Factorized linear discriminant analysis and its application in computational biology

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Abstract

A fundamental problem in computational biology is to find a suitable representation of the high-dimensional gene expression data that is consistent with the structural and functional properties of cell types, collectively called their phenotypes. This representation is often sought from a linear transformation of the original data, for the reasons of model interpretability and computational simplicity. Here we propose a novel method of linear dimensionality reduction to address this problem. This method, which we call factorized linear discriminant analysis (FLDA), seeks a linear transformation of gene expressions that varies highly with only one phenotypic feature and minimally with others. We further leverage our approach with a sparsity-based regularization algorithm, which selects a few genes important to a specific phenotypic feature or feature combination. We illustrated this approach by applying it to a single-cell transcriptome dataset of Drosophila T4/T5 neurons. A representation from FLDA captured structures in the data aligned with phenotypic features and revealed critical genes for each phenotype.

1 Introduction

Consider the following: in a group of single cells, the gene expression data for each cell is a high-dimensional vector. Therefore, every single cell can be represented as a point in the high-dimensional space of gene expression. These data points in the gene expression space form clusters, and each of these clusters can be identified and associated with a cell type, as a result of verification based on prior knowledge of cell types’ molecular markers [1–5]. Suppose that for each cell type, we know its phenotypic traits, from either previous studies or direct measurement [6–9], then we can label each cell type by its phenotypic characteristics. For example, neuronal cell types can be described by a set of phenotypic features such as dendritic and axonal laminations, electrophysiological properties, and connectivity [6,7,10]. These phenotypic features are often categorical.

The question is how to factorize the high-dimensional gene expression data into modules that are consistent with these phenotypes? Specifically, given phenotypic features of cell types, such as dendritic stratification and axonal termination, can we find an interpretable low-dimensional embedding of gene expression in which each axis represents one factor, aligned with either an individual phenotypic feature or an interaction of the features?

The above requires that variation along one of the axes in the embedding space causes the variation of only one phenotypic feature. In reality, this is hard to satisfy due to noise in the data, and we relax the constraint by letting data projected along one axis vary largely with only one phenotypic feature while

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minimally with others. In addition, we want to recover cell types in the low-dimensional embedding. We therefore ask that cells classified as the same type are still close to each other in the embedding space, while cells of different types are far apart.

Based on the assumptions above, we derive factorized linear discriminant analysis (FLDA), a supervised dimensionality reduction method. Inspired by multi-way analysis of variance (ANOVA) [11], this method factorizes data into components corresponding to phenotypic features and their interactions, and seeks a linear transformation that varies highly with one component but not with the others. The linear nature of this approach makes it simple to compute and easy to interpret. To extract a small set of genes important to each phenotype, we further introduce a sparse variant of the method by constraining the number of non-zero elements contributing to each linear projection. We illustrate this approach by applying FLDA to a single-cell RNA-Seq dataset of T4/T5 neurons in Drosophila [12], focusing on two phenotypes: dendritic location and axonal lamination.

2 Factorized linear discriminant analysis (FLDA)

As a start, let us consider a scenario where each cell type can be jointly defined by two phenotypic features, both of which are categorical. In other words, the sample space of cell types can be defined as a Cartesian product of the sample spaces of the two phenotypic features $I$ and $J$:

$$I \times J = \{(i, j) | i \in I, j \in J\}$$  \hspace{1cm} (1)

where $i$, $j$ indicate different categories of the two phenotypic features. Assume that in the data, we observed $n_{ij}$ cells in each cell type $(i, j)$, then the representation can also be described using a contingency table (Figure 1A,B). Note here that we allow the table to be partially filled.

We use $x_{ijk} (k \in 1, 2, ..., n_{ij})$ to denote the expression values of $g$ genes measured in the $k$th cell of the cell type $(i, j)$ ($x_{ijk} \in \mathbb{R}^g$). How to find linear projections $y_{ijk} = u^T x_{ijk} (u \in \mathbb{R}^g)$ and $z_{ijk} = v^T x_{ijk} (v \in \mathbb{R}^g)$ that are aligned with features $i$ and $j$ respectively (Figure 1C)? We first asked whether we could factorize, for example, $y_{ijk}$, with respect to components depending on features $i$ and $j$. Indeed, motivated by the linear factor models used in multi-way ANOVA and the idea of partitioning variance, we constructed an objective function as the following, and found $u^*$ that maximizes the objective (see detailed analysis in Appendix A):

$$u^* = \arg \max_{u \in \mathbb{R}^g} \frac{u^T N_A u}{u^T M^r u}$$  \hspace{1cm} (2)
When we have a complete table, and there are \( a \) categories for the feature \( i \) and \( b \) categories for the feature \( j \), we have

\[
N_A = M_A - \lambda_1 M_B - \lambda_2 M_{AB}
\]

(3)

where \( M_A \), \( M_B \), and \( M_{AB} \) are the covariance matrices explained by the feature \( i \), the feature \( j \), and the interaction of them. \( \lambda_1 \) and \( \lambda_2 \) are hyper-parameters controlling the relative weights of \( M_B \) and \( M_{AB} \) with respect to \( M_A \). \( M_e \) is the residual covariance matrix representing noise in gene expressions. Formal definitions of these terms are the following:

\[
M_A = \frac{1}{a-1} \sum_{i=1}^{a} \sum_{j=1}^{b} (m_{i.} - m_{..})(m_{i.} - m_{..})^T
\]

(4)

\[
M_B = \frac{1}{b-1} \sum_{i=1}^{a} \sum_{j=1}^{b} (m_{.j} - m_{..})(m_{.j} - m_{..})^T
\]

(5)

\[
M_{AB} = \frac{1}{(a-1)(b-1)} \sum_{i=1}^{a} \sum_{j=1}^{b} (m_{ij} - m_{i.} - m_{.j} + m_{..})^T
\]

(6)

\[
M_e = \frac{1}{N - ab} \sum_{i=1}^{a} \sum_{j=1}^{b} \left[ \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} (x_{ijk} - m_{ij})(x_{ijk} - m_{ij})^T \right]
\]

(7)

where

\[
m_{..} = \frac{1}{ab} \sum_{i=1}^{a} \sum_{j=1}^{b} m_{ij}
\]

(8)

\[
m_{i.} = \frac{1}{b} \sum_{j=1}^{b} m_{ij}
\]

(9)

\[
m_{.j} = \frac{1}{a} \sum_{i=1}^{a} m_{ij}
\]

(10)

in which

\[
m_{ij} = \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} x_{ijk}
\]

(11)

An analogous expression provides the linear projection \( v^* \) for the feature \( j \), and \( w^* \) for the interaction of both features \( i \) and \( j \). Similar arguments can be applied to the scenario of a partial table to find \( u^* \) or \( v^* \) as the linear projection for the feature \( i \) or \( j \) (see Appendix B for mathematical details).

Note that \( N_A \) is symmetric and \( M_e \) is positive definite. Therefore the optimization problem is a generalized eigenvalue problem [13]. When \( M_e \) is invertible, \( u^* \) is the eigenvector associated with the largest eigenvalue of \( M_e^{-1} N_A \). In general, if we want to embed \( x_{ijk} \) into a \( d \)-dimensional subspace aligned with the feature \( i \) \( (d < a) \), we can take the eigenvectors with the \( d \) largest eigenvalues of \( M_e^{-1} N_A \), which we call the top \( d \) factorized linear discriminant components (FLDs).

In the high dimensional setting where the number of genes is much larger than the number of cells, \( M_e \) is singular and non-invertible. Similar singular problems also showed up in other linear
dimensionality reduction methods, and solutions have been proposed. We considered the solution proposed in [14–16] by using a diagonal estimate of \( M_e \). This solution is also widely used in studies of computational biology [17–19].

Since multi-way ANOVA can handle contingency tables with more than two dimensions, our analysis can be easily generalized to more than two phenotypic features. In general, FLDA is suitable for data whose labels form a Cartesian product of multiple features.

3 Sparsity regularization of FLDA

For the application in computational biology, there is particular interest in finding a small group of genes that best determine one of the phenotypic features. This leads to finding axes that have only a few non-zero elements. To identify such a sparse solution, we solved the following optimization problem:

$$
\mathbf{u}^* = \arg \max_{\mathbf{u} \in \mathbb{R}^g} \frac{\mathbf{u}^T \mathbf{N_A} \mathbf{u}}{\mathbf{u}^T \mathbf{M_e} \mathbf{u}} \quad \text{subject to} \quad ||\mathbf{u}||_0 \leq l
$$

from which the number of non-zero elements of \( \mathbf{u}^* \) is less or equal to \( l \).

This is known as a sparse generalized eigenvalue problem, which has three challenges [20]: first, when the data are very high-dimensional, \( M_e \) can be singular and non-invertible; second, because of the normalization term \( \mathbf{u}^T \mathbf{M_e} \mathbf{u} \), many solutions for sparse eigenvalue problems cannot be directly applied; finally, this problem involves maximizing a convex objective over a nonconvex set, which is NP-hard.

To solve it, we used truncated Rayleigh flow (Rifle), a method specifically developed to solve sparse generalized eigenvalue problems. The algorithm of Rifle is composed of two steps [20]: first, to obtain an initial vector \( \mathbf{u}_0 \) that is close to \( \mathbf{u}^* \). We used the solution from the non-sparse FLDA as an initial estimate of \( \mathbf{u}_0 \); second, iteratively, to perform a gradient ascent step on the objective function, and then execute a truncation step that preserves the \( l \) entries of \( \mathbf{u} \) with the largest values and sets the remaining entries to 0. Pseudo-code for this algorithm is presented here:

```plaintext
procedure RIFLE(\( \mathbf{N_A}, \mathbf{M_e}, \mathbf{u}_0, l, \eta \))
    \( t = 1 \)  \( \triangleright \eta \) is the step size
    while not converge do
        \( \rho_{t-1} \leftarrow \frac{\mathbf{u}_{t-1}^T \mathbf{N_A} \mathbf{u}_{t-1}}{\mathbf{u}_{t-1}^T \mathbf{M_e} \mathbf{u}_{t-1}} \)
        \( C \leftarrow I + (\frac{\eta}{\rho_{t-1}})(\mathbf{N_A} - \rho_{t-1} \mathbf{M_e}) \)
        \( \mathbf{u}_t \leftarrow \frac{C\mathbf{u}_{t-1}}{||C\mathbf{u}_{t-1}||_2} \)
        Traverse \( \mathbf{u}_t \) by keeping the top \( l \) entries of \( \mathbf{u} \) with the largest values and setting the rest entries to 0
        \( \mathbf{u}_t \leftarrow \frac{\mathbf{u}_t}{||\mathbf{u}_t||_2} \)
        \( t \leftarrow t + 1 \)
    end while
    return \( \mathbf{u}_t \)
end procedure
```

As proved in [20], if there is a unique sparse leading generalized eigenvector, Rifle will converge linearly to it with the optimal statistical rate of convergence. The computational complexity of the second step is \( O(lg + g) \) for each iteration, therefore Rifle scales linearly with \( g \), the dimensionality of the original data. Based on the theoretical proof, to guarantee convergence, the hyperparameter \( \eta \) was selected to be sufficiently small such that \( \eta \lambda_{max}(\mathbf{M_e}) < 1 \), where \( \lambda_{max}(\mathbf{M_e}) \) is the largest eigenvalue of \( \mathbf{M_e} \). In our case, the other hyperparameter \( l \), indicating how many genes to be preserved, was empirically selected based on the design of a follow-up experiment. As mentioned later in Results, we chose \( l \) to be 20, a reasonable number of candidate genes to be tested in a biological study.
4 Related work: linear dimensionality reduction

FLDA is an approach for linear dimensionality reduction \[21\]. Formally, linear dimensionality reduction is defined as the following: given \(n\) data points each of \(g\) dimensions, \(X = [x_1, x_2, \ldots, x_n] \in \mathbb{R}^{g \times n}\), and a choice of reduced dimensionality \(r < g\), optimize an objective function \(f(.)\) to produce a linear projection \(U \in \mathbb{R}^{r \times g}\), and \(Y = UX \in \mathbb{R}^{r \times n}\) is the low-dimensional transformed data.

State-of-the-art methods for linear dimensionality reduction include principal component analysis (PCA), factor analysis (FA), linear multidimensional scaling (MDS), linear discriminant analysis (LDA), canonical correlations analysis (CCA), maximum autocorrelation factors (MAF), slow feature analysis (SFA), sufficient dimensionality reduction (SDR), locality preserving projections (LPP), and independent component analysis (ICA) \[21\]. These approaches can be roughly grouped for either unsupervised or supervised linear dimensionality reduction, and we discuss them separately.

4.1 Unsupervised methods for linear dimensionality reduction

Unsupervised linear dimensionality reduction, including PCA \[22\], ICA \[23\], FA \[24\] and more, project data into a low-dimensional space without using supervision labels. The problem of these unsupervised approaches is that axes of the low-dimensional space often do not represent the underlying ‘construct’ of the data and are therefore uninterpretable. This problem is even more prominent for gene expression data because the dimensionality is usually very high, with tens of thousands of genes, and expressions of a fair number of them can be very noisy. These noisy expressions cause large variance among individual cells, but in an unstructured way. Without supervision signals from the phenotypic features, unsupervised methods tend to select these genes to construct the low-dimensional space, which offers neither the desired alignment nor a good separation of cell type clusters. To illustrate this, we performed PCA on the gene expression data and compared it with FLDA. Briefly, we solved the following objective to find the linear projection:

\[
\begin{align*}
\mathbf{u}^* &= \arg \max_{\mathbf{u} \in \mathbb{R}^g} \frac{\mathbf{u}^T \mathbf{X} \mathbf{X}^T \mathbf{u}}{\mathbf{u}^T \mathbf{u}} \\
&= \mathbf{u}^* = \arg \max_{\mathbf{u} \in \mathbb{R}^g} \frac{\mathbf{u}^T \mathbf{X} \mathbf{X}^T \mathbf{u}}{\mathbf{u}^T \mathbf{u}}
\end{align*}
\]

The outcome of this comparison is shown in Results.

4.2 Supervised methods for linear dimensionality reduction

Supervised linear dimensionality reduction, represented by LDA \[25, 26\] and CCA \[27, 28\], can overcome the above issue. By including supervised signals of phenotypic features, we can devalue genes whose expressions are non-informative about the phenotypes.

4.2.1 Linear discriminant analysis (LDA)

LDA models the difference among data organized in pre-determined classes. Formally, LDA solves the following optimization problem:

\[
\begin{align*}
\mathbf{u}^* &= \arg \max_{\mathbf{u} \in \mathbb{R}^g} \frac{\mathbf{u}^T \mathbf{\Sigma}_b \mathbf{u}}{\mathbf{u}^T \mathbf{\Sigma}_e \mathbf{u}} \\
&= \mathbf{u}^* = \arg \max_{\mathbf{u} \in \mathbb{R}^g} \frac{\mathbf{u}^T \mathbf{\Sigma}_b \mathbf{u}}{\mathbf{u}^T \mathbf{\Sigma}_e \mathbf{u}}
\end{align*}
\]

where \(\mathbf{\Sigma}_b\) and \(\mathbf{\Sigma}_e\) are estimates of the between-class and within-class covariance matrices respectively. Different from FLDA, the representation of these classes is not explicitly formulated as a contingency table composed of multiple features. The consequence is that, when applied to the example problem in which cell types are organized into a two-dimensional contingency table with phenotypic features \(i\) and \(j\), in general, axes from LDA are not aligned with these two phenotypic features.

However, in the example above, we can perform two separate LDAs for the two features. This allows the axes from each LDA to align with its specific feature. We call this approach “2LDAs”. There are two limitations of this approach: first, it discards information about the component depending on the interaction of the two features which cannot be explained by a linear combination of them; second, it explicitly maximizes the segregation of cells with different feature levels which sometimes is not
consistent with a good separation of cell type clusters. Detailed comparisons between LDA, “2LDAs” and FLDA can be found in Results.

4.2.2 Canonical correlation analysis (CCA)

CCA projects two datasets $X_a \in \mathbb{R}^{g \times n}$ and $X_b \in \mathbb{R}^{d \times n}$ to $Y_a \in \mathbb{R}^{r \times n}$ and $Y_b \in \mathbb{R}^{r \times n}$, such that the correlation between $Y_a$ and $Y_b$ is maximized. Formally, it tries to maximize this objective:

$$
(u^*, v^*) = \arg \max_{u \in \mathbb{R}^g, v \in \mathbb{R}^d} \frac{u^T (X_a X_a^T)^{-1/2} X_a X_b^T (X_b X_b^T)^{-1/2} v}{(u^T u)^{-1/2} (v^T v)^{-1/2}}
$$

(15)

To apply CCA to our problem, we need to set $X_a$ to be the gene expression matrix, and $X_b$ to be the matrix of $d$ phenotypic features ($d = 2$ for two features as illustrated later). In contrast with FLDA, CCA finds a transformation of gene expressions aligned with a linear combination of phenotypic features, instead of a factorization of gene expressions corresponding to individual phenotypic features. This difference is quantified and shown in Results.

5 Experimental design

5.1 Datasets

In order to quantitatively compare FLDA with other linear dimensionality reduction methods, such as PCA, CCA, LDA, and the “2LDAs” approach, we created synthetic datasets. Four types of cells, each containing 25 examples, were generated from a Cartesian product of two features $i$ and $j$, organized in a 2x2 complete contingency table. Expressions of 10 genes were generated for these cells, in which the levels of Genes 1-8 were correlated with either the feature $i$, the feature $j$, or the interactions of them, and the levels of the remaining 2 genes were purely driven by noise (Figure 2A). Details of generating the data can be found in Appendix C.

To illustrate FLDA in analyzing single-cell transcriptome datasets for real biological problems, and demonstrate the merit of our approach in selecting a few important genes for each phenotype, we used a dataset of Drosophila T4/T5 neurons [12]. T4 and T5 neurons are very similar in terms of general morphology and physiological properties, but they differ by the location of their dendrites in the medulla and lobula, two distinct brain regions. T4 and T5 neurons each contain four subtypes, with each pair of the four laminating their axons in a specific layer in the lobula plate (Figure 3A). Therefore, we can use two phenotypic features to describe these neurons: the feature $i$ indicates the dendritic location at the medulla or lobula; the feature $j$ describes the axonal lamination at one of the four layers (a/b/c/d) (Figure 3B). In this experiment, we focused on the dataset containing expression data of 17492 genes from 3833 cells collected at a defined time during brain development.
Figure 3: FLDA on the dataset of T4/T5 neurons. (A) T4/T5 neuronal cell types and their dendritic and axonal phenotypes. (B) T4/T5 neurons can be organized in a complete contingency table. Here \( i \) indicates the dendritic location and \( j \) indicates the axonal termination. (C) SNR metric of each discriminant axis. (D) Projection of the data into the three-dimensional space consisting of the discriminant axis for the feature \( i \) (FLD\(_i\)) and the first and second discriminant axes for the feature \( j \) (FLD\(_j_1\) and FLD\(_j_2\)). (E-G) Projection of the data into the two-dimensional space made of FLD\(_i\) and FLD\(_j_1\) (E), FLD\(_j_1\) and FLD\(_j_2\) (F), or FLD\(_j_2\) and FLD\(_j_3\) (the third discriminant axis for the feature \( j \)) (G). Different cell types are indicated by different colors as in (A) and (D).

5.2 Data preprocessing

The T4/T5 neuron dataset was preprocessed as previously reported \[3, 5, 29, 12\]. Briefly, transcript counts within each column of the count matrix (genes×cells) were normalized to sum to the median number of transcripts per cell, resulting in the normalized counts Transcripts-per-median or TPM\(_{gc}\) for Gene \( g \) in Cell \( c \). We used the log-transformed expression data \( E_{gc} = \ln (TPM_{gc} + 1) \) for further analysis. We adopted a common approach in single-cell RNA-Seq studies that is based on fitting a relationship between mean and coefficient of variation \[30, 31, 12\] to select highly variable genes, and performed FLDA on the expression data with only these genes. In the experiment below, we set the hyper-parameters \( \lambda s \) in Equation (3) to 1.

5.3 Metrics

We included the following metrics to evaluate our method:

- A signal-to-noise ratio (SNR) measures how well each discriminant axis separates cell types compared with noise estimated from the variance within cell type clusters.
- The explained variance (EV) for each discriminant axis measures how much variance of the feature \( i \) or \( j \) is explained among the total variance explained by that axis.
- The mutual information (MI) between each discriminant axis and each feature quantifies how “informative” an axis is to a specific feature.
- Built on the calculation of MI, we included the modularity score which measures whether each discriminant axis depends on at most one feature \[32\].
The implementation details of these metrics can be found in Appendix D.

6 Results

6.1 Comparison of FLDA with other linear dimensionality reduction methods

To quantitatively compare the difference between FLDA and other alternative methods including PCA, CCA, LDA, and “2LDAs”, we measured the proposed metrics from analyses of the synthesized datasets (Figure 2A). Given that the synthesized data were organized in a 2x2 contingency table, each LDA of the “2LDAs” approach could find only one dimension for the specific feature i or j. Therefore, as a fair comparison, we only included the corresponding dimensions in FLDA (FLDi and FLDj) and the top two components of PCA, CCA, and LDA. The overall SNR values normalized by that of LDA and the overall modularity scores were plotted for data generated with different noise levels (Figure 2B,C). The performance of PCA is the worst among all these models because the unsupervised approach cannot prevent the noise from contaminating the signal. The supervised approaches in general have good SNRs, but LDA and CCA suffer from low modularity scores. This is expected because LDA maximizes the separation of cell type clusters but overlooks the alignment of the axes to the feature i or j, and CCA maximizes the correlation to a linear combination of phenotypic features instead of individual ones. By contrast, “2LDAs” achieves the highest modularity scores but has the worst SNR among the supervised approaches, because it tries to maximize the separation of cells with different feature levels, which is not necessarily consistent with maximizing the segregation of cell types. Both the SNR value and the modularity score of FLDA are close to the optimal, as it not only considers the alignment of axes to different features but also constrains the variance within cell types. A representative plot of the EV and MI metrics of these models is shown in Figure 5 reporting good alignment of axes to either the feature i or j in FLDA and ‘2LDAs”, but not in the others.

6.2 Application to a real problem in computational biology

A question of significance in biology is whether the diverse phenotypes of cell types are generated by combinations of modular transcriptional programs, and if so, what is the gene signature for each of the programs. To illustrate the ability of our approach in addressing this problem, we applied FLDA to the dataset of Drosophila T4/T5 neurons. The T4/T5 neurons could be organized in a 2x4 contingency table, therefore, FLDA was able to project the expression data into a subspace of seven dimensions, with one FLD aligned with dendritic location i (FLDi), three FLDs aligned with axonal termination j (FLDj1−3), and the remaining three representing the interaction of both phenotypes (FLDij1−3). We ranked these axes based on their SNR metrics and found that FLDj1, FLDi, and FLDj2 have much higher SNRs than the rest (Figure 3C). Indeed, data representations in the subspace consisting of these three dimensions show a clear separation of the eight neuronal cell types (Figure 3D). As expected, FLDi teases apart T4 from T5 neurons, whose dendrites are located at different brain regions (Figure 3E). Interestingly, FLDj1 separates T4/T5 neurons into two groups, a/b vs c/d, corresponding to the upper or lower lobula place, and FLDj2 divides them into another two, a/d vs b/c, indicating whether their axons laminate at the middle or lateral part of the lobula plate (Figure 3F). Among these three dimensions, FLDj1 has a much higher SNR than FLDi and FLDj2, suggesting a hierarchical structure in the genetic organization of T4/T5 neurons: they are first separated into either a/b or c/d types, and subsequently divided into each of the eight subtypes. In fact, this exactly matches the sequence of their cell fate determination, as revealed in a previous genetic study [33]. Finally, the last discriminant axis of the axonal feature FLDj3 separates the group a/c from b/d, suggesting its role in fine-tuning the axonal depth within the upper or lower lobula plate (Figure 3I).

To seek gene signatures for the discriminant components in FLDA, we applied the sparsity-based regularization to constrain the number of genes with non-zero weight coefficients. Here we set the number to 20, a reasonable number of candidate genes that might be tested in a follow-up biological study. We extracted a list of 20 genes each for the axis of FLDi or FLDj1. The relative importance of these genes to each axis is directly informed by their weight values (Figure 4A,C). Side-by-side, we plotted expression profiles of these genes in the eight neuronal cell types (Figure 4B,D). For both axes, the genes critical in separating cells with different feature levels are differentially expressed in corresponding cell types. We compared our gene lists with those obtained using conventional
Selected genes for the dendritic phenotype

Selected genes for the axonal phenotype

Figure 4: Critical genes extracted from the sparse algorithm. (A) Weight vector of the 20 genes selected for the dendritic phenotype (FLD\(_i\)). The weight value is indicated in the color bar with color indicating direction (red: positive and green: negative) and saturation indicating magnitude. (B) Expression patterns of the 20 genes from (A) in eight types of T4/T5 neurons. Dot size indicates the percentage of cells in which the gene was expressed, and color represents average scaled expression. (C) Weight vector of the 20 genes selected for the axonal phenotype (FLD\(_j\)). Legend as in (A). (D) Expression patterns of the 20 genes from (C) in eight types of T4/T5 neurons. Legend as in (B).

methods which were reported in [12]. Consistent with the report, we found indicator genes for dendritic location, such as \(TfAP-2\), \(dpr2\), \(dpr3\), \(twz\), \(CG34155\), and \(CG12065\), and those for axonal lamination such as \(klg\), \(bi\), \(pros\), \(mav\), \(beat-IIIb\), and \(Fas2\). In addition, we found genes that were not reported in this previous study. For example, our results suggest that the gene \(pHCl-1\) is important to the dendritic phenotype, and the gene \(Lac\) is critical to the axonal lamination. They are promising genetic targets to be tested in future experiments. Lastly, FLDA allowed us to examine the component that depends on the interaction of both features and identify its gene signature, which provides clues to transcriptional regulation of gene expressions in the T4/T5 neuronal cell types (Figures 6 and 7).

6.3 Perturbation analysis

As a supervised approach, FLDA depends on correct phenotype labels to extract meaningful information. But if the phenotypes are annotated incorrectly, can we use FLDA to raise a flag? We propose a perturbation analysis of FLDA to address this question, built on the assumption that among possible phenotype annotations, the projection of gene expression data based on correct labels leads to better metric measurements than incorrect ones. As detailed in Appendix E, we generated three kinds of incorrect labels for the dataset of T4/T5 neurons, corresponding to three common scenarios of mislabeling: the phenotypes of a cell type were mislabeled with those of another type; a singular phenotypic category was incorrectly split into two; two phenotypic categories are incorrectly merged into one. FLDA was applied to gene expressions of T4/T5 neurons but with these perturbed annotations. Proposed metrics such as the SNR value and modularity score were plotted in Figure 8. Indeed, the projection of gene expressions with correct annotation leads to the best SNR value and modularity score compared with incorrect annotations. This implies that this type of perturbation analysis is a useful practice in general: it raises the confidence that the original annotation is correct if FLDA on the perturbed annotations produces lower metric scores.
7 Discussion

We developed FLDA, a novel dimensionality reduction method in which the high-dimensional data, such as gene expressions, are linearly projected into a low-dimensional space, axes of which are aligned with features like phenotypes. We further leveraged FLDA with sparse regularization, to allow the selection of a small set of critical genes. We illustrate its application in computational biology by analyzing gene expression data of Drosophila T4/T5 neurons that are labeled by two phenotypic features. FLDA not only captured the structures in the data consistent with the phenotypic labels, but also revealed new genes for each phenotype that were not apparent in the previous report.

The approach is motivated by multi-way ANOVA, and thus it generalizes easily to more than two features. More generally, FLDA can be applied to any labeled data set for which the labels form a Cartesian product of multiple features. For example, this would include face images that can be jointly labeled by the age, gender, and other features of a person [34, 35].

Finally, FLDA provides a modular representation of the data aligned with the factors such as phenotypes. [32] argued that modularity together with explicitness could define disentangled representations. The linear nature of FLDA makes it an explicit model, therefore FLDA can potentially serve as a supervised approach to disentanglement [36–38].

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9 Appendix

9.1 A. Objective functions

Here we derive the objective functions used in our analysis. Again if \( x_{ijk} (k \in 1, 2, \ldots, n_{ij}) \) represents the expression values of \( g \) genes in each cell (\( x_{ijk} \in \mathbb{R}^g \)), we seek to find a linear projection \( y_{ijk} = u^T x_{ijk} \) that is aligned with the feature \( i \).

9.1.1 Inspiration from ANOVA

We asked what is the best way to factorize \( y_{ijk} \). Inspired by multi-way ANOVA [11], we identified three components: one depending on the feature \( i \), another depending on the feature \( j \), and the last one depending on the interaction of both features. We therefore followed the procedures of ANOVA to partition sums of squares and factorize \( y_{ijk} \) into these three components.

Let us first assume that all cell types defined by \( i \) and \( j \) contain the same number of cells. With cell types represented by a complete contingency table (Figure 1A), \( y_{ijk} \) can be linearly factorized using the model of two crossed factors. Formally, the linear factorization is the following:

\[
y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \beta)_{ij} + \epsilon_{ijk}
\]  

where \( y_{ijk} \) represents the coordinate of the \( k \)th cell in the category defined by \( i \) and \( j \); \( \mu \) is the average level of \( y \); \( \alpha_i \) is the component that depends on the feature \( i \), and \( \beta_j \) is the component that depends on the feature \( j \); \( (\alpha \beta)_{ij} \) describes the component that depends on the interaction of both features \( i \) and \( j \); \( \epsilon_{ijk} \sim \mathcal{N}(0, \sigma^2) \) is the residual of this factorization.

Let us say that the features \( i \) and \( j \) fall into \( a \) and \( b \) discrete categories respectively. Then without loss of generality, we can require:

\[
\sum_{i=1}^{a} \alpha_i = 0
\]  

(17)
\[ \sum_{j=1}^{b} \beta_j = 0 \quad (18) \]

\[ \sum_{i=1}^{a} (\alpha \beta)_{ij} = \sum_{j=1}^{b} (\alpha \beta)_{ij} = 0 \quad (19) \]

Corresponding to these, there are three null hypotheses:

\[ H_{01} : \alpha_i = 0 \quad (20) \]

\[ H_{02} : \beta_j = 0 \quad (21) \]

\[ H_{03} : (\alpha \beta)_{ij} = 0 \quad (22) \]

Here we want to reject \( H_{01} \) while accepting \( H_{02} \) and \( H_{03} \) in order that \( y_{ijk} \) is aligned with the feature \( i \).

Next, we partition the total sum of squares. If the number of cells within each cell type category is \( n \), and the total number of cells is \( N \), then we have

\[
\sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} (y_{ijk} - \bar{y}_{i..})^2 = bn \sum_{i=1}^{a} (\bar{y}_{i..} - \bar{y}_{...})^2 + an \sum_{j=1}^{b} (\bar{y}_{.j.} - \bar{y}_{...})^2 \\
+ n \sum_{i=1}^{a} \sum_{j=1}^{b} (\bar{y}_{ij} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 + \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} (y_{ijk} - \bar{y}_{ij..})^2 \quad (23)
\]

where \( \bar{y} \) is the average of \( y_{ijk} \) over the indices indicated by the dots. Equation (23) can be written as

\[ SS_T = SS_A + SS_B + SS_{AB} + SS_e \quad (24) \]

with each term having degrees of freedom \( N - 1, a - 1, b - 1, (a - 1)(b - 1), \) and \( N - ab \) respectively. Here \( SS_A, SS_B, SS_{AB}, \) and \( SS_e \) are partitioned sum of squares for the factors \( \alpha_i, \beta_j, (\alpha \beta)_{ij}, \) and the residual.

ANOVA rejects or accepts a null hypothesis by comparing its mean square (the partitioned sum of squares normalized by the degree of freedom) to that of the residual. This is done by constructing F-statistics for each factor as shown below:

\[ F_A = \frac{MS_A}{MS_e} = \frac{SS_A}{n-1} \frac{SS_e}{N-ab} \quad (25) \]

\[ F_B = \frac{MS_B}{MS_e} = \frac{SS_B}{b-1} \frac{SS_e}{N-ab} \quad (26) \]

\[ F_{AB} = \frac{MS_{AB}}{MS_e} = \frac{SS_{AB}}{(a-1)(b-1)} \frac{SS_e}{N-ab} \quad (27) \]

Under the null hypotheses, the F-statistics follow the F-distribution. Therefore, a null hypothesis is rejected when we observe the value of a F-statistic above a certain threshold calculated from the F-distribution. Here we want \( F_A \) to be large enough so that we can reject \( H_{01} \), but \( F_B \) and \( F_{AB} \)
to be small enough for us to accept $H_{02}$ and $H_{03}$. In other words, we want to maximize $F_A$ while minimizing $F_B$ and $F_{AB}$. Therefore, we propose maximizing an objective $L$:

$$L = F_A - \lambda_1 F_B - \lambda_2 F_{AB}$$

(28)

where $\lambda_1$ and $\lambda_2$ are hyper-parameters determining the relative weights of $F_B$ and $F_{AB}$ compared with $F_A$.

### 9.1.2 Objective functions under a complete contingency table

When the numbers of cells within categories defined by $i$ and $j$ ($n_{ij}$) are not all the same, the total sum of squares cannot be partitioned as in Equation (23). However, if we only care about distinctions between cell types instead of individual cells, we can use the mean value of each cell type cluster ($\bar{y}_{ij}$) to estimate the overall average value ($\bar{y}_{..}$), and the average value of each category $i$ ($\bar{y}_{i..}$) or $j$ ($\bar{y}_{.j}$). Therefore, Equation (23) can be modified as the following:

$$\sum_{i=1}^{a} \sum_{j=1}^{b} \left( \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} (y_{ijk} - \bar{y}_{ij})^2 \right) = \sum_{i=1}^{a} \sum_{j=1}^{b} (\bar{y}_{i..} - \bar{y}_{..})^2 + a \sum_{j=1}^{b} (\bar{y}_{.j} - \bar{y}_{..})^2$$

$$+ \sum_{i=1}^{a} \sum_{j=1}^{b} (\bar{y}_{ij} - \bar{y}_{i..} - \bar{y}_{.j} + \bar{y}_{..})^2 + \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n_{ij}} (y_{ijk} - \bar{y}_{ij})^2$$

(29)

where

$$\bar{y}_{ij} = \frac{\sum_{k=1}^{n_{ij}} y_{ijk}}{n_{ij}}$$

(30)

$$\bar{y}_{i..} = \frac{\sum_{j=1}^{b} \bar{y}_{ij}}{b}$$

(31)

$$\bar{y}_{.j} = \frac{\sum_{i=1}^{a} \bar{y}_{ij}}{a}$$

(32)

$$\bar{y}_{..} = \frac{\sum_{i=1}^{a} \sum_{j=1}^{b} \bar{y}_{ij}}{ab}$$

(33)

If we describe Equation (29) as:

$$\bar{SS}_T = \bar{SS}_A + \bar{SS}_B + \bar{SS}_{AB} + \bar{SS}_e$$

(34)

then following the same arguments, we want to maximize an objective function in the following format:

$$L = \frac{\bar{SS}_A}{a-1} - \lambda_1 \frac{\bar{SS}_B}{b-1} - \lambda_2 \frac{\bar{SS}_{AB}}{(a-1)(b-1)}$$

(35)

### 9.1.3 Objective functions under a partial contingency table

When we have a representation of a partial table, we can no longer separate out the component that depends on the interaction of both features. Therefore, we use another model, a linear model of two nested factors, to factorize $y_{ijk}$, which has the following format:

$$y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \epsilon_{ijk}$$

(36)
Note that we now have $\beta_{j(i)}$ instead of $\beta_j + (\alpha\beta)_{ij}$. In this model, we identify a primary factor, for instance, the feature denoted by $i$ which falls into $a$ categories, and the other (indexed by $j$) becomes a secondary factor, the number of whose levels $b_i$ depends on the level of the primary factor. We merge the component depending on the interaction of both features into that of the secondary factor as $\beta_{j(i)}$.

Similarly, we have

$$
\sum_{i=1}^{a} \sum_{j=1}^{b_i} \left( \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} (y_{ij(k)} - \bar{y}_{ij..})^2 \right) = \sum_{i=1}^{a} \sum_{j=1}^{b_i} \left( \bar{y}_{i..} - \bar{y}_{ij..} \right)^2
$$

(37)

which can be written as

$$
\tilde{SS}_T = \tilde{SS}_A + \tilde{SS}_B + \tilde{SS}_e
$$

(38)

with degrees of freedom $N - 1, a - 1, M - a$, and $N - M$ for each of the terms, where $M$ is:

$$
M = \sum_{i=1}^{a} b_i
$$

(39)

Therefore, we want to maximize the following objective:

$$
L = \frac{\tilde{SS}_A - \lambda \frac{\tilde{SS}_B}{M-a}}{\tilde{SS}_e \frac{N-M}{N-1}}
$$

(40)

9.2 B. FLDA with a partial contingency table

Here we provide the mathematical details of FLDA under the representation of a partial table. When we have a partial table, if the feature $i$ is the primary feature with $a$ levels, and the feature $j$ is the secondary feature with $b_i$ levels, then $N_A$ in Equation (2) is defined as follows:

$$
N_A = M_A - \lambda M_{B|A}
$$

(41)

where

$$
M_A = \frac{1}{a - 1} \sum_{i=1}^{a} \sum_{j=1}^{b_i} (m_{i..} - m_{i..})(m_{i..} - m_{i..})^T
$$

(42)

$$
M_{B|A} = \frac{1}{M - a} \sum_{i=1}^{a} \sum_{j=1}^{b_i} (m_{ij..} - m_{ij..})(m_{ij..} - m_{ij..})^T
$$

(43)

and $M$ is defined as in Equation (39). Correspondingly, $M_e$ in Equation (2) is defined as:

$$
M_e = \frac{1}{N - M} \sum_{i=1}^{a} \sum_{j=1}^{b_i} \sum_{k=1}^{n_{ij}} \left( x_{ij(k)} - m_{ij..} \right) \left( x_{ij(k)} - m_{ij..} \right)^T
$$

(44)
and

\[ m_{ii} = \frac{1}{M} \sum_{i=1}^{a} \sum_{j=1}^{b_i} m_{ij} \quad (45) \]

\[ m_{ij} = \frac{1}{b_i} \sum_{j=1}^{b_i} m_{ij} \quad (46) \]

The remaining mathematical arguments are the same as those for the complete table. In this scenario, because we don’t observe all possible combinations of features \( i \) and \( j \), we cannot find the linear projection for the interaction of both features.

### 9.3 Implementation details of data synthesis

To quantitatively compare FLDA with alternative approaches, we synthesized data of four cell types, each of which contained 25 cells. The four cell types were generated from a Cartesian product of two features \( i \) and \( j \), where \( i \in \{0, 1\} \) and \( j \in \{0, 1\} \). Expressions of 10 genes were generated for each cell. The expression value of the \( h \)th gene in the \( k \)th cell of the cell type \( ij \), \( g_{ijk}^h \), was defined as the following:

\[ g_{ijk}^1 = i + \epsilon_{ijk} \quad (47) \]

\[ g_{ijk}^2 = j + \epsilon_{ijk} \quad (48) \]

\[ g_{ijk}^3 = i \land j + \epsilon_{ijk} \quad (49) \]

\[ g_{ijk}^4 = i \lor j + \epsilon_{ijk} \quad (50) \]

\[ g_{ijk}^5 = 2i + \epsilon_{ijk} \quad (51) \]

\[ g_{ijk}^6 = 2j + \epsilon_{ijk} \quad (52) \]

\[ g_{ijk}^7 = 2i \land j + \epsilon_{ijk} \quad (53) \]

\[ g_{ijk}^8 = 2i \lor j + \epsilon_{ijk} \quad (54) \]

\[ g_{ijk}^9 = \epsilon_{ijk} \quad (55) \]

\[ g_{ijk}^{10} = 2 + \epsilon_{ijk} \quad (56) \]

where

\[ i \land j = \begin{cases} 1, & \text{if } i = 1, j = 1 \\ 0, & \text{otherwise} \end{cases} \quad (57) \]

and

\[ i \lor j = \begin{cases} 0, & \text{if } i = 0, j = 0 \\ 1, & \text{otherwise} \end{cases} \quad (58) \]
were interactions of the two features. Here $\epsilon_{ijk}$ was driven by Gaussian noise, namely,

$$\epsilon_{ijk} \sim \mathcal{N}(0, \sigma^2)$$  \hspace{1cm} (59)

We synthesized datasets of 5 different $\sigma$ values ($\sigma \in \{0.2, 0.4, 0.6, 0.8, 1.0\}$). This was repeated 10 times and metrics for each $\sigma$ value were calculated as the average across the 10 repeats.

9.4 D. Implementation details of the metrics used in the study

We measured the following metrics in our experiments:

9.4.1 Signal-to-Noise Ratio (SNR)

Because we care about the separation of cell types, we define the SNR metric as the ratio of the variance between cell types over the variance of the noise, which is estimated from within-cluster variance. For the entire embedding space, given $q$ cell types, if the coordinate of each cell is indicated by $c$, then we define the overall SNR metric as the following:

$$SNR_{overall} = \frac{\text{tr} (\sum_{p=1}^{q} n_p (\bar{c}_p - \bar{c}_.) (\bar{c}_p - \bar{c}_.)^T)}{\text{tr} (\sum_{p=1}^{q} \sum_{k=1}^{n_p} (c_{pk} - \bar{c}_p) (c_{pk} - \bar{c}_p)^T)}$$ \hspace{1cm} (60)

where $\bar{c}_p$ is the center of each cell type cluster, and $\bar{c}_.$ is the center of all data points.

Let $c$ denote the embedded coordinate along a specific dimension. The SNR metric for that axis is therefore:

$$SNR_i = \frac{\sum_{p=1}^{q} n_p (\bar{c}_p - \bar{c}_.)^2}{\sum_{p=1}^{q} \sum_{k=1}^{n_p} (c_{pk} - \bar{c}_p)^2}$$ \hspace{1cm} (61)

9.4.2 Explained Variance (EV)

We want to know whether the variation of a specific dimension is strongly explained by that of a specific feature. Therefore, we measure, for each axis, how much of the total explained variance is explained by the variance of the feature $i$ or $j$. Formally, given the embedded coordinate $y_{ijk}$, we calculate the EV as the following:

$$EV_i = \frac{\sum_{i=1}^{a} \sum_{j=1}^{b} n_{ij} (\bar{y}_{..} - \bar{y}_{..})^2}{\sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n_{ij}} (y_{ijk} - \bar{y}_{..})^2}$$ \hspace{1cm} (62)

$$EV_j = \frac{\sum_{i=1}^{a} \sum_{j=1}^{b} n_{ij} (\bar{y}_{..} - \bar{y}_{..})^2}{\sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n_{ij}} (y_{ijk} - \bar{y}_{..})^2}$$ \hspace{1cm} (63)

where $\bar{y}$ is the average of $y_{ijk}$ over the indices indicated by the dots.

9.4.3 Mutual Information (MI)

The MI between a discriminant axis $u$ and a feature quantifies how much information of the feature is obtained by observing data projected along that axis. It is calculated as the MI between data representations along the axis $y = u^T X$ and feature labels of the data $f$, where $X$ is the original gene expression matrix:

$$I(y, f) = H(y) + H(f) - H(y, f)$$

$$= - \sum_{y \in Y} p(y) \log_2 p(y) - \sum_{f \in F} p(f) \log_2 p(f) - \sum_{y \in Y} \sum_{f \in F} p(y, f) \log_2 p(y, f)$$ \hspace{1cm} (64)

Here $H$ indicates entropy. To calculate $H(y)$ and $H(y, f)$, we discretize $y$ into 10 bins.
9.4.4 Modularity

Ridgeway and Mozer (2018) argued that in a modular representation, each axis should depend on at most a single feature. Following the arguments in their paper, the modularity score is computed as follows: we first calculate the MI between each feature and each axis \((m_{if} \text{ denotes the MI between one axis } i \text{ and one feature } f)\). If an axis is perfectly modular, it will have high mutual information for only one feature and zeros for the others, we therefore compute a template \(t_{if}\) as the following:

\[
t_{if} = \begin{cases} 
\theta_i, & \text{if } f = \arg \max_g m_{ig} \\
0, & \text{otherwise}
\end{cases}
\]  

(65)

where \(\theta_i = \max_g m_{ig}\). We then calculate the deviation from the template as:

\[
\delta_i = \sum_f (m_{if} - t_{if})^2 / \theta_i^2(N-1)
\]  

(66)

where \(N\) is the number of features. The modularity score for the axis \(i\) is \(1 - \delta_i\). The mean of \(1 - \delta_i\) over \(i\) is defined as the overall modularity score.

9.5 E. Implementation details of annotation perturbation

To evaluate the effect of mislabeling phenotypic levels, we made use of the dataset of T4/T5 neurons, and generated three kinds of perturbation to the original labels:

First, we switched the phenotype labels of T4a neurons with one of the seven other types (T4b, T4c, T4d, T5a, T5b, T5c, T5d). In this scenario, phenotype labels of two cell types were incorrect, but the number of cell type clusters was the same. We had two levels of the dendritic phenotypes (T4/T5), and four levels of the axonal phenotypes (a/b/c/d). Therefore we kept one dimension for the dendritic feature, and three dimensions for the axonal feature.

Second, we merged the axonal phenotypic level a with another level (b/c/d), as an incorrect new level (a+b/a+c/a+d). In this scenario, we had three axonal phenotypes, therefore we kept two dimensions for the axonal feature.

Third, we randomly split each of the four axonal lamination labels (a/b/c/d) into two levels. For instance, among neurons with the original axonal level a, some of them were labeled with a level a1, and the others were labeled with a level a2. In this scenario, we had eight axonal phenotypes (a1/a2/b1/b2/c1/c2/d1/d2), and we kept seven dimensions for the axonal feature.

We performed FLDA on the dataset of T4/T5 neurons but with these perturbed annotations. Metrics from each of the perturbed annotations were measured and compared with those from the original annotation.

References


10 Additional Information
Figure 5: Representative plots (at $\sigma = 0.6$) of EV and MI metrics for FLDA and other models. (A,B) EV (A) and MI (B) metrics of FLDA. $\text{FLD}_i$ and $\text{FLD}_j$ indicate the factorized linear discriminants for features $i$ and $j$. (C,D) EV (C) and MI (D) metrics of 2LDAs. $\text{LD}_i$ and $\text{LD}_j$ indicate the linear discriminant components for features $i$ and $j$. (E,F) EV (E) and MI (F) metrics of LDA. $\text{LD}_1$ and $\text{LD}_2$ indicate the first two linear discriminant components. (G,H) EV (G) and MI (H) metrics of CCA. $\text{CCA}_1$ and $\text{CCA}_2$ indicate the first two canonical correlation axes. (I,J) EV (I) and MI (J) metrics of PCA. $\text{PC}_1$ and $\text{PC}_2$ indicate the first two principal components. EV$_i$ and EV$_j$ are the explained variance of features $i$ and $j$ along an axis, and MI$_i$ and MI$_j$ indicate the mutual inform between an axis and features $i$ and $j$ respectively. Values of EV and MI metrics are also indicated by the color bars on the right side.

Figure 6: Additional plots for FLDA on the dataset of T4/T5 neurons. (A, B) Projection of the original gene expression data into the two-dimensional space made of the first and second (FLD$_{ij1}$ and FLD$_{ij2}$) (A) or the second and third (FLD$_{ij2}$ and FLD$_{ij3}$) (B) discriminant axes for the component that depends on the combination of both features $i$ and $j$. Different cell types are indicated in different colors as in (B).
Figure 7: Additional plots for critical genes extracted from the sparse algorithm. (A) Weight vector of the 20 genes selected for the interaction of both dendritic and axonal features (FLD_{ij}). The weight value is indicated in the color bar with color indicating direction (red: positive and green: negative) and saturation indicating magnitude. (B) Expression patterns of the 20 genes from (A) in eight types of T4/T5 neurons. Dot size indicates the percentage of cells in which the gene was expressed, and color represents average scaled expression.

Figure 8: Evaluation of the effect of incorrect phenotype annotation on the dataset of T4/T5 neurons. (A,B) Normalized overall SNR metric (A) and overall modularity score (B) of FLDA after switching labels of T4a type with another neuronal type. (C,D) Normalized overall SNR metric (C) and overall modularity score (D) of FLDA after merging the axonal phenotypic level a with another phenotypic level (b/c/d). (E,F) Normalized overall SNR metric (E) and overall modularity score (F) of FLDA after splitting each axonal phenotypic level into two. Metrics under the original annotation are colored in green, and the values are indicated by the dashed lines. Here the SNR values are normalized with respect to that of the original annotation.