Nonnegative spatial factorization

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Abstract

Gaussian processes are widely used for the analysis of spatial data due to their nonparametric flexibility and ability to quantify uncertainty, and recently developed scalable approximations have facilitated application to massive datasets. For multivariate outcomes, linear models of coregionalization combine dimension reduction with spatial correlation. However, their real-valued latent factors and loadings are difficult to interpret because, unlike nonnegative models, they do not recover a parts-based representation. We present nonnegative spatial factorization (NSF), a spatially-aware probabilistic dimension reduction model that naturally encourages sparsity. We compare NSF to real-valued spatial factorizations such as MEFISTO (Velten et al., 2020) and nonspatial dimension reduction methods using simulations and high-dimensional spatial transcriptomics data. NSF identifies generalizable spatial patterns of gene expression. Since not all patterns of gene expression are spatial, we also propose a hybrid extension of NSF that combines spatial and nonspatial components, enabling quantification of spatial importance for both observations and features. A TensorFlow implementation of NSF is available from https://github.com/willtownes/nsf-paper

Keywords: spatial, multivariate, dimension reduction, nonnegative, Gaussian process, spatial transcriptomics

1. Introduction

Spatially-resolved transcriptomics (ST) has revolutionized the study of intact biological tissues (Moses and Pachter, 2021; Editors, 2021; Maynard et al., 2021). In contrast to single-cell RNA sequencing (scRNA-seq), which dissociates cells before sequencing each one, ST quantifies gene expression while preserving the spatial context of the cells within the tissue sample. Since the state and function of each cell is highly dependent upon interactions with its neighbors (Verma et al., 2021), measuring spatially-resolved transcription represents a crucial advance in our ability to understand cellular state and interactions.

Like scRNA-seq, ST data generally consist of discrete counts of transcripts from tens to thousands of genes, many of which are zero. Also, in both techniques there is typically no ground truth assignment of cell types. There are two basic strategies to measure spatial gene expression: microscopy and bead capture. Microscopy approaches have excellent spatial resolution, even at the sub-cellular level, but require specialized equipment and may not capture large numbers of cells or genes easily. Examples of microscopy protocols include seqFISH+ (Eng et al., 2019) and MERFISH (Xia et al., 2019). Protocols based on bead capture and sequencing tend to have coarser spatial resolution but cover larger spatial areas or larger numbers of genes.
use equipment and experimental procedures that are similar to single-cell RNA-seq (scRNA-seq). Examples of bead protocols include high definition spatial transcriptomics (HDST) (Vickovic et al., 2019), Slide-seqV2 (Stickels et al., 2021), and the Visium platform from 10x genomics.

Dimension reduction (DR) is a vital tool for unsupervised learning, and there has been a proliferation of DR methods for both scRNA-seq (Wolf et al., 2017; Butler et al., 2018; Sun et al., 2019) and ST (Palla et al., 2021; Dries et al., 2021). DR based on a Gaussian error assumption, such as principal components analysis (PCA) (Hotelling, 1933) and factor analysis (Bartholomew et al., 2011), is often computationally fast, but requires elaborate normalization procedures that may systematically distort the count data from sequencing technologies (Hicks et al., 2018; Townes et al., 2019a). For example, this has led to confusion about whether zero-inflated distributions are needed to analyze scRNA-seq data, or whether the high number of zeros is consistent with a simpler Poisson or negative binomial count distribution (Svensson, 2020; Kim et al., 2020; Sarkar and Stephens, 2020). To avoid normalization and its pitfalls, DR approaches such as scVI (Lopez et al., 2018), CPLVM (Jones et al., 2021), and GLM-PCA (Townes et al., 2019b) operate directly on raw counts of unique molecular identifiers (UMIs) by assuming appropriate likelihoods such as the Poisson or negative binomial.

Since ST data, like scRNA-seq, are high-dimensional molecule counts that map onto specific gene transcripts, in principle existing DR methods can be used to obtain a low-dimensional representation of gene expression at each spatial location. This is in fact the standard procedure recommended by tutorials from two popular packages: Seurat1 and Scanpy2. However, this application of scRNA-seq methods ignores the spatial coordinates that are the distinguishing feature of ST. We would instead like to retain spatial locality information while performing DR on these data.

Gaussian processes (GPs) are probability distributions over arbitrary functions on a continuous (e.g., spatial) domain (Rasmussen and Williams, 2005). GPs are a fundamental tool in spatial statistics (Banerjee et al., 2014; Cressie and Moores, 2021). Historically, GPs have been widely used in environmental applications with spatial structure (Finley et al., 2019). In the genomics (ST) context, spatialDE (Svensson et al., 2018) fits univariate GP models to ST data to identify which genes are spatially variable. Other examples of univariate GPs applied to ST data include the Bayesian hierarchical model Splotch (Åijö et al., 2019) and the scalable GPcounts (BinTayyash et al., 2021). While these methods make positive steps toward including spatial information in routine ST analyses, they do not provide dimension reduction. Genes do not act in isolation but interact with each other. This means there is substantial gene-gene correlation in multivariate ST data ignored by univariate approaches.

A multivariate approach to spatially-aware dimension reduction for ST data is provided by MEFISTO (Velten et al., 2020). The key concept of MEFISTO is to represent the high-dimensional gene expression features as a linear combination of a small number of independent GPs over the spatial domain. This is known in the statistics literature as a linear model of coregionalization (LMC) (Gelfand et al., 2004). Historically, LMC factorization required a conjugate (Gaussian) likelihood for computational tractability, which is not appropriate for ST count data. Following Yu et al. (2009), we refer to this model as Gaussian process factor analysis (GPFA). In neuroscience, attempts were made to relax the Gaussian assumption for application to functional MRI data, leading to count-GPFA Zhao and Park (2017).

Even with conjugate likelihoods and univariate outcomes, exact inference for GPs scales cubically with the number of observations (or spatial locations), which is often prohibitive for ST. For example, the recent Slide-seqV2 protocol can generate tens of thousands of observations (Stickels et al., 2021). Breakthroughs in variational inference for GPs have greatly improved scalability and enabled nonconjugate likelihoods through approximate inference with inducing points (IPs; see Leibfried et al. (2021) and van der Wilk et al. (2020) for overviews). GPFlow is a popular implementation supporting a variety of likelihoods (Matthews et al., 2017). MEFISTO also uses the variational IP

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1. https://satijalab.org/seurat/articles/spatial_vignette.html
2. https://scanpy-tutorials.readthedocs.io/en/latest/spatial/basic-analysis.html
strategy, and in principle is compatible with nonconjugate likelihoods, but in practice the authors recommend using a Gaussian likelihood (Velten et al., 2020). An alternative to IPs using polynomial approximate likelihoods (Huggins et al., 2017) has been proposed for count-GPFA (Keeley et al., 2020).

The latent, low-dimensional spatial factors discovered by LMC variants such as GPFA, count-GPFA, and MEFISTO are real-valued. Thus, they may be thought of as spatial analogs of PCA (when the data likelihood is Gaussian) and GLM-PCA (for non-Gaussian data likelihoods). In all cases, latent factors are combined linearly to predict the outcomes. We refer to the weights in these linear combinations as loadings, and note that, in the models described above, these loadings are assumed to be real-valued as well. We will use the terms real-valued spatial factorization (RSF) and factor analysis (FA) to refer to these spatial and nonspatial models, respectively. Both RSF and FA models tend to produce dense loadings, but MEFISTO counteracts this with sparsity-promoting priors on the loadings matrix. Sparse loadings are more interpretable than dense because, through nonzero values in the loadings, they assign a small number of relevant features to each component, rather than matching every feature to every component.

Another way to generate sparse loadings is to constrain the entire model to be nonnegative. In the non-spatial context, nonnegative matrix factorization (NMF) (Lee and Seung, 1999) and latent Dirichlet allocation (LDA) (Blei et al., 2003) are widely used to produce interpretable low-dimensional factorizations of high-dimensional count data (Carbonetto et al., 2021) including scRNA-seq (Elyanow et al., 2020; Sherman et al., 2020) and ST (Zeira et al., 2021). To quantify uncertainty, a Bayesian prior can be placed on latent factors and a Poisson or negative binomial data likelihood included to lead to probabilistic NMF (PNMF). The advantage of PNMF over real-valued alternatives is that, for geometric reasons, they produce parts-based representations rather than holistic representations. For example, when decomposing pixel-based representations of faces, PNMF decomposes a face into factors representing eyes, noses, mouths, and ears distinctly (Lee and Seung, 1999). On the other hand, real-valued alternatives produce eigenfaces, or representations of whole faces in each factor (Lee and Seung, 1999). Incorporating nonnegativity constraints into spatial models is not straightforward, though, since GPs are inherently real-valued.

The contributions of this work are threefold. First, we develop nonnegative spatial factorization (NSF), a model that allows spatially-aware dimension reduction using a Gaussian process prior over the spatial locations and with a Poisson or negative binomial likelihood for count data. Second, we combine this spatially-aware dimension reduction with nonspatial factors in a NSF hybrid model (NSFH) to partition variability into the spatial and nonspatial sources. Finally, we identify appropriate GP kernels and develop inference methods for the kernel parameters and latent variables to enable computationally tractable fitting of large field-of-view ST data.

This paper proceeds as follows. We first define the generative models of FA, PNMF, RSF, NSF, and NSFH. Secondly, we illustrate the ability of nonnegative factorizations to identify a parts-based representation using simulations. We then describe the basic features of the ST datasets and examine key results of a benchmarking comparison of different models. Next, we analyze three ST datasets from different technologies with NSFH and show how to interpret the spatial and nonspatial components. We conclude with a discussion of the implications of our results in ST data analysis and promising directions for future studies. Details on inference and parameter estimation, procedures for postprocessing nonnegative factor models and computing spatial importance scores, and data preprocessing are provided in the Methods section.

2. Results

2.1 Factor models for spatial count data

The data consist of a multivariate outcome $Y \in \mathbb{R}^{N \times J}$ and spatial coordinates $X \in \mathbb{R}^{N \times D}$. Let $i = 1, \ldots, N$ index the observations (e.g., cells, spots, or locations with a single $(x, y)$ coordinate
Table 1: Summary of probabilistic factor models for high-dimensional spatial count data. An X in the nonnegative, spatial, or nonspatial column indicates whether the model includes that type of latent factors. Likelihoods are listed with the default choice of each model first. gau: Gaussian or normal distribution, poi: Poisson, nb: negative binomial.

| abbrev | model                                              | nonnegative | spatial | nonspatial | likelihoods       |
|--------|----------------------------------------------------|-------------|---------|------------|------------------|
| FA     | factor analysis                                    | X           |         |            | gau              |
| PNMF   | probabilistic nonnegative matrix factorization     | X           |         |            | poi, nb, gau     |
| MEFISTO | MEFISTO                                           | X           |         |            | gau, poi         |
| RSF    | real-valued spatial factorization                  | X           |         |            | gau              |
| NSF    | nonnegative spatial factorization                  | X           | X       |            | poi, nb, gau     |
| NSFH   | nonnegative spatial factorization hybrid           | X           | X       | X          | poi, nb, gau     |

value), $j = 1, \ldots, J$ index the outcome features (e.g., genes), and $d = 1, \ldots, D$ index the spatial input dimensions.

2.1.1 Nonspatial models

In unsupervised dimension reduction such as PCA or NMF, the goal is to represent $Y$ (or a normalized version $\tilde{Y}$) as the product of two low-rank matrices $Y \approx FW'$, where the factors matrix $F$ has dimension $N \times L$ and the loadings matrix $W$ has dimension $J \times L$, with $L \ll J$. Let $l = 1, \ldots, L$ index over the components. A probabilistic version of PCA is factor analysis (FA):

$$\tilde{y}_{ij} \sim N(\mu_{ij}, \sigma_j^2)$$

$$\mu_{ij} = \sum_{l=1}^{L} w_{jl}f_{il}$$

$$f_{il} \sim N(m_l, s_l^2).$$

A probabilistic version of NMF is probabilistic nonnegative matrix factorization (PNMF):

$$y_{ij} \sim \text{Poi}(\nu_i \lambda_{ij})$$

$$\lambda_{ij} = \sum_{l=1}^{L} w_{jl}e_{il}$$

$$e_{il} \sim N(m_l, s_l^2),$$

where $\nu_i$ indicates a fixed size factor to account for differences in total counts per observation. In both of these unsupervised models, the prior on the factors $f_{il}$ assumes each observation is an independent draw and ignores spatial information $x_i$.

2.1.2 Spatial process factorization

In spatial process factorization, we assume that spatially adjacent observations should have correlated outcomes. We encode this assumption via a Gaussian process (GP) prior over the factors. We define real-valued spatial factorization (RSF) as

$$\tilde{y}_{ij} \sim N(\mu_{ij}, \sigma_j^2)$$

$$\mu_{ij} = \sum_{l=1}^{L} w_{jl}f_{il}$$

$$f_{il} = f_i(x_i) \sim GP(\mu_l(x_i), k_l(x_i, X)).$$
where $\mu_l(\cdot)$ indicates a parametric mean function and $k_l(\cdot, \cdot)$ a positive semidefinite covariance (kernel) function. In our implementation, we specify the mean function as a linear function of the spatial coordinates,

$$\mu_l(x_i) = \beta_{0l} + x_i^T \beta_{1l}.$$  

For the covariance function, we choose a Matérn kernel with fixed smoothness parameter $3/2$. We allow each component $l$ to have its own amplitude and length-scale parameters that we estimate from data. RSF is a spatial analog to factor analysis. MEFISTO has the same structure as RSF, but uses a squared exponential kernel instead of Matérn, and further places a sparsity-promoting prior on the loading weights $w_{jl}$. Our implementation is modular and can accept any positive semidefinite kernel. However, we found the Matérn kernel to have better numerical stability than the squared exponential in our experiments.

**Nonnegative spatial factorization (NSF)** is a spatial analog of probabilistic NMF (PNMF).

$$y_{ij} \sim \text{Poi}(\nu_i \lambda_{ij})$$

$$\lambda_{ij} = \sum_{l=1}^{L} w_{jl} f_{il}$$

$$f_{il} = f_l(x_i) \sim \text{GP}(\mu_l(x_i), k_l(x_i, X)).$$

For NSF, we use the same mean and kernel functions as RSF, but we additionally constrain the weights $w_{jl} \geq 0$.

We sought to quantify the relative importance of spatial versus nonspatial variation by combining NSF and PNMF into a semisupervised framework we refer to as the **nonnegative spatial factorization hybrid model (NSFH)**. NSFH consists of $L$ total factors, $T \leq L$ of which are spatial and $L - T$ are nonspatial. We recover NSF and PNMF as special cases when $T = L$ or $T = 0$, respectively. By default, we set $T = L/2$.

$$y_{ij} \sim \text{Poi}(\nu_i \lambda_{ij})$$

$$\lambda_{ij} = \sum_{l=1}^{T} w_{jl} f_{il} + \sum_{l=T+1}^{L} v_{jl} h_{il}$$

$$f_{il} = f_l(x_i) \sim \text{GP}(\mu_l(x_i), k_l(x_i, X))$$

$$h_{il} \sim \mathcal{N}(m_l, \sigma^2_l).$$

Our implementations of PNMF, NSF, and NSFH are modular with respect to the likelihood, so that the negative binomial or Gaussian distributions can be substituted for the Poisson. However, in our experiments we use the Poisson data likelihood.

**2.1.3 Postprocessing nonnegative factor models**

We postprocess fitted nonnegative models (PNMF, NSF, and NSFH) by projecting factors and loadings onto a simplex. This highlights features (genes) that are enriched in particular components rather than those with high expression across all components. In the NSFH model, we interpret the ratio of loadings weights for each feature across all spatial components as a spatial importance score. This is analogous to the proportion of variance explained in PCA. In particular, a score of 1 means that variation in a gene’s expression profile across all observations is completely captured by the spatial factors, whereas a 0 means that expression variation is completely captured by nonspatial factors. The gene-level scores can be used to identify spatially variable genes as pioneered by spatialDE (Svensson et al., 2018). We also compute observation-level scores by switching the role of the factors and loadings matrices; details are provided in the Methods.
2.2 Simulations: Nonnegative factorizations identify parts-based representation

To illustrate the ability of nonnegative models to recover a parts-based factorization, we simulated multivariate count data from two sets of spatial patterns. The “ggblocks” simulation was based on the Indian buffet process (IBP) Griffiths and Ghahramani (2011). The true factors consisted of four simple shapes in different spatial regions. In the “quilt” simulation, we created spatial patterns that overlapped in space. For both simulations, each of the 500 features was an independent negative binomial draw from one of the canonical patterns (Figures S1a,b and 1a,b). Real-valued models FA, MEFISTO, and RSF estimated latent factors consisting of linear combinations of the true factors (Figures S1c,e,f and 1c,e,f). Nonnegative models PNMF, NSF, and NSFH identified each pattern as a separate factor (Figures S1d,g,h and 1d,g,h). This suggests that the parts-based representation in PNMF is preserved in NSF and NSFH.

![Figure 1: Nonnegative factorizations recover a parts-based representation in “quilt” simulated multivariate spatial count data. (a) Each of 500 features was randomly assigned to one of four nonnegative spatial factors. (b) Negative binomial count data used for model fitting. (c) Real-valued factors learned from unsupervised (nonspatial) dimension reduction. (d) as (c) but using nonnegative components. (e) Real-valued, spatially-aware factors with squared exponential kernel. (f) as (e) but with Matérn kernel. (g) Nonnegative, spatially-aware factors. (h) as (g) but with additional three nonspatial factors.](image)
2.3 Application to spatial transcriptomics datasets

We examined the goodness-of-fit and interpretability of nonnegative spatial factorizations on three spatial transcriptomics datasets (Table 2). The Slide-seqV2 hippocampus data (Stickels et al., 2021) consists of 36,536 observations, each at a unique location. The XYEq liver data (Lee et al., 2021b) consists of 2,700 observations at 289 unique locations. Unlike the other protocols, each observation represents a single cell, but multiple cells are assigned to the same location. In other words, each spatial location in XYEq contains multiple distinguishable observations, whereas in the other protocols each spatial location contains a single observation. Finally, the 10x Visium mouse brain data consists of 2,487 observations from an anterior sagittal section, each at a unique location.

Each protocol represents a different trade-off between field-of-view (FOV) and spatial resolution. Slide-seqV2 has the smallest FOV and finest resolution, while XYEq has the largest FOV and the coarsest resolution. Visium is intermediate in both criteria, capturing more spatial locations than XYEq, but sacrificing single-cell resolution with each observation representing an average of multiple nearby cells.

Table 2: Spatial transcriptomics datasets. Slide-seqV2 and XYEq provide single-cell resolution, whereas each Visium observation is an average of multiple cells. XYEq combines multiple observations at each spatial location. obs: number of observations, resolution: center-to-center distance between spatial locations, FOV: field of view area.

| first author | year | tissue     | protocol | obs     | locations | resolution | FOV   |
|--------------|------|------------|----------|---------|-----------|------------|-------|
| Stickels     | 2021 | hippocampus| Slide-seqV2| 36,536  | 36,536    | 10 µm     | 7.4 mm² |
| Lee          | 2021 | liver/ tumor| XYZeq    | 2,700   | 289       | 500 µm    | 87.6 mm² |
| 10x Genomics | 2020 | brain- anterior| Visium  | 2,487   | 2,487     | 100 µm    | 42.3 mm² |

To assess the utility of nonnegative and spatial factors in describing spatial sequencing data, we systematically compared all (Table 1) on all three datasets. We split each dataset randomly into a training set (95% of observations) and validation set (5% of observations), and we fit each model with varying numbers of components. We quantified goodness-of-fit using Poisson deviance between the observed counts in the validation data and the predicted mean values from each model fit to the training data; a small deviance indicated that the model fit the data well.

2.3.1 Slide-seqV2 hippocampus data

On the Slide-seqV2 mouse hippocampus dataset, real-valued factor models had lower validation deviance (higher generalization accuracy) than nonnegative models (Figure 2a). This was to be expected since real-valued factors can encode more information than nonnegative factors. The unsupervised models (FA and PNMF) had higher deviance than their spatially-aware analogs. Surprisingly, RSF outperformed MEFISTO despite having nearly the same probabilistic structure. We attribute this difference to the choice of spatial covariance function (Stephenson et al., 2021)– MEFISTO uses a squared exponential kernel whereas RSF uses a Matérn(3/2) kernel. Matérn kernels produce spatial functions that are less smooth, which may better accommodate sharp transitions between adjacent biological tissue layers such as those of the brain. We were unable to fit MEFISTO models with more than six components because they ran out of memory.

In terms of sparsity, MEFISTO had the highest fraction of zero entries in the loadings matrix due to its sparsity promoting prior, followed by the nonnegative models NSFH, PNMF, and NSF (Figure S2a). Increasing the number of components also increased the sparsity. The time to convergence was comparable for all spatial models, with nonspatial models converging substantially faster (Figure S2b). Among nonnegative models, the negative binomial likelihood took longer to converge but did not reduce generalization error (Figure S2d). Both NSF and NSFH had similar deviances.
suggesting that including a mixture of spatial and nonspatial components (NSFH) did not degrade generalization in comparison to a strictly spatial model (NSF).

Figure 2: Benchmarking spatial and nonspatial factor models on Slide-seqV2 mouse hippocampus gene expression data. (a) Poisson deviance on held-out validation data. Lower deviance indicates better generalization accuracy. All spatial models used 2,000 inducing points. lik: likelihood, dim: number of latent dimensions (components), FA: factor analysis, RSF: real-valued spatial factorization, PNMF: probabilistic nonnegative matrix factorization, NSF: nonnegative spatial factorization, NSFH: NSF hybrid model. (b) Each feature (gene) was assigned a spatial importance score derived from NSFH fit with 20 components (10 spatial and 10 nonspatial). A score of 1 indicates spatial components explain all of the variation. (c) as (b) but with observations instead of features.

We examined the biological relevance of nonnegative factorization by focusing on the NSFH model with $M = 3000$ inducing points and $L = 20$ components (10 spatial and 10 nonspatial). Each factor was summarized by its (variational) approximate posterior mean. For each spatial factor this is a function in the $(x, y)$ spatial coordinate system. For each nonspatial factor the posterior is a vector with one value per observation. Spatial importance scores indicate that most genes are strongly spatial, although a small number are entirely nonspatial (Figure 2b). At the observation level, spatial scores were less extreme, suggesting that both spatial and nonspatial factors are needed to explain gene expression at each location (Figure 2c).

Spatial factors mapped to specific brain regions (Figure 3a) such as the choroid plexus (1), medial habenula (6), and dentate gyrus (8). Even the thin meninges layer was distinguishable (10), underscoring the high spatial resolution of the Slide-seqV2 protocol. We identified genes with the highest enrichment to individual components by examining the loadings matrix. Spatial gene expression patterns mirrored the spatial factors to which they were most associated (Figure 3a). Nonspatial factors were generally dispersed across the field of view (Figure 3c). Finally, we used the top genes for each component to identify cell types and biological processes (Table 3) using scfind$^3$ (Lee et al. 2021a) and the Panglao database$^4$ (Franzén et al. 2019). For example, spatial component 5 identified the corpus callosum, a white-matter region where myelination is crucial. Similarly, the top cell type for spatial component 10 capturing the meninges layer was meningeal cells. Generally, neurons and glia were the most common cell types across all components.

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3. https://scfind.sanger.ac.uk/
4. https://panglaodb.se
Figure 3: Nonnegative spatial factorization hybrid model (NSFH) combines spatial and nonspatial factors in Slide-seqV2 mouse hippocampus gene expression data. Field-of-view is a coronal section with left indicating the medial direction and right the lateral direction. (a) Heatmap (red=high, blue=low) of square-root transformed posterior mean of 10 spatial factors mapped into the \((x,y)\) coordinate space. (b) as (a) but mapping expression levels of top genes with strongest enrichment to each spatial component. (c) as (a) but mapping 10 nonspatial factors from the same model.
Table 3: Nonnegative spatial factorization hybrid model (NSFH) identifies biologically distinct components in Slide-seqV2 mouse hippocampus.

| dim | type | brain regions | cell types | genes | GO biological processes |
|-----|------|---------------|------------|-------|-------------------------|
| 1   | spat | choroid plexus of third ventricle | Choroid plexus cells | TTK, ENPP2, IFI27, TRPM3, STK39 | T cell migration, cellular response to chemokine |
| 2   | spat | thalamus | Interneurons | PRKCD, TNNT1, RAMP3, NTNG1, PDP1 | regulation of presynaptic cytosolic calcium ion concentration, proteoglycan metabolic process |
| 3   | spat | CA1-3 (Ammon’s Horn) pyramidal layer | Neurons | HPCA, NEUROD6, CRYM, WIPF3, CPNE6 | positive regulation of dendritic spine morphogenesis, postsynaptic modulation of chemical synaptic transmission |
| 4   | spat | cerebral cortex | Neurons | LAMP5, 3110035E14Rik, STX1A, MEF2C, EGR1 | myeloid leukocyte differentiation, hormone biosynthetic process |
| 5   | spat | fiber tracts/ corpus callosum | Oligodendrocytes | CLDN11, MAL, MAG, PLP1, MOG | myelination, central nervous system myelination |
| 6   | spat | medial habenula (thalamus) | Neurons | NWD2, TAC2, CALB2, NECA2, ZCCHC12 | steroid biosynthetic process, sex differentiation |
| 7   | spat | CA strata and dentate gyrus molecular layer | Astrocytes | DDN, SLC1A3, CST3, PSD, CABP7 | learning, response to amino acid |
| 8   | spat | dentate gyrus granule layer | Neurons | LRRRTM4, STXB6, SLC8A2, 2010300C02Rik, PLXNA4 | central nervous system projection neuron axonogenesis, regulation of cytoskeleton organization |
| 9   | spat | multiple | Astrocytes | SLC6A11, SPARC, SLC4A3, KCNJ10, ATP1A2 | negative regulation of blood coagulation, cellular amino acid catabolic process |
| 10  | spat | meninges | Meningeal cells | PTGDS, GFAP, APOD, FABP7, FYXD1 | nitric oxide mediated signal transduction, epithelial cell proliferation |
| 1   | nsp | | Neurons | MEG3, SNHG11, MIAT, CPNE7, TTCT14 | mRNA processing, RNA splicing |
| 2   | nsp | | GABAergic neurons | SST, NPY, GAD2, GAD1, CNR1 | neurotransmitter metabolic process, negative regulation of catecholamine secretion |
| 3   | nsp | | Neurons | CHGA, RAB3C, HSPA4L, CRKMT1, SYT4 | chemical synaptic transmission, regulation of short-term neuronal synaptic plasticity |
| 4   | nsp | | GABAergic neurons | PVALB, VAMP1, CPLX1, MT-ND1, SCRT1 | mitochondrial respiratory chain complex I assembly, aerobic respiration |
| 5   | nsp | | Astrocytes | MT-RNR2, MT-RNR1, 2900052N01Rik, MAP2, MT-ND5 | electron transport coupled proton transport, response to nicotine |
| 6   | nsp | | Astrocytes | GM3764, MALAT1, GPC5, LSAMP, TRPM3 | cell adhesion, synaptic membrane adhesion |
| 7   | nsp | | Neurons | NEFM, NEFH, MAP1B, VAMP1, SLC24A2 | cell adhesion, intermediate filament cytoskeleton organization |
| 8   | nsp | | Interneurons | NPTXR, SYN2, STMN2, NCA1D, YWHAH | mitotic cell cycle, thyroid gland development |
| 9   | nsp | | Neurons | NRG3, FGF14, CSMD1, DLG2, KCNIP4 | social behavior, positive regulation of synapse assembly |
| 10  | nsp | | | MIR6236, LARS2, CMSS1, HEXB, CAMK1D | translation, positive regulation of signal transduction by p53 class mediator |
2.3.2 XYZeq liver data

On the XYZeq mouse liver dataset, real-valued factor models again had lower validation deviance than nonnegative models and spatial models again outperformed their nonspatial analogs (Figure 2). The strictly spatial NSF model had slightly lower deviance than the hybrid spatial and nonspatial model NSFH.

![Graphs showing out-of-sample generalization error, gene spatial importance, and observation spatial importance.]

(a) Out-of-sample generalization error (b) Gene spatial importance (c) Observation spatial importance

Figure 4: Benchmarking spatial and nonspatial factor models on XYZeq mouse liver gene expression data. Lower deviance indicates higher generalization accuracy. All spatial models used 288 inducing points. lik: likelihood, dim: number of latent dimensions (components), FA: factor analysis, RSF: real-valued spatial factorization, PNMF: probabilistic nonnegative matrix factorization, NSF: nonnegative spatial factorization, NSFH: NSF hybrid model. (b) Each feature (gene) was assigned a spatial importance score derived from NSFH fit with 6 components (3 spatial and 3 nonspatial). A score of 1 indicates spatial components explain all of the variation. (c) as (b) but with observations instead of features.

Focusing on the NSFH model with $M = 288$ inducing points and $L = 6$ components, we found a strikingly bimodal distribution of spatial importance scores for both genes and observations (Figure 4b-c). However, like the Slide-seqV2 data, most scores were greater than 0.5, suggesting spatial variation was more explanatory than nonspatial overall. The first spatial factor identified normal liver tissue while the other spatial factors were associated with the tumor regions (Figure 5a). Genes associated with spatial component 1 indicated an enrichment of hepatocytes, while genes in the other components were associated with immune cells (Figure 5a, Table 4). The nonspatial factors again showed no distinct spatial patterns for these data (Figure 5c), although they were associated with particular cell types and biological processes (Table 4).

Table 4: Nonnegative spatial factorization hybrid model (NSFH) identifies biologically distinct components in XYZeq mouse liver.

| dim | type | cell types | genes | GO biological processes |
|-----|------|------------|-------|-------------------------|
| 1   | spat | Hepatocytes| $HNF1AOS1$, $CPS1$, $CYP2E1$, $AKR1C6$, $MUG2$ | cellular amino acid catabolic process, xenobiotic metabolic process |
| 2   | spat |            | $IL31RA$, $SEMA5A$, $TRPM3$, $PLCD1$, $FOXp4$ | positive regulation of cholesterol esterification, inclusion body assembly |
| 3   | spat | Macrophages| $LGALS1$, $S100A6$, $S100A4$, $RPL30$, $KLF6$ | translation, cytoplasmic translation |
| 1   | nsp  | Fibroblasts| $KIF26B$, $MEDAG$, $LAMA4$, $NGF$, $COL1A2$ | regulation of cellular response to vascular endothelial growth factor stimulus, collagen fibril organization |
| 2   | nsp  |            | $HMGA2$, $SLC35F1$, $FAM19A1$, $TENM4$, $HS8ST5$ | cell fate specification, specification of animal organ identity |
| 3   | nsp  | Macrophages| $ARDGAP15$, $DOCK10$, $MYO1F$, $LY86$, $HCK$ | negative regulation of leukocyte apoptotic process, negative regulation of immune response |
Figure 5: Nonnegative spatial factorization hybrid model (NSFH) combines spatial and nonspatial factors in XYZeq mouse liver gene expression data. (a) Heatmap (red=high, blue=low) of square-root transformed posterior mean of 3 spatial factors mapped into the (x, y) coordinate space. (b) as (a) but mapping expression levels of top genes with strongest enrichment to each spatial component. (c) as (a) but mapping 3 nonspatial factors from the same model.
2.3.3 Visium brain data

On the Visium mouse brain data, goodness-of-fit results in terms of validation deviance (lower values indicate better generalization accuracy) were markedly different than the other two datasets. First, real-valued models did not dominate nonnegative models. Both NSF and NSFH had lower deviance than factor analysis and MEFISTO. In fact, NSF had generalization accuracy comparable to the best-performing model (RSF). Furthermore, it was necessary to increase the number of components in NSFH to reduce the deviance to a level comparable with NSF, whereas in the other datasets deviance did not vary dramatically with the number of components, and NSFH had similar performance to NSF. However, consistent with the other datasets, nonspatial models generally had higher deviance than their spatial analogs, reinforcing the importance of including spatial information in out-of-sample prediction, interpolation, and generalization. Finally, the dramatic improvement in fit of RSF results over MEFISTO underscores the importance of choosing an appropriate GP kernel.

We next focused on interpretation of the NSFH model with $M = 2363$ inducing points and $L = 20$ components. A basic neuroanatomy diagram of mouse brain is provided for reference in Figure 6b. Spatial importance scores indicate that spatial factors are most explanatory for variation at both the gene and observation level (Figure 6c–d). Similar to the Slide-seqV2 data, most spatial factors mapped to specific brain regions (Figure 7a) such as the cerebral cortex (2), corpus callosum (4), and choroid plexus (10). Top genes for each spatial component again showed expression patterns overlapping with their associated factors (Figure 7c). While the majority of nonspatial factors were dispersed across the field-of-view (Figure 7c), a few of them did exhibit spatial localization to areas such as the hypothalamus (2) and hippocampus (4). This illustrates that the nonspatial factors are not antagonistic to spatial variation but should be thought of as spatially naive or agnostic. Given that Visium does not provide single-cell resolution, and this phenomenon was not observed in the other two datasets, we hypothesize that spatial patterns active in small numbers of observations may be more likely to be picked up as nonspatial factors under such conditions. Using the top genes for each component, we identified cell types and biological processes (Table 5). For example, spatial component 3 aligned to the basal ganglia, which is involved in the “response to amphetamine” biological process. Nonspatial component 10 had many genes associated with erythroid progenitor cells. The nonspatial patterns in this component suggest that this factor includes cell types in blood; however erythroid progenitor cells are not found in blood. We hypothesize these are actually erythrocytes, which have been shown to retain parts of the erythroid transcriptome despite the loss of the nucleus (Doss et al., 2015). As in the Slide-seqV2 hippocampus data, neurons and glia were the most common cell types across all components.
Figure 6: Benchmarking spatial and nonspatial factor models on Visium mouse brain gene expression data. Lower deviance indicates better generalization accuracy. All spatial models used $2,363$ inducing points. lik: likelihood, dim: number of latent dimensions (components), FA: factor analysis, RSF: real-valued spatial factorization, PNMF: probabilistic nonnegative matrix factorization, NSF: nonnegative spatial factorization, NSFH: NSF hybrid model. (b) Diagram of major brain regions, annotation by the authors, original image credit to 10x Genomics. (c) Each feature (gene) was assigned a spatial importance score derived from NSFH fit with 20 components (10 spatial and 10 nonspatial). A score of 1 indicates spatial components explain all of the variation. (d) as (c) but with observations instead of features.
Figure 7: Nonnegative spatial factorization hybrid model (NSFH) combines spatial and nonspatial factors in Visium mouse brain gene expression data. Field-of-view is a sagittal section with left indicating the anterior direction and right the posterior direction. (a) Heatmap (red=high, blue=low) of square-root transformed posterior mean of 10 spatial factors mapped into the \((x,y)\) coordinate space. (b) as (a) but mapping expression levels of top genes with strongest enrichment to each spatial component. (c) as (a) but mapping 10 nonspatial factors from the same model.
Table 5: Nonnegative spatial factorization hybrid model (NSFH) identifies biologically distinct components in Visium mouse brain.

| dim | type | brain regions                      | cell types       | genes                                      | GO biological processes                                                                 |
|-----|------|------------------------------------|------------------|--------------------------------------------|----------------------------------------------------------------------------------------|
| 1   | spat | multiple                           |                 | IGKC, COX6A2, TNNC1, CABP7, S100A9          | mitochondrial electron transport, NADH to ubiquinone, mitochondrial respiratory chain    |
|     |      |                                    |                  |                                            | complex I assembly                                                                       |
| 2   | spat | cerebral cortex                    | Interneurons     | CCK, DKK3, STX1A, NRN1, RTN4R              | axonogenesis, positive regulation of behavioral fear response                           |
| 3   | spat | basal ganglia                      | Neurons          | GPR88, PDE10A, RGS9, PPP1R1B, PENK         | response to amphetamine, striatum development                                          |
| 4   | spat | fiber tracts/corpus callosum       | Oligodendrocytes | PLP1, MAL, MOBP, MAG, CLDN11               | myelination, central nervous system myelination                                         |
| 5   | spat | olfactory granule layer            | Neurons          | GNG4, GPSM1, CPNE4, SHISA8, MYO16          | embryonic limb morphogenesis, proximal/distal pattern formation                         |
| 6   | spat | multiple                           | Interneurons     | NPTX1, CCN3, SLC30A3, RASL10A, LM03        | intracellular signal transduction, regulation of catalytic activity                    |
| 7   | spat | outer olfactory bulb               | Interneurons     | S100A5, DOC2G, CDHR1, CALB2, FABP7         | mesoderm formation, cellular response to glucose stimulus                               |
| 8   | spat | inner cerebral cortex              | Neurons          | HS3ST2, IGHM, CCN2, IGSP21, NR4A2          | isoprenoid biosynthetic process, cholesterol biosynthetic process                       |
| 9   | spat | multiple                           | GABAergic neurons| GAD1, SLC52A1, HAP1, STX6P6, CPNE7         | neurotransmitter metabolic process, regulation of gamma-aminobutyric acid secretion     |
| 10  | spat | choroid plexus of lateral ventricle| Choroid plexus   | TTR, ECRG4, ENPP2, KL, 2900040C04RIK       | hormone transport, retinol metabolic process                                           |
| 1   | nsp  |                                    | GABAergic neurons| PVALB, KCNAB3, CPLX1, VAMP1, SYT2           | positive regulation of potassium ion transmembrane transporter activity, neuromuscular   |
|     |      |                                    |                  |                                            | process                                                                                 |
| 2   | nsp  | hypothalamus                       | Neurons          | BAIAP3, NNTAT, HPCAL1, LYPD1, RESP18       | neurotrophin TRK receptor signaling pathway, muscle fiber development                   |
| 3   | nsp  |                                    | Neurons          | NEFM, LG12, DNER, PLCXD2, CARTPT           | regulation of synaptic vesicle fusion to presynaptic active zone membrane, neuronal action |
|     |      |                                    |                  |                                            | potential propagation                                                                    |
| 4   | nsp  | hippocampus                        | Neurons          | WIPF3, CPNE6, RGS14, ARPC5, CABP7          | Arp2/3 complex-mediated actin nucleation, calcineurin-NFAT signaling cascade            |
| 5   | nsp  |                                    | Neurons          | HS3ST4, RAB26, CLSTN2, TLE4, SNCA          | vacuolar acidification, protein glycosylation                                          |
| 6   | nsp  | vascular fibroblasts               |                 | LAR32, GM42418, VTN, PTN, JPY              | establishment of epithelial cell polarity, plasma lipoprotein particle organization     |
| 7   | nsp  |                                    | Neurons          | PLXND1, VSTM2L, CALB1, RGS7, MGAT5B        | heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules, short-term  |
|     |      |                                    |                  |                                            | memory                                                                                  |
| 8   | nsp  |                                    | GABAergic neurons| SST, NPY, RESP18, NOS1, PDYN               | neuropeptide signaling pathway, regulation of the force of heart contraction            |
| 9   | nsp  | Astrocytes                         |                 | TUBB2B, GM3764, NTRK2, MFGE8, PLPP3        | complement activation, negative regulation of growth                                    |
| 10  | nsp  | Erythrocytes                       |                 | HBA-A1, HBA-A2, HBB-BT, HBB-BS, ALAS2      | oxygen transport, cellular oxidant detoxification                                       |
3. Discussion

We have presented nonnegative spatial factorization (NSF), a probabilistic approach to spatially-aware dimension reduction on observations of count data based on Gaussian processes (GP). We also showed how to combine spatial and nonspatial factors with the NSF hybrid model (NSFH). On simulated data, NSF, NSFH, and the nonspatial model probabilistic NMF (PNMF) all recovered an interpretable parts-based representation, whereas real-valued factorizations such as MEFISTO (Velten et al., 2020) led to a less interpretable embedding. A key advantage of spatially-aware factorizations over unsupervised alternatives such as factor analysis (FA) and PNMF is generalizability; spatial factor models learn latent functions over the entire spatial domain rather than only at the observed locations. On a benchmarking task using three spatial transcriptomics (ST) datasets from three different technologies, NSF and NSFH had consistently lower out-of-sample prediction error than PNMF. We found that using a Matérn kernel reduced prediction error in our implementation of real-valued spatial factorization (RSF) when compared to MEFISTO, which uses a squared exponential kernel. We demonstrated how NSFH spatial and nonspatial components identify distinct regions in brain and liver tissue, cell types, and biological processes. Finally, we quantified the proportion of variation explained by spatial versus nonspatial components at both the gene and observation level using spatial importance scores.

A substantial limitation of the models studied here is the reliance on Euclidean distance as the metric for the GP kernel over the spatial domain. While this was appropriate for the particular datasets we explored, other ST datasets more closely resemble manifolds. An example is the embryonic tissue profiled by Lohoff et al. (2020). Under such conditions, standard GP kernels are inappropriate (Dunson et al., 2020). The recently proposed manifold GP (Borovitskiy et al., 2020) and graph GP (Borovitskiy et al., 2021) seem promising as alternatives. Either of these could be substituted for the standard GPs in our RSF, NSF, and NSFH models.

Historically, two major challenges for working with ST data have included integration with single-cell RNA-seq references (Lopez et al., 2019; Verma and Engelhardt 2020) and deconvolving observations that incorporate multiple cells (Cable et al., 2021; Lopez et al., 2021). Addressing these will be an important future direction for research into nonnegative spatial factor models. However, we anticipate that ongoing improvements in ST protocols will increase the number of genes detected per location while improving the spatial resolution to single-cell or even subcellular levels (Eng et al., 2019; Xia et al. 2019) while maintaining a wide field-of-view.

All of the spatial models we considered were based on linear combinations of GPs with variational inference using inducing points (Leibfried et al., 2021; van der Wilk et al., 2020). While this technique has greatly improved GP scalability by enabling minibatching and nonconjugate likelihoods, the computational complexity still scales cubically with the number of inducing points. Promising future directions for GP inference include the harmonic kernel decomposition (Sun et al., 2021), nearest-neighbor GPs (Finley et al., 2019), and random Fourier features (Hensman et al., 2017; Gundersen et al., 2020). While linearity and nonnegativity are advantageous for interpretability (Svensson et al., 2020), multivariate spatial factor models can also be formulated using nonlinear deep GPs (Wu et al., 2021).

We have focused on the application of nonnegative spatial factor models to genomics data. However, both NSF and NSFH are relevant to other types of multivariate spatial or temporal data. Examples include forestry and environmental remote sensing (Taylor-Rodriguez et al. 2019), wearable devices (Straczkiewicz et al., 2021), and neuroscience (Wu et al., 2017; Foti and Fox, 2019).
4. Methods

4.1 Nonnegative spatial factorization inference

4.1.1 Evidence lower bound objective (ELBO) function

The posterior distribution of NSF cannot be computed in closed form, so we resort to approximate inference using a variational distribution. We assume a set of inducing point locations \( z_m \) indexed by \( m = 1, \ldots, M \). If \( M = N \) we set \( z_m \) to be the spatial coordinates \( X \). Otherwise, for \( M < N \) we set \( z_m \) to be the center points of a k-mean clustering (with \( k = M \)) applied to \( X \). Let \( u_{ml} = f_l(z_m) \) be the inducing points, i.e., the Gaussian process evaluation of the inducing locations. We are interested in inference of the posterior of the latent variables \( u_{ml} \) and \( f_l \). Temporarily assume loadings \( w_{jl} \), likelihood shape and dispersion parameters, GP prior mean parameters \( \beta_l \), and kernel hyperparameters \( \theta_l \) are known. The posterior is given by

\[
p(U, F | Y; X, Z) = \frac{p(Y | U, F)p(U, F; X, Z)}{\int_{U,F} p(Y | U, F)p(U, F; X, Z)} = \frac{p(Y | F)p(F | U; X, Z)p(U; Z)}{\int_{U,F} p(Y | F)p(F | U; X, Z)p(U; Z)}.
\]

Note that the likelihood term depends on \( U \) only through \( F \), and we have decomposed the joint prior on \( U, F \) into a marginal prior of \( U \) and a conditional prior of \( F | U \). Following Salimbeni and Deisenroth (2017) and van der Wilk et al. (2020), the GP prior for inducing points is given by

\[
p(U; Z) = \prod_{l=1}^L p(u_l; Z)
p(u_l; Z) = N(\mu_l(Z), K_{uul})
\]

\[
[K_{uul}]_{m,m'} = k_l(z_m, z_{m'})
\]

Next, we specify the GP prior for the function values at the observed locations by conditioning on the inducing points.

\[
p(F | U; X, Z) = \prod_{l=1}^L p(f_l | u_l; X, Z)
p(f_l | u_l; X, Z) = N(\mu_{fl} | u_l, K_{fl} | u_l)
\]

\[
\mu_{fl} | u_l = \mu_l(X) + K_{ufl}^{-1}(u_l - \mu_l(Z))
\]

\[
K_{fl} | u_l = K_{ffl} - K_{ufl}K_{uul}^{-1}K_{ufl}
\]

\[
[K_{ufl}]_{m,i} = k_l(z_m, x_i)
\]

\[
[K_{ffl}]_{i,i'} = k_l(x_i, x_{i'})
\]

Note that \( K_{uul} \in \mathbb{R}^{M \times M}, K_{ffl} \in \mathbb{R}^{N \times N}, \) and \( K_{ufl} \in \mathbb{R}^{M \times N} \).

We use the following approximation to the true posterior to facilitate variational inference:

\[
q(U, F; X, Z) = p(F | U; X, Z)q(U; Z)
\]

\[
q(U; Z) = \prod_{l=1}^L q(u_l; Z)
\]

\[
q(u_l; Z) = N(\delta_l, \Omega_l).
\]
We will later need to draw samples of $F$ from this distribution. This is made easier by analytically marginalizing out $U$.

$$q(f_i | \delta_i, \Omega; X, Z) = \int_{u_i} p(f_i | u_i; X, Z)q(u_i; Z)$$
$$= \mathcal{N}(\mu_i, \Sigma_i),$$

where the marginal mean vector $\mu_i \in \mathbb{R}^N$ and covariance matrix $\Sigma_i \in \mathbb{R}^{N \times N}$ are given by

$$\mu_i = \mu_t(X) + K'_{u,fi}K^{-1}_{uul}(\delta_i - \mu_t(Z))$$
$$\Sigma_i = K_{fi} - K'_{u,fi}K^{-1}_{uul}(K_{uul} - \Omega_i)K^{-1}_{uul}K_{u,fi}.$$

Minimizing the KL divergence from the true posterior distribution to the approximating distribution is equivalent to maximizing the following evidence lower bound (ELBO) (Hensman et al., 2013, Salimbeni and Deisenroth, 2017, van der Wilk et al., 2020):

$$\mathcal{L} = E_{q(U,F)} \left[ \log \frac{p(Y | F)p(F | U; X, Z)p(U; Z)}{q(U)} \right]$$
$$= E_{q(U,F)} \left[ \log p(Y | F) \right] + E_{q(U,F)} \left[ \log \frac{p(F | U; X, Z)p(U; Z)}{p(F | U; X, Z)q(U; Z)} \right]$$
$$= (\mathcal{L}_1) - \sum_{l=1}^L E_{q(u_i)} \left[ \log \frac{q(u_i; Z)}{p(u_i; Z)} \right]$$
$$= \mathcal{L}_1 - \sum_{l=1}^L \text{KL} (q(u_i) || p(u_i)).$$

The KL divergence term from prior to approximate posterior has a closed-form expression since both are Gaussian (recall $M$ is the total number of inducing points):

$$\text{KL} (q(u_i) || p(u_i)) = \frac{1}{2} \left[ \log \frac{K_{uul}}{|\Omega_i|} - M + \text{tr} \left\{ K^{-1}_{uul}\Omega_i \right\} + (\delta_i - \mu_t(Z))^T K^{-1}_{uul}(\delta_i - \mu_t(Z)) \right].$$

Let $\zeta(y | \nu \lambda)$ be the log likelihood of an exponential family such as the Poisson or negative binomial distribution with mean $\nu \lambda$. In particular, for the Poisson distribution, $\zeta(y | \nu \lambda) = y \log(\nu \lambda) - \nu \lambda - \log y!$. Let $F[i,:]=(f_{i1}, \ldots, f_{iL})$. The expected log likelihood term in the ELBO is given by:

$$\mathcal{L}_1 = \sum_{i=1}^N \sum_{j=1}^J E_{q(U,F)} \left[ \zeta(y_{ij} | \nu_i \lambda_{ij}) \right]$$
$$= \sum_{i=1}^N \sum_{j=1}^J E_{q(F)} \left[ \zeta \left( y_{ij} \bigg| \sum_{l=1}^L w_{jli}f_{li} \right) \right]$$
$$= \sum_{i=1}^N \sum_{j=1}^J E_{q(F[i,:])} \left[ \zeta \left( y_{ij} \bigg| \sum_{l=1}^L w_{jli}f_{li} \right) \right].$$

The expectation in the above equation is intractable due to the nonlinear log likelihood function $\zeta(\cdot)$. However, we can simplify it in two ways. First, it only depends on $U$ through $F$, so the marginalized distribution $q(F)$ may be used instead of $q(U,F)$. Second, the log likelihood only depends on the marginal $f_{il}$ terms, as opposed to the multivariate $f_i = (f_{i1}, \ldots, f_{iL})$ or the multivariate $F[i,:]$. The
approximate posterior distribution is therefore
\[ q(F[i,:]) = \prod_{l=1}^{L} q(f_{il}) = \prod_{l=1}^{L} \mathcal{N} \left( \hat{\mu}_l, \delta_l \right), \]
where
\[ \alpha_l(x_i) = K_{uul}^{-1}(K_{ufl})_{i,:}, \]
\[ [\hat{\mu}_l]_i = \mu_l(x_i) + \alpha_l(x_i)'(\delta_l - \mu_l(Z)) \]
\[ \delta_l \]
\[ \delta_l = k_l(x_i, x_i) - \alpha_l(x_i)'(K_{uul} - \Omega_l)\alpha_l(x_i). \]

Despite these simplifications, the expectation still lacks a closed-form solution and is evaluated by approximation using Monte Carlo (MC) sampling (Salimbeni and Deisenroth 2017). The MC procedure draws \( S \) samples \( f_{il}^{(s)} \sim \mathcal{N} \left( [\hat{\mu}_l]_i, \delta_l \right) \) then evaluates
\[ E_{q(F[i,:])} \left[ \zeta \left( \sum_{l=1}^{L} w_{jl}e_{il}^{(s)} \right) \right] \approx \frac{1}{S} \sum_{s=1}^{S} \zeta \left( \sum_{l=1}^{L} w_{jl}e_{il}^{(s)} \right). \]

In practice, we found \( S = 3 \) to provide a reasonable balance between speed and numerical stability.

### 4.1.2 Parameter estimation

Using the ELBO as an objective function, we optimize all parameters using the Adam algorithm (Kingma and Ba 2014) with gradients computed by automatic differentiation in Tensorflow (Abadi et al. 2016). This includes the loadings weights \( w_{jl} \), mean function intercepts \( \beta_0 \) and slopes \( \beta_1 \), kernel length scale and amplitude parameters, variational location \( \delta_l \) and covariance \( \Omega_l \) parameters, and any shape or dispersion parameters associated with the likelihood (e.g., for negative binomial and Gaussian distributions).

To satisfy the nonnegativity constraint on \( w_{jl} \), we use a projected gradient approach. After each optimization step, any values that are negative are truncated to zero. All other parameter constraints are accommodated by monotone transformations. For example, the variational covariance matrices \( \Omega_l \) must all be positive definite, so we store and use the lower triangular Cholesky decomposition factors instead of the full covariance matrices themselves.

### 4.1.3 Real-valued spatial factorization inference

The inference procedure for RSF is identical to NSF except we do not exponentiate the sampled \( f_{il}^{(s)} \) terms prior to combining with the loadings \( w_{jl} \). Because the loadings are no longer constrained to be greater than or equal to zero, the truncation step is omitted during optimization. To facilitate comparisons with MEFISTO, we focused on a Gaussian likelihood and only applied RSF to normalized data with features centered to have zero mean.

### 4.2 Nonspatial count factorization inference

To fit PNMF and FA models, we adopt a mean field variational approximation (Blei et al., 2016) to the posterior distribution of the latent factors:
\[ q(f_{il}) = \mathcal{N}(\delta_{il}, \omega_{il}) \]

Focusing on PNMF, the ELBO is of the form
\[ \mathcal{L} = \sum_{i=1}^{N} \sum_{j=1}^{J} E_{q(F[i,:])} \left[ \zeta \left( \sum_{l=1}^{L} w_{jl}e_{il}^{(s)} \right) \right] - \sum_{i=1}^{N} \sum_{l=1}^{L} \text{KL} \left( q(f_{il}) \mid \mid p(f_{il}) \right). \]
The expectation in the first term is approximated by MC sampling just as in NSF. The second term involves two univariate Gaussians and has the closed form
\[
\text{KL}(q(f_{il}) \parallel p(f_{il})) = \frac{1}{2} \left[ \log \frac{s_{il}^2}{\omega_{il}} - 1 + \frac{\omega_{il}}{s_{il}^2} + \frac{(\delta_{il} - m_{il})^2}{s_{il}^2} \right].
\]

FA has an identical setup to NSF except without exponentiating the sampled \( f_{il} \sim q(f_{il}) \). Optimization of parameters is the same as in NSF, including the truncation of \( w_{jl} \) terms in PNMF.

4.3 Nonnegative spatial factorization hybrid (NSFH) model

Recall \( \nu_i \lambda_{ij} \) is the mean of the outcome \( y_{ij} \), which we assume is distributed as some exponential family likelihood, such as Gaussian, negative binomial, or Poisson. The NSFH model is specified as the combination of \( T \) spatial factors with \( L - T \) nonspatial factors
\[
\lambda_{ij} = \sum_{l=1}^{T} w_{jl} e^{f_{il}} + \sum_{l=T+1}^{L} v_{jl} e^{h_{il}}.
\]

To estimate the \( f_{il} \) terms, we use the same GP prior and variational inducing point approximate posterior as in NSF. To estimate the \( h_{il} \) terms, we use the same univariate Gaussian prior and mean field variational approximate posterior as in PNMF. The ELBO objective function is similar to NSF and PNMF:
\[
L = \sum_{i=1}^{N} \sum_{j=1}^{J} \mathbb{E}_{q(F[i,:], H[i,:])} \left[ \zeta \left( y_{ij} \Big| \sum_{l=1}^{T} w_{jl} e^{f_{il}} + \sum_{l=T+1}^{L} v_{jl} e^{h_{il}} \right) \right] \ldots
\]
\[
\ldots - \sum_{l=1}^{L} \text{KL}(q(u_l) \parallel p(u_l)) - \sum_{i=1}^{N} \sum_{l=1}^{L} \text{KL}(q(h_{il}) \parallel p(h_{il})).
\]

Due to the mean-field formulation, the variational distributions factorize over components:
\[
q(F[i,:], H[i,:]) = \prod_{i=1}^{T} q(f_{il}) \prod_{l=T+1}^{L} q(h_{il}).
\]

Thus, we approximated the expectation by independent MC samples of \( f_{il}^{(s)} \sim q(f_{il}) \) and \( h_{il}^{(s)} \sim q(h_{il}) \). The remaining two KL divergence terms are identical to those in NSF and PNMF and have the same closed form. We optimize all parameters using the same techniques described for NSF and PNMF.

4.4 Postprocessing nonnegative factorizations

Consider a generic nonnegative factorization \( \Lambda = FW' \) or equivalently \( \lambda_{ij} = \sum f_{il} w_{jl} \). We assume that the log-likelihood of data \( Y \) depends on the \( N \times L \) factors matrix \( F \) and \( J \times L \) loadings matrix \( W \) only through \( \Lambda \). The number of observations is \( N \), number of components is \( L \), and number of features is \( J \). For notational simplicity, here we use \( f_{il} \) to denote a nonnegative entry of a factor matrix rather than \( e^{f_{il}} \) used in other sections. In the case that the model is probabilistic, we assume \( f_{il} \) represents a posterior mean, posterior geometric mean, or other point estimate. We project \( F, W \)
onto the simplex while leaving the likelihood invariant.

\[ \bar{f} = \sum_{i=1}^{N} F[i,:] \in \mathbb{R}^L \]

\[ F \leftarrow F \ast \text{diag}(\bar{f})^{-1} \]

\[ W \leftarrow W \ast \text{diag}(\bar{f}) \]

\[ \bar{w} = \sum_{i=1}^{L} W[i,:] \in \mathbb{R}^J \]

\[ W \leftarrow \text{diag}(\bar{w})^{-1} \ast W. \]

Note that after this transformation \( \Lambda = FW' \ast \text{diag}(\bar{w}). \) We now have that the columns of \( F \) all sum to one and the rows of \( W \) all sum to one (i.e., they lie on the simplex). In the spatial transcriptomics context, the features are genes. A particular row of \( W \) represents a single gene’s soft clustering assignment to each of the \( L \) components. If \( w_{jl} = 1 \) this meant all of that gene’s expression could be predicted using only component \( l \), whereas if \( w_{jl} = 0 \) this meant that component \( l \) was irrelevant to gene \( j \). For a given component \( l \), we identified the top associated genes by sorting the \( w_{jl} \) values in decreasing order.

We refer to the above procedure as “SPDE-style” postprocessing due to its similarity to spatialDE (Svensson et al., 2018). An alternative postprocessing scheme is “LDA-style” (Blei et al., 2003; Carbonetto et al., 2021) where the roles of \( F \) and \( W \) are switched. This results in a loadings matrix whose columns sum to one (“topics”) and a factors matrix whose rows sum to one. LDA-style postprocessing provides a soft clustering of observations instead of features. We used SPDE-style postprocessing throughout this work with the sole exception of computing spatial importance scores for observations, described below.

### 4.4.1 NSFH Spatial Importance Scores

Let \( F \in \mathbb{R}^N_{+}^{\times T} \) represent the spatial factors matrix (rather than \( e^F \), with corresponding loadings \( W \in \mathbb{R}^J_{+}^{\times T} \). Similarly let \( H \in \mathbb{R}^N_{+}^{\times (L-T)} \) represent the nonspatial factors (rather than \( e^H \)) with corresponding loadings \( V \in \mathbb{R}^J_{+}^{\times (L-T)} \). Let \( A = [F,H] \in \mathbb{R}^N_{+}^{\times L} \) and \( B = [W,V] \in \mathbb{R}^J_{+}^{\times L} \).

To obtain spatial importance scores for features (genes), we applied SPDE-style postprocessing to \( A, B \). The score \( \gamma_j \) for feature \( j \) is given by the sum of the loadings weights across all the spatial components.

\[ W \leftarrow B_{[\cdot,1:T]} \]

\[ \gamma_j = \sum_{i=1}^{T} w_{ij}. \]

Due to the initial postprocessing, \( 0 \leq \gamma_j \leq 1 \) for all \( j \). If \( \gamma_j = 0 \) then all the variation in feature \( j \) was explained by the nonspatial factors. If \( \gamma_j = 1 \) then all the variation was explained by the spatial factors.

To obtain spatial scores for observations, we applied LDA-style postprocessing to \( A, B \). The score \( \rho_i \) for observation \( i \) is given by the sum of the factor values across all the spatial components.

\[ F \leftarrow A_{[\cdot,1:T]} \]

\[ \rho_i = \sum_{i=1}^{T} f_{id}. \]

As before, \( 0 \leq \rho_i \leq 1 \) for all \( i \). If \( \rho_i = 0 \) then all the variation in observation \( i \) was explained by the nonspatial factors. If \( \rho_i = 1 \) then all the variation was explained by the spatial factors.
4.5 Initialization

Real-valued models were initialized with singular value decomposition. Nonnegative models were initialized with the scikit-learn implementation of NMF (Pedregosa et al., 2011). For NSFH, we sorted the initial NMF factors and loadings in decreasing order of spatial autocorrelation using Moran’s I statistic (Moran, 1950) as implemented in squidpy (Palla et al., 2021). The first $T$ factors were assigned to the spatial component and the remaining $L-T$ factors to the nonspatial component.

4.6 Simulations

In the ggblocks simulation, each latent factor representing a canonical spatial pattern consisted of a $30 \times 30$ grid of locations (900 total spatial locations). The number of features (“genes”) was set to 500. Each gene was randomly assigned to one of the four patterns with uniform probabilities. The $900 \times 500$ mean matrix was defined as the sum of two nonnegative matrices: one spatial ($M_1$) and one nonspatial ($M_2$). Entries of $M_1$ were set to 11 in the active region (where a shape is visible) and 0 elsewhere. We then generated a set of three nonspatial factors each of length 900 by drawing from a Bernoulli distribution with probability 0.2. Each gene was randomly assigned to one of the three nonspatial factors with uniform probabilities. The entries of $M_2$ were then set to 9 in active cells and 0.1 elsewhere. The counts were then drawn from a negative binomial distribution with mean $M_1 + M_2$ and shape parameter 10 to promote overdispersion. For MEFISTO, RSF, and FA the count data were normalized to have the same total count at each spatial location, then log transformed with a pseudocount of one. Features were centered before applying each dimension reduction method. For PNMF, NSF, and NSFH the raw counts were used as input. The unsupervised methods (PNMF and FA) used only the $900 \times 500$ count matrix, while the supervised methods (NSF, NSFH, RSF, and MEFISTO) also used the $900 \times 2$ matrix of spatial coordinates. Since this was a smaller dataset, all spatial coordinates were used as inducing point locations to maximize accuracy. All models were fit with $L = 4$ components, except NSFH which was fit with $L = 7$ total components of which $T = 4$ were spatial and the rest nonspatial.

For the quilt simulation, we followed the same procedure as above, but the spatial patterns were $36 \times 36$, leading to 1296 total observations, each at a unique spatial location.

4.7 Data acquisition and preprocessing

For all datasets, after quality control filtering of observations, we selected the top 2000 informative genes using Poisson deviance as a criterion (Townes et al., 2019a; Street et al., 2021). Raw counts were used as input to nonnegative models (NSF, PNMF, NSFH) with size factors computed by the default Scanpy method as described below (Wolf et al., 2017). For real-valued models with Gaussian likelihoods (RSF, FA, MEFISTO), we followed the default Scanpy normalization for consistency with MEFISTO. The raw counts were normalized such that the total count per observation equaled the median of the total counts in the original data. The normalized counts were then log transformed with a pseudocount of one, and the features were centered to have mean zero. This scaled, log-normalized version of the data was then used for model fitting.

4.7.1 Visium mouse brain

The dataset “Mouse Brain Serial Section 1 (Sagittal-Anterior)” was downloaded from https://support.10xgenomics.com/spatial-gene-expression/datasets. To facilitate comparisons, preprocessing followed the MEFISTO tutorial (https://nbviewer.jupyter.org/github/bioFAM/MEFISTO_tutorials/blob/master/MEFISTO_ST.ipynb) (Velten et al., 2020). Observations (spots) with total counts less than 100 or mitochondrial counts greater than 20% were excluded.
4.7.2 Slide-seqV2 mouse hippocampus

This dataset was originally produced by Stickels et al. (2021). We obtained it through the SeuratData R package (Satija et al., 2019) and converted it to a Scanpy H5AD file (Wolf et al., 2017) using SeuratDisk (Hoffman, 2021). Observations (spots) with total counts less than 100 or mitochondrial counts greater than 20% were excluded.

4.7.3 XYZeq mouse liver

This dataset was originally produced by Lee et al. (2021b). We obtained it from the Gene Expression Omnibus (Edgar et al., 2002), accession number GSE164430. We focused on sample liver_slice_L20C1, which was featured in the original publication, and downloaded it as an H5AD file. The spatial coordinates were provided by the original authors. We did not exclude any observations (cells), since all had total counts greater than 100 and mitochondrial counts less than 20%.

4.8 Cell types and GO terms

For each dataset, we fit a NSFH model and applied SPDE-style postprocessing such that the loadings matrices had rows (representing genes) summing to one across all components. We then examined each column of the loadings matrix (representing a component) and identified the five genes with largest weights. We then manually searched for cell types on scfind (https://scfind.sanger.ac.uk/) (Lee et al. 2021a). If no results were found, we next searched the Panglao database (https://panglaodb.se) (Franzén et al., 2019). We identified brain regions in the Slide-seqV2 hippocampus and Visium brain datasets by referring to the interactive Allen Brain Atlas (https://atlas.brain-map.org) (Wang et al., 2020). GO annotations for all genes were downloaded from the BioMart ENSEMBL database (release 104, May 2021) using the biomaRt package in Bioconductor (version 3.13). Enriched terms were identified using the topGO Bioconductor package with the default algorithm “weight01” and statistic “fisher”, considering the top 100 genes for each component against a background of all other genes.

4.9 Software versions and hardware

We implemented all models using Python 3.8.10, tensorflow 2.5.0, tensorflow probability 0.13.0. Other Python packages used include scanpy 1.8.0, squidpy 1.1.0, scikit-learn 0.24.2, pandas 1.2.5, numpy 1.19.5, scipy 1.7.0. We used the MEFISTO implementation from the mofapy2 Python package, installed from the GitHub development branch at commit 8f6ffcb5b18d22b3f4ff2a06bcb92f2806afed0. Graphics were generated using either matplotlib 3.4.2 in Python or ggplot2 3.3.5 (Wickham, 2016) in R (version 4.1.0). The R packages Seurat 0.4.3 (Hao et al., 2021), SeuratData 0.2.1, and SeuratDisk 0.0.0.9019 were used for some initial data manipulations. Computationally-intensive model fitting was done on Princeton’s Della cluster. Each model was assigned 12 CPU cores. We provided the following total memory per dataset: 180 Gb for Slide-seq V2, 72 Gb for Visium, and 48 Gb for XYZeq.

Code for reproducing the analyses of this manuscript is available from https://github.com/willtownes/nsf-paper.

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Supplemental Figures

Figure S1: Nonnegative factorizations recover a parts-based representation in “ggblocks” simulated multivariate spatial count data. (a) Each of 500 features was randomly assigned to one of four nonnegative spatial factors. (b) Negative binomial count data used for model fitting. (c) Real-valued factors learned from unsupervised (nonspatial) dimension reduction. (d) as (c) but using nonnegative components. (e) Real-valued, spatially-aware factors with squared exponential kernel. (f) as (e) but with Matérn kernel. (g) Nonnegative, spatially-aware factors. (h) as (g) but with additional three nonspatial factors.
Figure S2: Benchmarking spatial and nonspatial factor models on Slide-seqV2 mouse hippocampus gene expression data. FA: factor analysis, RSF: real-valued spatial factorization, PNMF: probabilistic nonnegative matrix factorization, NSF: nonnegative spatial factorization, NSFH: NSF hybrid model, lik: likelihood, gau: Gaussian, poi: Poisson, nb: negative binomial. (a) Sparsity of loadings matrix increases with larger numbers of components (dim) in nonnegative models PNMF, NSFH, and NSF. (b) Nonnegative spatial models NSF and NSFH converge faster than MEFISTO but not as fast as nonspatial models FA and PNMF. (c) Negative binomial and Poisson likelihoods provide similar generalization accuracy (lower deviance) in nonnegative models. (d) Negative binomial likelihood is more computationally expensive than Poisson likelihood in nonnegative models.