**Nonlinear Dimensionality Reduction of Single Cell Data using Autoencoders**

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Introduction

Single cell technologies, such as single cell RNAseq and mass cytometry, have allowed for extremely high resolution data to be collected about tissues. These technologies can help elucidate genetic and metabolic pathways at the cellular level, therefore having the potential to vastly increase our knowledge of cellular differentiation, disease progression, and other topics. However, due to its high resolution, single cell datasets often have a large number of dimensions and tens of thousands of samples, making the data difficult to work with. Simply applying PCA is not an excellent solution as it struggles to account for the underlying structure of the data. Therefore, many have turned to nonlinear dimensionality reduction techniques to interpret and work with single cell datasets.

Two commonly used nonlinear dimensionality reduction techniques are t-distributed stochastic neighbour embeddings (tSNE) and uniform manifold approximation and projection (UMAP).

tSNE is essentially an unsupervised, nonlinear dimensionality reduction method that works by calculating similarity measures between points in a high dimensional space and low dimensionality space and then trying to optimize the two similarity measures with a KL-divergence cost function. The benefits of tSNE over PCA is that tSNE is capable of learning nonlinearly structured data such as the famous Swiss Roll Dataset because of tSNE’s emphasis on preserving pairwise distances and local similarities. The main drawbacks of tSNE are its inability to preserve global structure and its long runtime.

UMAP is a newer similar unsupervised, nonlinear dimensionality reduction method similar to tSNE that is argued by its author Leland McInnes to fix upon many of the pitfalls of tSNE. UMAP is far faster than tSNE for high dimensional data because it does not apply normalization like tSNE does as well as applied stochastic gradient descent instead of gradient descent like tSNE. Furthermore, UMAP can preserve global structure by using cross entropy as the cost function instead of KL-divergence like tSNE does.

Another approach to nonlinear dimensionality reduction is the use of autoencoders (AEs), a type of neural network that learns to reconstruct data through a low dimensional bottleneck. An AE consists of an encoder network that learns a low dimensional representation of the original data, and a decoder network that learns to recreate the original data from the latent space. AEs have been wildly successful in computer vision, and were able to learn low dimensional representations of high dimensional image data and accurately reconstruct new images from latent spaces. Because of their neural network architecture, AEs come with several advantages over other dimensionality reduction techniques. Firstly, AEs are highly parallelizable, dramatically speeding up the training process to learn latent spaces. Furthermore, the encoder network can be extracted from the AE after training and used to rapidly generate low dimensional representations of new samples outside the training dataset. Online training, continual improvement, and transfer learning can all be performed with AEs as well, which make them well suited to a variety of applications. AEs are also highly customizable as the designer is able to modify the network to account for any underlying assumptions or structure of the data in order to generate more accurate reconstructions from the latent representations. [Include some stuff about previous use of AEs in single cell data].

Data

There were two datasets for which we analyzed the performance of the different dimensionality reduction methods on. The first dataset we analyzed is the Samusik01 dataset which was one of the datasets analyzed in the paper “Dimensionality reduction for visualizing single-cell data using UMAP” by Becht et al which we are replicating for this report . The Samusik01 dataset is the first bone marrow sample analyzed by Samusik et al in the paper “Automated mapping of phenotype space with single-cell data”. It is a mass cytometry (CyTOF)  dataset taken from C57BL/6 mice consisting of over 86,000 events, 38 parameters, and 24 different cell populations. The Samusik dataset also featured cell annotations from the authors allowing for more meaningful interpretations of the generated embeddings. The dataset is available in a zipped format for download directly from the authors using the link: <https://web.stanford.edu/~samusik/Panorama%20BM%201-10.zip>. The dataset can also be downloaded directly from our public Github in the folder: <https://github.com/robbywaxman/GDAFinalProject/tree/main/SamusikData>.

The second dataset we analyzed is the Levine dataset from the paper “Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis” by Levine et al. The Levine dataset is a mass cytometry (CyTOF) dataset consisting of protein expression levels from healthy human bone marrow mononuclear cells (BMCs) from two healthy individuals. The whole dataset contains over 250,000 events, 32 parameters, and 14 different cell populations along with cell annotations from the authors, but for our analysis we filtered out the unassigned cells leaving just  over 100,000 cells. This dataset is available for download from the Github link: <https://github.com/lmweber/benchmark-data-Levine-32-dim/tree/master/data>. The dataset can also be downloaded directly from our public Github in the folder: <https://github.com/robbywaxman/GDAFinalProject/tree/main/LevineData>.

Methods

Before embeddings were generated, data was first pre-processed by selecting only the dimensions relevant for study and applying a hyperbolic arcsine transformation as is standard for mass cytometry data [cite?]. After preprocessing, dimensionality reduction was performed using PCA, tSNE, UMAP, and three different AE models. PCA and tSNE were performed using the implementations available in scikit-learn [cite], and UMAP was performed using the umap-learn implementation [cite]. The three AE models included a two layer feedforward model (FF2), a six-layer feedforward model (FF6), and a variational autoencoder (VAE). AE models were built with Keras and were trained on either samusik01 or levine data until convergence. FF2 and FF6 were trained to minimize the reconstruction error (mean squared error) of the data, whereas VAE minimized both reconstruction error and the KL divergence of the encoder and decoder probability distributions [citation?].

The embeddings generated by each model were evaluated on the separation of data classes, the average normalized mutual information of k-means clustering performed on the embeddings of data subsamples and k-means clustering performed on total datasets, the preservation of correlation of coordinates in subsamples versus in the embedding of the full dataset, the preservation of pairwise distances, and the runtime to generate the embeddings. Separation of data classes was measured using the same method as [OG paper]. A random forest classifier was trained to predict cell type from each type of embedding, and accuracy on held out data in a 5-fold cross validation setting was recorded. To validate the robustness of the separation of data classes, we also trained and tested a support vector machine (SVM) classifier and a K-nearest neighbors (KNN) classifier in the same way. To further measure the preservation of local structure, k-means clustering (with k = 10) was performed on the embeddings of five data subsamples of size 20,000 as well as on the embedding of the full dataset and the average normalized mutual information was calculated. This is a slight deviation from the method used in Becht et al as the parameters had to be adjusted to match the smaller dataset sizes in this analysis. The reproducibility of embeddings on a large scale was measured using the same method as [OG paper]. The correlation of coordinates on random subsamples of varying sizes versus embeddings of the full datasets were measured while accounting for symmetries across the axes. Preservation of pairwise distances was measured by correlating pairwise distances of random samples taken from original data and pairwise distances of those same samples in the embeddings. Finally, time to generate embeddings for various sample sizes, ranging from 100 samples to 50,000 samples, was measured for each algorithm - [Robby add your metric for when an AE was “done”].

Results

Figure 1. Latent space visualizations of Samusik01 and Levine datasets. Top six images are embeddings of the samusik01 dataset, and bottom six images are embeddings of the levine dataset. Embeddings are organized as such: A - PCA, B - tSNE, C - UMAP, D - FF2, E - FF6, F - VAE.

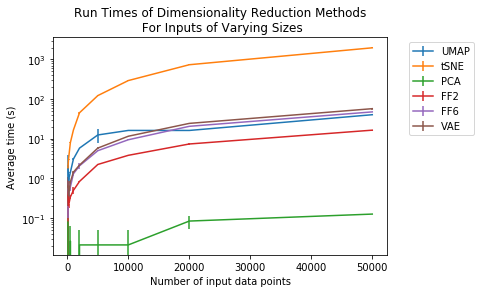
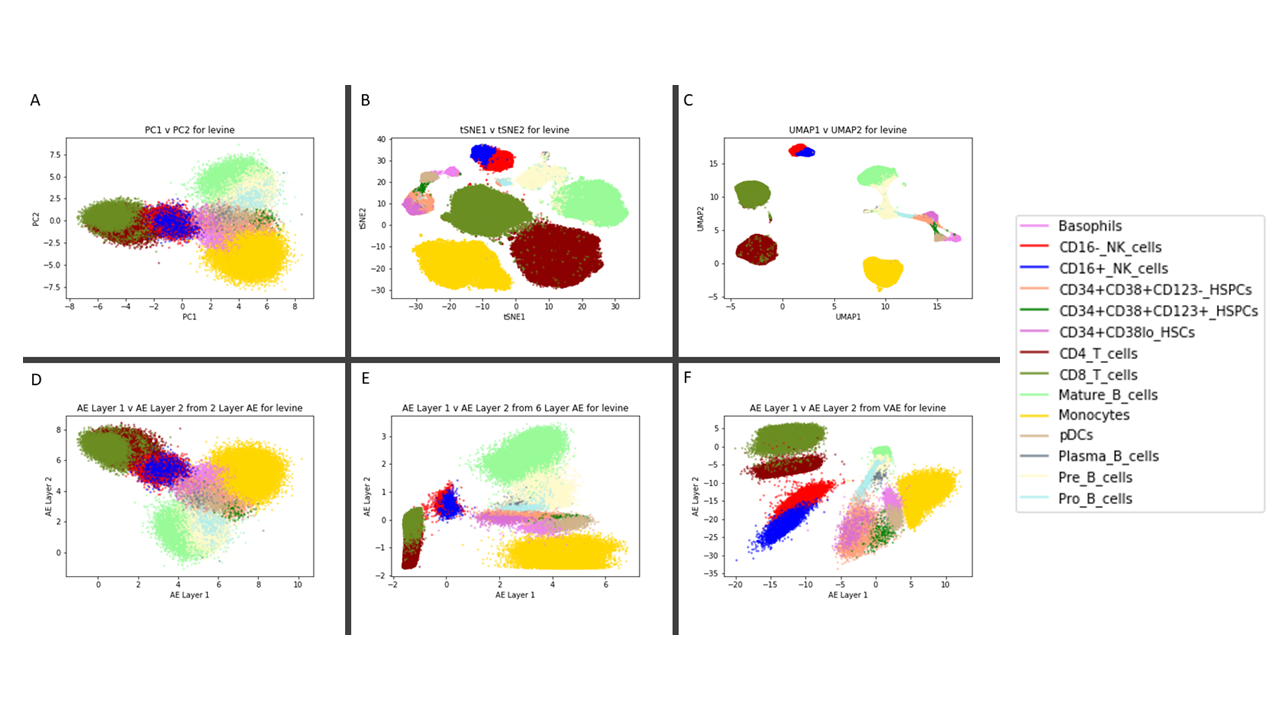
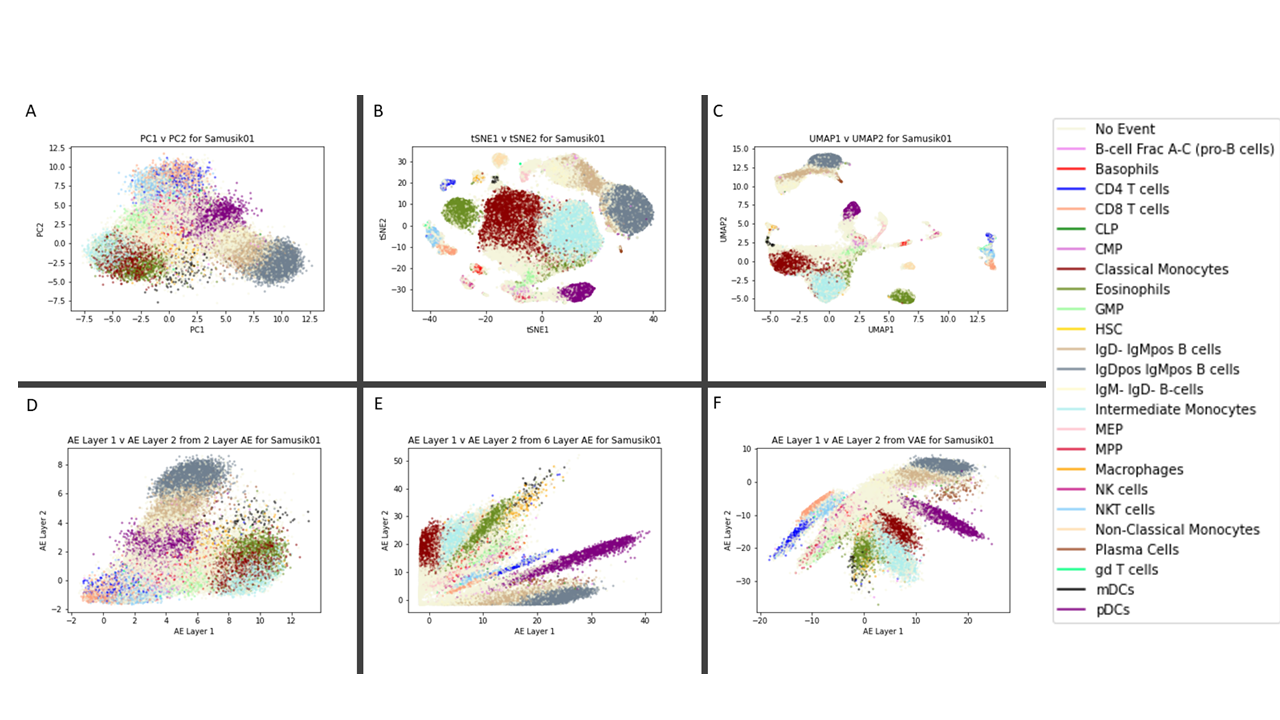


Figure 2. Runtimes of Dimensionality Reduction Algorithms.

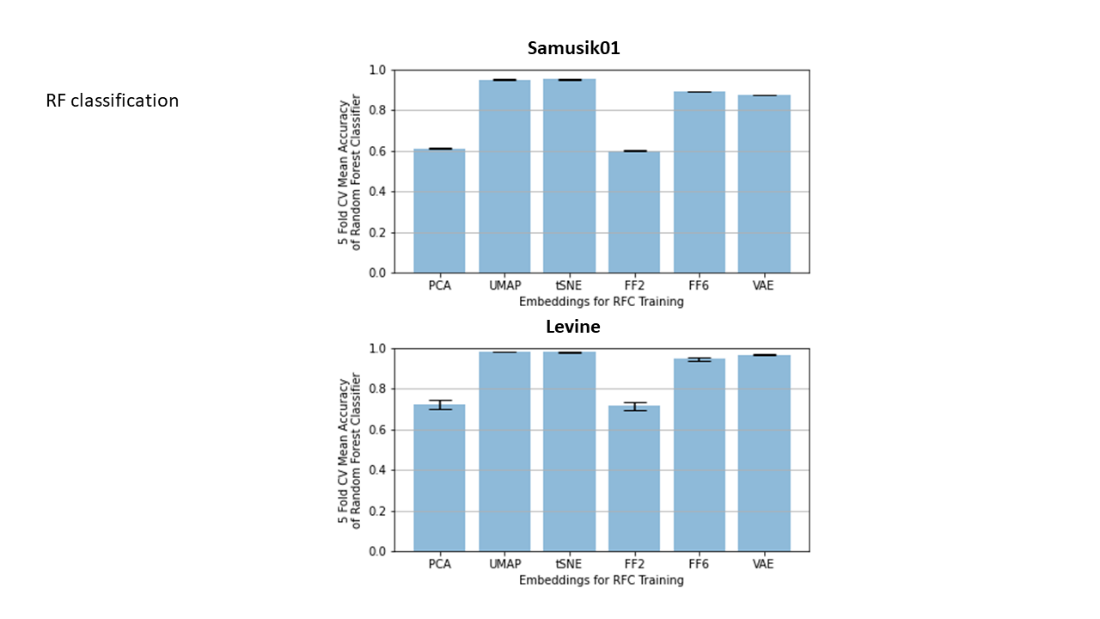


Figure 3. Random Forest Classifier Accuracy on Embeddings.

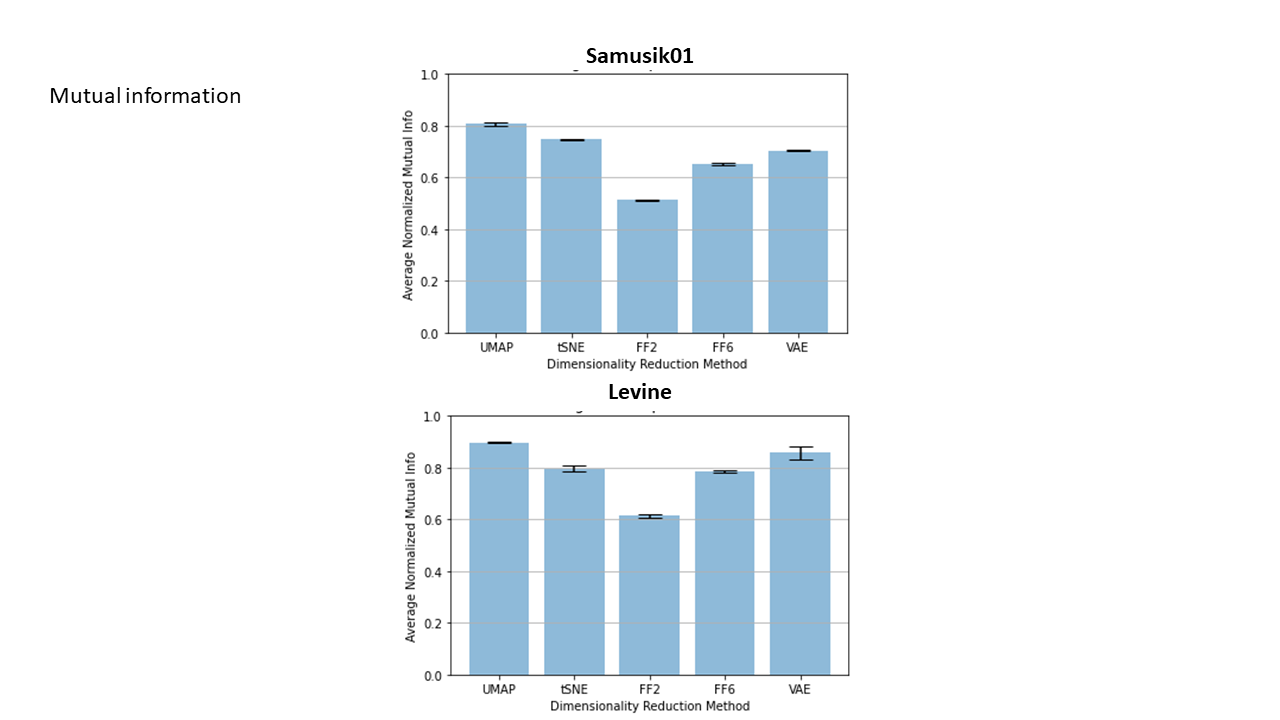


Figure 4. Normalized Mutual Information for each embedding.

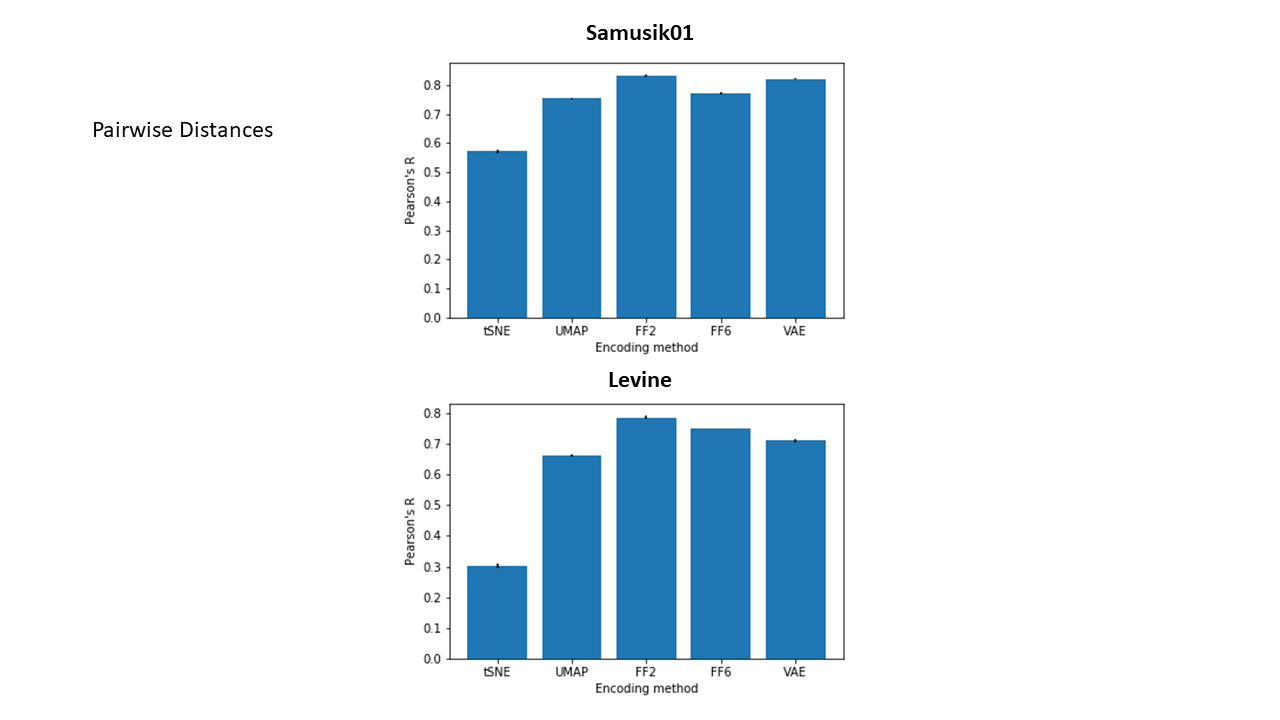


Figure 5. Correlation of pair-wise distances in original dataset and embeddings.