Spike-and-Slab Lasso Biclustering

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Abstract

Biclustering methods aim to group samples using only subsets of their associated features. In this way, biclustering methods differ from traditional clustering methods, which utilize the entire set of features to group samples. Motivating applications for biclustering include genomics data, where the goal is to cluster patients or samples by their gene expression profiles; and recommender systems, which seek to group customers based on their product preferences. Biclusters of interest often manifest as rank-1 submatrices of the data matrix. This submatrix detection problem can be viewed as a factor analysis problem in which both the factors and loadings are sparse. In this paper, we propose a new biclustering method called Spike-and-Slab Lasso Biclustering (SSLB) which utilizes the Spike-and-Slab Lasso of Ročková and George (2018) to find such a sparse factorization of the data matrix. SSLB also incorporates an Indian Buffet Process prior to automatically choose the number of biclusters. Many biclustering methods make assumptions about the size of the latent biclusters; either assuming that the biclusters are all of the same size, or that the biclusters are very large or very small. In contrast, SSLB can adapt to find biclusters which have a continuum of sizes. SSLB is implemented via a fast EM algorithm with a variational step. In a variety of simulation settings, SSLB outperforms other biclustering methods. We apply SSLB to both a microarray dataset and a single-cell RNA-sequencing dataset and highlight that SSLB can recover biologically meaningful structures in the data. The SSLB software is available as an R/C++ package at https://github.com/gemoran/SSLB.

1 Introduction

Biclustering has emerged as a popular tool for simultaneously grouping samples and their associated features. Standard clustering methods typically group the samples based on

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their entire set of features; however, this may be problematic in large datasets where many of the features are not expected to play a role in distinguishing the groups. For example, in gene expression data it is expected that only a small fraction of genes are differentially expressed across groups of interest. If samples are required to be similar over all genes to belong to the same cluster, such groups may be missed. Biclustering methods mitigate this problem by simultaneously finding subsets of samples and corresponding subsets of features on which the samples have similar values. In this way, biclustering methods perform a two-way subset selection for clustering. Along with gene expression data (Cheng and Church, 2000), biclustering methods have also been applied to recommender systems, which seek to group consumers based on their ratings of different products (De Castro et al., 2007; Zhu et al., 2016); neuroscience (Fan et al., 2010); and agriculture (Mucherino et al., 2009). Biclustering also yields more interpretable results than clustering; by finding features that are associated with group membership, biclustering methods have identified novel biological modules (Xiong et al., 2013).

In this paper, we propose a new method for biclustering, which we call Spike-and-Slab Lasso Biclustering (SSLB). Before introducing our method and discussing related work, we first outline as a motivating example the gene expression microarray dataset of Van De Vijver et al. (2002); Van’t Veer et al. (2002), which we will revisit in Section 4. This data consists of the expression levels of \( G = 24,158 \) genes from the breast cancer tumors of \( N = 337 \) patients with stage I or II breast cancer. Like many cancers, breast cancer is a heterogenous disease; patients are typically classed into sub-types based on their expression of hormone receptors (estrogen and progesterone) and human epidermal growth factor 2 (HER2) (Howlader et al., 2014). The different sub-types have contrasting prognoses and require sub-type specific treatment regimens (Howlader et al., 2014). Because the different sub-types are defined by differing gene expression levels, recovering these sub-types is a problem of biclustering. As such, this dataset has often been used as a benchmark for biclustering methods. This is particularly the case the data also features the estrogen receptor (ER) status of patients, as determined by immunohistochemical staining, not gene expression levels, which may be used as a check that the method has recovered biologically meaningful clusters.

The left side of Figure 1 shows the gene expression dataset before any clustering. We applied SSLB and re-ordered this gene expression matrix to correspond to the resulting SSLB bicluster; the re-ordered matrix is shown in the middle of Figure 1. As a validation of this SSLB bicluster, we plot the clinical ER status of the patients to the right of the ordered matrix. We emphasize that this clinical information was not used to run SSLB, which is an unsupervised method. Encouragingly, the bicluster found by SSLB corresponds very well to the ER status of the patients, highlighting that SSLB can recover biologically meaningful signal in the data.
1.1 Our Approach: Spike-and-Slab Lasso Biclustering

We now describe our proposed method, Spike-and-Slab Lasso Biclustering (SSLB). The observed data is the matrix of samples by features, denoted by

\[ Y = [y_1, \ldots, y_N]^T \in \mathbb{R}^{N \times G}, \]

where \( Y_{ij} \) is the measurement of feature \( j \) in sample \( i \) for \( i = 1, \ldots, N \), and \( j = 1, \ldots, G \). The goal is to find submatrices of the data matrix (up to permutation of rows and columns) for which the elements \( Y_{ij} \) are “similar”. The row and column indices of such a submatrix are then referred to as a “bicluster”. Our method assumes that biclusters manifest as rank-1 submatrices of the data matrix, \( Y \). That is, we seek submatrices on which the rows (columns) are correlated. Intuitively, we seek samples which exhibit the same behavior (to potentially different degrees) over a subset of features.

This assumption corresponds to a factor analysis model where both the factors and the loadings are sparse. That is, we assume that \( Y \) has the following structure:

\[ Y = \sum_{k=1}^{K} x^k \beta^k + E, \]  

(1.1)

where \( X = [x^1, \ldots, x^K] \in \mathbb{R}^{N \times K} \) is the factor matrix, \( B = [\beta^1, \ldots, \beta^K] \in \mathbb{R}^{G \times K} \) is the loadings matrix and \( E = [e_1, \ldots, e_N]^T \in \mathbb{R}^{N \times G} \) is a matrix of Gaussian noise with \( e_i \sim \)
Figure 2: Mean of a data matrix with two biclusters: $E[Y] = x^1 \beta^{1T} + x^2 \beta^{2T}$.

$N_{G}(0, \Sigma)$ where $\Sigma = \text{diag}\{\sigma^2_j\}_{j=1}^G$ for $i = 1, \ldots, N$. We allow for the number of biclusters, $K$ to be unknown. We use the convention that the superscript $x^k$ refers to the $k$th column of $X$, and the subscript $x_i$ to refers to the $i$th row of $X$. The mean of a data matrix with two rank-1 biclusters is shown in Figure 2. An example of a data matrix with $K = 10$ biclusters is shown in Figure 3.

The benefits of utilizing a factor model for biclustering are threefold. Firstly, it is interpretable. Using gene expression data as an example: the genes (i.e. features) in a bicluster may be expressed at different levels to drive a biological process. This expression pattern in turn may be weaker or stronger in different samples, as determined by the sample-specific multiplicative effect. Secondly, there are many applications in which features and samples have been shown to be well approximated by such multiplicative effect models (Hochreiter et al., 2010). Thirdly, the definition allows for the specification of the model (1.1), allowing for systematic analysis of the noise variance and, in possible future work, coherent inclusion of prior information regarding the features or the samples.

In (1.1), $x_{ik}$ is non-zero if sample $i$ belongs to bicluster $k$ and $\beta_{jk}$ is non-zero if feature $j$ belongs to bicluster $k$. As such, the problem of finding the biclusters in this framework can be viewed as a two-way subset selection problem: identifying biclusters corresponds to finding the support of $x^k$ and $\beta^K$. To address this problem, we adopt a Bayesian framework and place sparsity-inducing Spike-and-Slab Lasso priors (Ročková and George, 2018) on each of the columns of the factor matrix, $X$, and of the loadings matrix, $B$. The Spike-and-Slab Lasso was introduced by Ročková and George (2018) for variable selection in linear regression and has subsequently been used in grouped regression (Bai et al., 2019), multivariate regression (Deshpande et al., 2019) and sparse factor analysis (Ročková and George, 2016). A difference here from Ročková and George (2016) is that we induce sparsity in both the factor matrix and the loadings matrix, instead of only the loadings matrix. A benefit of the Spike-and-Slab Lasso is that it can adapt to the underlying levels of sparsity (or lack thereof) in the data. As we will show, this allows the method to find biclusters of a range of different sizes.
To determine the number of biclusters, $K$, we use a Bayesian nonparametric strategy. Specifically, we use an Indian Buffet Process prior (IBP, Griffiths and Ghahramani, 2011) on the “size” of each bicluster, which ensures that each new bicluster is smaller than the previous one. We also allow for the IBP prior to be extended to a Pitman-Yor IBP (Teh et al., 2007), which drives the size of consecutive biclusters to decrease as a power law. This extension may be appropriate in applications where one expects a larger number of biclusters of a smaller size.

For implementation, we develop a fast, deterministic EM algorithm with a variational step to find the modal estimates of $X$ and $B$. Biclustering is in general NP-hard (Peeters, 2003). The Spike-and-Slab Lasso prior ameliorates such computational difficulties as it uses a continuous relaxation of bicluster membership.

We note that the factorization (1.1) is similar to the singular value decomposition (SVD) of $Y$. However, the SVD assumption forces the columns of $X$ and $B$ to be orthogonal, a requirement which is relaxed here, as is done in factor analysis more generally. A benefit of not requiring orthogonality is that it allows for biclusters to overlap, enabling samples and features to belong to more than one bicluster. Further, samples and features do not have to belong to any biclusters.

A potential issue with not requiring orthogonality is that the model (1.1) is not identifiable up to rotation: that is, $XB^T = (PX)(PB)^T$ for any rotation matrix $P$. However, by anchoring our priors on a sparse factorization of $Y$, the space of rotation matrices is “softly constrained” to mitigate the identifiability issue.

1.2 Related Work

A number of biclustering methods have utilized the same factor analysis model (1.1) with alternate sparsity-inducing priors for the factor and loading matrices. The first method
to do so was Factor Analysis for Bicluster Acquisition (FABIA, Hochreiter et al., 2010) who placed single Laplace priors on both $x^k$ and $\beta^k$. However, the posterior resulting from Laplacian priors does not place enough mass on sparse solutions in variable selection problems (Castillo et al., 2015). This is because such a single Laplace prior has one variance parameter and so cannot both shrink negligible values to zero and maintain the larger signal. As a result, the estimates of $X$ and $B$ from FABIA are not sparse; the authors recommend a heuristic thresholding rule to then determine bicluster membership. In contrast, the Spike-and-Slab Lasso performs selective shrinkage on the latent variables; indeed the Spike-and-Slab Lasso concentrates at the optimal rate for sparse models (Ročková et al., 2018). Further, our method gives an indicator of bicluster membership, precluding the need for an arbitrary thresholding strategy. Finally, FABIA does not automatically select the number of biclusters, requiring this to be set in advance.

Gao et al. (2016) also begin with the model (1.1) for their method, BicMix. They allow for the components $x^k$ and $\beta^k$ to be either sparse, or dense to account for potential confounders. To achieve strong regularization on the sparse components, the authors utilize a three parameter beta distribution (Armagan et al., 2011), a generalization of the horseshoe prior (Carvalho et al., 2010). Whilst this dichotomous framework may be appropriate in some applications, in other cases it may be more appropriate to allow for a continuum of sparsity levels. Such a continuum is achieved in our model as the Spike-and-Slab Lasso prior is indexed by a continuous parameter which controls the proportion of non-zero values in each bicluster. Further, the Spike-and-Slab Lasso automatically thresholds negligible values to zero; such thresholding does not occur automatically for the horseshoe prior and generalizations thereof. Gao et al. (2016) also allow for the number of biclusters, $K$, to be unknown by starting with an overestimate of $K$, imposing strong regularization on $X$ and $B$, and then removing zero columns. This strategy is similar to to our Bayesian nonparametric strategy; the difference is that the IBP prior which we utilize increases the strength of the regularization of $X$ and $B$ as a function of the column number $k$, as opposed to BicMix which applies the same regularization to each column.

Recently, Denitto et al. (2017) proposed the similarly named method “Spike and Slab Biclustering”. Despite this likeness, there are a number of differences between our methods. Firstly, Denitto et al. (2017) utilize Gaussians distributions for both their spike and slab priors, whereas we use Laplacian priors. In Bayesian variable selection, the slab distribution requires tails at least as heavy as the Laplace for optimal posterior concentration (Castillo and van der Vaart, 2012). Furthermore, Denitto et al. (2017) do not use sparsity inducing priors, nor use a non-parametric strategy to estimate the number of biclusters and instead require this number to be set in advance.

Up to this point, we have reviewed only biclustering methods which utilize the factor model (1.1). In the literature, there have been a variety of methods which use different notions of similarity to define biclusters. Generally speaking, these notions of similarity can be
grouped into four categories, as outlined by Madeira and Oliveira (2004).

The first category assumes that biclusters manifest as submatrices of constant values (for example, Hartigan, 1972; Shabalin et al., 2009; Prelić et al., 2006). The second category extends this constant submatrix assumption to accommodate additive row and column bicluster-specific effects in a similar manner to two-way ANOVA (see, for example, Cheng and Church, 2000; Lazzeroni and Owen, 2002; Gu and Liu, 2008). The third category assumes multiplicative row and column effects, instead of additive. That is, biclusters are assumed to manifest as rank-1 submatrices in the data matrix, up to permutations of rows and columns. This category includes the factor analysis methods discussed above, as well as methods which utilize Pearson’s correlation as a criterion for bicluster membership (Bozdağ et al., 2009; Bhattacharyya and Cui, 2017) and Rangan et al. (2018), which uses a loop-counting method to find rank-1 submatrices. Methods in the fourth category do not assume a model for the data matrix but instead search for patterns in the data matrix. Such patterns may be viewed as generalizations of the additive or multiplicative assumptions. For example, the Iterative Signature Algorithm (ISA, Bergmann et al., 2003) finds submatrices in which all rows and all columns are above a certain threshold. Ben-Dor et al. (2003) generalize the multiplicative effects assumption to find subsets of features which have the same order on a subset of samples, which can be thought of as a slightly more flexible correlation structure.

In addition to how they define biclusters, methods can also be classified according to other criteria, including: the types of algorithms they utilize to find such biclusters; the assumptions they make regarding the noise distribution; and whether features and samples are allowed to belong to more than one bicluster, to name a few. For more detailed reviews of biclustering methods, see Madeira and Oliveira (2004); Prelić et al. (2006); Bozdağ et al. (2010); Eren et al. (2012); Padilha and Campello (2017).

2 Hierarchical Model for SSLB

In this section, we outline the Spike-and-Slab Lasso Biclustering (SSLB) model in greater detail. We adopt the factor analysis model in (1.1). To allow for uncertainty in the number of biclusters, $K$, we initialize the factor and loading matrices with an overestimate, $K^*$. The IBP prior discourages biclusters with negligible signal from entering consideration, and so the estimated factor and loading matrices will contain columns of all zeroes, provided $K^*$ is a true overestimate. After removing these zero columns, the number of remaining columns is the estimated number of biclusters.

We also restrict $X$ and $B$ to be matrices with at least two non-zero entries per column (Frühwirth-Schnatter and Lopes, 2010; Ročková and George, 2016). This avoids a singleton column in either $X$ or $B$ which would be unidentifiable with regard to the noise matrix $\Sigma$. 

in the marginal covariance of \( Y \) (after marginalizing over either \( B \) or \( X \), respectively).

2.1 Hierarchical structure for loadings \( B \)

For each column \( \beta^k \), we have a Spike-and-Slab Lasso prior. That is, each \( \beta_{jk} \) is drawn \textit{a priori} from either a Laplacian “spike” parameterized by \( \lambda_0 \) and is consequently negligible, or a Laplacian “slab” parameterized by \( \lambda_1 \) and thus can be large:

\[
\pi(\beta_{jk}|\gamma_{jk}, \lambda_0, \lambda_1) = (1 - \gamma_{jk}) \psi(\beta_{jk}|\lambda_0) + \gamma_{jk} \psi(\beta_{jk}|\lambda_1), \quad 1 \leq j \leq G, \ 1 \leq k \leq K^*, \quad (2.1)
\]

where the Laplace density is denoted by \( \psi(\beta|\lambda) = \frac{1}{\lambda} e^{-\lambda|\beta|} \) and \( \gamma_{jk} \) is a binary indicator variable. Here, \( \gamma_{jk} = 1 \) if feature \( j \) is active in bicluster \( k \), and \( \gamma_{jk} = 0 \) if feature \( j \) has a negligible contribution to bicluster \( k \). We allow for uncertainty in bicluster membership by using the common Beta-Bernoulli prior for the latent indicators:

\[
\gamma_{jk}|\theta_k \sim \text{Bernoulli}(\theta_k), \\
\theta_k \sim \text{Beta}(a,b). \quad (2.2)
\]

It is important to emphasize here the “sparsity-indexing” parameter \( \theta_k \). Due to the Beta-Bernoulli prior, it has a natural interpretation as the percentage of non-zero elements in the column \( \beta^k \). By allowing \( \theta_k \) to vary continuously, the method can adapt to differing levels of sparsity in each of the different columns of \( B = [\beta^1, \ldots, \beta^K] \).

Here, we can use a finite approximation to the IBP by setting the hyperparameters of the Beta prior in (2.2) to: \( a \propto 1/K^* \), \( b = 1 \). This ensures that in the limit as \( K^* \to \infty \), this prior is the IBP. While this is the default choice for these hyperparameters, we note that they can be easily tailored to the problem at hand. For instance, a choice of \( a = 1/G \), \( b = 1/G \) will result in the prior mass concentrating around \( \theta = 0 \) and \( \theta = 1 \), which may be preferred when both very dense and very sparse biclusters are expected.

2.2 Hierarchical structure for factors \( X \)

To find biclusters, we also want sparsity in the columns of \( X \). To this end, we place a Spike-and-Slab Lasso prior on each \( x_{ik} \). However, we require an alternate formulation of the Spike-and-Slab Lasso prior to Section 2.1 for the \( x_{ik} \) in order to yield a tractable EM algorithm. This is accomplished by introducing auxiliary variables \( \{\tau_{ik}\}^{N,K*}_{i,k=1} \) for the variance of each \( x_{ik} \):

\[
x_{ik}|\tau_{ik} \sim N(0, \tau_{ik}) \quad 1 \leq i \leq N, \ 1 \leq k \leq K^*. \quad (2.3)
\]
Then, the $\tau_{ik}$ are each assigned a mixture of exponentials prior, where $\tau_{ik}$ is drawn a priori from either an exponential “spike” parameterized by $\lambda_2^2$ and consequently is small, or from an exponential “slab” parameterized by $\lambda_0^2$ and hence can be large:

$$
\pi(\tau_{ik} | \tilde{\gamma}_{ik}) = \tilde{\gamma}_{ik} \frac{\lambda_2^2}{2} e^{-\frac{\lambda_2^2}{2} \tau_{ik}} + (1 - \tilde{\gamma}_{ik}) \frac{\lambda_0^2}{2} e^{-\frac{\lambda_0^2}{2} \tau_{ik}}
$$

(2.4)

where $\tilde{\gamma}_{ik}$ is a binary indicator variable. This augmentation strategy uses the fact that the Laplace distribution can be represented as a scale mixture of a normal with an exponential mixing density; marginalizing over the $\tau_{ik}$ yields the usual Spike-and-Slab Lasso prior in (2.1).

We place independent Bernoulli priors on each of the $\tilde{\gamma}_{ik}$ binary indicators. Similarly as before, $\tilde{\gamma}_{ik} = 1$ if sample $i$ is active in bicluster $k$, and $\tilde{\gamma}_{ik} = 0$ if sample $i$ has a negligible contribution to bicluster $k$. The Bernoulli priors are parameterized by the “sparsity indexing” parameters $\tilde{\theta}_k$. Instead of placing a Beta prior on the $\tilde{\theta}_k$ as for the hierarchical model for the loadings $B$, we use an Indian Buffet Process prior with an optional Pitman-Yor extension. This is achieved using the stick-breaking construction of Teh et al. (2007):

$$
\tilde{\gamma}_{ik} \sim \text{Bernoulli}(\tilde{\theta}_{(k)}),
$$

$$
\tilde{\theta}_{(k)} = \prod_{l=1}^{k} \nu(l),
$$

$$
\nu(k) \sim \text{Beta}(\tilde{\alpha} + kd, 1 - d), \quad \text{where } d \in [0, 1), \quad \tilde{\alpha} > -d.
$$

(2.5)

When $d = 0$, the above formulation is the usual IBP prior. When $0 < d < 1$, the ordered sparsity weights, $\tilde{\theta}_{(k)}$, decrease in expectation as an $O(k^{-1/d})$ power-law (Teh et al., 2007). This may be useful in applications where there are expected to be more, but smaller, biclusters.

We note that we only utilize this stick-breaking formulation of the IBP prior for the sparsity weights for the factors, $X$, and not the loadings, $B$. This is because this formulation requires ordering the columns of $X$ from most dense to least dense. There is no reason to assume that the bicluster with the largest number of samples (i.e. non-zero $x_{ik}$) would also have the largest number of features (i.e. non-zero $\beta_{jk}$). That is, the most dense column of $X$ should not be forced to line up with the most dense column of $B$, which would be the case if we used a similar stick-breaking construction for the priors of $B$.

In the simulation studies in Section 3, we will also consider the finite approximation to the IBP for comparison. Similarly as for the loadings $B$, this formulation has a Beta prior on the sparsity weights, $\theta_k \sim \text{Beta}(\tilde{\alpha}, \tilde{\beta})$ with $\tilde{\alpha} \propto 1/K^*$ and $\tilde{\beta} = 1$.

To complete the model, we place an inverse gamma prior on the elements of the covariance matrix, $\Sigma$:

$$
\sigma_j^2 \sim IG \left( \frac{\eta}{2}, \frac{\xi}{2} \right).
$$

(2.6)
Finally, we use the notation $T = \{ \tau_{ik} \}_{i,k=1}^{N,K^*} \in \mathbb{R}^{N \times K^*}$, $\tilde{\Gamma} = \{ \tilde{\gamma}_{ik} \}_{i,k=1}^{N,K^*}$ and $D_i = \text{diag}\{ \tau^{-1}_{i1}, \ldots, \tau^{-1}_{iK^*} \}$.

### 2.3 Implementation

We develop an EM algorithm with a variational step to quickly target modes of the posterior. In the E-Step, we compute the expectation of the factors $X$ and factor indicators $\tilde{\Gamma}$, conditional on the data and current values of the rest of the parameters. This step is rendered tractable by the augmentation strategy outlined in Section 2.2. In the M-Step, we marginalize over the loading indicators, $\Gamma$, and use a coordinate ascent strategy to find the modes of $B$ (Ročková and George, 2018). For this algorithm, we also use the variance updates detailed by Moran et al. (2018). To maximize the parameters of the IBP prior, we implement a variational step with closed form updates inspired by Doshi et al. (2009). Further details of the algorithm are given in Section A of the Appendix.

We adopt a dynamic posterior exploration strategy for finding the modes of $B$ (Ročková and George, 2018). Specifically, we hold the slab parameters $\lambda_1$ fixed and then gradually increase the spike parameter $\lambda_0$ along a “ladder” of values, propagating the solutions forward as “warm starts” for the next largest spike values in the ladder. As outlined by Ročková and George (2018), holding the slab parameter fixed serves to stabilize the large coefficients; this is in contrast to the Lasso, which shrinks the larger coefficients along with the small. Meanwhile, gradually increasing $\lambda_0$ over a ladder of values progressively thresholds negligible coefficients to zero.

For the factor matrix, $X$, we modify this strategy slightly. As we are calculating the conditional mean of $X$, values of $x_{ik}$ that were previously zero may re-enter the bicluster for very large $\tilde{\lambda}_0$. This phenomenon is illustrated in the following simple example: suppose the true value is $x_{ik} = 0.005$. Then, the contribution of sample $i$ is essentially negligible and so $x_{ik}$ should reasonably “belong” to the spike. However, if spike parameter is $\lambda_0 = 200$, it is actually unlikely that $x_{ik}$ was drawn from the spike distribution; this is because this $\tilde{\lambda}_0$ corresponds to an extremely small spike variance of $5 \times 10^{-5}$. This phenomenon is an example of Lindley’s paradox. Whilst this phenomenon occurs for both $B$ and $X$, we estimate the mode of $B$ which does have this problem, unlike the mean. For estimation of the mean of $X$, we implement a stopping rule for $\tilde{\lambda}_0$. We have found that an effective data-driven strategy is to “freeze” $\tilde{\lambda}_0$ at the value at which $X$ is the most sparse, whilst continuing to increase $\lambda_0$ (the spike parameter for $B$). To conclude the discussion on the dynamic posterior exploration strategy, we note that we increase $\lambda_0$ and $\tilde{\lambda}_0$ concurrently (up until the point where $\tilde{\lambda}_0$ is fixed).

We also implement a re-scaling step for the columns of $X$ and $B$. Whilst sparsity-inducing priors mitigate to some extent the identifiability problems of the likelihood in regard to rotation, the scale of the columns of the factor and loadings matrices remains unidenti-
fiable. That is, \( x^k \beta^k T \) is equivalent to \((c_k^{-1} x^k)(c_k \beta^k)^T\) for any constant \( c_k \in \mathbb{R} \). The focus of biclustering, however, is to find the non-zero elements of these matrices; it is the covarying subsets that are of interest, and not their magnitude. As the scale is not of particular interest, we re-scale \( X \) and \( B \) at each step of the EM algorithm to ensure that the corresponding columns have the same norm. That is, for each \( k = 1, \ldots, K \), we set

\[
\begin{align*}
  c_k &\leftarrow \sqrt{\| x^k \|_1 / \| \beta^k \|_1}, \\
x^k &\leftarrow 1 / c_k x^k, \\
\beta^k &\leftarrow c_k \beta^k.
\end{align*}
\]

(2.7)

The re-scaling step is also important to ensure that the default choices of regularization parameters \( \lambda_0, \tilde{\lambda}_0 \) are appropriate; if \( X \) and \( B \) have vastly different scales, then one matrix may be over-thresholded whilst the other is under-thresholded.

The complexity of the SSLB algorithm is \( O(NK^*^3 + GK^*) \), assuming that the initial number of biclusters, \( K^* \), is less than both the number of samples, \( N \), and the number of features, \( G \). The first term comes from the E-Step for \( X \), where the \( K^* \times K^* \) matrix \( V^i \) needs to be inverted for \( i = 1, \ldots, N \). The second term comes from the M-Step for \( B \), where the coordinate ascent algorithm has complexity \( K^* \) and is applied to each of the \( G \) rows. However, the E-Step and M-Step are trivially parallelizable across the samples and features, respectively. Such a parallelization would yield an improved complexity of \( O(K^*^3) \).

### 2.4 Automatic Thresholding

A key benefit of SSLB is that it automatically thresholds negligible elements of the loadings matrix, \( B \), to zero. This allows for a direct interpretation of bicluster membership: if the estimated \( \hat{\beta}_{jk} \neq 0 \), then feature \( j \) is included in bicluster \( k \). To determine bicluster membership for the samples, SSLB calculates the posterior mean of the indicator variables, \( \tilde{\Gamma} \). The indicator \( \tilde{\gamma}_{ik} \) may be interpreted as the posterior probability that sample \( i \) belongs to bicluster \( k \). If this posterior probability is greater than 0.5, we include sample \( i \) in bicluster \( k \). More precisely, we implement the following thresholding rule after convergence of the SSLB algorithm:

\[
\begin{align*}
\tilde{x}_{ik} &= \begin{cases} \\
\tilde{x}_{ik} & \text{if } E[\tilde{\gamma}_{ik}|Y, T^*, \tilde{\theta}^*] > 0.5, \\
0 & \text{if } E[\tilde{\gamma}_{ik}|Y, T^*, \tilde{\theta}^*] \leq 0.5,
\end{cases} \\
&\quad 1 \leq i \leq N, \ 1 \leq k \leq K^*
\end{align*}
\]

(2.8)

where \( T^* \) and \( \tilde{\theta}^* \) are the solutions obtained after convergence of the EM algorithm. That is, if the posterior probability of \( x_{ik} \) belonging to the “spike” is greater than 0.5, it is thresholded to zero.

The natural thresholding scheme that arises from the SSLB model is in contrast to both FABIA and BicMix. FABIA utilizes an ad-hoc post-processing thresholding step, while
the three-parameter beta prior of BicMix does not exactly threshold small values of the factors and loadings to zero.

2.5 Default Settings

The default hyper-parameters settings are as follows. For both the loadings and the factors, $B$ and $X$, the slab parameters are set to $\lambda_1, \tilde{\lambda}_1 = 1$ and the increasing ladder of spike parameters are set to $\lambda_0, \tilde{\lambda}_0 \in \{1, 5, 10, 50, 100, 500, 1000, 10000, 100000, 1000000\}$. Note, however, that $\tilde{\lambda}_0$ is halted at a data-driven value as described earlier in this section.

To determine the hyper-parameters of the variances, $\{\sigma_j\}_{j=1}^G$, we use an informal empirical Bayes strategy, motivated by Chipman et al. (2010). We denote the sample variances of the columns of the data matrix, $Y$, by $\{s_j^2\}_{j=1}^G$. Then, the intuition for our strategy is as follows: if we assume that most biclusters are sparse, then small values of the $s_j^2$ are essentially “pure noise” and contain no signal. Hence, the prior for the error variances should be centered around a small value of $s_j^2$. In addition, we recommend using a small value of the degrees of freedom parameter, $\eta$, to allow for larger prior uncertainty. As a default, we take $\eta = 3$. More specifically, we calculate the 5% quantile of the $s_j^2$ and find the value of $\xi$ such that this 5% quantile is the median of the prior distribution.

We initialize the parameters of SSLB as follows. Each entry of $B$ is generated independently from a standard normal distribution. The entries of $T$, the matrix of auxiliary variance parameters, are set to 100, representing an initial relatively non-informative prior on $X$. The sparsity weights, $\theta_k$, are initialized at 0.5. The IBP parameters, $\nu$, are generated independently from a Beta(1, 1) distribution and then ordered from largest to smallest.

For the initialization of $K$, we recommend $K^* = 50$ as an initial overestimate. If SSLB obtains a final estimate of $\hat{K} = 50$ biclusters, this is an indication that the initial choice $K^* = 50$ underestimated the true number of biclusters; in this case, we recommend running SSLB again with a larger initial $K^*$.

3 Simulation Studies

In this section, we compare the performance of SSLB to the methods of BicMix and FABIA in two simulation settings. Similarly to Gao et al. (2016), the simulation studies we present illustrate the performance of our method on settings with different levels of sparsity in the biclusters. Specifically, the first simulation study considers matrices with only sparse biclusters while the second simulation study considers both sparse and dense biclusters.
3.1 Simulation 1

We first consider a simulated example with $N = 300$, $G = 1000$ and $K = 15$ biclusters. The data was simulated using settings very similar to the FABIA paper (Hochreiter et al., 2010). Specifically, the data matrix $Y$ was generated as $XB^T + E$, where each entry of the noise matrix $E$ is sampled from an independent standard normal distribution. For each column $x^k$, we draw the number of samples in bicluster $k$ uniformly from $\{5, \ldots, 20\}$. The indices of these elements were randomly selected and then assigned a value from $N(\pm 2, 1)$, with the sign of the mean chosen randomly. The elements of $x^k$ not in the bicluster had values drawn from $N(0, 0.2^2)$. The columns $\beta^k$ were generated similarly, except the number of elements in each bicluster was drawn from $\{10, \ldots, 50\}$. We allow biclusters to share at most five samples and at least fifteen features. For both SSLB and BicMix, we set the initial overestimate of the number of biclusters to be $K^* = 30$. For FABIA, which requires the number of biclusters to be set in advance, we set the number of biclusters to the truth, $K = 15$.

For each of the methods, we recorded the following metrics: (i) relevance and recovery (Prelić et al., 2006); and (ii) consensus (Hochreiter et al., 2010) (see Section B of the Appendix for precise definitions). Relevance measures how similar on average the biclusters found by a method are to the true biclusters (where similarity is defined by the Jaccard index). Recovery instead measures how similar the true biclusters are to the found biclusters on average. However, if many duplicated biclusters are found by a method, this will not be reflected in either the relevance or recovery scores. To provide a meaningful metric in such circumstances, Hochreiter et al. (2010) developed the consensus score. The consensus score is similar to the recovery score, but penalizes overestimation of the true number of biclusters.

In this simulation study, we compared three implementations of Spike-and-Slab Lasso Biclustering: (i) SSLB with the Pitman-Yor extension where $\tilde{\alpha} = 1$ and $d = 0.5$ (SSLB-PY); (ii) SSLB with the stick-breaking IBP prior for the factors where $\tilde{\alpha} = 1$ (SSLB-IBP), and (iii) SSLB with the finite approximation to the IBP prior (i.e. Beta-Binomial) for the factors where $\tilde{a} = 1/K^*$ and $\tilde{b} = 1$ (SSLB-BB). For each implementation, we used the default settings as outlined in Section 2.5. For the loadings matrix, $B$, we set the Beta-Binomial hyperparameters to be $a = 1/K^*, b = 1$.

BicMix\(^1\) was implemented using the default parameters. Following Gao et al. (2016), we thresholded values less than $10^{-10}$. FABIA was implemented using the fabia R package (Hochreiter et al., 2010) with the default parameters and recommended post-processing thresholding step.

For one realization from the above simulation setting, Figure 4 displays the support of the

\(^1\)Code obtained from bee hive.cs.princeton.edu/software
Figure 4: Simulation 1: Factor matrices, $X$, and loading matrices, $B$, found by each of the methods. Only the support of the matrix is displayed: a red value indicates a non-zero element; a grey value indicates a zero element.

estimated factor and loadings matrices, $X$ and $B$, found by each of SSLB-IBP, BicMix and FABIA. SSLB-IBP finds the true bicluster structure with few false positives, while BicMix finds many more false positives due to small values not being exactly thresholded to zero by the three-parameter beta prior. FABIA recovers much of the sparse structure, but also splits some of the biclusters into two or more columns.

To further quantify the performance of each of the methods, we generated 50 realizations of the simulated data and calculated the consensus (Figure 5a), relevance and recovery scores (Figure 5b) for each method. All implementations of SSLB have higher consensus, relevance and recovery scores than the other methods.

Table 1 displays the estimated number of biclusters, $\hat{K}$, from SSLB and BicMix. Both
the IBP and Pitman-Yor implementations of SSLB are centered at the truth. We can see empirically the benefit of using the stick-breaking construction for the IBP prior here; the SSLB-BB formulation with the finite IBP approximation slightly overestimates the true number of biclusters. Meanwhile, BicMix slightly underestimates the true number of biclusters.

Table 1: Mean estimated number of biclusters, $\hat{K}$, over 50 replications. Standard errors are shown in parentheses.

<table>
<thead>
<tr>
<th>Method</th>
<th>Simulation 1</th>
<th>Simulation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Truth</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>SSLB-IBP</td>
<td>15.0 (0.09)</td>
<td>9.9 (0.14)</td>
</tr>
<tr>
<td>SSLB-PY</td>
<td>15.0 (0.09)</td>
<td>10.1 (0.15)</td>
</tr>
<tr>
<td>SSLB-BB</td>
<td>16.4 (0.24)</td>
<td>10.3 (0.14)</td>
</tr>
<tr>
<td>BicMix</td>
<td>14.5 (0.18)</td>
<td>8.7 (0.10)</td>
</tr>
</tbody>
</table>

3.2 Simulation 2

We now assess how well SSLB can find both sparse and dense biclusters with a simulation study inspired by that of Gao et al. (2016). We again take $N = 300$, $G = 1000$ and $K = 15$. For both the factor and loading matrices, five columns are dense and ten columns are sparse. The sparse columns (corresponding to sparse biclusters) are generated as Simulation 1. The dense columns (corresponding to dense biclusters) are generated as independent $N(0, 2^2)$. We allow for one dense column in $X$ to correspond to a sparse column in $B$ and vice versa; this results in $K = 9$ biclusters which are sparse in both $X$ and $B$. 

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The goal for this simulation study is to recover the sparse biclusters while removing the effect of the dense biclusters, which are acting as confounders. As such, we calculate the recovery, relevance and consensus scores for the sparse biclusters found by each of the methods only. For SSLB and FABIA, we determine a “sparse” bicluster to be one where both columns $x^k$ and $\beta^k$ have less than 50% of values being non-zero. BicMix provides a binary indicator for whether $x^k$ and $\beta^k$ are sparse or dense; we kept BicMix biclusters for which both $x^k$ and $\beta^k$ were sparse.

For one realization from the above simulation setting, Figure 6 displays the support of the estimated factor and loadings matrices, $X$ and $B$, found by SSLB-IBP, BicMix and FABIA. SSLB finds nine of the ten true sparse biclusters with few false positives, successfully adapting to the dense and sparse structure. BicMix also finds nine out of ten true biclusters, albeit with more false positives. Meanwhile, FABIA is unable to adapt to the dichotomous bicluster structure and consequently does not recover any of the true sparse biclusters.

To further quantify the performance of each of the methods, we then generated 50 realizations of the simulated data and calculated the consensus (Figure 5a), relevance and recovery scores (Figure 5b) for each method. Again, all implementations of SSLB have higher consensus, relevance and recovery scores than the other methods. It appears that SSLB is overestimating the number of biclusters by one (Table 1). However, this additional bicluster that SSLB finds is not spurious; it is the bicluster where the true $x^k$ is sparse and $\beta^k$ is dense. In SSLB, the estimated $\beta^k$ is not completely dense and so is included in the count.

4 Breast Cancer Microarray Dataset

We now return to the breast cancer microarray dataset from Section 1 and show how we obtain the ordered matrix in Figure 1. The dataset\textsuperscript{2} consists of the expression levels of $G = 24,158$ genes from the breast cancer tumors of $N = 337$ patients with stage I or II breast cancer (Van De Vijver et al., 2002; Van’t Veer et al., 2002). Gao et al. (2016) also used this dataset to illustrate the performance of their biclustering method, BicMix. We followed a similar data processing pipeline to Gao et al. (2016) (details in Section C). However, unlike Gao et al. (2016), we did not project the quantiles of the expression levels to a standard normal. We chose not to do so to assess the ability of SSLB to capture biological signal in the presence of possible confounders. Removing of unwanted variation via matrix factorization (specifically, via singular value decomposition) has been shown to be an effective technique by previous authors (Leek and Storey, 2007), albeit not in the context of biclustering.

We ran SSLB-IBP with the initial number of biclusters set to $K^* = 50$. For the loadings,

\textsuperscript{2}Data sourced from R package \texttt{breastCancerNKi} (Schroeder et al., 2011)
**Figure 6:** Simulation 2: Factor matrices, $X$, and loading matrices, $B$, found by each of the methods. Only the support of the matrix is displayed: a red value indicates a non-zero element; a grey value indicates a zero element.

(a) Truth  
(b) SSLB-IBP  
(c) BicMix  
(d) FABIA  
(e) Truth  
(f) SSLB-IBP  
(g) BicMix  
(h) FABIA

**Figure 7:** Simulation 2: (a) Boxplots of the consensus scores. (b) Relevance versus recovery scores.
Figure 8: Left: SSLB factor matrix where each row corresponds to a patient and each column corresponds to a bicluster. A patient belongs to a bicluster if they have a non-zero value in that column. Rows are ordered by clinical ER status; within ER status, rows are ordered by factor values in biclusters 1 and 2. Only the first 10 biclusters (ordered by size) are shown for improved visualization; full factor matrix is displayed in Section D. Middle: submatrix of gene expression values where rows correspond to all samples (ordered by ER status) and columns correspond to genes in Bicluster 1 (re-ordered according to their loadings in Bicluster 1). Expression values with magnitude greater than 0.25 have had magnitude set to 0.25 for improved visualization. Right: Clinical Estrogen Receptor (ER) status (Blue = ER-, Red = ER+).

B, we set the Beta-Binomial hyperparameters to $a = 1/(GK^*)$ and $b = 1$. This division by $G$ places an added emphasis on sparsity. For the factors, $X$, we set the IBP hyperparameter to $\tilde{\alpha} = 1/N$ with $d = 0$. For the remaining parameters, we use the default settings outlined in Section 2.5. SSLB-IBP found $\hat{K} = 30$ biclusters (Figure 8).

4.1 SSLB identifies subtypes of breast cancer

Breast cancers can be broadly grouped into subtypes based on the expression levels of two genes: ESR1, which encodes an estrogen receptor (ER), and ERBB2, which encodes the human epidermal growth factor receptor 2 (HER2) (Howlader et al., 2014). A patient is deemed ER-positive (-negative) if they have relatively high (low) expression levels of ESR1. HER2 status is similarly defined by the expression of ERBB2. The expression levels of these genes determine four subtypes of breast cancer: (i) ER+/HER2+, (ii) ER+/HER2-, (iii) ER-/HER2+ and (iv) ER-/HER2-. These subtypes have been shown to be valuable prognostic indicators and are used to determine the treatment protocol for patients (Howlader et al., 2014). The clinical ER status of patients (determined by immunohistochemical staining, not gene expression levels) was provided with the dataset.
ER+/HER2+  | ER+/HER2-  | ER-/HER2+  | ER-/HER2-
--- | --- | --- | ---
Onitilo et al. (2009) | 10.2% | 68.9% | 7.5% | 13.4%
SSLB | 7.7% | 70.3% | 8.9% | 13.1%

Table 2: Proportion of breast cancer patients in each of the subtypes determined by ER and HER2 status from (i) the study of Onitilo et al. (2009); and (ii) SSLB.

and so can provide a measure of validation for the biclusters that SSLB found. The HER2 status of patients was not recorded, however.

SSLB found four biclusters with significantly different means in the factors between the clinically ER-negative and ER-positive patients\(^3\). The patients with negative factors in SSLB bicluster 1 are almost all patients whose clinical status was recorded as ER-negative (Figure 8). We then investigated the genes in this bicluster and found ESR1, the gene encoding an estrogen receptor, was down-regulated for these patients. There are five patients with clinical ER-positive status who were in the ER-negative bicluster found by SSLB. However, the down-regulation of the ESR1 gene in this patients suggests that the original clinical characterization was a misclassification. In the original paper analyzing this data, Van De Vijver et al. (2002) also found five patients had a discrepancy between their clinical ER-status and gene expression determined ER status, concluding that the latter classification was correct.

The gene ERBB2 is present in SSLB biclusters 1 and 2. In both biclusters, ERBB2 is up-regulated for patients with positive factors and down-regulated for patients with negative factors. For patients with negative bicluster 1 and zero bicluster 2 factors, ESR1 and ERBB2 are both down-regulated, indicating ER-/HER2- status. Meanwhile, patients with negative bicluster 1 and positive bicluster 2 factors are likely ER-/HER2+. Turning to the ER-positive patients (with zero bicluster 1 values), those with positive bicluster 2 values are potentially ER+/HER2+. Finally, ER-positive patients with negative bicluster 2 factors are likely ER+/HER2-. We note that a number of patients are in neither bicluster 1 or 2; we hypothesize that these patients are also ER+/HER2- as this is the most common breast cancer subtype (Onitilo et al., 2009). The proportions of patients in each subtype found by SSLB matches fairly well with reported subtype proportions in the literature (Table 2).

After determining these groups, we then investigated whether genes known to play a role in these subtypes were present in the biclusters. In particular, genes considered to be indicators (or markers) of ER+ status are KRT8, GATA-3, XBP-1, FOXA1 and ADH1B (Zhang et al., 2014). Four of these five marker genes were down-regulated in bicluster 1, and consequently were relatively over-expressed for the ER+ patients (p-value 0.002, Fisher’s exact test). The gene GRB7 is located adjacent to the ERBB2 (HER2) gene and

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\(^3\)Biclusters 1, 2, 5 and 22 had p-values, $6.1 \times 10^{-50}$, $2.2 \times 10^{-9}$, $1.0 \times 10^{-5}$ and $7.2 \times 10^{-6}$, respectively, from a Wilcoxon rank-sum test with Bonferroni significance level $0.01/\hat{K}$.
as such is often co-expressed with ERBB2; we indeed found that GRB7 was up-regulated in bicluster 2 (as well as down-regulated for the HER2- patients in bicluster 1).

\section*{4.2 Gene Ontology Enrichment Analysis}

We next conducted gene ontology enrichment analysis on the genes found by SSLB using the R package \texttt{clusterProfiler} \citep{Yu2012}. This software conducts an overrepresentation test to determine whether genes which coordinate the same biological process are significantly co-occurring. If a subset of genes is found to be overrepresented in a set, the set is said to be “enriched” for the biological process in which those genes are active. With a false discovery rate (FDR) threshold of 0.05, we found that the genes which were up-regulated in SSLB bicluster 1 (corresponding to the ER-negative patients) were enriched for 124 biological processes. Many of these were related to cell proliferation, including the G1/S transition of mitotic cell cycle. As cancer is fundamentally the un-regulated growth of cells, such proliferation signatures are commonly found in tumor samples \citep{Whitfield2006}. Another biological process for which the ER-negative bicluster is enriched is: response to leukemia inhibitory factor. Leukemia inhibitory factor has actually been shown to stimulate cell proliferation in breast cancer \citep{Kellokumpu-Lehtinen1996}. An enrichment map summarizing the most statistically significant processes is displayed in Figure 14a (Section D of the Appendix).

The genes up-regulated in the HER2+ patients in SSLB bicluster 2 were enriched for 495 biological processes (again with FDR threshold of 0.05). The enrichment map summarizing these processes is displayed in Figure 14b (Section D of the Appendix). In particular, these genes were enriched for the Wnt signaling pathway, the over-expression of which has been implicated in the development of cancer \citep{Zhan2017}. Further, stem cell proliferation was enriched in this bicluster; stem cells have been implicated as possible originators of tumors, and may in some cases potentially drive tumorigenesis \citep{Reya2001}.

Overall, 86.6\% of the biclusters found by SSLB were enriched for biological processes. Further investigation of the remaining biclusters and their potential clinical utility may be interesting future work.

\section*{4.3 Comparison with BicMix and FABIA}

We ran BicMix on this data using the default settings; however, BicMix found zero biclusters. This is in contrast to the results of \citep{Gao2016} on this dataset: the difference here is because we did not use quantile normalization, unlike Gao et al. (2016) who projected the quantiles of the gene expression levels to the standard normal distribution.

We ran FABIA on this dataset using the default settings for two different bicluster ini-
Figure 9: Left: Clinical Estrogen Receptor (ER) status (Blue = ER-, Red = ER+). Middle: FABIA factor matrix (initial $K^* = 10$) with rows ordered by clinical ER status. Right: FABIA factor matrix (initial $K^* = 50$) with rows ordered by clinical ER status.

In the $K = 10$ setting, FABIA found five biclusters that had a significantly different mean between ER+ and ER- patients ($p$-values $3.9 \times 10^{-24}$, $2.5 \times 10^{-12}$, $9.2 \times 10^{-10}$, $4.8 \times 10^{-8}$, $1.7 \times 10^{-7}$ from Wilcoxon rank-sum test with Bonferroni significance level 0.01/10). Unlike SSLB, however, FABIA does not find a bicluster with almost exclusively ER-negative patients.

In the $K = 50$ setting, FABIA found three biclusters that had a significantly different mean between ER+ and ER- patients ($p$-values $2.3 \times 10^{-5}$, $5.7 \times 10^{-5}$, $2.0 \times 10^{-4}$ from Wilcoxon rank-sum test with Bonferroni significance level 0.01/50). We can see that with a larger number of initial biclusters, the ER signal is diluted across multiple biclusters. As a result, the conclusions of FABIA seem to be highly dependent on the initial number of biclusters. Further, for this larger value of $K$, FABIA also does not find a bicluster consisting of almost exclusively ER-negative patients. In contrast, SSLB was initialized with 50 biclusters and then determined $\hat{K} = 30$ biclusters were sufficient, and found a bicluster consisting of almost all ER-negative patients (apart from the five patients whose clinical measurement was most likely misclassified).
5 Mouse Cortex and Hippocampus scRNA-seq Dataset

For a second application, we assess the performance of SSLB on the data of Zeisel et al. (2015) (hereafter referred to as Z15). Z15 used single-cell RNA-sequencing (scRNA-seq) to obtain counts of RNA molecules in 3005 cells from the mouse somatosensory cortex and hippocampal CA1 region. The goal of the study was to characterize the RNA-expression levels in different cell-types of the mouse brain. Previously, cell types in the brain have been defined by alternative features such as location, morphology, and electrophysiological characteristics, combined with molecular markers Zeisel et al. (2015). Defining cell-types instead by expression levels requires clustering both the cells and the genes particularly associated with that cell cluster and as such is a biclustering problem.

Z15 developed a biclustering algorithm called BackSPIN which identified nine major types of cells in the mouse brain based on their transcription profiles: (i) interneurons; (ii) S1 pyramidal neurons; (iii) CA1 pyramidal neurons; (iv) oligodendrocytes; (v) microglia cells; (vi) endothelial cells; (vii) astrocytes; (viii) ependymal cells; and (ix) mural cells. By repeatedly applying BackSPIN on these biclusters, Z15 found a further 47 subclasses of cells. Here, we apply SSLB to the same dataset. A benefit of SSLB is that it can find classes and subclasses simultaneously without having to iteratively re-apply the method.

The scRNA-seq dataset made available by Z15 consists of RNA molecule counts for 19,972 genes in 3005 individual cells. Following these authors, we (i) removed genes with less than 25 molecules in total over all cells; (ii) removed genes that were not correlated with more than 5 other genes; and (iii) retained the top 5000 most biologically variable genes. Further details of these processing steps are given in Section E of the Appendix. Although more sophisticated methods for removing technical variability in scRNA-seq data have been developed in recent years (for example, Huang et al., 2018), we follow the steps of Z15 to enable a direct comparison of our biclustering results.

After processing the data, the subset we used for biclustering is a matrix containing the RNA counts of $G = 5000$ genes in $N = 3005$ individual cells. We note that as a matrix of counts, this data is perhaps best modeled by a Poisson distribution, instead of assuming normally distributed residuals as in SSLB. However, Poisson-distributed data with a large rate parameter is approximately normal. As we are considering the most variable genes (with high RNA molecule counts), such a normal approximation is not too unreasonable. Despite this, there are still a high proportion of zero entries in the matrix and so this application may be seen as a test of the robustness of SSLB to model misspecification.

We ran SSLB-IBP with the initial number of biclusters set to $K^* = 100$. as in Section 4, we set the Beta-Binomial hyperparameters to $a = 1/(GK^*)$ and $b = 1$, and the IBP hyperparameter to $\tilde{\alpha} = 1/N$ with $d = 0$. For the remaining parameters, we use the default settings outlined in Section 2.5. SSLB returned $\hat{K} = 95$ biclusters.

\footnote{http://linnarssonlab.org/cortex}
Figure 10: Zeisel dataset: SSLB results

(a) Left: Cell types found by Z15. Middle: Cell subtypes found by Z15. The rows colored black were not assigned a subtype by Z15. Right: SSLB factor matrix with rows ordered to correspond to the Z15 cell types. Each row corresponds to a cell and each column corresponds to a bicluster. A cell belongs to a bicluster if they have a non-zero value in the bicluster (column). Factor values have been capped for improved visualization.

(b) “Zoom in” on S1 Pyramidal cells with subtypes annotated by Z15. Top: subtypes of S1 Pyramidal cells. Bottom: Column 10 of the SSLB factor matrix, corresponding to the cells in bicluster 10. SSLB groups a subset of the uncategorized “(none)” cells as of the S1PyrL23 subtype. (Colors have been modified from Figure 10a for improved visualization.)

5.1 SSLB recovers major cell types

SSLB recovered the nine major cell classes identified by Z15, finding a specific bicluster for each class except for the microglia class, which SSLB split into two biclusters (Figure...
For each class, Z15 also identified one or two potential marker genes; that is, a gene that is almost exclusively expressed in that cell class. Encouragingly, the SSLB biclusters corresponding to the major cell classes all contained the associated marker gene for that cell class. More specifically:

- The interneuron gene marker *Pnoc* was found in three SSLB biclusters, one corresponding to the major interneuron cell class and the others to subclasses of interneurons.

- The S1 pyramidal neuron marker genes *Gm11549* and *Tbr1* were present in two biclusters, one corresponding to the major S1 pyramidal neuron cell class and the other to a subclass of S1 pyramidal neurons. *Tbr1* was also found in a bicluster containing cells from four different cell types, a potential false positive.

- The CA1 pyramidal neuron marker *Spink8* was found in three biclusters. Two of these biclusters corresponded to the major CA1 pyramidal neuron cell class and a subclass of CA1 pyramidal neurons, respectively. The third bicluster contained CA1 pyramidal, S1 pyramidal and interneuron cells, suggesting that *Spink8* may not necessarily be an exclusive marker for CA1 pyramidal neurons.

- The oligodendrocyte marker *Hapln2* was active in three SSLB biclusters, all corresponding to either the major oligodendrocyte cell class or a subclass of oligodendrocytes. Interestingly, one of these biclusters contained 17 cells, all oligodendrocytes, but did not correspond to one of the Z15 identified subclasses; as such, this bicluster may correspond to a yet-to-be classified subtype of oligodendrocytes. Figure 17 shows the biological processes that are enriched in this bicluster, which can be broadly grouped into two categories: (i) processes related to oligodendrocyte-specific functions, including myelination, and (ii) cell metabolic processes.

- The endothelial cell marker *Ly6c1* was found in four SSLB biclusters, two corresponding to the major endothelial group or a subclass. The other two biclusters were mostly all endothelial cells, but contained some astrocytes and microglia cells also.

- The mural cell marker *Acta2* was active in three SSLB biclusters. One bicluster corresponded to the main mural bicluster and another to a bicluster with almost all mural cells. The third bicluster contained mostly endothelial cells, with a few oligodendrocyte, microglia, astrocyte and mural cells, indicating that either *Acta2* is not exclusively expressed in mural cells, or a potential false positive of SSLB.

In addition to the nine main cell types, SSLB found two biclusters (biclusters 1 and 2) which contained many interneurons, S1 pyramidal neurons and CA1 pyramidal neurons. This is unsurprising as these cell types are all subsets of neurons, and so we would expect them to have more similar expression profiles than the other (non-neuronal) brain cells. We
conducted gene ontology enrichment analysis on the genes SSLB found in these biclusters. With an FDR threshold of 0.05, bicluster 1 was enriched for 154 biological processes, the majority of which were related to cell metabolic processes and synaptic activity, as may be expected for neurons (Figure 16a). Bicluster 2 was similarly enriched for processes relating to synaptic activity, including axonal transport and synaptic signaling (Figure 16b).

The results of SSLB yield a number of observations that may warrant further scientific investigation. Firstly, while SSLB recovered the major cell types, it grouped together a number of the 47 sub-categories found by Z15. This was particularly the case for the interneuron cells, where SSLB found 5 subtypes (Z15 found 16), and the S1 pyramidal cells, where SSLB found 3 subtypes (Z15 found 12). It may be the case that SSLB has trouble finding more granular clusters, or potentially there really are fewer cell subtypes than identified by Z15.

Although SSLB collapsed many of the interneuron and S1 pyramidal subtypes, it found many more subtypes of microglia and ependymal cells than Z15. This suggests that there could be a great deal of heterogeneity in expression levels in these classes of cells, a phenomenon which may prove to be of scientific interest.

There are a number of cells which Z15 did not assign to a subtype (colored in black in Figure 10a). Interestingly, SSLB grouped a number of the previously unclassified S1 pyramidal cells into the “S1PyrL23” subtype (Figure 10b).

Finally, we conducted gene ontology enrichment analysis\(^5\) for all of the biclusters found by SSLB. In this analysis, 83% of the biclusters identified by SSLB were enriched for at least one biological process.

5.2 Comparison with BicMix and FABIA

We also applied both BicMix and FABIA to the Zeisel dataset. We used the default settings for both methods with initial number of clusters \(K^* = 100\). For BicMix, we thresholded values less than \(10^{-10}\) as recommended by Gao et al. (2016). For FABIA, we implemented the recommended post-processing thresholding step (Hochreiter et al., 2010). BicMix found \(\hat{K} = 94\) biclusters (Figure 11) while FABIA found \(\hat{K} = 99\) biclusters (Figure 15 in Section F of the Appendix). BicMix finds many of the smaller subtypes defined by Z15 but assigns the major cell type signals to dense biclusters. This is a result of the dichotomous nature of BicMix; it finds either completely dense or very sparse biclusters. In contrast, SSLB can adapt to the underlying sparsity, allowing it to also estimate such "medium"-sized biclusters. Meanwhile, FABIA finds many larger biclusters but does not

\(^5\)Using \texttt{clusterProfiler} with FDR threshold of 0.05. We took the 5000 genes obtained after processing as the “background” genes for the overrepresentation test instead of the original number of 19,972 to avoid selection bias.
do well at recovering the more granular cell subtypes. This is due to FABIA having the same thresholding parameter for each bicluster; it is unable to adapt to the differing levels of sparsity.

Figure 11: Factor matrix found by BicMix. On the side of the factor matrix are the cell types and subtypes found by Z15, respectively. The rows of the factor matrices have been ordered to correspond to the Zeisel cell types. Factor values have been capped for improved visualization.

6 Conclusion

In this paper, we introduced a new method for biclustering called Spike-and-Slab Lasso Biclustering (SSLB). SSLB finds subsets of samples which co-vary on subset of features. These paired subsets manifest as rank-1 submatrices in the data, referred to as “biclusters” in this setting. To find these biclusters, SSLB performs two-way subset selection to conduct doubly-sparse factor analysis in which both the loadings and the factors are sparse. To induce this sparsity in the loadings and factors, SSLB uses the Spike-and-Slab Lasso prior
of Ročková and George (2018). This prior is combined with an Indian Buffet Process prior to automatically choose the number of biclusters. SSLB utilizes a fast EM algorithm with a variational step to find the modes of the posterior. This EM algorithm is rendered tractable by a novel augmentation of the Spike-and-Slab Lasso prior.

SSLB features a number of benefits over similar biclustering methods. Firstly, the adaptivity inherent in the Spike-and-Slab Lasso prior allows for SSLB to find a continuum of biclusters of different sizes. This is in contrast to other biclustering methods which have more restrictive assumptions on the sizes of the biclusters. Secondly, the Spike-and-Slab Lasso prior automatically thresholds negligible bicluster values to zero; this is unlike other biclustering methods which require post-processing thresholding steps.

SSLB out-performs a number of alternative biclustering methods on a variety of simulated data. On the breast cancer microarray dataset of Van De Vijver et al. (2002); Van’t Veer et al. (2002), SSLB finds biclusters corresponding to different subtypes of breast cancer. These biclusters also contained genes which were enriched for a variety of biological processes related to breast cancer. Finally, we applied SSLB to the mouse cortex and hippocampus single-cell RNA-sequencing dataset of Zeisel et al. (2015). SSLB recovered all the major cell classes found by Zeisel et al. (2015) as well as many of the cell subclasses. This performance was achieved despite the non-Gaussianity of the residual noise in the data, highlighting the potential robustness of SSLB to model misspecification. However, it would be interesting to explicitly extend SSLB to non-Gaussian residual noise models in future work. The SSLB software is available as an R/C++ package at https://github.com/gemoran/SSLB. Code to reproduce the results in this paper can also be found at https://github.com/gemoran/SSLB-examples.

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### A SSLB Algorithm

In this section, we provide details for the EM algorithm we use to find the modes of the posterior. Before outlining the EM algorithm, we first marginalize over the binary indicator variables $\Gamma$ (associated with the loadings $B$) to yield the non-separable Spike-and-Slab Lasso prior (Ročková and George, 2018). For each column $\beta_k$, the log of this prior (up to an additive constant) is:

$$\log \pi(\beta_k) = \sum_{j=1}^{G} -\lambda_1|\beta_{jk}| + \log[p^*(0; \theta_{jk})/p^*(\beta_{jk}; \theta_{jk})], \tag{A.1}$$

where $p^*(\beta; \theta) = \theta \psi(\beta|\lambda_1) + (1 - \theta) \psi(\beta|\lambda_0)$ \tag{A.2}

and $\theta_{jk} = E[\theta_k|\beta_{k\setminus j}]$ where $\beta_{k\setminus j}$ denotes the vector $\beta_k$ with the $j$th element removed.

When $G$ is large, $\beta_{k\setminus j}$ is very similar to $\beta_k$, so this expectation may be approximated by $E[\theta_k|\beta_k]$.

We are now in a position to describe the EM algorithm. We find the expectation of $X$ and factor indicators $\bar{\Gamma}$ with respect to the complete log posterior and then maximize the
Now, due to the separability of the parameters in the posterior, we may write
\[ Q(\Delta) = \mathbb{E}_{\textbf{X},\Gamma|\Delta^{(o)}, Y} \left[ \log \pi(\Delta, \textbf{X}, \Gamma|Y) \right], \] (A.3)
where we have used the notation \( \Delta = \{ \textbf{B}, \Sigma, \textbf{T}, \nu \} \) to denote the parameters over which we will maximize. For convenience, we will use the notation \( \mathbb{E}_{\textbf{X},\Gamma|\Delta^{(o)}, Y}(Z) = \langle Z \rangle \).

Now, due to the separability of the parameters in the posterior, we may write
\[ Q(\Delta) = Q_1(\textbf{B}, \Sigma) + Q_2(\textbf{T}, \nu) + Q_3(\nu) + C, \] (A.4)
where \( Q_1(\textbf{B}, \Sigma) = \langle \pi(\textbf{B}, \Sigma, \Gamma, \textbf{X}|Y) \rangle \), \( Q_2(\tau, \nu) = \langle \pi(\textbf{X}, \textbf{T}, \Gamma, \nu|Y) \rangle \), \( Q_3(\nu) = \langle \pi(\nu, \Gamma|Y) \rangle \) and \( C \in \mathbb{R} \) is a constant.

The first term of the above objective function is:
\[ Q_1(\textbf{B}, \Sigma) = C - \frac{1}{2} \sum_{i=1}^{N} \left\{ (\textbf{y}_i - \textbf{B}\langle \textbf{x}_i \rangle)^T \Sigma^{-1} (\textbf{y}_i - \textbf{B}\langle \textbf{x}_i \rangle) + \text{tr}[\textbf{B}'\Sigma^{-1}\textbf{B} (\langle \textbf{x}_i \rangle - \langle \textbf{x}_i \rangle')^T] \right\} \\ - \sum_{k=1}^{K^*} \log \pi(\beta_k) - \frac{N + \eta + 2}{2} \sum_{j=1}^{G} \log \sigma_j^2 - \frac{\eta \xi}{2 \sigma_j^2}, \]
where \( \pi(\beta_k) \) is defined in (A.1). Next,
\[ Q_2(\textbf{T}) = -\frac{1}{2} \sum_{i=1}^{N} \left\{ (\langle \textbf{x}_i \rangle^T \textbf{D}_i \langle \textbf{x}_i \rangle + \text{tr}[\textbf{D}_i (\langle \textbf{x}_i \rangle - \langle \textbf{x}_i \rangle')^T] \right\} - \frac{1}{2} \sum_{i=1}^{N} \sum_{k=1}^{K^*} \log \tau_{ik} \\ - \frac{1}{2} \sum_{i=1}^{N} \sum_{k=1}^{K^*} \left[ (\bar{\gamma}_{ik})^2 + (1 - (\bar{\gamma}_{ik})) \bar{\lambda}_0^2 \right] \tau_{ik}. \] (A.5)

and finally,
\[ Q_3(\nu) = \sum_{k=1}^{K^*} \left[ (\bar{\gamma}_k) \log \prod_{l=1}^{k} \nu_l + (N - (\bar{\gamma}_k)) \log \left( 1 - \prod_{l=1}^{K} \nu_l \right) \right] \\ + \sum_{k=1}^{K^*} [(\bar{\alpha} + kd - 1) \log \nu_k - d \log(1 - \nu_k)]. \] (A.6)

where \( \langle \bar{\gamma}_k \rangle = \sum_{i=1}^{N} (\bar{\gamma}_{ik}) \).

**A.0.1 E-Step**

The conditional posterior distribution of \( \textbf{x}_i \) is given by:
\[ \pi(\textbf{x}_i|\textbf{B}^{(t)}, \Sigma^{(t)}, \textbf{T}^{(t)}, \textbf{y}_i) \sim N(\textbf{V}^{(t)} \Sigma^{(t)^{-1}} \textbf{y}_i, \textbf{V}^{(t)}), \] (A.7)
where \( \mathbf{V}^i = [\mathbf{B}^{(t)}_i][\Sigma^{(t)}]^{-1}\mathbf{B}^{(t)} + \mathbf{D}_i^{(t)}]^{-1} \). Further, let \( \mathbf{V} = \sum_{i=1}^{N} \mathbf{V}^i \).

We now determine the update for the indicators of the factors, \( \tilde{\Gamma} \). Note that conditional on \( \tau_{ik}; \tilde{\gamma}_{ik} \) is independent of \( x_{ik} \). We have:

\[
\langle \tilde{\gamma}_{ik} \rangle = P(\tilde{\gamma}_{ik} = 1|\mathbf{T}, \tilde{\theta})
= \frac{\pi(\tau_{ik}|\tilde{\gamma}_{ik} = 1)\pi(\tilde{\gamma}_{ik} = 1|\theta_k)}{\pi(\tau_{ik}|\tilde{\gamma}_{ik} = 1)\pi(\tilde{\gamma}_{ik} = 1|\theta_k) + \pi(\tau_{ik}|\tilde{\gamma}_{ik} = 0)\pi(\tilde{\gamma}_{ik} = 0|\theta_k)}
= \frac{\tilde{\theta}_k \lambda^2 \exp(-\lambda^2 \tau_{ik}/2)}{\tilde{\theta}_k \lambda^2 \exp(-\lambda^2 \tau_{ik}/2) + (1 - \tilde{\theta}_k) \lambda^2 \exp(-\lambda^2 \tau_{ik}/2)}.
\]  

(A.8)

A.0.2 M-Step

Let \( y^1, \ldots, y^G \) be the columns of \( \mathbf{Y} \). Denote \( \langle \mathbf{X} \rangle = [(x_1), \ldots, (x_N)] \) and let \( \beta_1, \ldots, \beta_G \) be the rows of \( \mathbf{B} \). Then

\[
Q_1(\mathbf{B}, \Sigma) = \sum_{j=1}^{G} Q_j(\beta_j, \sigma_j)
\]

(A.9)

where

\[
Q_j(\beta_j, \sigma_j) = -\frac{1}{2\sigma_j^2} \|y^j - \mathbf{X}\beta_j\|^2 - \frac{1}{2\sigma_j^2} \beta_j^T \mathbf{V} \beta_j - \sum_{k=1}^{K^*} \log \pi(\beta_k) - \frac{N + \eta + 2}{2} \log \sigma_j^2 - \frac{\eta \xi + 2}{2\sigma_j^2}
\]

(A.10)

To find a maximum of (A.10) with regard to \( \beta_j \), we use the refined thresholding scheme of Rocková and George (2018) with the extension to the unknown variance case given in Moran et al. (2018). Evaluation of \( \log \pi(\beta_k) \) requires the expectation of \( \theta_k \) given the previous values of the loadings, \( \beta_k^{(t-1)} \); this yields the following update for \( \theta_k \) (Rocková and George, 2018):

\[
\theta_k^{(t)} = \frac{a + \|\beta_k^{(t-1)}\|_0}{a + b + G}.
\]

(A.11)

The update for \( \sigma_j^2 \) is:

\[
\sigma_j^{2(t)} = \frac{\|y^j - \mathbf{X}\beta_j^{(t)}\|^2 + \beta_j^{(t)T} \mathbf{V} \beta_j^{(t)} + \eta \xi}{N + \eta + 2}.
\]

(A.12)
The update for \( \tau_{ik} \) is given by:

\[
\tau_{ik}^{(t)} = -1 + \sqrt{1 + 4\tilde{\lambda}_{ik}((x_{ik})^2 + V_{kk})}
\]

(A.13)

where \( \tilde{\lambda}_{ik} = \langle \tilde{\gamma}_{ik} \rangle \tilde{\lambda}_1^2 + (1 - \langle \tilde{\gamma}_{ik} \rangle)\tilde{\lambda}_0^2 \).

We now consider the update for the IBP stick-breaking parameters \( \nu \). This involves finding the \( \nu \) that maximize the objective in equation \( Q_3(\nu) \). The difficulty in maximizing this objective is the non-linear term \( \log \left( 1 - \prod_{l=1}^{k} \nu_l \right) \). We find a lower bound for this term using a variational approximation inspired by Doshi et al. (2009).

This approximation begins with writing the non-linear term as a telescoping sum. Then, we introduce a parameter \( q_k = (q_{k1}, \ldots, q_{kk}) \) where \( \sum_{m=1}^{k} q_{km} = 1 \), which allows the use of Jensen’s inequality:

\[
\log \left( 1 - \prod_{l=1}^{k} \nu_l \right) = \log \left( \sum_{m=1}^{k} (1 - \nu_m) \prod_{l=1}^{m-1} \nu_l \right) \\
= \log \left( \sum_{m=1}^{k} q_{km} \frac{(1 - \nu_m) \prod_{l=1}^{m-1} \nu_l}{q_{km}} \right) \\
\geq \sum_{m=1}^{k} q_{km} \left[ \log(1 - \nu_m) + \sum_{l=1}^{m-1} \log \nu_l \right] - \sum_{m=1}^{k} q_{km} \log q_{km}. 
\]

(A.14)

To make the bound (A.14) as tight as possible, we maximize over the parameter \( q_k \) to obtain updates \( \hat{q}_{(t)} \):

\[
\hat{q}_{km}^{(t)} = \frac{(1 - \nu_{m}^{(t-1)}) \prod_{l=1}^{m-1} \nu_l^{(t-1)}}{1 - \prod_{l=1}^{k} \nu_l^{(t-1)}}. 
\]

(A.15)

The lower bound for the objective function for \( \nu \) at iteration \( t \) is now:

\[
Q_3(\nu) \geq \sum_{k=1}^{K^*} \left[ \langle \tilde{\gamma}_k \rangle \sum_{l=1}^{k} \log \nu_l + (N - \langle \tilde{\gamma}_k \rangle) \left[ \sum_{m=1}^{k} q_{km}^{(t)} \left( \log(1 - \nu_m) + \sum_{l=1}^{m-1} \log \nu_l \right) \right] \right] \\
+ \sum_{k=1}^{K^*} [\bar{\alpha} + kd - 1] \log \nu_k - d \log(1 - \nu_k). 
\]

(A.16)

Maximizing the lower bound (A.16) over \( \nu \) then yields closed form updates:

\[
\nu_k^{(t)} = \frac{r_k^{(t)}}{r_k^{(t)} + s_k^{(t)}}. 
\]

(A.17)
where
\[
    r_k^{(t)} = \frac{K^*}{m=k} \sum (\tilde{\gamma}_k) + \frac{K^*}{m=k+1} (N - \langle \tilde{\gamma}_k \rangle) \left( \sum_{i=k+1}^{m} q_{mi}^{(t)} \right) + \bar{\alpha} + kd - 1 \tag{A.18}
\]
\[
    s_k^{(t)} = \frac{K^*}{m=k} (N - \langle \tilde{\gamma}_k \rangle) q_{mk}^{(t)} - d. \tag{A.19}
\]

B Bicluster Quality Metrics

Here we provide the formulas for the (i) relevance; (ii) recovery; and (iii) consensus scores used to evaluate biclusters in the simulation studies. Each of these scores use the Jaccard index, a measure of similarity between two sets \( A \) and \( B \), defined as:
\[
    J(A, B) = \left| \frac{A \cap B}{A \cup B} \right|. \tag{B.1}
\]

The Jaccard index naturally penalizes methods which find spurious bicluster elements. The relevance and recovery scores were proposed by Prelić et al. (2006) and are defined below. Denote bicluster \( C_k \) as the set non-zero entries of the vectorized matrix \( x_k^{(t)} \beta_k^{(t)} \). Let \( M_t \) be the set of true biclusters and let \( M_f \) be the set of biclusters found by a particular method. Then the relevance and recovery scores are given by:

\[
    \text{Relevance} = \frac{1}{|M_f|} \sum_{C_1 \in M_f} \max_{C_2 \in M_t} J(C_1, C_2),
\]
\[
    \text{Recovery} = \frac{1}{|M_t|} \sum_{C_2 \in M_t} \max_{C_1 \in M_f} J(C_1, C_2).
\]

The consensus score of Hochreiter et al. (2010) is computed as follows.

1. Compute the Jaccard similarity matrix, where the \((i, j)\)th entry is the Jaccard similarity score (B.1) between the \(i\)th bicluster in \( M_t \) and the \(j\)th bicluster in \( M_f \);
2. Find the optimal assignment (based on the highest Jaccard scores) of the true set of biclusters to the found set of biclusters using the Hungarian algorithm (Munkres, 1957);
3. Sum the similarity scores of the assigned biclusters and divide by \( \max\{|M_t|, |M_f|\} \).
C Processing Breast Cancer Data

Here, we provide more details on the processing of the breast cancer dataset in Section 4. We first removed genes with more than 10% of values missing and imputed the remaining missing values with $k$ nearest neighbors ($k = 10$), implemented using the R package `impute` (Hastie et al., 2018). We chose not to project the quantiles of the gene expression levels to the standard normal distribution, as done by Gao et al. (2016). This is because the unnormalized gene expression values were mostly clustered around zero with heavy tails (Figure 12a). Although SSLB assumes that the errors are normally distributed, the gene loadings $\{\beta_{jk}\}_{j,k=1}^{G,K}$ are assumed to be drawn a priori from either a Laplacian spike concentrated around zero or a Laplacian slab. We assume that such a mixture model is flexible enough to model the gene expression levels exemplified in Figure 12a.

![Graphs](image)

**Figure 12:** Histogram of (a) unnormalized expression values for gene $SUHW2$, (b) quantile normalized expression values for gene $SUHW2$ with standard normal distribution as reference. For both histograms, a standard normal density is overlaid.

D Supplementary Figures for Breast Cancer Dataset

Here, we provide supplementary figures for the analysis of the breast cancer microarray dataset in Section 4. Enrichment maps (Figure 14) were created using the R package `enrichplot` (Yu, 2018) and display the top 30 biological processes (with lowest FDR $q$-values satisfying threshold of 0.05) found in the gene ontology enrichment analysis as described in Section 4.2.
Figure 13: SSLB factor matrix where each row corresponds to a patient and each column corresponds to a bicluster. A patient belongs to a bicluster if they have a non-zero value in that column. Rows are ordered by clinical ER status; within ER status, rows are ordered by factor values in biclusters 1 and 2. All 30 biclusters found by SSLB are shown.

E Processing Zeisel Dataset

Here, we describe how we processed the data in Section 5. We followed the same pipeline as Z15 but provide the details here for completeness.

Many RNA-seq studies normalize the raw count data to the unit RPKM (Reads Per Kilo-base of transcript per Million mapped reads), which accounts for longer genes having more transcripts mapped to them simply due to their length (and not meaningful biological variability). This was unnecessary for this dataset as only the 5’ end of each RNA was sequenced and thus the read number was not proportional to gene length (Islam et al., 2014). Additionally, many single-cell RNA-seq studies account for differing cell sizes as larger cells have more RNA. However, this normalization was not done for this dataset as such information is informative in clustering different cell types.

The scRNA-seq data is provided by Z15 at http://linnarssonlab.org/cortex and consists of molecule counts for 19,972 genes in 3005 individual cells.

Following Z15, we:

1. Removed all genes that have less than 25 molecules in total over all cells

2. Calculated correlation matrix over the genes and define a threshold as 90th percentile of this matrix ($\rho = 0.2091$). Removed all genes which have less than 5 other genes which correlate more than this threshold.
Figure 14: Breast cancer data; enrichment maps for SSLB genes (a) up-regulated in ER-negative patients, and (b) up-regulated in HER2+ patients. Nodes represent biological processes; size of node reflects number of genes in process which were found by the method. Edges connect genes that are active in different biological processes.
The next step of data processing was to identify the noisiest genes. Assuming that most of the variability of the genes across the cells can be attributed to the underlying biological processes, these genes are the ones which are most informative for clustering of cells. The strategy of Z15 was to search for genes whose noise - measured by coefficient of variation (CV, standard deviation divided by mean) - was high compared to a Poisson distribution with inflated CV. The rationale for this was outlined in Islam et al. (2014) which used the same single-cell RNA-seq protocol as Z15 but for mouse embryonic stem cells. First, Islam et al. (2014) noted that the technical noise distribution of ERCC (External RNA Controls Consortium) spike-in molecules (which have no biological variability) followed that of a Poisson, but its CV was inflated by constant factor. The CVs of endogenous genes were inflated above those of the ERCCs, suggesting that this variation is driven by biological factors rather than the variation induced by loss of transcripts in cDNA synthesis.

Z15 implemented the same procedure to identify genes with the greatest biological variability. We followed this procedure: for the genes remaining after the aforementioned data cleaning steps, the mean and CV was calculated. The noise model

$$\log_2(CV) = \log_2(mean^\alpha + k)$$

was fit using the software ceftools\(^6\). The best fit was found to be $\alpha = -0.55$ and $k = 0.64$. Next all genes were ranked by their distance from the fit line and the top 5000 genes with the largest distance were selected as informative for further clustering.

Finally, we normalized the gene counts using quantile normalization (using the R package preprocessCore (Bolstad, 2018)). Note we used the commonly used “average distribution” as the reference distribution to which to project the quantiles of the raw gene expression levels. The average distribution is obtained by taking the average of each quantile across the samples (Bolstad et al., 2003).

**F Supplementary Figures for Zeisel Dataset**

Here, we provide supplementary figures for the analysis of the mouse single-cell RNA sequencing dataset in Section 5. Enrichment maps (Figures 16 and 17) were created using the R package enrichplot (Yu, 2018) and display the top 30 biological processes (with lowest FDR $q$-values satisfying threshold of 0.05) found in the gene ontology enrichment analysis as described in Section 5.1.

\(^6\)https://github.com/linnarsson-lab/ceftools
Figure 15: Zeisel dataset: Factor matrix found by SSLB (top) and FABIA (bottom). On the side of the factor matrix are the cell types and subtypes found by Z15, respectively. The rows of the factor matrices have been ordered to correspond to the Zeisel cell types. Factor values have been capped for improved visualization.
Figure 16: Zeisel dataset: enrichment maps for SSLB genes in (a) bicluster 1 and (b) bicluster 2. Each bicluster contains a mixture of interneurons, S1 pyramidal neurons and CA1 pyramidal neurons. Nodes represent biological processes; size of node reflects number of genes in process which were found by the method. Edges connect genes that are active in different biological processes.
Figure 17: Zeisel dataset: enrichment map for genes in SSLB bicluster 44. Bicluster 44 contains 17 oligodendrocyte cells. Nodes represent biological processes; size of node reflects number of genes in process which were found by the method. Edges connect genes that are active in different biological processes.