**Bayesian clustering and deconvolution of expression profiles in spatial transcriptomics**

**Abstract**

**Introduction**

Single-cell RNA sequencing (scRNA-seq) achieves high-throughput and high-resolution profiling of gene expression, but sample preparation results in the loss of spatial information. Knowledge of the spatial location of transcripts can provide vital insights into biological function and pathology. Fortunately, technological advances have allowed for high-throughput profiling of gene expression while retaining spatial information (Stahl 2016). Such spatially resolved transcriptomic profiling allows analyses to be made within the context of the biological tissue. Studies done on the Spatial Transcriptomics (ST) platform and the closely related Visium platform have already generated insights into diverse areas such as tumor heterogeneity (Thrane 2018, Berglund 2018), brain function (Maynard 2020), and the pathophysiology of sepsis (Janosevic 2020). The primary technological limitation of these spatial gene expression platforms is resolution, with the unit of observation being spots that are 100μm in diameter on the ST platform and 55μm in diameter on the Visium platform. While it is possible that a spot contains only a single cell, typically multiple cells will contribute to the captured gene expression at a spot. The number of cells within a spot may range from 1 to 200 depending on the biological tissue and the technological platform (Saiselet 2020).

Presently, there is a need for statistical methods developed for the analysis of spatial gene expression data that efficiently leverage the available spatial information. Clustering is an important step in the spatial gene expression analysis pipeline that allows downstream analyses such as cell type or tissue annotation and differential expression to provide unbiased biological insights. Existing analyses of spatial gene expression data often rely primarily on clustering methods for non-spatial RNA-seq data (Thrane 2018, Maynard 2020). The additional spatial information available from ST and Visium can help address the challenges to analysis brought by sparsity and noise by smoothing over adjacent spots, which are more likely to have similar transcriptomic profiles. Zhu et al. (2018) proposed a hidden Markov random field model (HMRF) for clustering of low-resolution *in situ* hybridization data into distinct spatial domains by jointly modeling gene expression and the spatial neighborhood structure. HMRF was later adapted for use with high-throughput spatial transcriptomics data through the selection of spatially differentially expressed genes prior to clustering (Dries 2020). Here, we propose BayesSpace, a fully Bayesian spatial clustering method that models a low-dimensional representation of the gene expression matrix and encourages neighboring spots to belong to the same cluster via a spatial prior. From a modeling perspective, BayesSpace allows for a more flexible specification of the error term. From a user perspective, BayesSpace is accessible in that it takes as input the widely used SingleCellExperiment object as input, does not require the additional task of preselection of marker genes, and involves minimal parameter tuning.

In addition, there is a need for spatial gene expression methods that address the relatively low resolution of the technology. Existing spatial gene expression deconvolution methods build upon bulk RNA-seq deconvolution methods and depend on additional scRNA-seq data (Andersson 2019, Cable 2020). While integration with scRNA-seq is appealing, it may be costly if using matched samples or introduce bias if using publicly available references. Furthermore, the deconvolved mixtures are still only spatially resolved at the original scale of the ST or Visium technology. We extend BayesSpace to deconvolve spots without external scRNA-seq data by leveraging the spatial neighborhood structure to resolve expression at a sub-spot level.

PLACEHOLDER FOR FIGURE 1: (A) diagram of analysis workflow, B) clustering cartoon C) deconvolution cartoon)

**Methods**

*Clustering model*

For each spot , a low -dimensional representation (e.g. principal components) of the gene expression vector can be obtained. We assume that

where the errors . Here, denotes the latent cluster that belongs to, and denote the mean vector and precision matrix for cluster , and denotes the weight of . We place the following priors on and :

where denotes a fixed degrees of freedom parameter to control the heaviness of tails. We also assume and are independent. We can update and via Gibbs sampling from the posterior distributions:

Given spots and a set cluster number , the cluster label vector can take values in and the Metropolis-Hastings algorithm can be used to explore this parameter space. For each spot , a new cluster label is proposed from and accepted or rejected based on the ratio of posterior distributions , where the likelihood is given by and the spatial smoothing prior is given by the Potts model:

where denotes all spots that are neighbors of , and is a fixed parameter controlling the strength of the smoothing. Given that ST and Visium spots are arranged on a regular lattice, there is a natural way to define spatial neighbors. ST spots can have up to 4 neighbors while Visium spots can have up to 6.

Estimation of the parameters is done using a Markov chain Monte Carlo (MCMC) method. We initialize using a non-spatial clustering method such as *k*-means or mclust. Then, iteratively and sequentially, each , and is updated via Gibbs sampling and each is updated via Metropolis-Hastings as described above. After iterating for a fixed number of iterations, the mode of the chain for each is assigned as the cluster label for the corresponding spot .

Note that , a multivariate *t*-distribution with a fixed degrees of freedom which allows for a more robust error model. Two possible simplifications of the model include 1) forcing all clusters to have the same precision matrix and 2) fixing all weights to be , resulting in Gaussian marginal errors.

*Deconvolution model*

Relative to the clustering method, the model specification and parameter estimation is largely similar for deconvolution, though the unit of analysis is now a sub-spot rather than a spot. Each ST spot can be segmented into 9 sub-spots while each Visium spot can be segmented into 7 sub-spots. Since gene expression is not observed at the sub-spot level, it is modeled as another latent variable that is iteratively updated. The latent expression of each sub-spot that is part of spot is denoted , initialized to be ,and then updated via Metropolis-Hastings. In each iteration and for each spot, the new proposal is given by for each sub-spot such that where is a small fixed parameter and .In effect, this jitters the latent expression value of each sub-spot within a spot while keeping the total expression of the spot fixed. The proposal is accepted or rejected based on the conditional likelihood of the proposal given the other parameters. A weak Gaussian prior is placed on the latent expression to ensure that the jittered values do not drift too far away from . Aside from replacing with , all other steps of the MCMC algorithm remain the same as in the clustering method.

**Results**

PLACEHOLDER FOR FIGURE 2: brain layer maynard 2020 data, ARI as evaluation metric, spatial clustering vs. k-means, knn, mclust, HMRF

PLACEHOLDER FOR FIGURE 3: Benchmark of simulated data: spatial clustering vs. k-means, knn, mclust, HMRF

PLACEHOLDER FOR FIGURE 4: Deconvolution w/ real melanoma data from Thrane2018 and simulated data from Thrane2018 melanoma)

PLACEHOLDER FOR FIGURE 5: Integration with scRNAseq data (MIBI and/or Moncada pancreatic cancer data)

**Discussion**

**Conclusion**

**References**