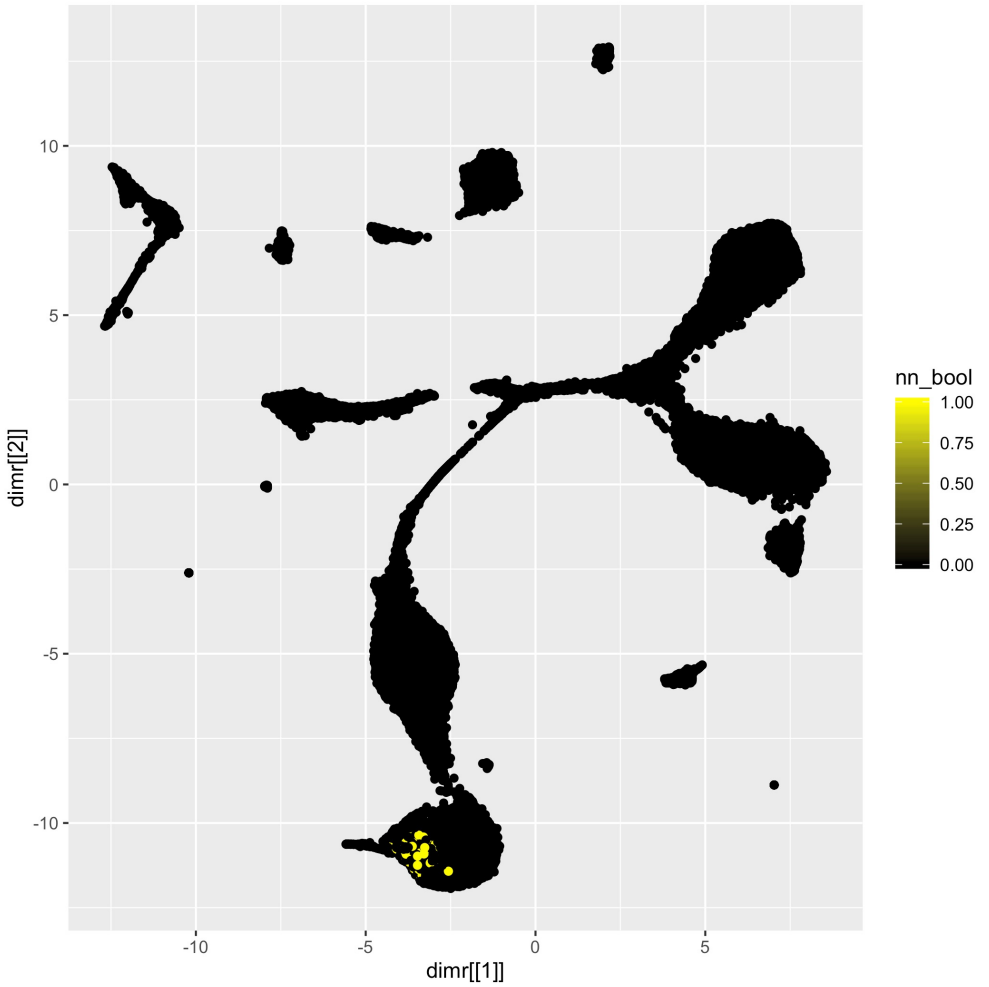


KNN identity for UMAP, k = 1000, cell 4

Dimr neighbors

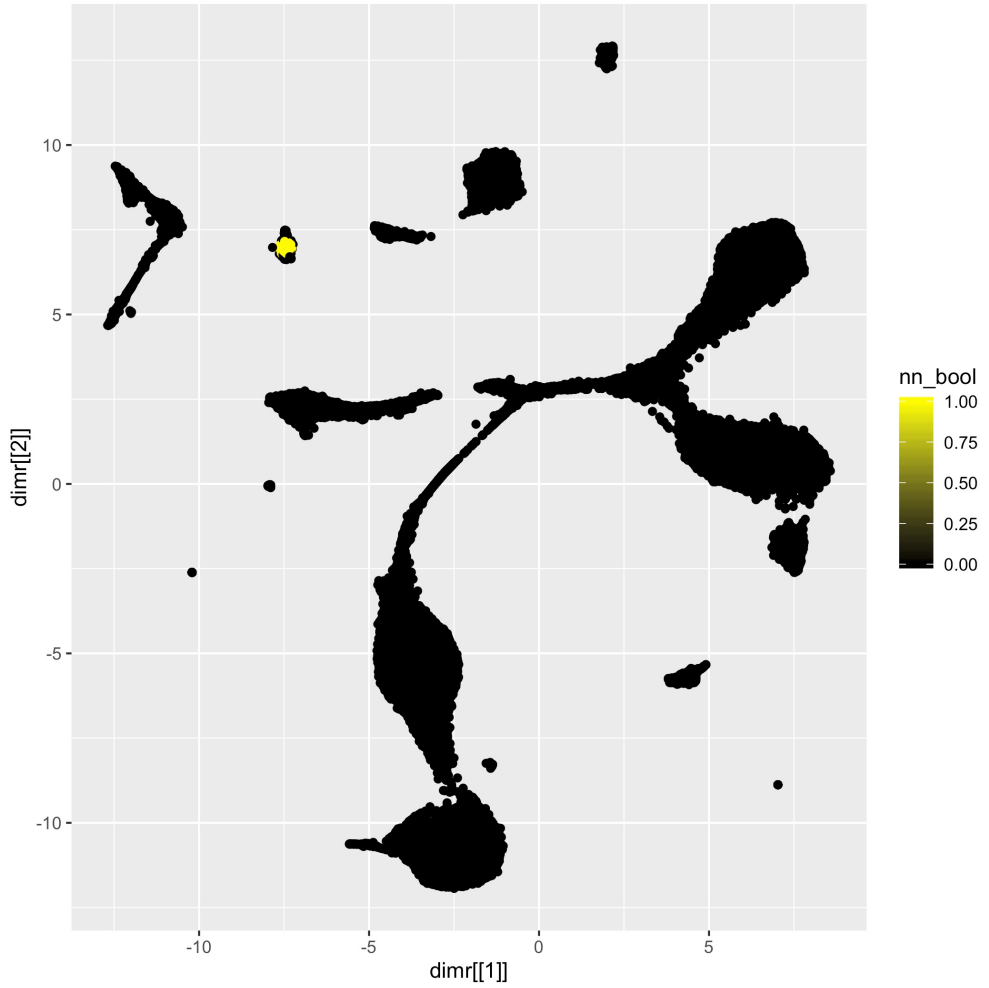


High-D neighbors

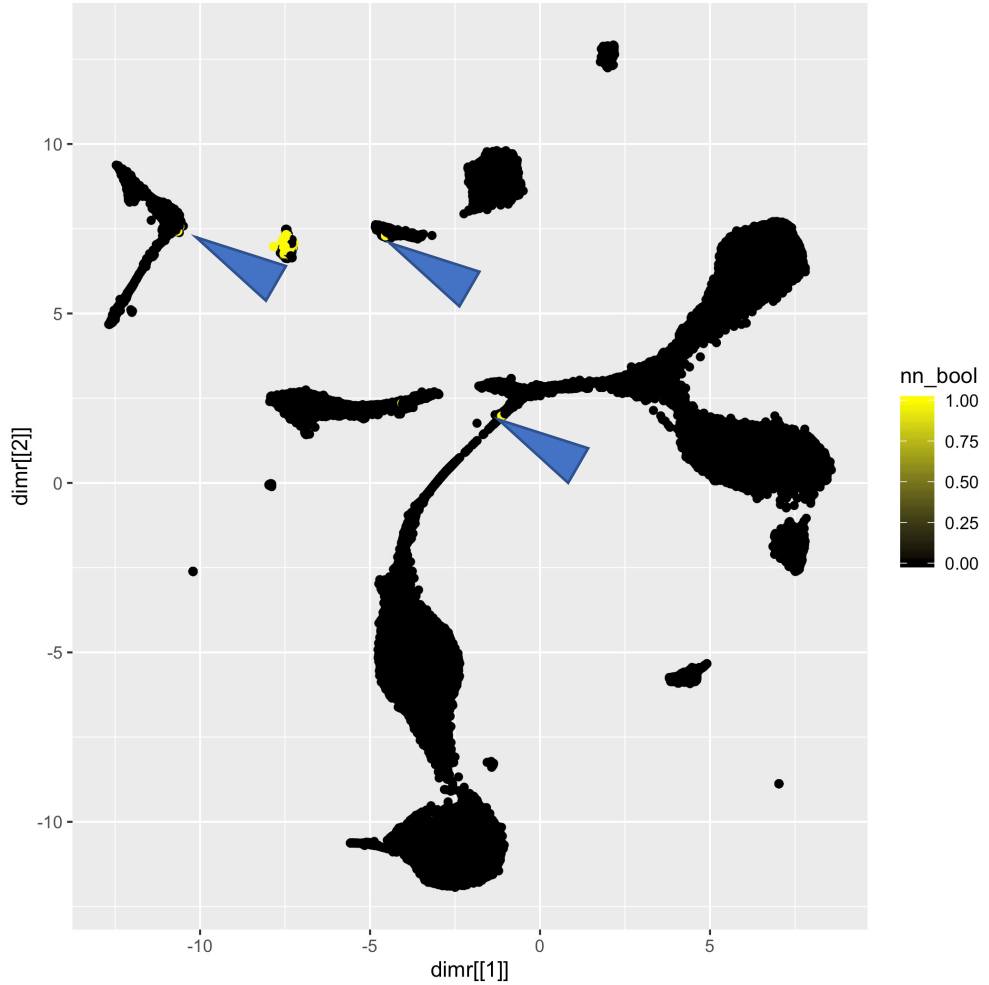


KNN identity for UMAP, k = 1000, cell 6

Dimr neighbors



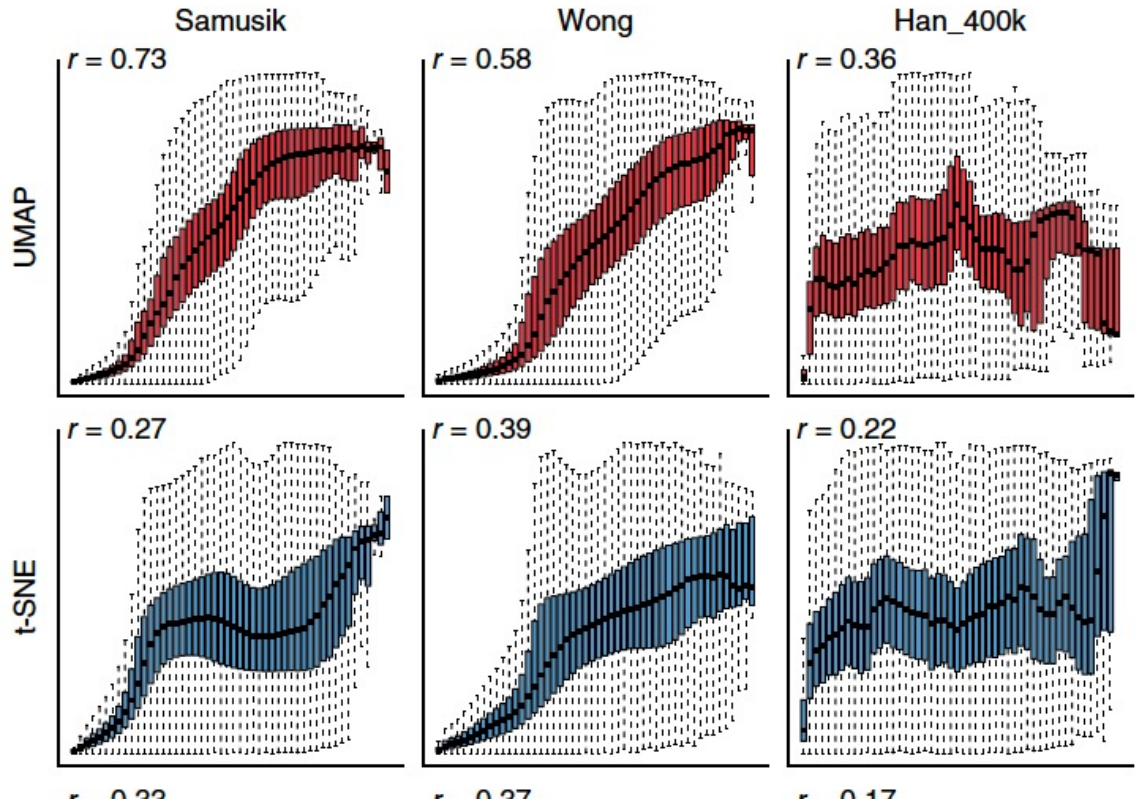
High-D neighbors



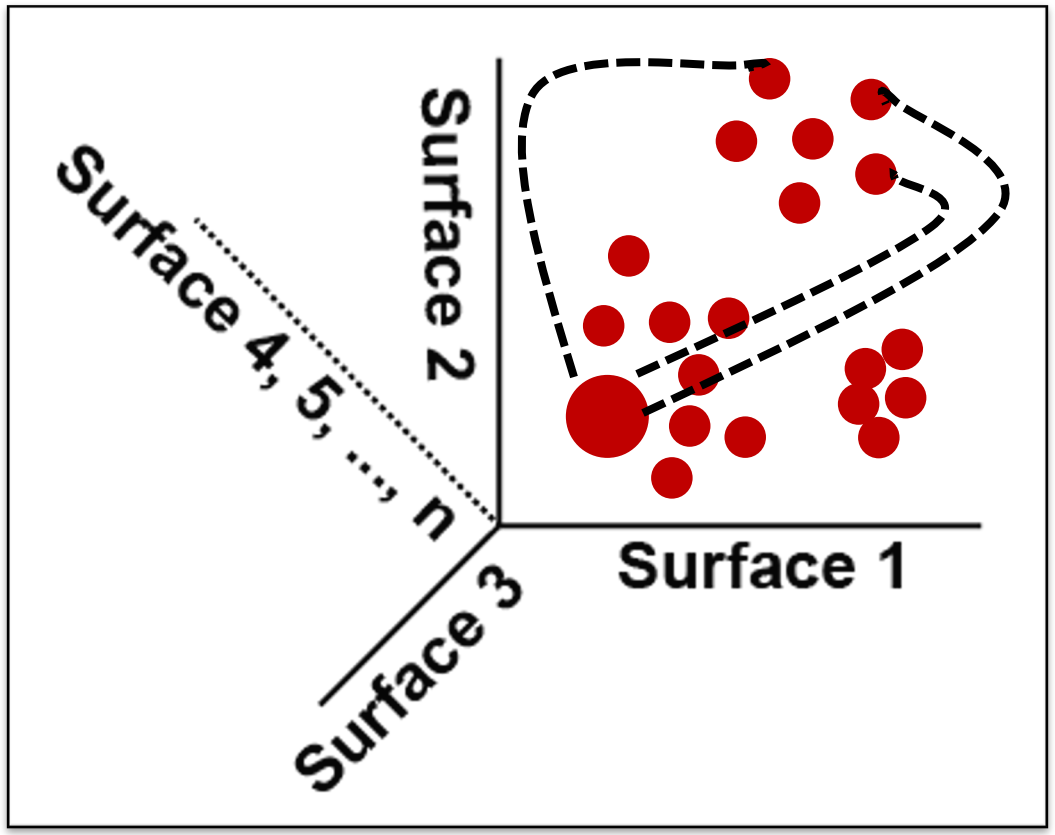
Global preservation, measured by pairwise distances

Dimensionality reduction for visualizing single-cell data using UMAP

Etienne Becht[1], Leland McInnes[2], John Healy[2], Charles-Antoine Dutertre[1], Immanuel W H Kwok[1], Lai Guan Ng[1], Florent Ginhoux[1] & Evan W Newell[1,3]



K-**farthest** neighbors (KFN) to determine global preservation of dimension reduction maps



Find KFN for each cell from high-dim space

Find KFN for each cell from a 2-D embedding (eg tSNE)

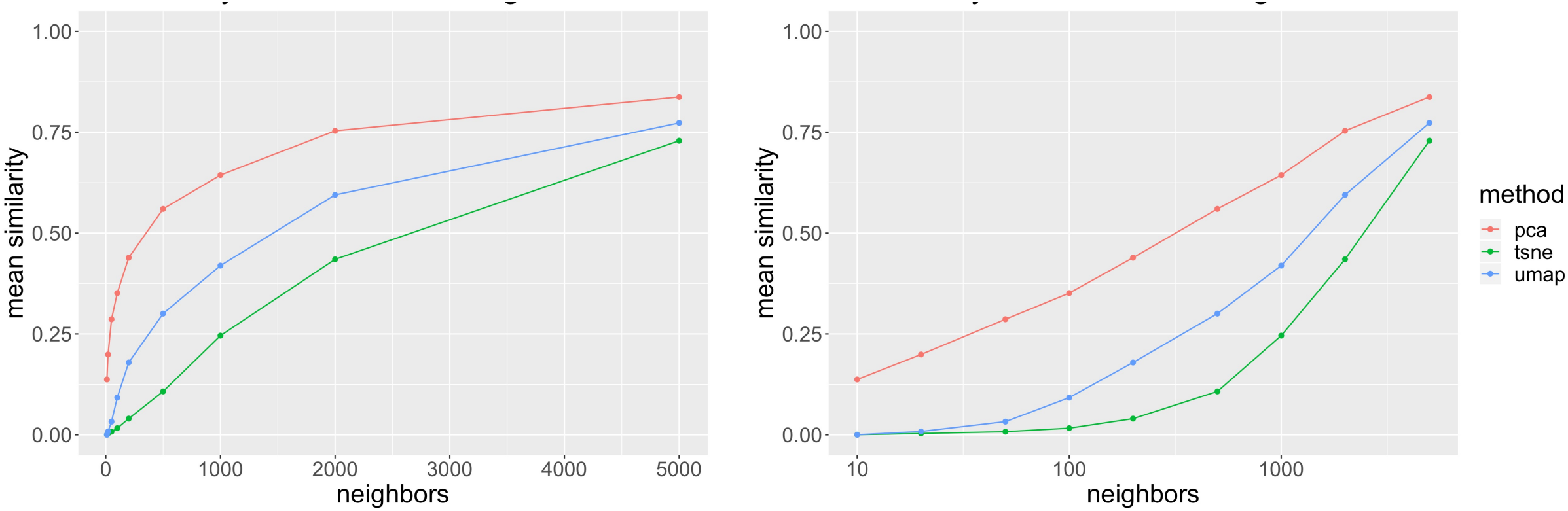
Compare KFN identities from the 2-D embedding and high-dim space

Repeat across a wide range of values for K

Bioconductor package: Sconify

Global KFN comparison between PCA, t-SNE and UMAP (10k cell subsample)

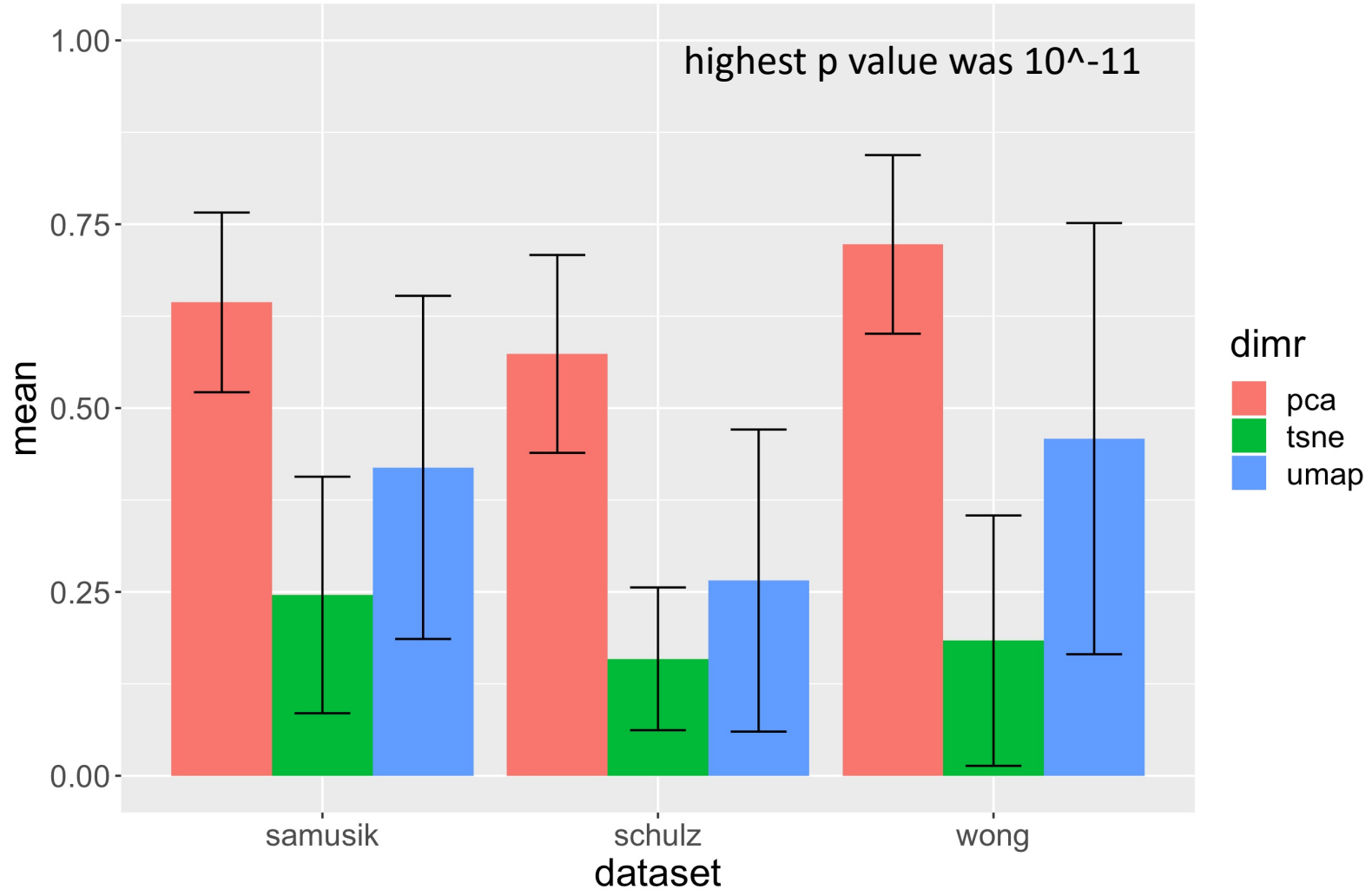
X axis is on a log scale



therefore concerns itself primarily with accurately representing local structure. While we believe that UMAP can capture more global structure than these other techniques, it remains true that if global structure is of primary interest then UMAP may not be the best choice for dimension reduction.

Mcinness *et al*, *Arxiv* 2018 (the UMAP paper)

Across 3 datasets, bar plots with error bars and p values



Cells: 10k
k = 1000

Part 3 conclusions

- Nearest neighborhoods computed from high-D space and dimension reduction space occupy similar regions
- Positioning of the islands relative to each other could be arbitrary
- K-farthest neighborhood (KFN) preservation reveals global structure preservation: PCA > UMAP > t-SNE

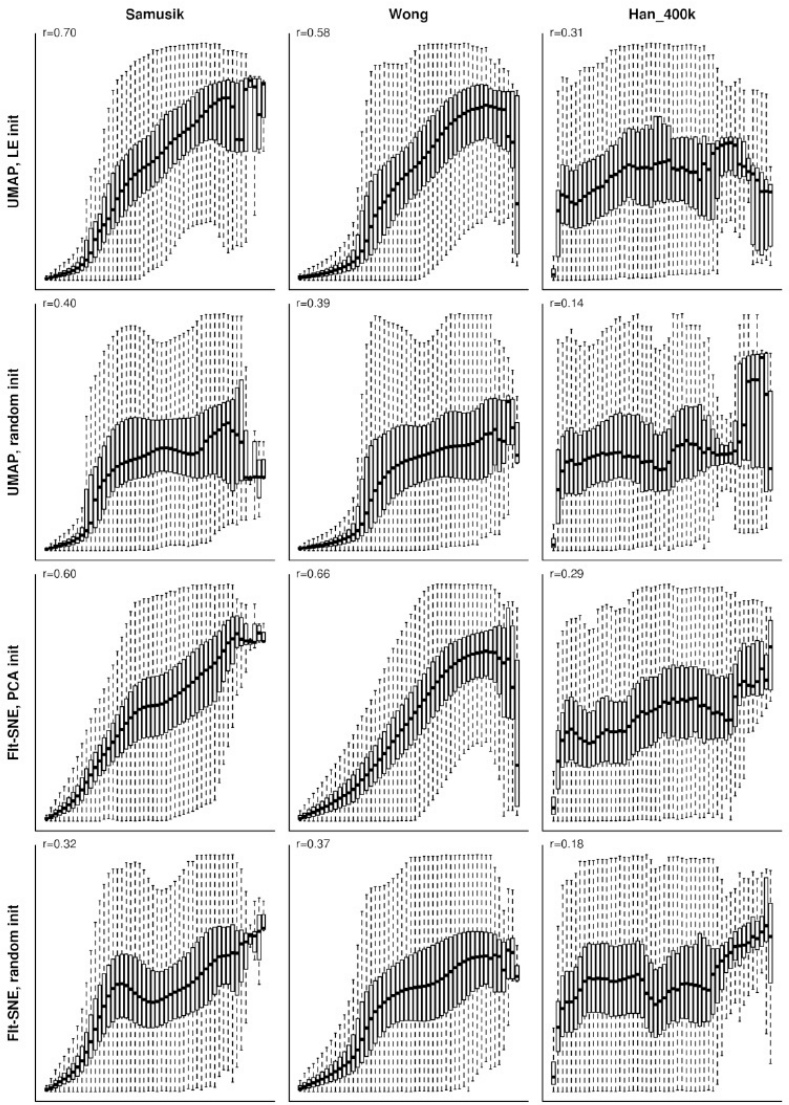
Next steps: initialization matters

UMAP does not preserve global structure any better than t-SNE when using the same initialization

Dmitry Kobak[1] and George C. Linderman[2]

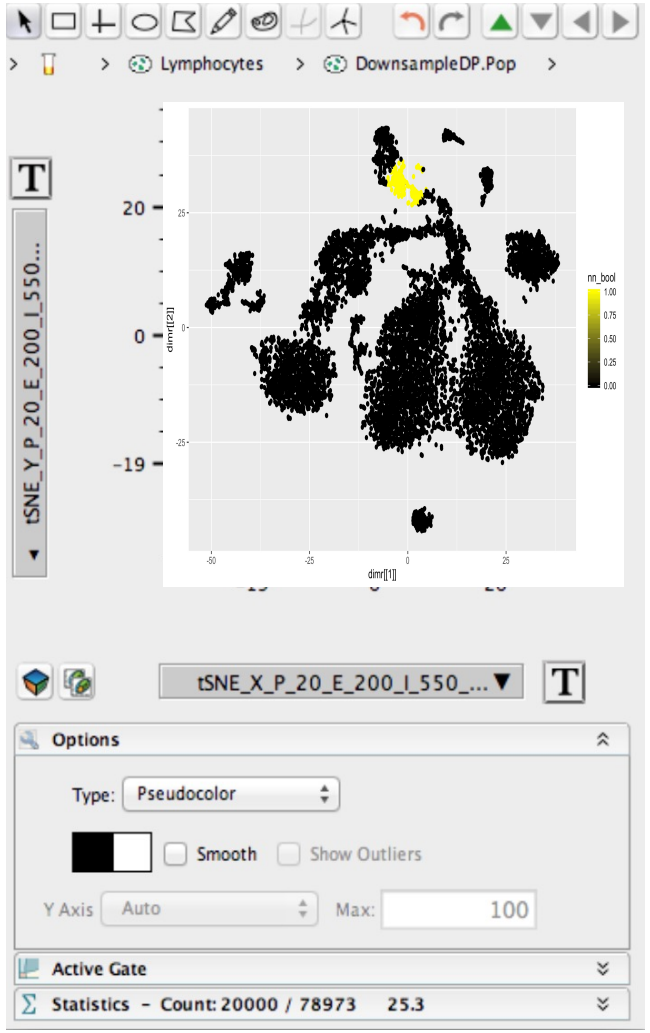
[1] *Institute for Ophthalmic Research, University of Tübingen, Germany*
[2] *Applied Mathematics Program, Yale University, New Haven, CT, USA*
dmitry.kobak@uni-tuebingen.de, george.linderman@yale.edu

(BioRxiv)

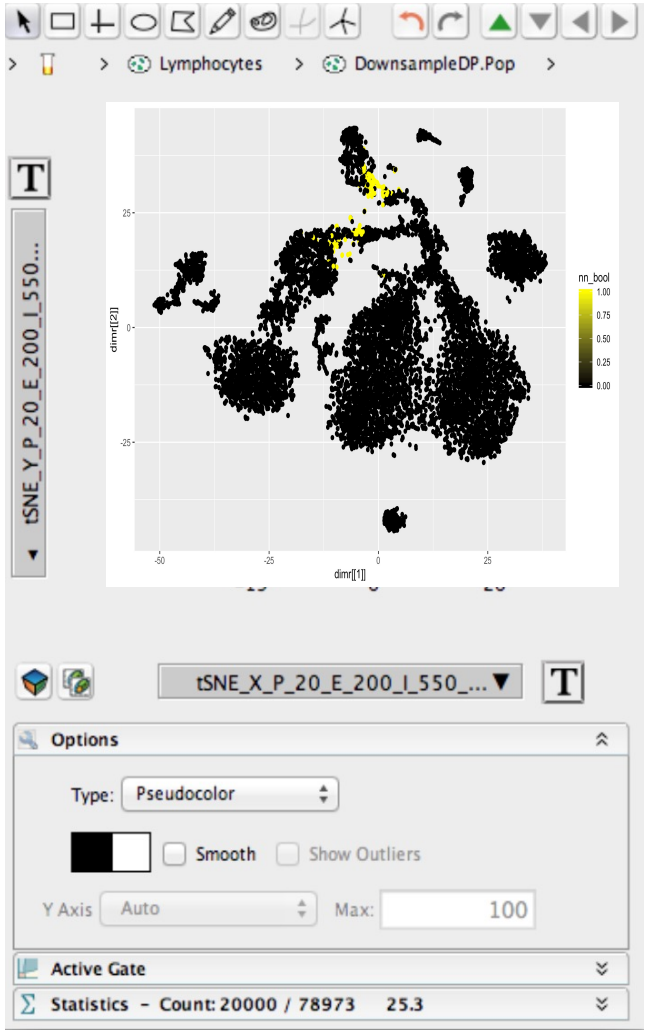


Toward a ”safe” manual gating interface for dimension reduction maps

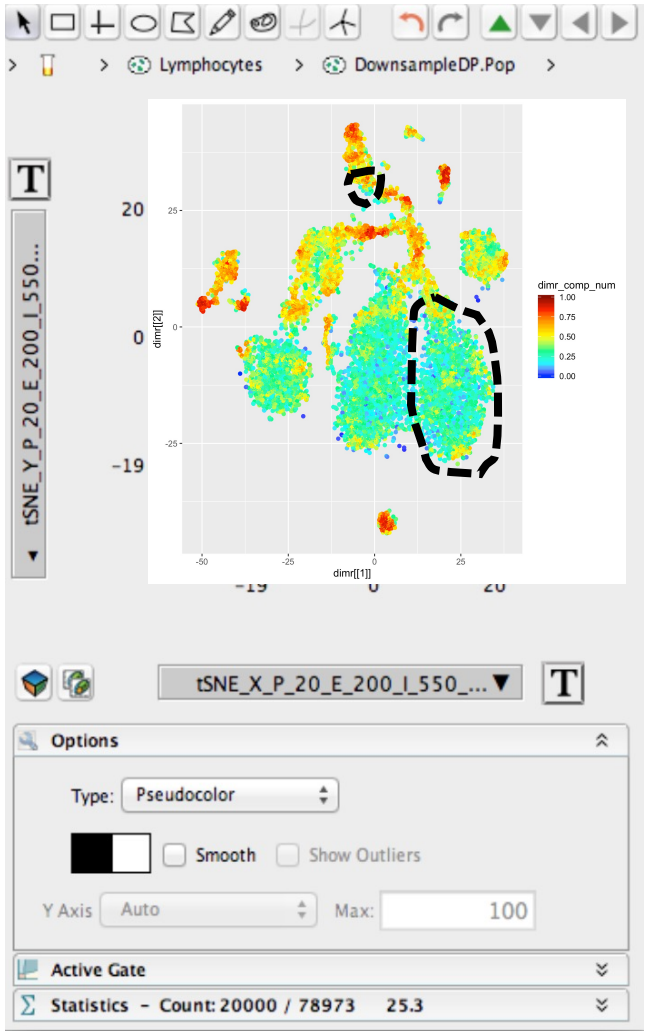
Identity color (dimr)



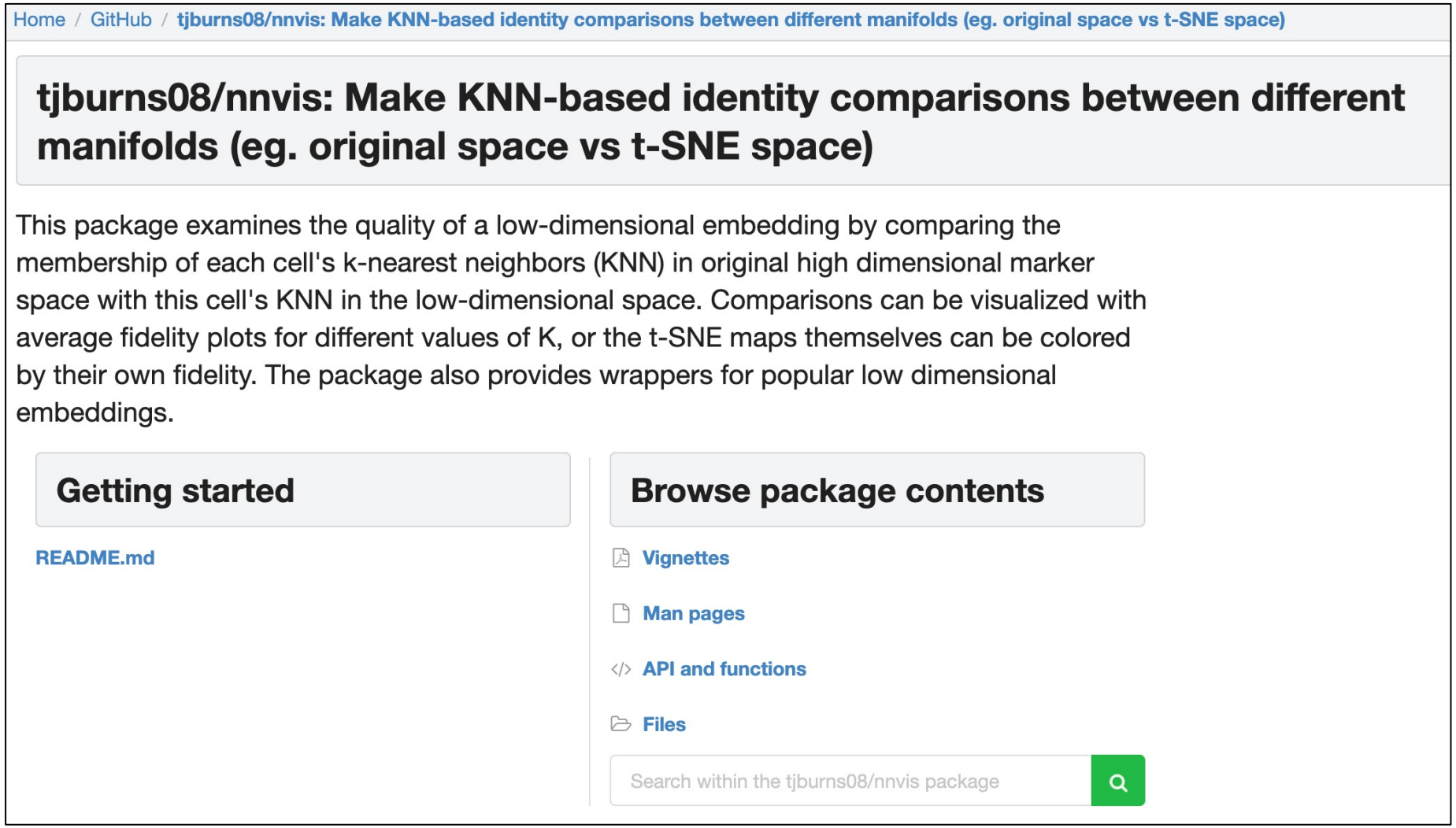
Identity color (high-d)



Identity comparison



nnvis: an R package to do neighbor-based preservation analysis on your dimension reductions



Acknowledgments

Heike Hirseland Antonia Niedobitek René Riedel

Sarah Gräßle Marie Urbicht Silke Stanislawiak

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Deutsches Rheuma-Forschungszentrum
Ein Institut der Leibniz-Gemeinschaft

Sabine Baumgart **Andreas Grützkau** Pawel Durek

Henrik Mei **Axel Schulz**