Statistical Inference for Cell Type Deconvolution

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Abstract

Integrating data from different platforms, such as bulk and single-cell RNA sequencing, is crucial for improving the accuracy and interpretability of complex biological analyses like cell type deconvolution. However, this task is complicated by measurement and biological heterogeneity between target and reference datasets. For the problem of cell type deconvolution, existing methods often neglect the correlation and uncertainty in cell type proportion estimates, possibly leading to an additional concern of false positives in downstream comparisons across multiple individuals. We introduce MEAD, a comprehensive statistical framework that not only estimates cell type proportions but also provides asymptotically valid statistical inference on the estimates. One of our key contributions is the identifiability result, which rigorously establishes the conditions under which cell type proportions are identifiable despite arbitrary heterogeneity of measurement biases between platforms. MEAD also supports the comparison of cell type proportions across individuals after deconvolution, accounting for gene-gene correlations and biological variability. Through simulations and real-data analysis, MEAD demonstrates superior reliability for inferring cell type compositions in complex biological systems.

Keywords: error-in-variable models, single-cell sequencing, transfer learning

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1. Introduction

Integrating data from diverse sources is a common challenge in practical applications, especially when addressing complex problems with limited or unavailable data. In such cases, leveraging information from other datasets—often collected from different individuals or using different technologies—is crucial to fill information gaps within a specific dataset. While this approach offers a cost-effective alternative to collecting entirely new data, it is important to recognize that integrated datasets often introduce more complex biases and randomness compared to freshly collected data. If the inherent heterogeneity across datasets is overlooked, researchers may face an increased risk of false positives during data analysis and statistical inference.

Solutions to this general challenge are often model- and context-specific. In this paper, we focus on a particular problem that arises in cell-type deconvolution in genetics, represented by:

\[ y_i = \tilde{X}_i p_i + \epsilon_i. \]  

Our interest lies in estimating \( p_i \), but the predictors \( \tilde{X}_i \) are unavailable, necessitating the use of external data sources.

Cell-type deconvolution is a computational approach aimed at estimating the proportions of different cell types (Dong et al., 2021; Menden et al., 2020; Newman et al., 2019; Wang et al., 2019), represented as \( p_i = (p_{i1}, \cdots, p_{iK}) \in [0,1]^K \) where \( K \) is the number of cell types, within a tissue of each target individual \( i \), to gain insights into changes in cell type composition across groups of individuals (Figure 1). The composition of cell types plays a critical role in the development of common diseases (Fridman et al., 2012; Mendizabal et al., 2019). However, directly measuring the proportions of each cell type across many individuals remains challenging with current experimental technologies (Jew et al., 2020; O’sullivan et al., 2019). In cell-type deconvolution, bulk RNA sequencing (RNA-seq) provides noisy measurement \( y_i \in \mathbb{R}^G \) of the mixed gene expressions for every target individual, where \( G \) is the number of measured genes. However, there are no direct measurements of cell-type-specific gene expressions \( \tilde{X}_i \in \mathbb{R}^{G \times K} \) for these individuals, deconvolution methods rely on data from other individuals and alternative technologies, such as single-cell RNA sequencing (scRNA-seq), to approximate \( \tilde{X}_i \). Similar
problems also arise in other applications when decomposing mixtures, such as the inference of admixture population and ancestry (Alexander et al., 2009).

Although this concept seems straightforward and the problem involves only linear models, several challenges complicate statistical inference and uncertainty quantification in real-world applications. First, there can be substantial differences between the unknown $\tilde{X}_i$ and the approximated $\tilde{X}_i$ from external sources, due to both technical and biological heterogeneity. Specifically, true gene expression levels vary significantly across the target and reference individuals, and there are also platform-specific biases in measuring gene expressions. In consequence, the approximation of $\tilde{X}_i$ may both be noisy and biased. Recent methods (Cable et al., 2022; Jew et al., 2020) have been developed to account for unknown gene-specific differences between the target and reference platforms. However, cell type proportions may become unidentifiable if arbitrary cross-platform, gene-specific measurement biases are allowed.

Second, specific data structures and preprocessing steps can complicate the inference of estimated cell types. To solve model (1) for any target individual $i$, one can treat the genes as “samples”. However, genes are correlated and often have substantially different scales and noise levels. Additionally, raw gene expression measurements are often normalized as a preprocessing step, introducing further dependencies across genes.

Figure 1: Overview of cell type deconvolution
Third, the parameter $p_i$, representing cell-type proportions, must satisfy the constraints of being non-negative and summing to one, i.e. $\sum_k p_{ik} = 1$. These constraints complicate the estimation process but can help mitigate issues related to scaling differences between the approximated and true $\tilde{X}_i$.

Finally, in cell-type deconvolution, researchers often focus on downstream analyses that examine changes in cell-type proportions across multiple individuals. In this step, the estimated proportions are frequently treated as true values, ignoring both the uncertainty in the estimates and the correlations of the estimates across individuals, since the same reference data is used. It remains unclear whether such simplifications introduce false positives in downstream analyses.

In this paper, we introduce MEAD (Measurement Error Adjusted Deconvolution), a novel method that addresses these challenges and offers asymptotically valid inference for assessing individual-level cell-type proportions as well as quantifying changes in cell-type proportions across multiple individuals. Our method ensures more robust inference than methods that rely on specific family class of data distributions. One of our contributions is showing that the naive approach in downstream analyses—which simply treats the estimated cell-type proportions as the true proportions when comparing the change across multiple individuals—is reliable when the number of target individuals $N$ is relatively small compared to the number of genes, as the noise across the downstream samples dominates estimation errors in $\hat{p}_i$. However, if $N$ is relatively large such that $N/G^2 \rightarrow 0$ while $N/G$ does not vanish, then the naive approach ignoring estimation errors in $\hat{p}_i$ can introduce false positives. Surprisingly, we find that the naive approach is still reliable even when $N$ is large if one is only interested in testing for the global null that none of the proportions change with any features of interest. While we specifically proved this result for MEAD, our simulations show that it appears to be a general rule for other cell-type deconvolution methods as well.

Another contribution is that we establish necessary and sufficient conditions for identifying $p_i$ when gene-specific measuring biases differ arbitrarily across sequencing platforms. We show that merely having multiple target individuals is not enough to guarantee the separation of gene-specific cross-platform bias ratios from the cell type proportions. The problem is only
identifiable under specific constraints of the cell-specific gene expression matrix.

2. Model Setup and Identification

Let $K$ denote the number of cell types, and $G$ the number of genes measured in both bulk RNA-seq and scRNA-seq data. Define $N$ as the total number of target individuals in the bulk RNA-seq dataset requiring deconvolution, and $M$ as the number of reference individuals. While $N$ can often be large, potentially including hundreds of individuals, the number of reference individuals $M$ is typically quite limited since most scRNA-seq experiments sample only a small number of subjects. In our notation, unbolded lowercase letters represent scalars, bold lowercase letters represent vectors, and bold uppercase letters represent matrices.

For a target individual $i$ measured in bulk RNA-seq, the observations follow the model:

$$
y_i = \gamma_i \text{diag}(\alpha) X_i p_i + \epsilon_i, \quad \mathbb{E}(\epsilon_i | X_i) = 0.
$$

(2)

Here, matrix $X_i \in \mathbb{R}^{G \times K}$ represents the true cell-type specific average gene expression, and terms $\gamma_i$ and $\alpha = (\alpha_1, \cdots, \alpha_G)$ represent the subject-specific and gene-specific biases in the measured gene expressions, respectively (Wang et al., 2018). Variations in $\alpha_g$ across genes can arise due to gene-specific factors such as gene length and GC content, both of which affect sequencing efficiency (Benjamini and Speed, 2012). The scalar $\gamma_i$ accounts for differences in sequencing depth between subjects and variability in tissue sample size. Compared to the general model (1), the matrix $\tilde{X}_i = \gamma_i \text{diag}(\alpha) X_i$ captures both the true underlying gene expression of individual $i$ and the technical (measurement) biases in bulk RNA-seq data.

For a reference individual $j$ measured in scRNA-seq, the observations can be summarized as following the model:

$$
Z^r_j = \gamma^r_j \text{diag}(\alpha^r) X^r_j + E^r_j, \quad \mathbb{E}(E^r_j | X^r_j) = 0.
$$

(3)

Although scRNA-seq measures gene expression at the level of individual cells, we define the cell-type-specific observations $Z^r_j \in \mathbb{R}^{G \times K}$ as the observed average gene expression within each cell type (see Section S2.1 for its connection with cell-level measurements). The matrix $X^r_j \in \mathbb{R}^{G \times K}$ represents the true cell-type specific average gene expression for the reference individual.
Similar to the bulk RNA-seq model, terms \( \gamma_j \) and \( \alpha^r = (\alpha^r_1, \cdots, \alpha^r_G) \) account for subject-specific and gene-specific biases in gene expression measurements. It is important to note that, due to differences in sequencing technologies, the gene-specific biases \( \alpha^r_g \) in the scRNA-seq data may differ from \( \alpha_g \) in the bulk RNA-seq data for most genes (Cable et al., 2022; Jew et al., 2020).

2.1. Identifiability allowing arbitrary cross-platform bias ratios

We now introduce several assumptions necessary for the identification of the cell-type proportions \( p_i \). Some of these assumptions are implicitly used in other cell-type deconvolution methods, often in stronger forms. Unlike most methods, our identification conditions accommodate differences between cross-platform measuring biases \( \alpha \) and \( \alpha^r \), treating these biases as fixed, while allowing true cell-type-specific gene expression levels to be random across individuals.

The first key assumption establishes a connection between the true cell-type-specific gene expressions of the target and reference individuals. A natural assumption is that both cohorts are sampled from the same population.

**Assumption 1** (Homogeneous population). Both the bulk and reference individuals are randomly sampled from the same population. Specifically,

\[
X_1, \cdots, X_N \text{i.i.d.} \sim F_X, \quad X_1^r, \cdots, X_M^r \text{i.i.d.} \sim F_X.
\]

For any \( X \in F_X \), denote its mean as \( U^\star = \mathbb{E} [X] \). Additionally, the measurement noise terms \( \epsilon_1, \cdots, \epsilon_N \) and \( E_1, \cdots, E_M \) are mutually independent from each other.

Assumption 1 implies unbiased sampling from the population, which might not always hold in practice. Potential relaxations of this assumption are discussed in Section 8.

Define a biased population-level cell-type gene expression matrix \( U \stackrel{\Delta}{=} (\mu_{gk})_{G \times K} = \text{diag}(\alpha^r)U^\star \in \mathbb{R}^{G \times K} \). Given model (3) of the reference data, it is straightforward to show that \( U \) is identifiable up to a scaling constant (Corollary S1). Without loss of generality, we assume that \( \sum_{k=1}^K \sum_{g=1}^G \mu_{gk} = KG \) for a proper scaling of the population-level gene expression matrix.
Based on Assumptions 1, we can rewrite model (2) as

$$y_i = \gamma_i \Lambda U p_i + \epsilon_i'$$

(4)

where $\Lambda = \text{diag}(\lambda_1, \cdots, \lambda_G)$ with $\lambda_g = \alpha_g / \alpha_g'$ representing the gene-specific measurement bias ratios between the two sequencing platforms. The noise term $\epsilon_i'$ still satisfies $E(\epsilon_i') = \gamma_i \text{diag}(\alpha) E(X_i - U^*) p_i = 0$. Denote $P = [p_1, \cdots, p_N]$, Equation (4) indicates that each column of the matrix $\Lambda U P$ is identifiable up to a scaling factor.

Since the cell type proportions $P$ are constrained such that for each individual $i$, $\sum_k p_{ik} = 1$, the problem of identifying $P$ can be rephrased as separating $B = (\beta_1, \cdots, \beta_N)$ and $\Lambda$ where $\beta_i = \gamma_i p_i$ when both $\Lambda U B$ and $U$ are known. The following theorem provides a rigorous statement regarding the necessary and sufficient conditions:

**Theorem 1.** Under Assumption 1 and the condition $\text{rank}(P) = K$, the cell-type proportions matrix $P = [p_1, \cdots, p_N] \in \mathbb{R}^{K \times N}$ is identifiable if and only if the following two conditions hold:

a. The matrix $U$ has full rank $K$;

b. For any partition $\{I_1, I_2, \cdots, I_t\}$ of the genes $\{1, 2, \cdots, G\}$ with $t \geq 2$, denote $U_{I_k}$ as the $|I_k| \times K$ sub-matrix of $U$ corresponding to rows in $I_k$, we have $\sum_{s=1}^t \text{rank}(U_{I_s}) > K$.

The first condition ensures that sufficient informative genes are available to estimate the cell-type proportions, a standard requirement in linear regression. For the second condition, intuitively, if the genes can be partitioned so that each partition contains non-overlapping information for different cell types, it becomes impossible to distinguish between $\Lambda$ and $P$. For example, if all genes are perfect marker genes for the involved cell types, $P$ cannot be identified.

**Example (counter-example).** Consider a matrix $U$ where only marker genes for each cell type are selected, a typical suggestion in many deconvolution methods (Chen et al., 2018; Newman et al., 2019). Specifically, assume that the marker genes are perfect, meaning $u_{gk} > 0$ while $u_{gk'} = 0$ for any $k' \neq k$ if gene $g \in I_k$, where $I_k$ is the set of marker genes for cell type $k$.

To show that $P$ is not identifiable, let $\delta = (\delta_1, \cdots, \delta_K)$ be any non-negative vector. Define a
new gene-bias ratio matrix $\tilde{\Lambda} = \text{diag}(\tilde{\lambda}_1, \ldots, \tilde{\lambda}_G)$ with $\tilde{\lambda}_g = \lambda_g / \delta_k$ if $g \in I_k$. Also, define new cell-type proportions $\tilde{\mathbf{p}}_i = (\tilde{p}_{i1}, \ldots, \tilde{p}_{iK})$ as $\tilde{p}_{ik} = \delta_k p_{ik} / \sum_{l=1}^{K} \delta_l p_{il}$, and a new individual-specific scaling factor $\tilde{\gamma}_i = \gamma_i \sum_{l=1}^{K} \delta_l p_{il}$. Then, we have

$$\tilde{\gamma}_i \tilde{\Lambda} U \tilde{\mathbf{p}}_i = \gamma_i \Lambda U \mathbf{p}_i, \quad \forall i = 1, 2, \ldots, N.$$  

This indicates that $\mathbf{P}$ is not identifiable.

Finally, the assumption $\text{rank}(\mathbf{P}) = K$ in Theorem 1 implicitly requires that $N$ is at least $K$. This condition, previously highlighted in Jew et al. (2020) and Cable et al. (2022), emphasizes the need for a sufficient number of target individuals to effectively estimate $\mathbf{P}$ under arbitrary gene-specific platform bias. Theorem 1 underscores that satisfying $N \geq K$ alone is insufficient—the selection of genes used in deconvolution also plays a crucial role.

### 2.2. The final model with an independent prior on bias ratios

Although the identification conditions in Theorem 1 allow arbitrary gene-specific bias ratios $\{\lambda_g, g = 1, 2, \ldots, G\}$, accurately estimating these ratios and addressing their uncertainty is challenging, especially when dealing with a large number of genes. To simplify the inference framework, we introduce an additional assumption that treats the bias ratios $\lambda_g$ as non-informative, following Cable et al. (2022). It is more flexible than most existing methods, which often assume constant bias ratios across all genes.

**Assumption 2** (Non-informative gene-specific bias ratios). The gene-specific bias ratios $\{\lambda_g = \alpha_g / \alpha_g^T\}$ have an independent prior with mean $\lambda_0$ and variance $\sigma_0^2$, following $\lambda_g \overset{i.i.d.}{\sim} [\lambda_0, \sigma_0^2]$.

Since the proportion vector $\mathbf{p}_i$ for individual $i$ must satisfy $\mathbf{p}_i^T \mathbf{1} = 1$, and the scalar $\gamma_i$ is also a free parameter that can adjust for scaling differences, we assume without loss of generality that $\lambda_0 = 1$ in Assumption 2. Additionally, as $U$ is identifiable up to a scaling constant, we assume $\sum_{k=1}^{K} \sum_{g=1}^{G} \mu_{gk} = KG$ for a proper scaling of the population-level gene expression matrix. Define $\mathbf{\beta}_i = \gamma_i \mathbf{p}_i$. This allows us to further simplify the model for target individual $i$ as:

$$\mathbf{y}_i = U \mathbf{\beta}_i + \mathbf{e}_i, \quad \mathbf{p}_i = \mathbf{\beta}_i / ||\mathbf{\beta}_i||_1, \quad (5)$$
where the error term \( e_i = (\Lambda - I)U\beta_i + \epsilon'_i \) accounts for both biological variability and measurement error in the target data. Under assumptions 1-2, we have \( \mathbb{E}[e_i] = 0 \).

Similarly, we can simplify the model for reference individual \( j \) as:

\[
Z'_j = \gamma'_j U + \hat{E}'_j
\]

where the noise matrix \( \hat{E}'_j = \text{diag}(\alpha'_r)X_rj - U \) satisfies \( \mathbb{E}[\hat{E}'_j] = 0 \).

Assumption 2 implies that the gene-specific bias ratios do not depend on the expression levels \( U \), thus can be incorporated into the error term of the final model (5). Under this assumption, the bias ratios \( \lambda_g \) do not introduce systematic bias in the estimates of \( \beta_i \), but they increase their uncertainty and introduce extra dependence of the estimated proportions across individuals. For example, the errors \( e_i \) are no longer independent across target individuals, which complicates the analysis when comparing multiple individuals. Furthermore, our final models do not assume a specific distribution family for the observed data and are flexible to accommodate heterogeneity and correlations across genes or cell types within each target or reference individual.

### 3. Model Estimation

We first derive an estimate \( \hat{U} \) from the reference data. Then, the estimation of the coefficients \( \beta_i \) in model (5) becomes an error-in-variable linear regression problem, characterized by heterogeneous and correlated noise, as well as non-negative coefficients. To address these challenges, we develop a new procedure for estimating the cell-type proportions \( p_i \). While our procedure follows the general steps of existing deconvolution methods, it includes specific adjustments to account for the bias introduced by the estimation error in \( \hat{U} \).

#### 3.1. Estimation of \( U \)

For the reference data, let \( z'^{r}_{jgk} \) denote the \((g, k)\)-th entry of matrix \( Z'_j \), and \( z'^r_{jg} \) represent the \( g \)-th row of \( Z'_j \). Also, denote the row vector \( \mu_g = (\mu_{g1}, \cdots, \mu_{gK}) \), which is the \( g \)-th row of \( U \). A natural estimator of \( \mu_g \) is the sample average with estimated individual-specific scaling factors:

\[
\hat{\gamma}_j = \frac{\sum_{k=1}^{K} \sum_{g=1}^{G} z'^{r}_{jgk}}{KG}, \quad \hat{\mu}_g = \frac{1}{M} \sum_{j=1}^{M} \frac{z'^{r}_{jg}}{\hat{\gamma}_j}.
\]
Let \( \hat{U} = (\hat{\mu}_1, \cdots, \hat{\mu}_G)^T \) and define \( V_g = \text{Cov} [\hat{\mu}_g] \) as the variance of each \( \hat{\mu}_g \). Since the noise terms \( \tilde{E}_j \) in model (6) are independent across \( j \), we estimate the covariance \( V_g \) using the sample covariance of the set \( \{ z_{jg}^r / \tilde{\gamma}_j, j = 1, 2, \cdots, M \} \):

\[
\hat{V}_g = \frac{1}{M(M-1)} \sum_{j=1}^M \left( \frac{z_{jg}^r}{\tilde{\gamma}_j} - \hat{\mu}_g \right) \left( \frac{z_{jg}^r}{\tilde{\gamma}_j} - \hat{\mu}_g \right)^T.
\]

**Remark 1.** Both \( \hat{\mu}_g \) and \( \hat{V}_g \) are not unbiased estimators, since the true scaling factors \( \gamma_j \) are unknown. However, as we will show in Section 4, under appropriate assumptions, \( \hat{\gamma}_j \) is a consistent estimator of \( \gamma_j \). Consequently, \( \hat{\mu}_g \) and \( \hat{V}_g \) will be asymptotically unbiased as \( G \to \infty \).

### 3.2. Estimation of the cell type proportions

For the target data, model (5) takes the form of a linear regression if we treat each gene as a “sample”. As the reference data provides a noisy estimate of \( U \), estimating \( \beta_i \) becomes an error-in-variables regression problem (Fuller, 2009). However, unlike the classical errors-in-variables regression problem, there can be substantial differences across genes, and they are often not independent. To provide an efficient point estimator of \( \beta_i \), it is important to weigh the genes differently (Wang et al., 2019). Specifically, we consider using a non-random weight matrix \( W = \text{diag}(w) \), where \( w = (w_1, \cdots, w_G) \). Many existing deconvolution methods focus on selecting an appropriate \( W \) (Newman et al., 2019; Wang et al., 2019). In Section 6.1, we will introduce our empirical approach for determining \( W \) and explain how it relates to, and differs from, existing methods. Here, we focus on the estimation of \( \beta_i \) given a pre-chosen \( W \).

Following a classic errors-in-variable regression approach (Fuller, 2009), we derive bias-corrected estimating equations for \( \beta_i \) as:

\[
\phi(\beta_i) = \hat{U}^T W y_i - (\hat{U}^T W \hat{U} - \hat{V}) \beta_i = 0
\]

where \( \hat{V} = \sum_{g=1}^G w_g \hat{V}_g \). If the estimators \( \hat{\mu}_g \) and \( \hat{V}_g \) are asymptotically unbiased for \( \mu_g \) and \( V_g \) as \( G \to \infty \), then we also have the asymptotic validity, meaning \( \mathbb{E} [\phi(\beta_i)] \to 0 \) at the true value of \( \beta_i \). A key feature of these estimating equations is that their asymptotic validity is robust to the heterogeneity and correlations across genes.
Without any constraints, Equation (7) has a simple analytical solution:

\[
\hat{\beta}_i = (\hat{U}^T W \hat{U} - \hat{V})^{-1} \hat{U}^T W y_i.
\] (8)

Since the elements of \(\beta_i\) should be non-negative, we obtain a non-negative estimator \(\hat{\beta}^*_i\) either by setting \(\hat{\beta}^*_i = \hat{\beta}_i \lor 0\), or by solving the non-negative least squares problem using a bias-adjusted quadratic loss function:

\[
\hat{\beta}^*_i = \arg\min_{\beta_i \succeq 0} (y_i - \hat{U}_i \beta_i)^T W (y_i - \hat{U}_i \beta_i) - \beta_i^T \hat{V} \beta_i.
\]

In either case, \(\hat{\beta}^*_i \neq \hat{\beta}_i\) only when any element \(\hat{\beta}_{ik} < 0\) in \(\hat{\beta}_i\). The cell type proportions \(p_i\) are then estimated as

\[
\hat{p}_i = \frac{\hat{\beta}^*_i}{|\hat{\beta}^*_i|_1}.
\] (9)

4. Statistical inference for a single target individual

In this section, we analyze the theoretical properties of the estimator \(\hat{p}_i\) and discuss the construction of the confidence interval for \(p_{ik}\). We focus on a particular target individual \(i\), with a comparison across multiple individuals deferred to Section 5. Typically, the number of genes \(G\) is large, while the number of reference individuals \(M\) and cell types \(K\) are relatively small. Therefore, we focus on the asymptotic regime where \(G \to \infty\), while \(M\) and \(K\) remain fixed.

As mentioned earlier, Model (5)-(6) accommodate potential dependencies among genes. However, we still need additional assumptions on the dependence structure to establish desirable theoretical properties for the estimator \(\hat{p}_i\). To address this, we introduce the notion of an “almost sparse” gene-gene dependence structure, which is based on the following definition of a dependency graph.

**Definition 1** (Dependency graph, Chen and Shao (2004)). Consider a set of random variables \(\{X_i, i \in \mathcal{V}\}\) indexed by the vertices of a graph \(\mathcal{G} = (\mathcal{V}, \mathcal{E})\). \(\mathcal{G}\) is said to be a dependency graph if, for any pair of disjoint sets \(\Gamma_1\) and \(\Gamma_2\) in \(\mathcal{V}\) such that no edge in \(\mathcal{E}\) has one endpoint in \(\Gamma_1\) and the other in \(\Gamma_2\), the sets of random variables \(\{X_i, i \in \Gamma_1\}\) and \(\{X_i, i \in \Gamma_2\}\) are independent.
Let \( \mathbf{e}_i = (e_{i1}, \ldots, e_{iG}) \) in model (5) and \( \mathbf{E}_j = (e_{j1}, \ldots, e_{jG})^T \) in model (6). We assume that these random error terms of most genes adhere to a dependency graph with a fixed degree, implying that each gene depends on a limited number of other genes. However, we allow for a small subset of genes that may have arbitrary dependencies with other genes, incorporating the presence of both structured dependencies and a few genes with more complex interdependencies.

**Assumption 3** (Dependence structure across genes). There exists a subset of genes \( V \subset \{1, 2, \ldots, G\} \) such that the noise in either target or reference individuals, \( \{(e_{ig}, \forall i), (e_{jg}, \forall j)\}, g \in V\), forms a dependency graph \( \mathcal{G} \). The maximal degree \( D \) of \( \mathcal{G} \) satisfies \( D \leq s \) for some constant \( s \), and \( |V^c|/\sqrt{G} \to 0 \) as \( G \to \infty \).

**4.1. Consistency**

Rather than imposing assumptions about the parametric form of the data distributions, we assume that the observed data have bounded moments across genes. This assumption ensures that the variability in gene expression is not dominated by a small subset of genes.

**Assumption 4** (Bounded moments). For models (5)-(6), assume

a. As \( G \to \infty \), \( \frac{1}{G} \mathbf{U}^T \mathbf{W} \mathbf{U} \to \Omega \), where \( \Omega > 0 \) is a positive definite matrix and \( \mathbf{W} \) is the chosen non-random weight matrix.

b. There exists some \( \delta > 0 \) and a constant \( C \) such that \( \max_{i,g} \mathbb{E}[y_{ig}^{4+\delta}] \leq C \), \( \max_{j,g,k} \mathbb{E}[(z_{jgk}^r)^{4+\delta}] \leq C \), and \( \max_{g} \mathbb{E}[\lambda_g^{4+\delta}] \leq C \). In other words, the \((4+\delta)\)th moments of all these variables are uniformly bounded above by \( C \). Additionally, the biased population-level gene expressions are bounded, i.e., \( \max_{g} \|\mu_g\|_1 \leq C \).

c. The weights \( w_g \) are uniformly bounded, with \( 0 \leq w_g \leq C \) for any gene \( g \).

With these conditions, we can establish the consistency of the estimator \( \hat{p}_i \) as follows:

**Theorem 2.** Under Assumptions 1-4, with \( M \) and \( K \) fixed and \( G \to \infty \), for any reference individual \( j \in \{1, \ldots, M\} \), we have:

\[
\hat{\gamma}_j \overset{p}{\to} \gamma_j, \quad \hat{\Omega} \overset{d}{=} \frac{1}{G}(\hat{\mathbf{U}}^T \mathbf{W} \hat{\mathbf{U}} - \hat{\mathbf{V}}) \overset{p}{\to} \Omega,
\]
and for any target individual $i$,

$$\hat{\mathbf{p}}_i \xrightarrow{p} \mathbf{p}_i.$$  \hfill (11)

### 4.2. Asymptotic normality

Given that we allow gene-gene correlations while treating each gene as a “sample”, an additional technical condition is required to ensure that the variance $\text{Cov} \left[ \sqrt{G} \hat{\mathbf{p}}_i \right]$ increases sufficiently as $G \to \infty$. This condition enables us to study the asymptotic distribution of $\hat{\mathbf{p}}_i$.

Define $\bar{\epsilon}_g = \frac{1}{M} \sum_{j=1}^{M} (\epsilon_{jg}^r / \gamma_j^r)$. Also, define

$$\mathbf{H}_g = \sum_{g=1}^{G} w_g \left[ \epsilon_g^r (\epsilon_g^r + \mu_g)^T - \text{Cov}_M (\epsilon_g^r) \right], \quad s_i = \sum_{g=1}^{G} w_g e_{ig} \epsilon_g^r$$

where $\text{Cov}_M (\epsilon_g^r) \overset{\Delta}{=} \left( \sum_{j=1}^{M} (\epsilon_{jg}^r - \bar{\epsilon}_g^r) (\epsilon_{jg}^r - \bar{\epsilon}_g^r)^T \right) / [M(M-1)]$.

We introduce the following assumption:

**Assumption 5** (Non-collapsing variance). When $G \to \infty$, we have $\lim_{G \to \infty} \text{Cov} \left[ \text{vec} \left( \mathbf{H}_g \right) \right] / G > 0$, and for each target individual $i$, $\lim_{G \to \infty} \text{Cov} \left[ s_i \right] / G$ exists.

Empirical studies suggest that most gene-gene correlations are positive. As a result, the variances $\text{Cov} \left[ \text{vec} \left( \mathbf{H}_g \right) \right]$ and $\text{Cov} \left[ s_i \right]$ are typically larger than they would be if the genes were independent, making this assumption practical and reasonable. Assumption 5 ensures that the variance of the estimating equation $\phi(\mathbf{p})$. Specifically, we have the following result:

**Lemma 1.** Under Assumptions 1-5, we have

$$\Sigma_i \overset{\Delta}{=} \lim_{G \to \infty} \frac{\text{Cov} \left[ \phi(\mathbf{p}_i) \right]}{G} > 0$$  \hfill (12)

for each target individual $i$, which further ensures that $\lim_{G \to \infty} \text{Cov} \left[ \sqrt{G} \hat{\mathbf{p}}_i \right] > 0$.

Building on this, and using the central limit theorem for dependent random variables with local dependence from Chen and Shao (2004), we have the following result:

**Theorem 3.** Under Assumptions 1-5, for each target individual $i$, if $p_{ik} > 0$ for all $k$, then the
estimate \( \hat{p}_i \) is asymptotically normal as \( G \to \infty \). Specifically,

\[
\sqrt{G}(\hat{p}_i - p_i) \overset{d}{\to} N(0, \nabla g(\beta_i)^T \Omega^{-1} \Sigma_i \Omega^{-1} \nabla g(\beta_i))
\]  

(13)

where \( \nabla g(x) \) is the Jacobian matrix of the standardizing function \( g(x) = x/(x^T 1) \).

Theorem 3 provides key insights into the standard error of \( \hat{p}_i \). If genes exhibit higher positive correlations, the covariance matrix \( \Sigma_i \) will be larger, leading to increased variability in \( \hat{p}_i \). Similarly, if gene expressions are more homogeneous across different cell types, the inverse of \( \Omega \) will be larger, which also increases the variability of \( \hat{p}_i \).

**Remark 2.** Theorem 3 relies on the condition that all \( p_{ik} > 0 \), as this allows us to apply the following bound: \( \mathbb{P} \left[ \hat{\beta}_k \neq \hat{\beta}_k \right] \leq \sum_{k=1}^K \mathbb{P} \left[ \hat{\beta}_{ik} < 0 \right] \xrightarrow{G \to \infty} 0 \), where \( \hat{\beta}_k^* \) is defined in Section 3.2 via either of the two methods provided. In practical applications, however, some cell types may have exactly zero proportions, leading to an asymptotically non-Gaussian distribution of \( \hat{p}_i \).

To address this issue, we have also implemented an alternative approach by defining a biased estimator \( \hat{p}_i^{(a)} = \hat{\beta}_i^{(a)}/|\hat{\beta}_i^{(a)}|_1 \) where the Softplus transformation \( \hat{\beta}_k^{(a)} = \log[1 + \exp(a\hat{\beta}_{ik})]/a \) is applied, with \( a \) being some tuning parameter. For any finite \( a \), \( \hat{p}_i^{(a)} \) serves as a biased estimator for \( p_i \) but the biases decreases when \( a \to \infty \) as \( \hat{p}_i^{(a)} \) converges to the truncated estimator. This estimator still follows an asymptotically normal distribution even when some cell types have zero proportions. Our empirical results demonstrate that confidence intervals constructed using the asymptotic normality of \( \hat{p}_i^{(a)} \) are generally shorter while still having reasonable coverage in practice. Further details can be found in Section S2.2.

To construct valid confidence intervals for each \( p_{ik} \) based on the asymptotic normality of \( \hat{p}_i \), it is essential to accurately estimate its asymptotic covariance matrix. Intuitively, we can estimate the asymptotic covariance matrix as:

\[
\text{Cov} \left[ \sqrt{G} \hat{p}_i \right] = \nabla g(\hat{\beta}_i)^T \hat{\Omega}^{-1} \hat{\Sigma}_i \hat{\Omega}^{-1} \nabla g(\hat{\beta}_i)
\]

where the consistency of both \( \hat{\beta}_i^* \) and \( \hat{\Omega} \) is guaranteed by Theorem 2, ensuring that these estimates converge to their true values.
The primary challenge lies in obtaining a good estimate of $\Sigma_i$ when genes are correlated. As the function $\phi(\beta_i)$ can be written as $\phi(\beta_i) = \sum_g \phi_g(\beta_i)$ where

$$
\phi_g(\beta_i) = w_g \hat{\mu}_g y_{ig} - (w_g \hat{\mu}_g \hat{\mu}_g - w_g \hat{V}_g) \beta_i,
$$
to estimate $\Sigma_i = \lim_{G \to \infty} \text{Cov} \left[ \sum_g \phi_g(\beta_i) \right] / G$, we can define a sandwich-type estimator:

$$\hat{\Sigma}_i = \frac{1}{G} \left( \sum_{g=1}^G \phi_g(\hat{\beta}_i^*) \phi_g(\hat{\beta}_i^*)^T + \sum_{(g_1, g_2) \in A} \phi_{g_1}(\hat{\beta}_i^*) \phi_{g_2}(\hat{\beta}_i^*)^T \right)
$$

where the set $A = \{(g_1, g_2) : (e_{ig_1}, e'_{jg_1}) \text{ and } (e_{ig_2}, e'_{jg_2}) \text{ are not independent}\}$.

In Section 6, we will discuss how we estimate $A$ (Section 6.2) and apply finite-sample corrections (Section 6.3) to obtain better coverage for our confidence intervals in practice.

5. Statistical Inference across multiple individuals

In practical applications, estimating cell type proportions for individual samples is often an intermediate step. The ultimate goal is usually to investigate variations in cell type proportions across multiple target individuals with different characteristics. These analyses aim to uncover how cell type proportions differ across groups, such as comparing individuals with a specific disease to healthy controls. Researchers may also explore relationships between cell type proportions and continuous traits like age, blood pressure, or genetic factors (Fadista et al., 2014).

Many of these downstream analyses can be framed as a linear regression problem, to regress the true cell type proportions on features of the target individuals:

$$
p_i = p_0 + A^T f_i + \delta_i, \quad i = 1, 2, \cdots N.
$$

Here, $f_i \in \mathbb{R}^S$ such as disease status, treatment assignment, or genotypes. The vector $p_0$ is a nuisance parameter representing the intercept, while we aim to estimate the coefficient matrix $A \in \mathbb{R}^{S \times K}$, which captures how cell type proportions change with these features.

In contrast to Theorem 3, where cell type proportions $p_i$ are treated as fixed, the model here
assumes that $p_i$ are random variables that vary across individuals. For each individual as fixed quantities. We reconcile these perspectives by viewing the previous model and inference as conditional on $p_i$. To account for the randomness of $p_i$, we assume that the noise terms $\delta_i$ are drawn randomly from the population:

**Assumption 6.** In model (14), the noise term satisfies $\delta_i \overset{i.i.d.}{\sim} [0, \Sigma]$ with some unknown covariance matrix $\Sigma \neq 0$, and mutually independent from $\epsilon'_i$ and $\Lambda$ in model (4).

To estimate $A$, we assume without loss of generality that $f_i$ is centered (i.e., $\sum_i f_i = 0$). If the true proportions $p_i$ were observed, the ordinary least squares (OLS) estimator for $A$ would be $A_N = (F^T F)^{-1} F^T P$ where $F = (f_1, \cdots, f_N)^T$ and $P = (p_1, \cdots, p_N)^T$. Since the cell type proportions are estimated, we use the plug-in estimator: $\hat{A} = (F^T F)^{-1} F^T \hat{P}$.

To obtain confidence intervals for $A$, a common, but naive, approach is to treat the estimated proportions $\hat{p}_i$ as the true values and perform standard linear regression inference. However, this method overlooks two key issues: (1) the estimation error in $\hat{p}_i$ may lead to inflated uncertainty in $\hat{A}$, and (2) the correlation among the estimated proportions, as all target individuals share the same reference data. Ignoring these factors can lead to invalid confidence intervals. We assess the impact of estimation errors in $\hat{p}_i$ on downstream analysis under the following assumptions:

**Assumption 7.** We assume the following conditions hold:

- a. Covariates $f_i$ satisfy $\lim_{N \to \infty} \sum_i f_i f_i^T / N > 0$ and $\max_i \|f_i\|_2 \leq C_1$ for some constant $C_1$.

- b. The scaling parameters $\gamma_i$ for the target individuals are bounded below such that $\min_i \gamma_i \geq C_2$ for some $C_2 > 0$, and bounded above such that $\max_i \gamma_i \leq C_3$ for some constant $C_3$. Additionally, $\min_{i,k} p_{ik} \geq C_2 > 0$.

Assumption 7a is standard for linear regression, ensuring that the covariates have sufficient variability for estimation. Assumption 7b is typically satisfied in practice, particularly in RNA-sequencing studies. In these studies, samples are often included only if their total RNA counts exceed a threshold, and there are upper limits due to sequencing budget constraints. This leads to natural lower and upper bounds on the scaling parameters $\gamma_i$. Additionally, as discussed in Remark 2, the assumption $\min_{i,k} p_{ik} \geq C_2 > 0$ ensures that our non-negative refinement $\hat{\beta}_i^*$ is
asymptotically equivalent to $\hat{\beta}_i$, simplifying our analysis.

We decompose $\tilde{A} - A$ into two terms: $\tilde{A} - A = (\tilde{A} - A_N) + (A_N - A)$. The second term, $A_N - A$, represents the error from standard linear regression even when the true cell type proportions are observed. This term is typically of order $O_p(1/\sqrt{N})$.

The first term, $\tilde{A} - A_N$, arises from the estimation of cell type proportions. If the estimated proportions $\hat{p}_i$ are consistent and the number of target individuals $N$ is not too large (specifically, $N/G \to 0$ as $G \to \infty$, where $G$ is the number of genes), we find that the error introduced by the estimation of cell type proportions becomes negligible compared to the standard linear regression error $\tilde{A} - A_N = o_p(1/\sqrt{N})$. In other words, the variability due to estimating cell type proportions is negligible in this regime. More formally:

**Theorem 4.** Under Assumptions 1-7, if the number of target individuals satisfy $N/G \to 0$ when $G \to \infty$, then the term 

$$\tilde{A} - A_N = O_p\left(\frac{1}{\sqrt{G}}\right) = o_p\left(\frac{1}{\sqrt{N}}\right).$$

If $N \to \infty$, we also have $\sqrt{N} \text{vec}(\tilde{A} - A) \stackrel{d}{\to} N\left(0, \Sigma \otimes (F^TF)^{-1}\right)$.

Theorem 4 demonstrates that the naive approach of treating the estimated proportions as true values remains valid under certain conditions. Specifically, when the number of genes $G$ is much larger than the number of target individuals $N$, standard linear regression with a plug-in $\hat{P}$ provides reliable estimates and confidence intervals for $A$. Since the number of genes $G$ is typically at the scale of 10,000, the assumption of $N/G \to 0$ should be reasonable in applications where the number of target individuals is less than a few hundred.

However, if $N$ becomes large relative to $G$, the estimation error in $\hat{p}_i$ becomes less ignorable, and the naive approach may no longer be valid. Thus, under a more relaxed assumption that $N/G^2 \to 0$ as $G \to \infty$, we investigate the asymptotic properties of $\tilde{A} - A_N$. A special case occurs when the global null hypothesis—stating that there is no relationship between the cell type proportions and any covariates in model (14)—is true.

**Theorem 5.** Under Assumptions 1-7, if $N/G^2 \to 0$ as $G \to \infty$, then the term $\tilde{A} - A_N = \ldots$
$O_p(1/\sqrt{G})$ still holds. Further, if the global null $H_0 : \mathbf{A} = \mathbf{0}$ is true, then

$$\hat{\mathbf{A}} - \mathbf{A}_N = O_p\left(\frac{1}{\sqrt{N\sqrt{G}}}\right) + O_p\left(\frac{1}{G}\right) = o_p\left(\frac{1}{\sqrt{N}}\right),$$

which indicates that $\sqrt{N} \text{vec} (\hat{\mathbf{A}} - \mathbf{A}) \overset{d}{\rightarrow} \mathcal{N} (\mathbf{0}, \Sigma \otimes (\mathbf{F}^T\mathbf{F})^{-1})$ if $N \rightarrow \infty$ as in Theorem 4.

The result shows that, surprisingly, under the global null, the estimation error due to $\mathbf{p}_i$ becomes negligible, even when $N$ is large. In contrast, when there is a nonzero linear relationship between the proportion of any cell type and any covariate, the variability introduced by the estimation of cell type proportions becomes non-negligible. The naive method that ignores the measurement error in $\hat{\mathbf{p}}_i$ will lead to asymptotically invalid confidence intervals for elements in $\mathbf{A}$.

Based on the theoretical findings, if the primary goal is to test the global null hypothesis $\mathbf{A} = \mathbf{0}$—that is, to determine whether there is any change in cell type proportions with respect to covariates—ignoring the uncertainties in the estimated proportions is generally acceptable. However, if the researcher’s objective extends beyond the global null hypothesis and involves examining specific relationships between the cell type proportions and the covariates, caution is necessary, particularly when the number of target individuals is on the order of a few hundred or more. In such cases, ignoring the uncertainties in the estimated proportions may lead to an increased risk of false positive findings, as will be demonstrated in Section 7.2.

6. Practical considerations

6.1. Choice of the weight matrix $W$

Given the variability in gene expressions, setting equal weights to all genes may lead to very inefficient estimators. One common approach is to identify a subset of marker genes (Chen et al., 2018) by conducting differential analyses across cell types in the reference data and selecting the top differentially expressed genes. One rationale for this method is to remove genes that are less informative for estimating $\mathbf{p}_i$. However, based on the Gauss-Markov theorem on linear regressions, simply removing a subset of genes does not necessarily improve the efficiency of estimating $\mathbf{p}_i$ (thus $\mathbf{p}_i$) when all samples have equal noise.

A more effective criterion is to choose weights based on the variability of the noise term for each
For instance, the MuSiC method (Wang et al., 2019) estimates the variability for each gene as $\sigma^2_g = \text{Var} \left[ y_{ig} - \mu_T^g \beta_i \right]$ and sets the weight $w_g = 1/\sigma^2_g$. However, our estimating equation (7) cannot directly utilize MuSiC’s weights, as MuSiC makes use of the residuals $y_{ig} - \hat{\mu}_g^T \hat{\beta}_i$ to compute $\hat{\sigma}^2_g$, which depends heavily on the observed $y_{ig}$. This dependence can greatly bias our estimating equation (7).

To address this limitation, we propose a novel method for determining $w_g$. Similar to MuSiC, we set $w_g = 1/\sigma^2_g$, but we estimate $\sigma_g$ solely using the reference scRNA-seq data. In bulk RNA-sequencing, biological randomness often dominates technical noise. Since the matrix $V_g$ from the reference data captures biological variability across cell types, we can roughly approximate $\sigma^2_g$ by leveraging $V_g$. A straightforward approach is to define $s^2_g = 1^T \tilde{V}_g 1$ which approximates the average biological variability across all cell types, and set the weights $w_g = 1/s^2_g$. To avoid extremely small or large weights, we refine this estimate using an empirical Bayes approach.

Specifically, we approximate the variability of the sample variances $s^2_g$ with a scaled Chi-square distribution: $s^2_g \sim \sigma^2_g \chi^2_d/d$ with degrees of freedom $d = M - 1$. We then employ the empirical Bayes method Vash (Lu and Stephens, 2016), which assumes that $\sigma^2_g \sim \sum_k \pi_k \text{InvGamma}(a_k, b_k)$, a mixture of inverse-Gamma distributions. Using Vash, we obtain the “posterior mean” estimate $\tilde{s}^2_g$ of $\sigma^2_g$, shrinking the naive estimates $s^2_g$ towards the mean of $s^2_g$ across genes. This approach helps avoid extreme weight values. Our final weights of each gene are set as $w_g = 1/\tilde{s}^2_g$.

### 6.2. Estimation of gene-gene dependence set $A$

Identifying the set of dependent gene pairs, $A$, is challenging as the specific pairs of dependent genes are typically unknown. To estimate $A$, we rely on the covariance matrix of the target samples and approximate the non-zero elements, which reflect gene-gene dependencies. Since we often have a limited number of subjects to estimate gene-gene correlations for cell-type specific expressions $z^*_{r,ig}$ across reference individuals, we primarily derive $A$ from the target samples.

Theoretically, under the sparse gene-gene dependence assumption 3, the non-zero elements of the covariance matrix can be consistently estimated using thresholding procedures (Cai and Liu, 2011), provided that the number of samples $N$ is sufficiently large. However, in most applications, $N$ is typically much smaller than the number of genes $G$, making these thresholding methods
ineffective. Instead, we employ a multiple testing procedure proposed by Cai and Liu (2016) to identify non-zero correlation entries in a more reliable manner.

Specifically, let the correlation matrix of each $y_i$ be $R = (\rho_{g_1g_2})_{G \times G}$, where $\rho_{g_1g_2}$ represents the correlation between genes $g_1$ and $g_2$. We aim to test the null hypotheses $H_{0,g_1g_2} : \rho_{g_1g_2} = 0$ for all gene pairs. Following Cai and Liu (2016), the test statistics for $H_{0,g_1g_2}$ is defined as $T_{g_1g_2} = \left( \sum_{i=1}^{N}(y_{ig_1} - \bar{y}_{g_1})(y_{ig_2} - \bar{y}_{g_2}) \right) / \sqrt{N\hat{\theta}_{g_1g_2}}$ where $\bar{y}_{g}$ is the sample mean of gene $g$, and $\hat{\theta}_{g_1g_2}$ is defined as $\hat{\theta}_{g_1g_2} = \sum_{i=1}^{N}((y_{ig_1} - \bar{y}_{g_1})(y_{ig_2} - \bar{y}_{g_2}) - \hat{\sigma}^2_{g_1g_2})^2 / N$ with $\hat{\sigma}^2_{g_1g_2}$ being the sample covariance between gene $g_1$ and $g_2$. Define $b_G = \sqrt{4 \log G - 2 \log(\log G)}$ and given a desired false discovery rate (FDR) level $\alpha$, we reject $H_{0,g_1g_2}$ if $|T_{g_1g_2}| \geq \hat{t}$ where

$$\hat{t} = \inf \left\{ 0 \leq t \leq b_G : \frac{(2 - 2\Phi(t))(G^2 - G)/2}{\max \left( \sum_{1 \leq g_1 < g_2 \leq G} I(|T_{g_1g_2}| \geq t), 1 \right)} \leq \alpha \right\}$$

with $\Phi(t)$ being the cumulative distribution function of the standard normal distribution.

If $N$ is still too small, we can also leverage publicly available bulk RNA-seq data, such as the GTEx database (Lonsdale et al., 2013), for the same tissue to identify the top gene-gene pairs with non-zero correlations. Our simulation studies show that the coverage of our confidence intervals is not very sensitive to the choice of FDR level $\alpha$ (Table S1).

6.3. Finite-sample correction

Our proposed sandwich estimator $\hat{\Sigma}_i$ for the covariance matrix of $\hat{\beta}_i$ tends to underestimate the true covariance, primarily because we substitute $\hat{\beta}^{*}_i$ for the true $\beta_i$. Although the number of genes $G$ is large, the effective sample size can still be small due to the fact that many gene-gene dependence pairs exhibit positive correlations. This issue is common in sandwich estimators (Long and Ervin, 2000), making finite-sample corrections necessary in the estimation of $\Sigma_i$.

One of the most widely used finite-sample corrections for sandwich estimators is the HC3 method (MacKinnon and White, 1985), which utilizes the jackknife estimator of $\beta_i$. The Jackknife estimator involves omitting one gene $g$ at a time and using the remaining genes to compute a
new estimator $\hat{\beta}_{ig}$. By iterating this process across genes, it estimates $\Sigma_i$ as:

$$\hat{\Sigma}_i = \frac{1}{G} \left( \sum_{g=1}^{G} \phi_g(\hat{\beta}_{ig}^*) \phi_g(\hat{\beta}_{ig}^*)^T + \sum_{(g_1,g_2) \in A} \phi_{g_1}(\hat{\beta}_{ig_1}^*) \phi_{g_2}(\hat{\beta}_{ig_2}^*)^T \right).$$  \hspace{1cm} (15)

As defined earlier, $\phi_g(\beta_i) = w_g \mu_g^T y_{ig} - (w_g \mu_g^T \mu_g - w_g \nu_g) \beta_i$ is the gene-specific estimating equation. For independent genes, this leave-one-out method effectively reduces the correlation between $y_{ig}$ and $\hat{\beta}_{ig}^*$. However, in our case, where genes are correlated, $y_{ig}$ and $\hat{\beta}_{ig}^*$ remain correlated, making the standard HC3 correction ineffective.

To account for the correlation structure between genes, we propose a clustering-based $C$-fold cross-validation method, with a default value of $C = 10$. The first step involves using the k-medoids clustering algorithm (Rdusseeun and Kaufman, 1987) on a dissimilarity matrix $11^T - A$.

Here, $A$ is a $G \times G$ matrix, where $a_{g_1g_2} = 1$ if $(g_1,g_2) \in A$ or $g_1 = g_2$, and $a_{g_1g_2} = 0$ otherwise. The purpose of this clustering step is to group the most correlated gene pairs within the same fold, ensuring that dependencies are preserved within each fold.

After forming $C$ clusters, each cluster is treated as a separate fold in the cross-validation process. For each fold $s$, we leave it out and compute a new estimator $\hat{\beta}_{is}^*$ for $\beta_i$ using only genes in the remaining fold. To minimize correlations, we exclude genes that have dependent pairs with genes belonging to fold $s$ in the set $A$. This step further reduces the correlation between $y_{is}$ and $\hat{\beta}_{is}^*$ when gene $g$ belongs to fold $s$. Finally, the corrected covariance estimator $\Sigma_i$ takes the same form as in equation (15), where we set $\hat{\beta}_{is}^*$ to $\hat{\beta}_{is}^*$ if gene $g$ belongs to fold $s$.

After incorporating these practical considerations, the flowchart of the final MEAD method is summarized in Figure 2. Though this final procedure will be too complicated to be fully analyzed theoretically, intuitively, the practical adjustments should not significantly affect the validity of our inference procedure. For instance, while we use weights that are dependent on the reference data, they are still independent from the target data, thus should not severely impact the asymptotic validity of our estimating equation (7). In Section 7, we will demonstrate that the statistical inference provided by MEAD maintains good empirical coverage in both simulations and real data.
7. Simulations and real data analysis

We evaluate the performance of MEAD using both synthetic and real genetic data. In the first simulation, we generate synthetic data by sampling both reference and bulk RNA-seq data from a parametric distribution that incorporates a known gene-gene dependence structure. In the second analysis, based on real data, we directly sample individuals from a population-scale scRNA-seq dataset focused on neuron differentiation (Jerber et al., 2021).

We benchmark MEAD’s performance against ordinary least squares (OLS) using all available genes, as well as two commonly used cell type deconvolution methods: CIBERSORT (Newman et al., 2015) and MuSiC (Wang et al., 2019). Additionally, we compare MEAD to RNA-Sieve (Erdmann-Pham et al., 2021), which, to our knowledge, is the only other method that provides confidence intervals for estimated proportions.

7.1. Synthetic data

We generate synthetic data based on a parametric model with parameters estimated from the scRNA-seq dataset Xin et al. (2016). After excluding four individuals with missing cell types, we compute the mean observed gene expressions per individual and cell type for the remaining 14 individuals. The “true” population mean $\mathbf{U}$ is set as the average of these means, and the “true” covariance matrix across cell types for any gene $\mathbf{V}_g = \text{diag}(\sigma^2_{g1}, \ldots, \sigma^2_{gK}) \in \mathbb{R}^{K \times K}$ is a diagonal matrix where each diagonal element is set as the sample variance of the corresponding gene and
cell type across the 14 individuals. We filter out lowly expressed genes (nonzero expressions in less than 5% of the cells) and highly expressed genes (expression levels above the 95th percentile within each cell type and individual), leaving 9496 genes for further analysis.

To introduce gene-gene dependence structures, we generate the “true” gene expressions \( X_j \) for each simulated individual \( j \) (either target or reference) using a multivariate log-normal distribution. Specifically, for each cell type \( k \), the generated gene expressions \( x_{jk} = (x_{j1k}, \ldots, x_{jGk}) \) satisfy that \( \mathbb{E} [x_{jk}] = \mu_k \) where \( \mu_k \) is the \( k \)-th column of \( U \), \( \text{Var} [x_{jk}] = \sigma^2_{gk} \) and \( \text{Corr} [\log x_{jk}] = R \).

The banded correlation matrix \( R \) has entries \( \rho_{g_1 g_2} = \max \left( 1 - \frac{|g_1 - g_2|}{d}, 0 \right) \) and a bandwidth \( d = 500 \), ensuring that each gene is correlated with 500 other genes. For reference individuals, we generate observed counts \( z_{rkjg} \sim \text{Gamma}(5, 5/x_{jk}) \) for each cell type, so that \( \mathbb{E} [z_{rkjg}] = x_{jk} \) and \( \text{Var} [z_{rkjg}] = x_{jk}^2 / 5 \). For target individuals, the observed counts \( y_{ig} \) are generated from a Poisson distribution:

\[
y_{ig} \sim \text{Poisson} \left( s_i \frac{\sum_k \lambda_g x_{igk} p_{ik}}{\sum_{g'} \sum_k x_{ig'k} p_{ik}} \right), \tag{16}
\]

where the library size \( s_i = 500 \times G \), and the cross-platform bias ratios \( \lambda_g \overset{i.i.d.}{\sim} \mathcal{N}(1, 0.1) \). The cell type proportions \( p_i \) are drawn independently from a Dirichlet distribution with parameters \( \alpha = ap_0 \), where \( p_0 = (0.5, 0.3, 0.1, 0.1) \) represents the population average, and \( a = 10 \) introduces variation across individuals. We set the number of target individuals to \( N = 50 \) and the number of reference individuals to \( M = 10 \). Each experiment is repeated \( B = 100 \) times.

We compare MEAD using three different weight choices against OLS, MuSiC, and CIBERSORT. The weight choices are: equal weights \( (w_g = 1) \), weight 1 on marker genes obtained from Newman et al. (2019) and 0 for others, and the weighting approach proposed in Section 6.1. The same marker genes are used for CIBERSORT. Table 1 presents the root mean square errors (RMSE) for each method. Among the methods, MuSiC achieves the smallest RMSE in this simulation, but MEAD with similar weighting performs comparably. Assigning weights based on marker genes does not significantly reduce the error for MEAD or CIBERSORT. However, the weighting scheme proposed for MEAD reduces the RMSE from 0.338 to 0.073.

We also assess the coverage of CIs for each target individual’s cell type proportions, using equal weights or the weighting approach in Section 6.1 (Table 2), and compare with OLS, with
the CI calculated using the Sandwich estimator with HC3 adjustment. Without considering gene-gene correlations, the coverages of MEAD or OLS both fall far below the nominal level. Then, we explore the impact of adjusting for gene-gene correlations and applying the finite-sample correction using cross-validation, as described in Section 6.3. To estimate gene-gene dependence pairs, we generate additional 100 target samples with random cell type proportions, and apply the proposed method in Section 6.2 with FDR levels of $\alpha = 0.1, 0.3$ or 0.5 (Tables 2 and Table S1). The coverage improves substantially after adjusting for gene-gene correlations, and further improves with the cross-validation method described in Section 6.2, both of which are insensitive to the choice of FDR level when identifying correlated gene pairs. Additionally, Figure 3 illustrates the confidence intervals for each cell type and target individual in one random simulation, demonstrating reasonable interval lengths without excessive conservatism.

![Figure 3: 95% CI of simulated target individuals from one random simulation. Nominal FDR level for selecting the gene-gene dependence pairs is set to $\alpha = 0.1$. The target samples are sorted for each cell type in ascending order according to the true cell type proportions. The grey shaded areas represent the confidence intervals, and the red dots indicate the true proportions.](image)

7.2. Real data

Next, we benchmark MEAD using a population-scale scRNA-seq dataset from Jerber et al. (2021), which profiles neuron development across over 250,000 cells from 175 individuals. The original paper identified seven cell types, but for our analysis, we combined two unknown neuron subtypes due to the small size of one subtype (only identified with at least 10 cells in 16 individuals), leaving us with six cell types. We exclude individuals who have fewer than 10 cells for any cell type and only retain genes that exhibit non-zero observed expressions in all individuals. Additionally, we removed 6 genes ($TMSB4X$, $MALAT1$, $TUBA1B$, $TUBA1A$, $DLK1$, $TTR$) due to abnormal expression patterns. The final dataset includes 12,400 genes and 97 individuals.
We repeat the experiment $B = 100$ times. In each iteration, 11 individuals were randomly selected as the reference, while the remaining 86 individuals were used to create $N$ target samples. We sample with replacement if $N > 86$. We divide the target individuals into two groups of equal size. In the global null scenario, the mean cell type proportions for both groups are set to $\mathbf{p}_1 = \mathbf{p}_2 = (0.3, 0.2, 0.15, 0.15, 0.1, 0.1)$. In the alternative scenario, the mean cell type proportions differ between the groups: $\mathbf{p}_1 = (0.15, 0.15, 0.1, 0.1, 0.2, 0.3)$ and $\mathbf{p}_2 = (0.1, 0.1, 0.2, 0.3, 0.15, 0.15)$. The true cell type proportions for each target individual $i$ are drawn from a Dirichlet distribution, with the parameter $\alpha$ is set to $\alpha = a\mathbf{p}_1$ for group 1, and $\alpha = a\mathbf{p}_2$ for group 2, where the scaling parameter $a = 5$ (more variation) or $a = 20$ (less variation) controls the variation in cell type proportions.

For the reference data, we compute $Z_j^r$ by averaging the normalized single-cell gene expression values within each cell type for the 11 reference individuals. To generate $\mathbf{X}_i$ for each target individual $i$, we average the normalized observed gene expression values within each cell type. Then, we generate $y_{ig}$ following the same Poisson distribution as in (16) with the cross-platform bias ratios $\lambda_g \overset{i.i.d.}{\sim} \mathcal{N}(1, 0.1)$. We estimate gene-gene dependence pairs by pooling all cells from each of the 97 individuals to create 97 individuals into pseudo-bulk samples, applying the multiple testing procedure from Section 6.2 with an FDR level $\alpha = 0.3$.

Figure 4ab shows the RMSE of five methods in the scenario of $N = 86$ and $a = 5$ (Similar results in Figure S1 for $a = 20$), presenting both the estimated individual-level cell type proportions under the global null scenario (equal group means, 4a), and the RMSE of the estimated mean group difference under the alternative scenario (different group means, 4b). MEAD is more accurate overall compared with other methods.

Table 3 and Table S2 compare the coverage of 95% CIs for each individual’s cell type proportions between MEAD and RNA-Sieve. MEAD consistently achieves better coverage, close to the nominal 95%, while RNA-Sieve’s coverage is significantly lower. RNA-Sieve only accounts for measurement errors in single-cell RNA data and variability across cells, whereas MEAD also adjusts for biological variability across individuals and gene-gene correlations. Additionally, Figure S3 illustrates the confidence intervals for each cell type and target individual in one
Figure 4: Comparison of RMSE and coverage across methods when \( a = 5 \). a) RMSE for estimating cell type proportions under the global null scenario. b) RMSE for estimating cell type proportion differences between two groups under the alternative scenario. c) Coverage of 95% CIs of the two group difference by cell types under the alternative scenario with different cell types between groups.

random split, demonstrating the reasonable interval lengths provided by MEAD.

We also examine the coverage of CIs when estimating changes in mean cell type proportions between two groups using a naive two-sample t-test on the estimated proportions. Figure 4c and Figure S2 show the CI coverage for each cell type, including the oracle case where true proportions are known. MEAD consistently shows better coverage for mean differences, comparable to the oracle case. Other methods exhibit good CI coverage for most cell types when \( a = 5 \), while their performance is worse when \( a = 20 \), where the variability of cell type proportions across individuals is smaller.

Table 4 further examines how CI coverage changes as the number of target individuals increases from \( N = 86 \) to \( N = 1000 \). When the global null hypothesis \( H_0 : \mathbf{A} = \mathbf{0} \) (no difference between groups) holds, all CIs exhibit good coverage as \( N \) increases. However, when group means differ (alternative hypothesis), CI coverage decreases across all methods as \( N \) grows, consistent with
the theoretical results in Theorems 4 and 5.

The two case studies above are based on synthetic cross-platform measurement bias differences, where the bias ratios $\lambda_g$ are sampled from a normal distribution $\lambda_g \sim \mathcal{N}(1, 0.1)$. To further assess the performance of our method under real cross-platform bias ratios, where Assumption 2 may not hold, we conducted an additional analysis using scRNA-seq data from two different platforms (Segerstolpe et al., 2016; Xin et al., 2016). Details of the analysis are provided in Section S2.3.

8. Discussion

In this paper, we introduced MEAD, a method for estimating and inferring cell type proportions in cell type deconvolution. Unlike many existing methods that only provide point estimates, MEAD offers asymptotically valid confidence intervals for both individual cell type proportions and changes in proportions across multiple individuals.

We recognize that the assumption that target and reference individuals are drawn from the same population may not always hold in real-world scenarios. For example, the target data may have a higher proportion of individuals with a specific disease than the reference data. To address this, Assumption 1 can be relaxed to assume that the target and reference individuals are sampled from the same sub-population, conditional on certain individual characteristics. This adjustment allows us to estimate mean gene expression matrices for different sub-populations without significantly altering the estimation and inference procedures.

Our discussion also touched on the use of marker genes in cell type deconvolution. Contrary to common practice, we showed both analytically and empirically that selecting marker genes does not necessarily improve estimation accuracy. This aligns with findings in recent empirical studies (Cobos et al., 2020; Tsoucas et al., 2019; Wang et al., 2019).

Lastly, we briefly discussed the impact of missing cell types and the decomposition of a cell type into multiple subtypes. If a cell type $k$ in model (5) is not measured in the reference data, let $U^{(k)}$ represent the matrix $U$ without the $k$-th column. Then, we can only identify $\beta^{(k)}$, the coefficients of the linear projection of $U\beta$ in model (5) onto $U^{(k)}$. The gap between $\beta$ and $\beta^{(k)}$ grows with the size of $\beta_k$ and the similarity between $\mu_k$ and the other columns of $U$. 

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Decomposing one cell type into multiple subtypes can also influence deconvolution results. As suggested by (9) in Theorem 3, splitting a cell type into subtypes reduces gene-gene correlations within each subtype, which decreases $\Sigma_i$ while increasing $\Omega^{-1}$ as the similarity between cell types grows. The trade-off between these effects depends on the application and parameter values, a topic that extends beyond the scope of this paper.

|       | OLS | MEAD (equal weights) | MEAD (marker) | MEAD | MuSiC | CIBERSORT |
|-------|-----|----------------------|---------------|------|-------|-----------|
| RMSE  | 0.080 | 0.093 | 0.089 | 0.073 | 0.059 | 0.094 |

Table 1: RMSE comparisons on simulated data.

| Method  | Correlation Considered | Known Cor | Estimated Cor |
|---------|-------------------------|-----------|---------------|
| OLS     | No                      | 0.29(0.053) |               |
| MEAD (equal weights) | No                      | 0.40(0.080) |               |
| MEAD    | No                      | 0.40(0.089) |               |
| MEAD    | Yes                     | 0.91(0.030) | 0.93(0.048)   |
| MEAD+cv | Yes                     | 0.95(0.021) | 0.97(0.028)   |

Table 2: CI Coverage in simulation. Nominal FDR level for selecting the gene-gene dependence pairs is set to $\alpha = 0.1$. The Coverage reported is averaged over all target individuals. We report both the mean coverage over repeated simulations and its standard deviation in the parenthesis.

|       | DA | Epen1 | Sert | FPP | P | FPP | U | Neur |
|-------|----|-------|------|-----|---|-----|---|------|
| MEAD  | 0.93 | 0.89 | 0.87 | 0.89 | 0.92 | 0.88 |
| RNA-Sieve | 0.11 | 0.18 | 0.12 | 0.16 | 0.23 | 0.13 |

Table 3: Coverage of 95% CIs for each individual’s proportions under the global null with $a = 5$.

|       | Equal group means | Different group means |
|-------|-------------------|----------------------|
|       | $N = 86$ | $N = 500$ | $N = 1000$ | $N = 86$ | $N = 500$ | $N = 1000$ |
| True p | 0.940 | 0.958 | 0.943 | 0.955 | 0.972 | 0.957 |
| MuSiC  | 0.963 | 0.961 | 0.938 | 0.877 | 0.600 | 0.468 |
| CIBERSORT | 0.955 | 0.961 | 0.945 | 0.913 | 0.711 | 0.610 |
| RNA-Sieve | 0.958 | – | – | 0.853 | – | – |
| MEAD  | 0.968 | 0.957 | 0.937 | 0.927 | 0.861 | 0.810 |

Table 4: Mean coverage of the 95% CIs of the group difference with growing $N$ when $a = 5$. RNA-Sieve is not performed for larger samples due to its high computational cost.

**Data and Code Availability**

All data used are publicly available. The scRNA-seq pancreas dataset from Xin et al. (2016) is available at [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE81608](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE81608). The scRNA-seq data from Jerber et al. (2021) is available at [https://zenodo.org/record/4333872](https://zenodo.org/record/4333872).
The code for reproducing results in this paper is accessible at https://github.com/DongyueXie/MEAD-paper, and the R package is available at https://github.com/DongyueXie/MEAD.

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SUPPLEMENTARY MATERIALS FOR “STATISTICAL INFERENCE FOR CELL TYPE DECONVOLUTION”

S1. Supplementary tables and figures

| Method            | Correlation considered | Coverage                |
|-------------------|------------------------|-------------------------|
| OLS               | No                     | 0.29(0.053)             |
| MEAD (equal weights) | No                     | 0.40(0.080)             |
| MEAD              | No                     | 0.40(0.089)             |

Table S1: Mean coverage of 95% CIs on synthetic data for each individual’s cell type proportions with different FDR levels in choosing the gene-gene dependence pairs.

| Method   | Known Cor | Estimated Cor FDR=0.1 | Estimated Cor FDR=0.3 | Estimated Cor FDR=0.5 |
|----------|-----------|-----------------------|-----------------------|-----------------------|
| MEAD     | Yes       | 0.91(0.030)           | 0.93(0.048)           | 0.96(0.030)           | 0.94(0.032)           |
| MEAD+cv  | Yes       | 0.95(0.021)           | 0.97(0.028)           | 0.98(0.017)           | 0.98(0.019)           |

Table S2: Coverage of the 95% CIs for each individual’s cell type proportions when two group means are equal and $a = 20$.

| Method   | DA | Epen1 | Sert | FPP | P | FPP | U_Neur |
|----------|----|-------|------|-----|---|-----|--------|
| MEAD     | 0.92 | 0.88  | 0.83 | 0.87 | 0.91 | 0.86 |
| RNA-Sieve | 0.06 | 0.14  | 0.07 | 0.11 | 0.24 | 0.09 |
Figure S1: Comparison of RMSE across methods when $a = 20$. Left: RMSE comparisons when estimating cell type proportions under the scenario of equal group means. Right: RMSE comparisons when estimating cell type proportion differences between two groups under the scenario of different group means.

Figure S2: Coverage of the 95% CIs for each individual’s cell type proportions for the two group difference by cell types when $a = 20$. The Two sample t-test is applied to the estimated cell proportions for each method under the alternative scenario with different cell types between groups.
Figure S3: Confidence intervals of cell-type proportions in 86 target individuals from one random split of the individuals. Nominal FDR level for selecting the gene-gene dependence pairs is set to $\alpha = 0.3$. The target samples are sorted for each cell type in ascending order according to the true cell type proportions. The grey shaded areas represent the confidence intervals, and read dotted lines indicate the true proportions.
S2. Supplementary text

S2.1. Cell level model for the reference data

In Section 2 of the main text, for a reference individual $j$, we started with the cell-type level model:

$$Z^r_j = \gamma^r_j \text{diag}(\alpha^r) X^r_j + E^r_j, \quad \mathbb{E} \left( E^r_j \mid X^r_j \right) = 0.$$  

As scRNA-seq measures gene expressions for individual cells, we now provide how the cell-type level model is derived from the raw scRNA-seq measurements.

Specifically, denote $y^r_{jc} \in \mathbb{R}^G$ as the observed scRNA-seq counts for cell $c$ in individual $j$. Then we have

$$y^r_{jc} = \gamma^r_{jc} \text{diag}(\alpha^r) \tilde{x}^r_{jc} + e^r_{jc}, \quad \mathbb{E} \left( e^r_{jc} \mid \tilde{x}^r_{jc}, X^r_j \right) = 0 \tag{S1}$$

Here, $\tilde{x}^r_{jc}$ is the true gene expression level in individual $j$ and cell $c$ and the term $\gamma^r_{jc}$ represents the centered measurement errors. The gene-specific biases $\alpha^r$ are the same in model (3) in the main text. The term $e^r_{jc}$ represents the centered measurement errors.

Let $Z^r_j = (z^r_{j1}, \cdots, z^r_{jK})$ where each $z^r_{jk}$ represents the observed average gene expression within cell type $k$. Also, let $X^r_j = (x^r_{j1}, \cdots, x^r_{jK})$ where each $x^r_{jk}$ represents the true average gene expression within cell type $k$. Define the set of cells belonging to cell type $k$ in individual $j$ as $C_{jk}$. Now we require the following assumptions to derive model (3) in the main text from model (S1).

**Assumption S1** (Unbiased sampling of the cells). scRNA-seq provides an unbiased sampling of cells within each cell type. Specifically, for a captured cell $c$ from reference individual $j$, it satisfies $\mathbb{E} \left[ \tilde{x}^r_{jc} \mid X^r_j \right] = x^r_{jk}$ if $c \in C_{jk}$.

**Assumption S2** (Random cell-specific efficiencies). For any reference individual $j$ and any cell $c$,

$$\gamma^r_{jc} \perp \tilde{x}^r_{jc} \mid X^r_j.$$
Also, regardless of the cell type of cell \( c \), \( \mathbb{E} [\gamma_{jc}] = \gamma_j^r \) always holds where \( \gamma_j^r \) is the subject-specific measurement bias for individual \( j \), as defined in model (3) of the main text.

**Remark S1.** In practice, there may exist cell-type specific bias in the scaling factors. Then, one may relax the assumption to \( \gamma_{jc}^r = \gamma_j^r/\delta_k \) for some \( \delta_k \) to allow heterogeneity across cell types. However, we can then only identify biased cell type proportions defined as \( \tilde{p}_{ik} = \delta_k p_{ik} / \sum_k \delta_k p_{ik'} \).

See also a similar discussion in Wang et al. (2019).

Given Assumptions S1-S2, if cell \( c \in C_{jk} \), we can rewrite the model for \( y_{jc}^r \) as

\[
y_{jc}^r = \gamma_j^r \text{diag}(\alpha^r) x_{jk}^r + \tilde{e}_{jc}^r
\]

where \( \mathbb{E} [\tilde{e}_{jc}^r | X_j^r] = \mathbb{E} [(\gamma_{jc}^r - \gamma_j^r) \text{diag}(\alpha^r) \tilde{x}_{jc}^r + \gamma_j^r \text{diag}(\alpha^r) (\tilde{x}_{jc}^r - x_{jk}^r) + e_{jc}^r | X_j^r] = 0 \). Now we define the observed average gene expression within cell type \( k \) as

\[
z_{jk}^r = \frac{1}{|C_{jk}|} \sum_{c \in C_{jk}} y_{jc}^r = \gamma_j^r \text{diag}(\alpha^r) x_{jk}^r + \frac{1}{|C_{jk}|} \sum_{c \in C_{jk}} \tilde{e}_{jc}^r.
\]

**Remark S2.** In the paper, we estimate an individual-level common scaling factor \( \hat{\gamma}_j \) for \( \gamma_j^r \) while a more common scaling approach for scRNA-seq data is to work with the normalized gene expressions \( \tilde{y}_{jc}^r = y_{jc}^r / \hat{\gamma}_{jc} \) which applies different scaling factors (library size) \( \hat{\gamma}_{jc} = (y_{jc}^r)^T 1 \) to different cells. We avoid using cell-specific scaling factors for easier theoretical analysis and to avoid biases due to differences in cell size across cell types (Wang et al., 2019).

### S2.2. Cell type proportion estimation based on the Softplus transformation

As discussed in Remark 2 of the main text, we define the estimator of \( p_i \) after applying the Softplus transformation on the estimated \( \hat{\beta}_i \):

\[
\hat{\beta}_{ik}^{(a)} = h_a(\hat{\beta}_{ik}) \triangleq \frac{1}{a} \log(1 + e^{a\tilde{\beta}_{ik}}), \quad \hat{\beta}_{i}^{(a)} = \frac{\hat{\beta}_{i}^{(a)}}{|\hat{\beta}_{i}^{(a)}|_1}
\]
where $\hat{\beta}_i^{(a)} = (\hat{\beta}_{i1}^{(a)} \cdots \hat{\beta}_{iK}^{(a)})$, and $a$ is a tuning parameter. As $a \to \infty$, it is easy to show that $h_a(\hat{\beta}_{ik}) \xrightarrow{a \to \infty} \max(\hat{\beta}_{ik}, 0)$, which implies:

$$\hat{p}_i^{(a)} \xrightarrow{a \to \infty} \frac{\hat{\beta}_i^*}{|\hat{\beta}_i^*|_1},$$

where $\hat{\beta}_i^* = \hat{\beta}_i \vee 0$.

Since $h_a(\cdot)$ is a smooth function, given the CLT of $\hat{\beta}_i$ as shown in Theorem 3, we can derive the CLT for $\hat{p}_i^{(a)}$ as:

$$\sqrt{G}(\hat{p}_i^{(a)} - p_i^{(a)}) \xrightarrow{d} N\left(0, \nabla g(\beta_i^{(a)})^T \Gamma \Omega^{-1} \Sigma_i \Omega^{-1} \Gamma \nabla g(\beta_i^{(a)})\right)$$

where each element of $\beta_i^{(a)}$ is defined as $\beta_{ik}^{(a)} = h(\beta_{ik})$, and $p_i^{(a)} = g(\beta_i^{(a)})$. The asymptotic covariance matrix involves a diagonal matrix $\Gamma = \text{diag}(\gamma_{11}, \cdots, \gamma_{KK}) \in \mathbb{R}^{K \times K}$, where

$$\gamma_{ii} = h'(\beta_{ik}) = \frac{e^{a\beta_{ik}}}{1 + e^{a\beta_{ik}}}.$$

We can use the asymptotic normality of $\hat{p}_i^{(a)}$ to construct confidence intervals for the cell type proportions, by estimating the asymptotic covariance matrix as

$$\widehat{\text{Cov}} \left[ \sqrt{G} \hat{p}_i^{(a)} \right] = \nabla g(\beta_i^{(a)})^T \widehat{\Gamma} \widehat{\Omega}^{-1} \widehat{\Sigma}_i \widehat{\Omega}^{-1} \widehat{\Gamma} \nabla g(\beta_i^{(a)}).$$

The practical performance of the confidence intervals based on the Softplus transformation depends on a suitable choice of the tuning parameter $a$. If $a$ is too small, the estimator will be biased, as $p_i^{(a)}$ is different from $p_i$. On the other hand, if $a$ is too large, the normal approximation for the distribution of $\hat{p}_i^{(a)}$ may become inaccurate, especially when some elements of $\hat{\beta}_i$ is negative. Additionally, notice that $\hat{\Gamma} \preceq I$ for any value of $a$ and $\hat{\beta}_i$, we will always have $\widehat{\text{Cov}} \left[ \sqrt{G} \hat{p}_i^{(a)} \right] \preceq \widehat{\text{Cov}} \left[ \sqrt{G} \hat{p}_i \right]$ when $a$ is large. Thus, while the two point estimators should be similar when $a$ is large, the inference based on our original estimator should always be more conservative than the one derived from the Softplus transformation.

In our implementation of the Softplus estimator, we found that the absolute scale of $\hat{\beta}_{ik}$ is
typically large, thus by default we set $a = 10$ (we tried $a = 1000$ in our simulation study and find that the results were very similar). To handle potential numerical overflow for large value of $x$, we reformulated the Softplus function as follows:

$$\frac{1}{a} \log(1 + e^{ax}) = \begin{cases} 
  x + \frac{1}{a} \log(1 + e^{-ax}) & \text{if } x > 0, \\
  \frac{1}{a} \log(1 + e^{ax}) & \text{if } x \leq 0.
\end{cases}$$

Figure S4: 95% CI from the Softplus estimator for 4 cell types in 50 simulated target individuals from the same random simulation as Figure 3.

| Method               | Correlation considered | Coverage               | Known Cor               | Estimated Cor |
|----------------------|------------------------|------------------------|-------------------------|---------------|
| OLS                  | No                     | 0.29(0.053)            |                         |               |
| MEAD(equal weights)  | No                     | 0.37(0.065)            |                         |               |
| MEAD                 | No                     | 0.37(0.072)            |                         |               |
| MEAD                 | Yes                    | 0.88(0.035)            | 0.91(0.045)            | 0.94(0.031) | 0.92(0.032) |
| MEAD+cv              | Yes                    | 0.93(0.030)            | 0.95(0.029)            | 0.97(0.020) | 0.96(0.020) |

Table S3: Mean coverage of 95% CIs from the Softplus estimator on synthetic data for each individual’s cell type proportions with different FDR levels in choosing the gene-gene dependence pairs.

For the empirical evaluation of the Softplus transformed estimator, we repeated the simulation study from Section 7.1, using the same settings but substituting the original estimator with the Softplus transformed estimator for cell type proportion estimation and inference. Figure S4 displays the confidence intervals from the same random simulation as in Figure S4. Confidence intervals for non-zero estimates were nearly identical for both the original and Softplus estimators. However, for small proportions where $\hat{\beta}_{ik}$ took negative values, the confidence intervals
using the Softplus transformed estimator were narrower. While the overall coverage slightly decreased under the Softplus transformation, it remained within a reasonable range, as shown in Table S3.

**S2.3. Additional case studies with real cross-platform measurement biases differences**

In addition to the case studies in Section 7, we performed an additional deconvolution analysis involving two different sequencing platforms, following Wang et al. (2019), for human pancreatic islets. Specifically, we constructed pseudo-bulk samples as target individuals by averaging cell-specific gene expression data for each individual from Xin et al. (2016). This dataset has 18 individuals (12 healthy and 6 with Type 2 diabetes (T2D)). The reference data were obtained from Segerstolpe et al. (2016), where scRNA-seq data were collected from 10 individuals (6 healthy and 4 T2D patients). Following MuSiC, we only use the 6 healthy individuals as the reference individuals. Since the scRNA-seq data from Xin et al. (2016) were sequenced using the Illumina HiSeq 2500 protocol, and the data from Segerstolpe et al. (2016) were sequenced using the Smart-seq2 protocol, the two platforms can potentially have systematic differences on the gene-specific measurement biases.

After preprocessing, we retain 17858 genes and 4 cell types (alpha, beta, delta, gamma) for the reference dataset. We compared MEAD against MuSiC and NNLS (Non-negative Least Squares, as implemented in the MuSiC package) for estimation accuracy. As shown in Table S4 and Figure S5, MEAD demonstrated comparable performance with MuSiC in providing point estimates of cell type proportions. Figure S6 illustrates the confidence intervals for each cell type proportion estimated by MEAD. Among all $18 \times 4 = 72$ cell type proportions, 92% were covered by our confidence intervals at the nominal confidence level 0.95.

|          | MEAD | MuSiC | NNLS |
|----------|------|-------|------|
| RMSE     | 0.113| 0.099 | 0.172|
| MAD      | 0.084| 0.064 | 0.117|

Table S4: Comparison of RMSE and MAD (mean absolute deviation) on estimated cell type proportions in the cross-platform deconvolution study.
Figure S5: Heatmap showing estimated cell type proportions in pseudo-bulk data from the cross-platform deconvolution study.

Figure S6: Confidence intervals for cell type proportions in the pseudo-bulk data from the cross-platform deconvolution study.
S2.4. Proofs

In model (5) of the main text, the noise term $e_i$ contains the platform biases $\Lambda$ that is shared across all the target individuals, thus these noise terms are not independent across $i$. When we are comparing multiple target individuals as discussed in Section 5, we need to specifically account for such dependence. Thus in the proof, to simplify the description we decompose $y_i$ following model (4) in the main text where

$$y_i = \Lambda U \beta_i + e'_i \quad (S3)$$

**Corollary S1.** Under Assumptions 1, the scaling factors $\tilde{\gamma}^r_j$ for all reference individuals and the biased population-level expressions $U$ are identifiable up to a common scaling factor. Specifically, if there exists another set of parameters \{\tilde{\gamma}^r_j, j = 1, \cdots M\} and $\tilde{U}$ that yield the same distribution of the observed reference data, then there exists a constant $c$ such that

$$\tilde{\gamma}^r_j = c \gamma^r_j, \quad \tilde{U} = \frac{1}{c} U$$

for all $j = 1, \cdots, M$.

**Proof.** We can simplify model (3) for a reference individual $j$ as

$$Z^r_j = \gamma^r_j U + \tilde{E}^r_j \quad (S4)$$

where

$$E \left[ \tilde{E}^r_j \right] = E \left[ E^r_j + \gamma^r_j \left( \text{diag}(\alpha^r) X^r_j - U \right) \right] = 0$$

Notice that (S4) is similar to a two-way ANOVA model. To show the identification of $\gamma^r_j$ up to a constant $c$, take an average across all entries of $Z^r_j$ in (S4):

$$\bar{z}^r_{j..} = \gamma^r_j \bar{\mu}.. + \bar{e}^r_{j..}$$

where $\bar{\mu}.. = \sum_{j,k} \mu_{jk} / KG$. Since $E(e^r_{j..}) = 0$ and $\bar{\mu}..$ is a shared constant among all reference
individuals, if there exists another set of parameters \( \{ \tilde{\gamma}_j^r, j = 1, \cdots, M \} \) and \( \tilde{U} \) that result in the same distribution of \( \{ Z^r_j, j = 1, \cdots, M \} \), then \( \gamma_j^r \tilde{\mu} = \tilde{\gamma}_j^r \tilde{\mu} \), or alternatively

\[
\tilde{\gamma}_j^r = \frac{\tilde{\mu}}{\tilde{\mu}} \gamma_j^r
\]

showing the identification of \( \gamma_j^r U \) for any \( j \), we have

\[
\tilde{U} = \frac{\tilde{\mu}}{\tilde{\mu}} U
\]

which completes the proof.

\[\Box\]

S2.4.1. Proof of Theorem 1

Similar to the proof of Corollary S1, in model (4), the matrix \( \Lambda U P \) can be identified up to a scaling factor. If \( \text{rank}(\Lambda U) < K \), then \( P \) is not identifiable even when \( \Lambda U \) is identifiable up to a scaling constant, so \( \text{rank}(\Lambda U) = K \) is a necessary condition for the identifiability of \( P \).

Without loss of generality, we assume that \( G \geq K \) and for any \( g, \alpha_g \neq 0 \) and \( \mu_{gk} \neq 0 \) for at least one \( k \). Since under Assumptions 1, \( U \) is identifiable up to a scaling factor, to prove Theorem 1, we only need to show that if \( \Lambda_1 U P_1 = \Lambda_2 U P_2 \) and \( \text{rank}(\Lambda_1 U P_1) = K \) then there exists some constant \( c \) such that \( \Lambda_2 = c \Lambda_1, P_2 = \frac{1}{c} P_1 \) if and only if for any disjoint partition \( \{ I_1, I_2, \cdots, I_t \} \) of \( \{ 1, 2, \cdots, G \} = \bigcup_{s=1}^t I_s \) with \( t \geq 2 \), we have \( \sum_{s=1}^t \text{rank}(U_{I_s}) > K \). Matrix \( P_1 \) and \( P_2 \) do not need to satisfy the constraint that each column has sum 1 as we can always rescale the unobserved \( \gamma_i \) in each target individual so that \( \mathbf{p}_i^T \mathbf{1} = 1 \) in model (4).

To show this, notice that since \( \Lambda_1 U, \Lambda_2 U, P_1, P_2 \) all have full rank, we have \( \text{span}(P_1) = \text{span}(P_2) \). Hence there exists an invertible matrix \( V \in \mathbb{R}^{K \times K} \) such that \( P_2^T = P_1^T V \). Now we only need to prove that there does not exist \( V \neq c_0 I \) for any constant \( c_0 \) if and only if the inequality \( \sum_{s=1}^t \text{rank}(U_{I_s}) > K \) holds for any disjoint partition \( \{ 1, 2, \cdots, G \} = \bigcup_{s=1}^t I_s \) with \( t \geq 2 \).

Denote \( \mu_g \) as each row vector of the matrix \( U \), and let the \( g \)th diagonal element of \( \Lambda_1 \) and \( \Lambda_2 \)
be $\lambda_{g1}$ and $\lambda_{g2}$. Then $\Lambda_1 U P_1 = \Lambda_2 U P_2$ is equivalent to

$$\lambda_{g1} P_1^T \mu_g = \lambda_{g2} P_2^T \mu_g = \lambda_{g1,2} P_1^T V \mu_g,$$

which leads to

$$P_1^T \left( V \mu_g - \frac{\lambda_{g1}}{\lambda_{g2}} \mu_g \right) = 0.$$

Since $P_1$ has full rank, we have $V \mu_g - \frac{\lambda_{g1}}{\lambda_{g2}} \mu_g = 0$, so $\{\lambda_{g1}/\lambda_{g2}\}$ and $\{\mu_g\}$ are eigenvalues and eigenvectors of $V$.

"if": If the condition on partitions holds and there exists $V \neq c_0 I$, then $\lambda_{g1}/\lambda_{g2}$ also takes different value $s_1, ..., s_D$ where $D \geq 2$. Denote the submatrix $U_d = U_{\{g : \alpha_g = s_d\}}$, then we will have $\sum_{d=1}^D \text{rank}(U_d) \leq K$. To see this, notice that the matrix $V$ is similar to its Jordan canonical form $J$. Also, $\text{rank}(U_d)$ is at most the geometric dimension of eigenvalue $s_d$, which equals to the number of Jordan blocks corresponding to $s_d$. Let $J_i$ be the $i$th Jordan block, then $\sum_{d=1}^D \text{rank}(U_d) \leq \sum_i \text{rank}(J_i) = K$. This contradicts with the condition on the partitions.

"only if": assume that there exists some partition with $\sum_{s=1}^t \text{rank}(U_{I_s}) \leq K$. As $\sum_s \text{rank}(U_{I_s}) \geq \text{rank}(U) = K$, we actually have $\sum_{s=1}^t \text{rank}(U_{I_s}) = K$. Let $\tilde{U}_s \in \mathbb{R}^{K \times n_s}$ be the matrix whose columns form the orthogonal basis of $\{\mu_g : g \in I_s\}$. Then $\sum_s n_s = K$ and we can construct a rank $K$ matrix $\tilde{U}_0 = (\tilde{U}_1, ..., \tilde{U}_t) \in \mathbb{R}^{K \times K}$. Let $D = \text{diag}(d_1, \cdots, d_1, \cdots, d_t, \cdots, d_t)$ be a $K$-dimensional diagonal matrix where each $d_s$ replicates $n_s$ times and $d_1, \cdots, d_t$ are not all equal. Then we can construct $V = \tilde{U} D \tilde{U}^{-1}$. As columns of $\tilde{U}$ are eigenvectors of $V$, and columns of each $\tilde{U}_s$ share the same eigenvalue, each $\mu_g$ is also an eigenvector of $V$. So if the condition on the partitions does not hold, for any values of $\{d_1, \cdots, d_t\}$ we can construct a matrix $V$ satisfying $V \mu_g - d_s \mu_g = 0$ if $\mu_g$ is a row of $U_{I_s}$. We also have $V \neq c_0 I$ for any $c_0$ as long as $d_1, \cdots, d_t$ are not all equal.
S2.4.2. Proof of Theorem 2

Notice that by definition in model (S3),

\[
\phi(\beta_i) = \hat{U}^T W y_i - (\hat{U}^T W \hat{U} - \sum_{g=1}^{G} w_g \hat{V}_g) \beta_i \\
\quad = \hat{U}^T W \epsilon'_i - \left( \hat{U}^T W (\hat{U} - \Lambda U) - \sum_{g=1}^{G} w_g \hat{V}_g \right) \beta_i
\]

Define \( H = \hat{U}^T W (\hat{U} - \Lambda U) - \sum_{g=1}^{G} w_g \hat{V}_g \), then by definition

\[
\phi(\beta_i) = \hat{U}^T W \epsilon'_i - H \beta_i.
\]

At the same time, we define oracle “estimators” with know scaling factors:

\[
\hat{U}^* = (\hat{\mu}_1^*, \ldots, \hat{\mu}_G^*)^T
\]

where \( \hat{\mu}_g^* = \frac{1}{M} \sum_{j=1}^{M} z_{jg}^r / \gamma_j^r \). Additionally, we denote

\[
\hat{V}_g^* = \frac{1}{M(M-1)} \sum_{j=1}^{M} \left( \frac{z_{jg}^r}{\gamma_j^r} - \hat{\mu}_g^* \right) \left( \frac{z_{jg}^r}{\gamma_j^r} - \hat{\mu}_g^* \right)^T
\]

\[
\hat{\Omega}^* = \frac{1}{G} \left( \sum_g w_g \hat{\mu}_g^* \hat{\mu}_g^{*T} - \sum_g w_g \hat{V}_g^* \right)
\]

\[
H^* = \hat{U}^{*T} W (\hat{U}^* - \Lambda U) - \sum_{g=1}^{G} w_g \hat{V}_g^*.
\]

**Lemma S1.** Under Assumptions 1-2, we have \( \mathbb{E} \left[ \hat{V}_g^* \right] = V_g \) for each \( g = 1, 2, \ldots, G \).

**Lemma S2.** Under the assumptions of Theorem 2, we have

\[
\hat{\Omega}^* - \Omega = O_p \left( \frac{1}{\sqrt{G}} \right), \quad \hat{U}^{*T} W \epsilon'_i = O_p(\sqrt{G}), \quad H^* = O_p(\sqrt{G})
\]
**Proof of Lemma S2.** By definition and using Lemma S1, it is straightforward that

\[
E\left[\hat{\Omega}^*\right] = \Omega, \quad E\left[\hat{U}^{*T}W \epsilon_i^*\right] = 0, \quad E[H^*] = 0.
\]

Denote \(\epsilon_i' = (\epsilon_i', \ldots, \epsilon_iG')\). Then we can rewrite as

\[
\hat{U}^{*T}W \epsilon_i' = \sum_{g=1}^{G} w_g \hat{\mu}_g^* \epsilon_{ig}'
\]

\[
H^* = \sum_{g} w_g \left( \hat{\mu}_g^* (\hat{\mu}_g^* - \lambda_g \mu_g)^T - \hat{V}_g^* \right).
\]

Under Assumption 4b of bounded moments, for any \(k_1 \leq K\) and \(k_2 \leq K\), \(\text{Var} [\hat{\mu}_{gk_1}^* \hat{\mu}_{gk_2}^*]\), \(\text{Var} [\hat{\mu}_g^*]\) and \(\text{Var} [\lambda_g \mu_g^*]\) are all uniformly bounded across \(g\) (note: \(V_{g,k1,k2}\) denotes the \((k_1,k_2)\)th element of \(V_g\)). Thus

\[
\max_g \text{Cov} \left[ \text{vec} \left( \hat{\mu}_g^* (\hat{\mu}_g^*)^T \right) \right] \leq \text{const.}, \quad \max_g \text{Cov} \left[ \text{vec} \left( \hat{V}_g^* \right) \right] = O(1)
\]

This indicates that

\[
\max_g \text{Cov} \left[ \text{vec} \left( \hat{\mu}_g^* (\hat{\mu}_g^*)^T - \hat{V}_g^* \right) \right] = O(1)
\]

and

\[
\max_g \text{Cov} \left[ \text{vec} \left( \hat{\mu}_g^* (\hat{\mu}_g^* - \lambda_g \mu_g)^T - \hat{V}_g^* \right) \right] = O(1).
\]

In addition, as \(\epsilon_i' \perp \hat{U}\), under Assumption 4b

\[
\max_g \text{Cov} \left[ \hat{\mu}_g^* \epsilon_{ig} \right] = \max_g E \left[ \epsilon_{ig}^2 \right] E \left[ \hat{\mu}_g^* (\hat{\mu}_g^*)^T \right] = O(1).
\]

Thus, combining with Assumption 3 on the gene-gene dependence structure and Assumption 4c, we have

\[
\text{Cov} \left[ \sum_{g \in \mathcal{V}} w_g \hat{\mu}_g^* \epsilon_{ig} \right] = O(G), \quad \text{Cov} \left[ \text{vec} \left( \sum_{g \in \mathcal{V}} w_g \left( \hat{\mu}_g^* (\hat{\mu}_g^*)^T - \hat{V}_g^* \right) \right) \right] = O(G)
\]

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\[
\text{Cov} \left[ \text{vec} \left( \sum_{g \in V_c} w_g \left( \hat{\mu}_g^* (\hat{\mu}_g^* - \lambda_g \mu_g)^T - \hat{V}_g^* \right) \right) \right] = O(G).
\]

On the other hand, as $|V_c| = o\left( \sqrt{G} \right)$, thus

\[
\text{Cov} \left[ \sum_{g \in V_c} w_g \hat{\mu}_g^* \epsilon_{ijg} \right] = o(G), \quad \text{Cov} \left[ \text{vec} \left( \sum_{g \in V_c} w_g \left( \hat{\mu}_g^* (\hat{\mu}_g^* - \lambda_g \mu_g)^T - \hat{V}_g^* \right) \right) \right] = o(G)
\]

\[
\text{Cov} \left[ \text{vec} \left( \sum_{g \in V_c} w_g \left( \hat{\mu}_g^* (\hat{\mu}_g^* - \lambda_g \mu_g)^T - \hat{V}_g^* \right) \right) \right] = o(G).
\]

Thus,

\[
\text{Cov} \left[ \hat{\Psi}^* \right] = O \left( \frac{1}{\sqrt{G}} \right), \quad \text{Cov} \left[ \text{vec} \left( \hat{U}^T W \epsilon_i^j \right) \right] = O(G), \quad \text{Cov} \left[ \text{vec} \left( \Psi^* \right) \right] = O(G)
\]

So using Chebyshev’s inequality,

\[
\hat{\Psi}^* - \Psi^* = O_p \left( \frac{1}{\sqrt{G}} \right), \quad \hat{U}^T W \epsilon_i^j = O_p(\sqrt{G}), \quad H^* = O_p(\sqrt{G}).
\]

\[\square\]

**Lemma S3.** Under the assumptions of Theorem 2, we have

\[
\hat{\Psi} - \Psi^* = O_p \left( \frac{1}{\sqrt{G}} \right), \quad \hat{U}^T W \epsilon_i^j - \Psi^* U W \epsilon_i^j = O_p(1)
\]

\[
H - H^* = -\sum_{j=1}^{M} \frac{1}{\gamma_j^T} \gamma_j (\hat{\gamma}_j - \hat{\gamma}_j^T) U^T W U + O_p(1)
\]

with

\[
\hat{\gamma}_j = \gamma_j^T + O_p \left( \frac{1}{\sqrt{G}} \right)
\]

**Proof of Lemma S3.** First, we show that for each reference individual $j$, the estimate $\hat{\gamma}_j - \gamma_j^T = O_p(1/\sqrt{G})$ when $G \to \infty$. Notice that Under Assumption 4b and Assumption 3

\[
\text{Var} \left( \hat{\gamma}_j \right) = \frac{1}{G^2} \text{Var} \left[ \sum_{g=1}^{G} \sum_{k=1}^{K} \frac{z_{gjk}}{K} \right] = O \left( \frac{1}{G} \right).
\]
As $E[\gamma_j] = \gamma_j^*$, we have $\hat{\gamma}_j - \gamma_j^* = O_p(1/\sqrt{G})$.

Next, by definition

$$\sum_g w_g \hat{\mu}_g \hat{\mu}_g^T - \sum_g w_g \hat{\mu}_g^* (\hat{\mu}_g^*)^T$$

$$= \frac{1}{M^2} \sum_{j_1,j_2=1}^M \left( \frac{\gamma_{j_1}^* \gamma_{j_2}^*}{\gamma_{j_1} \gamma_{j_2}} - 1 \right) \sum_{g=1}^G w_g z_{j_1g}^r (z_{j_2g}^r)^T$$

$$= \frac{1}{M^2} \sum_{j_1,j_2=1}^M \left( \frac{\gamma_{j_1}^* \gamma_{j_2}^*}{\gamma_{j_1} \gamma_{j_2}} - 1 \right) \left( \sum_{g=1}^G w_g \frac{z_{j_1g}^r (z_{j_2g}^r)^T}{\gamma_{j_1} \gamma_{j_2}} - \sum_g w_g \mu_g \mu_g^T \right) + \frac{1}{M^2} \sum_{j_1,j_2=1}^M \left( \frac{\gamma_{j_1}^* \gamma_{j_2}^*}{\gamma_{j_1} \gamma_{j_2}} - 1 \right) U^T W U.$$

Using the same logic as in the proof of Lemma S2, we have

$$\sum_{g=1}^G w_g \frac{z_{j_1g}^r (z_{j_2g}^r)^T}{\gamma_{j_1} \gamma_{j_2}} - \sum_g w_g \mu_g \mu_g^T = O_p(\sqrt{G})$$

with $E \left[ \frac{z_{j_1g}^r (z_{j_2g}^r)^T}{\gamma_{j_1} \gamma_{j_2}} \right] = \mu_g \mu_g^T$. As $\frac{\gamma_{j_1}^* \gamma_{j_2}^*}{\gamma_{j_1} \gamma_{j_2}} - 1 = O_p(1/\sqrt{G})$ and $M$ is fixed,

$$\sum_g w_g \hat{\mu}_g \hat{\mu}_g^T - \sum_g w_g \hat{\mu}_g^* (\hat{\mu}_g^*)^T = \frac{1}{M^2} \sum_{j_1,j_2=1}^M \left( \frac{\gamma_{j_1} \gamma_{j_2}}{\gamma_{j_1} \gamma_{j_2}} - 1 \right) U^T W U + O_p(1).$$

Similarly, as

$$\sum_g w_g \hat{\nu}_g - \sum_g w_g \hat{\nu}_g^*$$

$$= \frac{1}{M(M-1)} \sum_{j=1}^M \left( \frac{(\gamma_j^*)^2}{\gamma_j^2} - 1 \right) \sum_g w_g \frac{z_{jg}^r (z_{jg}^r)^T}{\gamma_j^r \gamma_j} - \frac{1}{M-1} \sum_g w_g \left( \hat{\mu}_g \hat{\mu}_g^T - \hat{\mu}_g^* \hat{\mu}_g^{**} \right)$$

where under Assumption 4b of bounded moments,

$$\sum_{j=1}^M \left( \frac{(\gamma_j^*)^2}{\gamma_j^2} - 1 \right) \sum_g w_g \frac{z_{jg}^r (z_{jg}^r)^T}{\gamma_j^r}$$

$$= \sum_{j=1}^M \left( \frac{(\gamma_j^*)^2}{\gamma_j^2} - 1 \right) \left( \sum_g w_g \frac{z_{jg}^r (z_{jg}^r)^T}{\gamma_j^r} - \sum_g w_g \mu_g \mu_g^T \right) + \sum_{j=1}^M \left( \frac{(\gamma_j^*)^2}{\gamma_j^2} - 1 \right) U^T W U$$

$$= \sum_{j=1}^M \left( \frac{(\gamma_j^*)^2}{\gamma_j^2} - 1 \right) U^T W U + O_p(1).$$
Also,

\[ \hat{U}^T W \Lambda U - \hat{U}^* W \Lambda U \]

\[ = \frac{1}{M} \sum_{j=1}^{M} \left( \frac{\gamma_j^r}{\gamma_j} - 1 \right) \sum_{g=1}^{G} w_g \lambda_g \frac{z_{jg}^r}{\gamma_j} \mu_g^T \]

\[ = \frac{1}{M} \sum_{j=1}^{M} \left( \frac{\gamma_j^r}{\gamma_j} - 1 \right) \left( \sum_{g=1}^{G} w_g \lambda_g \frac{z_{jg}^r}{\gamma_j} \mu_g^T - \sum_{g} w_g \mu_g \mu_g^T \right) + \frac{1}{M} \sum_{j=1}^{M} \left( \frac{\gamma_j^r}{\gamma_j} - 1 \right) U^T W U \]

\[ = \frac{1}{M} \sum_{j=1}^{M} \left( \frac{\gamma_j^r}{\gamma_j} - 1 \right) U^T W U + O_p(1). \]

Combining all above, we get

\[ \hat{\Omega} - \Omega^* = O_p \left( \frac{1}{\sqrt{G}} \right) \]

\[ \hat{H} - H^* = h(\gamma_1, \ldots, \gamma_m) U^T W U + O_p(1) \]

where the function

\[ h(x_1, \ldots, x_M) = \frac{1}{M(M-1)} \sum_{j_1 \neq j_2} \frac{\gamma_{j_1}^r \gamma_{j_2}^r}{x_{j_1} x_{j_2}} - \frac{1}{M} \sum_{j} \frac{\gamma_j^r}{x_j} \]

Taking the derivative, we find that for this function we have

\[ \frac{\partial h}{\partial x_j}(\gamma_1, \ldots, \gamma_M) = -\frac{1}{\gamma_j^r}. \]

So if we take Taylor expansion of \( h(\cdot) \) at the true value \( (\gamma_1^r, \ldots, \gamma_m^r) \), then we have

\[ h(\hat{\gamma}_1, \ldots, \hat{\gamma}_M) = h(\gamma_1^r, \ldots, \gamma_M^r) - \sum_{j=1}^{M} \frac{1}{\gamma_j^r} (\hat{\gamma}_j - \gamma_j^r) + O_p \left( \frac{1}{G} \right) \]

As \( h(\gamma_1^r, \ldots, \gamma_M^r) = 0 \), we further have

\[ \hat{H} - H^* = -\sum_{j=1}^{M} \frac{1}{\gamma_j^r} (\hat{\gamma}_j - \gamma_j^r) U^T W U + O_p(1). \]
Finally,
\[ \hat{U}^T W \epsilon_i - \hat{U}^* T W \epsilon'_i = \frac{1}{M} \sum_{j=1}^{M} \left( \frac{\gamma_j}{\hat{\gamma}_j} - 1 \right) \sum_{g=1}^{G} w_{ig} \epsilon_{ig} z_{jg}^{i}. \]

As \( \epsilon_{ig} \perp z_{jg} \) as they come from two different individuals, we have \( \mathbb{E} \left[ \epsilon_{ig} z_{jg}^{i} \right] = 0 \). So \( \sum_{g=1}^{G} w_{ig} \epsilon_{ig} z_{jg}^{i} = O_p(\sqrt{G}) \) and

\[ \hat{U}^T W \epsilon'_i - \hat{U}^* T W \epsilon'_i = O_p(1). \]

\[ \square \]

**Proof of Theorem 2.** Lemma S3 guarantees that \( \hat{\gamma}_j \xrightarrow{p} \gamma_j^* \) for any \( j \) when \( G \to \infty \). Using Lemma S2 and Lemma S3, we also have

\[ \hat{\Omega} = \Omega - \hat{\Omega}^* + \hat{\Omega}^* \xrightarrow{p} \Omega \succ 0 \]

By the Continuous mapping theorem, additionally we have \( \hat{\Omega}^{-1} \xrightarrow{p} \Omega^{-1} \).

Now we show that \( \hat{\beta}_i \xrightarrow{p} \beta_i \) where \( \hat{\beta}_i \) is either the truncation estimator \( \hat{\beta}_i = \hat{\beta}_i \lor 0 \) or the constrained estimator from non-negative least squares. For the truncation estimator \( \hat{\beta}_i = \hat{\beta}_i \lor 0 \), since we have

\[ \frac{1}{G} \phi(\beta_i) = \frac{1}{G} \hat{U}^T W \epsilon'_i - \frac{1}{G} H \beta_i \xrightarrow{p} 0, \]

then

\[ \hat{\beta}_i - \beta_i = \frac{1}{G} \hat{\Omega}^{-1} \phi(\beta_i) \xrightarrow{p} 0. \]

Thus, \( \hat{\beta}_i = \hat{\beta}_i \lor 0 \xrightarrow{p} \beta_i \lor 0 = \beta_i \).

For the constrained estimator from non-negative least squares where

\[ \hat{\beta}_i = \arg \min_{\beta_i \geq 0} (y_i - \hat{U} \beta_i)^T W (y_i - \hat{U} \beta_i) - \beta_i^T \hat{V} \beta_i \overset{\Delta}{=} \arg \min_{\beta_i \geq 0} l(\beta_i), \]
plug in (S3) for \( y_i \) and denote the true \( \beta_i \) as \( \beta_{0i} \), we have

\[
l_i(\beta_i) = \tilde{l}(\beta_i) + 2(\Lambda U \beta_{0i} - \hat{U} \beta_i)^T W \epsilon_i' + \text{const} = \tilde{l}(\beta_i) + (\beta_{0i} - \beta_i)^T \hat{H}^T W \epsilon_i' + \text{const}
\]

\[
= \tilde{l}(\beta_i) + O_p(\sqrt{G})\|\beta_{0i} - \beta_i\|_2 + \text{const}
\]

where \( \tilde{l}(\beta_i) = (\Lambda U \beta_{0i} - \hat{U} \beta_i)^T W (\Lambda U \beta_{0i} - \hat{U} \beta_i) - \beta_i^T \hat{V} \beta_i \). Additionally, expand \( \Lambda U \beta_{0i} - \hat{U} \beta_i = \Lambda U (\beta_{0i} - \beta_i) + (\Lambda U - \hat{U}) \beta_i \), we have

\[
\tilde{l}(\beta_i) = (\beta_{0i} - \beta_i)^T U^T \Lambda W \Lambda U (\beta_{0i} - \beta_i) + (\beta_{0i} - \beta_i)^T U^T \Lambda W (\Lambda U - \hat{U}) \beta_i
\]

\[
+ \beta_i^T [(\Lambda U - \hat{U})^T W (\Lambda U - \hat{U}) - \hat{V}] \beta_i
\]

Given results in Lemma S2 and Lemma S3, we have

\[
U^T \Lambda W (\Lambda U - \hat{U}) = O_p(\sqrt{G}), \quad (\Lambda U - \hat{U})^T W (\Lambda U - \hat{U}) - \hat{V} = O_p(\sqrt{G}).
\]

Additionally, as \( \lambda_g \) are i.i.d. across \( g \) with mean 1, it is easy to show that

\[
U^T \Lambda W \Lambda U = U^T W U + O_p(\sqrt{G}).
\]

Thus, we have \( \tilde{l}(\beta_i) = (\beta_{0i} - \beta_i)^T U^T W U (\beta_{0i} - \beta_i) + O_p(\sqrt{G})\|\beta_{0i} - \beta_i\|_2 + O_p(\sqrt{G})\|\beta_{0i} - \beta_i\|_2^2 + \text{const} \), indicating that

\[
l_i(\beta_i) = (\beta_{0i} - \beta_i)^T U^T W U (\beta_{0i} - \beta_i) + O_p(\sqrt{G})\|\beta_{0i} - \beta_i\|_2 + O_p(\sqrt{G})\|\beta_{0i} - \beta_i\|_2^2 + \text{const}.
\]

Thus, as \( U^T W U^T / G \xrightarrow{G \to \infty} \Omega \succ 0 \) under Assumption 4a, for any \( \epsilon > 0 \), when \( G \to \infty \) we have

\[
P \left[ \| \hat{\beta}_i^* - \beta_{0i} \|_2 \leq \epsilon \right] \geq P \left[ l(\beta_{0i}) < \min_{\| \beta_i - \beta_{0i} \|_2 \geq \epsilon} l(\beta_i) \right]
\]

\[
= P \left[ (\beta_{0i} - \beta_i)^T U^T W U (\beta_{0i} - \beta_i) + O_p(1/\sqrt{G})\|\beta_{0i} - \beta_i\|_2 + O_p(1/\sqrt{G})\|\beta_{0i} - \beta_i\|_2^2 > 0 \right]
\]

\[
= P \left[ (\beta_{0i} - \beta_i)^T \Omega (\beta_{0i} - \beta_i) + O_p(1/\sqrt{G})\|\beta_{0i} - \beta_i\|_2 + O_p(1)\|\beta_{0i} - \beta_i\|_2^2 > 0 \right]
\]

\[
\geq P \left[ \lambda_{\min}(\Omega) \epsilon^2 - |O_p(1/\sqrt{G})\epsilon| - |O_p(1/\sqrt{G})\epsilon^2| > 0 \right] \to 1
\]

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where \( \lambda_{\text{min}}(\Omega) > 0 \) is a minimum eigenvalue of matrix \( \Omega \). Thus

\[
\hat{\beta}_i^* \overset{p}{\to} \beta_{0i}.
\]

Finally, by the Continuous mapping theorem, we have

\[
\hat{p}_i = \frac{\hat{\beta}_i^*}{\beta_i^T 1} \overset{p}{\to} \frac{\beta_i}{\beta_i^T 1} = p_i
\]

when \( G \to \infty \) for any target individual \( i \). \( \square \)

\section*{S2.4.3. Proof of Lemma 1}

First, notice that by definition of \( \hat{U}^{*T} \) and \( \hat{V}_g^* \), and following model (6),

\[
H^r = \hat{U}^{*T} W (\hat{U}^* - U) - \sum_{g=1}^{G} w_g \hat{V}_g^*.
\]

On the other hand, due to the equivalence between model (S3) and model (5), we have

\[
\hat{U}^{*T} W e_i' - H^r \beta_i = \hat{U}^{*T} W e_i - H^r \beta_i = s_i - H^r \beta_i.
\]

As \( \hat{U} \) and \( H^r \) only depends on the reference data, we have \( e_i \perp \perp (\hat{U}, H^r) \). Also \( \mathbb{E}[e_i] = 0 \), thus

\[
\text{Cov} [s_i, H^r \beta_i] = 0.
\]

Thus, under Assumption 5, we have

\[
\lim_{G \to \infty} \frac{\text{Cov} [\hat{U}^{*T} W e_i' - H^r \beta_i]}{G} = \lim_{G \to \infty} \frac{\text{Cov} [s_i - H^r \beta_i]}{G} = \lim_{G \to \infty} \frac{\text{Cov} [s_i] + \text{Cov} [H^r \beta_i]}{G} > 0.
\]
On the other hand, using Lemma S3, we have

\[
\phi(\beta_i) = \hat{U}^T W \epsilon_i - H \beta_i \\
= \hat{U}^*^T W \epsilon_i - H^* \beta_i + \sum_j \frac{1}{\gamma_j} (\hat{\gamma}_j - \gamma_j^*) U^T W U \beta_i + O_p(1).
\]

Given Assumption 4 and Lemma S3, we have

\[
\sum_j \frac{1}{\gamma_j} (\hat{\gamma}_j - \gamma_j^*) U^T W U \beta_i = o_p(G),
\]

thus

\[
\Sigma_i = \lim_{G \to \infty} \frac{\text{Cov} [\phi(\beta_i)]}{G} = \lim_{G \to \infty} \frac{\text{Cov} \left[ \hat{U}^*^T W \epsilon_i - H^* \beta_i \right]}{G} \succ 0.
\]

Since \( \hat{\beta}_i - \beta_i = \frac{1}{G} \hat{\Omega}^{-1} \phi(\beta_i) \), we have

\[
\lim_{G \to \infty} \text{Cov} \left[ \sqrt{G} \hat{\beta}_i \right] \succ 0,
\]

which also holds if \( \beta_i \) is replace by \( p_i \).

**S2.4.4. Proof of Theorem 3**

We need the following lemmas.

**Lemma S4** (Theorem 2.7 of Chen and Shao). Let \( \{X_i, i \in V\} \) be random variables indexed by the vertices of a dependency graph and let \( D \) be the maximum degree. Put \( W = \sum_{i \in V} X_i \). Assume that \( \mathbb{E}[W^2] = 1, \mathbb{E}[X_i] = 0 \) and \( \mathbb{E}[|X_i|^p] \leq \theta^p \) for \( i \in V \) and for some \( \theta > 0 \). Then

\[
\sum_z \| \mathbb{P}[W \leq z] - \Phi(z) \| \leq 75 D^{5(p-1)} |V| \theta^p
\]

**Lemma S5.** Under the assumptions of Theorem 3, for each target individual \( i \) the score function \( \phi(\beta_i) \) satisfy

\[
\frac{1}{\sqrt{G}} \phi(\beta_i) \xrightarrow{d} \mathcal{N}(0, \Sigma_i).
\]
Proof of Lemma S5. Using Lemma S3, we have

\[ \phi(\beta_i) = \hat{U}^T W e'_i - H \beta_i \]

\[ = \hat{U}^* T W e'_i - H^* \beta_i + \sum_j \frac{1}{\gamma_j^2} (\hat{\beta}_j - \gamma_j^2) U^T W U \beta_i + O_p(1). \]

Notice that by Assumption 4a, \( U^T W U - G \Omega = o(G) \), so

\[ \frac{1}{G} \left( \hat{U}^* T W e'_i - H^* \beta_i \right) + \sum_j \frac{1}{\gamma_j^2} (\hat{\beta}_j - \gamma_j^2) \Omega \beta_i = \frac{1}{G} \sum_{g=1}^G \eta_g + o_p\left( \frac{1}{\sqrt{G}} \right) \]

where

\[ \eta_g \Delta= w_g e'_i m_g - w_g (m_g (m_g - \lambda_g \mu_g)^T - \hat{V}_g^* \beta_i + \sum_j \frac{1}{\gamma_j^2} \sum_k z_{gjk} - \sum_k \gamma_j^2 \mu_{gk} \Omega_{\beta_i} \]

Thus

\[ \frac{1}{G} \phi(\beta_i) = \frac{1}{G} \sum_{g=1}^G \eta_g + o_p\left( \frac{1}{\sqrt{G}} \right) \]

Each \( \mathbb{E}[\eta_g] = 0 \) and using Lemma 1, we have \( \lim_{G \to \infty} \text{Var} \left[ \sum_{g=1}^G \eta_g \right] / G = \lim_{G \to \infty} \text{Var} \left[ \phi(\beta_i) \right] / G = \Sigma_i \succ 0 \). Also, similar to our argument in the proof of Lemma S2, under Assumption 4bc, let \( \eta_g = (\eta_g 1, \ldots, \eta_g K) \), then \( \mathbb{E} \left[ \eta_{g_k}^{2+\delta/2} \right] \) is uniformly bounded across all genes \( g \).

Further under Assumption 3, \( \text{Cov} \left[ \sum_{g \in \mathcal{V}} \eta_g \right] = o(G) \) as \( |\mathcal{V}| = o(\sqrt{G}) \). Thus \( \sum_{g \in \mathcal{V}} \eta_g = o_p(\sqrt{G}) \) and

\[ \frac{1}{G} \phi(\beta_i) = \frac{1}{G} \sum_{g \in \mathcal{V}} \eta_g + o_p \left( \frac{1}{\sqrt{G}} \right) . \]

Now let \( t \in \mathbb{R}^K \) be a non-random vector with \( \|t\|_2 = 1 \). Then under Assumption 3, \( \{\eta_g^T t, g \in \mathcal{V}\} \)
forms a dependency graph with maximum degree \( D = O(1) \). Additionally, we have \( \max_{g} \mathbb{E} \left[ (\eta_{g_k} T t)^{2+\delta/2} \right] \leq c \) for some constant \( c \). Also, as \( \text{Var} \left[ \sum_{g \in \mathcal{V}} \eta_g^T t / G = \text{Var} \left[ \phi(\beta_i) t / G + o(1) \right] \), we have

\[ \lim_{G \to \infty} \text{Var} \left[ \sum_{g \in \mathcal{V}} \eta_g^T t \right] / G = t^T \Sigma_t t > 0. \]
Using Lemma S4, we have
\[
\frac{1}{\sqrt{G}} \left( \sum_{g \in V} \eta_g^T t \right) \xrightarrow{d} N(0, t^T \Sigma_i t).
\]
Then, using the Cramer-wold theorem, we can obtain
\[
\frac{1}{\sqrt{G}} \sum_{g \in V} \eta_g \xrightarrow{d} N(0, \Sigma_i).
\]
which implies that
\[
\frac{1}{\sqrt{G}} \phi(\beta_i) \xrightarrow{d} N(0, \Sigma_i).
\]

\[
\square
\]

Proof of Theorem 3. Notice that
\[
\sqrt{G} (\beta_i - \beta_i) = \hat{\Omega}^{-1} \phi(\beta_i)/\sqrt{G}
\]
Then combining Theorem 2 and Lemma S5, we have
\[
\sqrt{G}(\hat{\beta}_i - \beta_i) \xrightarrow{d} N(0, \Omega^{-1}\Sigma_i\Omega^{-1})
\]
Next, notice that for either the truncated on the constrained estimator, \(\hat{\beta}_i^* \neq \hat{\beta}_i\) only when at least one \(\hat{\beta}_{ik} < 0\). For a target individual \(i\), if \(p_{ik} > 0\) for any \(k\), then \(\beta_{ik} > 0\) for any \(k\). Thus, for any \(\epsilon > 0\),
\[
P \left[ \| \sqrt{G}(\hat{\beta}_i^* - \hat{\beta}_i) \|_2 > \epsilon \right] \leq P \left[ \hat{\beta}_i^* \neq \hat{\beta}_i \right] \leq \sum_{k=1}^{K} P \left[ \hat{\beta}_{ik} < 0 \right] G \to 0
\]
where the last limit is due to the consistency of \(\hat{\beta}_{ik}\). This indicates that \(\sqrt{G}(\hat{\beta}_i^* - \hat{\beta}_i) \xrightarrow{p} 0\), thus
\[
\sqrt{G}(\hat{\beta}_i^* - \beta_i) \xrightarrow{d} N(0, \Omega^{-1}\Sigma_i\Omega^{-1})
\]
Finally, the cell type proportions $\hat{p}_i = g(\hat{\beta}_i^*)$ is the standardized $\hat{\beta}_i^*$. By delta method, we have

$$\sqrt{G}(\hat{p}_i - p_i) \xrightarrow{d} N(0, \nabla g(\beta_i)^T \Omega^{-1} \Sigma_i \Omega^{-1} \nabla g(\beta_i)).$$

(S5)

S2.4.5. Proof of Theorem 4

**Lemma S6.** Let the normalization function be $g(z) = \frac{z}{z_1^T 1}$ where $z$ is some non-negative k-dimensional vector. Then for any $z$ and any $z_0$ satisfying $\|z_0\|_1 > \delta$ with some $\delta > 0$, there exists some $L_0 > 0$ satisfying

$$\|g(z) - g(z_0)\|_2 \leq L_0 \|z - z_0\|_2$$

and some $L_1 > 0$ satisfying that

$$\|g(z) - g(z_0) - \nabla g(z_0)(z - z_0)\|_2 \leq L_1 \|z - z_0\|_2^2$$

**Proof.** By definition, we can calculate that

$$\nabla g(z_0) = \frac{1}{z_0^T 1} \left( I - \frac{z_0 1^T}{z_0^T 1} \right)$$

where $I$ is a $k \times k$ identity matrix. Then we have

$$g(z) - g(z_0) - \nabla g(z_0)(z - z_0) = \frac{(z - z_0)^T 1}{z_0^T 1} (g(z_0) - g(z)).$$

(S6)

In general, notice that for any two $k$-dimensional non-negative vectors $x$ and $y$

$$\|g(x) - g(y)\|_2^2 = \frac{\sum_l (\sum_k y_k x_l - \sum_k x_k y_l)^2}{(\sum_k x_k)^2 (\sum_k y_k)^2}$$

$$= \frac{\sum_l \left( \sum_k y_k (x_l - y_l) + (\sum_k y_k - \sum_k x_k) y_l \right)^2}{(\sum_k x_k)^2 (\sum_k y_k)^2}$$

$$\leq 2 \frac{(\sum_k y_k)^2 \sum_l (x_l - y_l)^2 + (\sum_k y_k - \sum_k x_k)^2 \sum_l y_l^2}{(\sum_k x_k)^2 (\sum_k y_k)^2}$$

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Notice that $\sum_i y_i^2 < (\sum_k y_k)^2$ as $\mathbf{y}$ is non-negative and $(\sum_k y_k - \sum_k x_k)^2 \leq k \sum_k (y_k - x_k)^2$, we have

$$\|g(\mathbf{x}) - g(\mathbf{y})\|_2^2 \leq \|\mathbf{x} - \mathbf{y}\|_2^2 \frac{2(1 + k)}{\|\mathbf{x}\|_1}$$

As $\|\mathbf{z}_0\|_1$ is bounded below, there exists some $L_0 > 0$ so that $\|g(\mathbf{z}_0) - g(\mathbf{z})\|_2 \leq L_0 \|\mathbf{z} - \mathbf{z}_0\|_2$.

As $\|\mathbf{z} - \mathbf{z}_0\|_1 \leq \sqrt{k} \|\mathbf{z} - \mathbf{z}_0\|_2$, from (S6) we also have

$$\|g(\mathbf{z}) - g(\mathbf{z}_0) - \nabla g(\mathbf{z}_0)(\mathbf{z} - \mathbf{z}_0)\|_2 \leq \frac{L_0 \sqrt{k}}{\delta} \|\mathbf{z} - \mathbf{z}_0\|_2$$

$\blacksquare$

**Lemma S7.** Under Assumptions 1-5 and Assumptions 7, if $N/G^2 \rightarrow 0$ when $G \rightarrow \infty$, we then have

$$\frac{1}{N} \sum_{i=1}^{N} \|\phi(\beta_i)\|_2 = O_p(\sqrt{G}), \quad \frac{1}{N} \sum_{i=1}^{N} \|\phi(\beta_i)\|^2_2 = O_p(G), \quad \max_i \|\phi(\beta_i)\|_2 = o_p(G)$$

**Proof of Lemma S7.** Recall that $\phi(\beta_i) = \mathbf{U}^T \mathbf{W} \epsilon'_i - \mathbf{H} \beta_i$, so

$$\|\phi(\beta_i)\|_2 \leq \|\mathbf{U}^T \mathbf{W} \epsilon'_i\|_2 + \|\mathbf{H}\|_2 \|\beta_i\|_2$$

$$\|\phi(\beta_i)\|_2^2 \leq 2 \|\mathbf{U}^T \mathbf{W} \epsilon'_i\|_2^2 + 2 \|\mathbf{H}\|_2^2 \|\beta_i\|_2^2$$

Using Lemma S2 and Lemma S3, we have $\|\mathbf{H}\|_2 = O_p\left(\sqrt{G}\right)$. Also, as $\beta_i$ is always non-negative, $\|\beta_i\|_2^2 \leq \|\beta_i\|_1^2 = \gamma_i^2$ since by definition $\beta_i = \gamma_i \mathbf{p}_i$. Under Assumption 7b,

$$\frac{1}{N} \sum_{i=1}^{N} \|\beta_i\|_2 \leq \frac{1}{N} \sum_{i=1}^{N} \gamma_i = O_p(1), \quad \frac{1}{N} \sum_{i=1}^{N} \|\beta_i\|_2^2 \leq \frac{1}{N} \sum_{i=1}^{N} \gamma_i^2 = O_p(1), \quad \max_i \|\beta_i\|_2 \leq \max_i \gamma_i = O_p(1).$$

So $\|\mathbf{H}\|_2 \max_i \|\beta_i\|_2 = o_p(G)$, $\|\mathbf{H}\|_2 \sum_{i=1}^{N} \|\beta_i\|_2 / N = O_p\left(\sqrt{G}\right)$ and $\|\mathbf{H}\|_2^2 \sum_{i=1}^{N} \|\beta_i\|_2^2 / N = O_p(G)$.
Next, consider $\hat{U}^*^T W \epsilon_i' = \sum_g w_g \epsilon_i' \hat{\mu}_g$ where $\hat{U}^*$ is defined as in Lemma S2. Under Assumptions 4bc,
\[
\max_{i,g} \mathbb{E} \left[ \|w_g \epsilon_i' \hat{\mu}_g\|_2^4 \right] \leq \max_{i,g} \left( w_g^4 \mathbb{E} \left[ \epsilon_i'^4 \right] \mathbb{E} \left[ \|\hat{\mu}_g\|_2^4 \right] \right) \leq c
\]
for some constant $c$. So all lower moments are also uniformly bounded. In addition, we have the inequality
\[
\max_i \mathbb{E} \left[ \left\| \hat{U}^T W \epsilon_i' \right\|_2^4 \right] \leq \max_i 8 \left( \mathbb{E} \left[ \left\| \sum_{g \in V} w_g \epsilon_i' \hat{\mu}_g\right\|_2^4 \right] + \mathbb{E} \left[ \left\| \sum_{g \in V^c} w_g \epsilon_i' \hat{\mu}_g\right\|_2^4 \right] \right).
\]
Since $|V^c| = O(\sqrt{G})$, so we have $\max_i \mathbb{E} \left[ \left\| \sum_{g \in V^c} w_g \epsilon_i' \hat{\mu}_g\right\|_2^4 \right] = O(G^2)$. Also, under the sparse dependence structure in Assumption 3 and the fact that $\epsilon_i'$ is mutually independent from $\hat{U}^*$ with $\mathbb{E} [\epsilon_i''] = 0$, we have
\[
\mathbb{E} \left[ \left\| \sum_{g \in V} w_g \epsilon_i' \hat{\mu}_g\right\|_2^4 \right] = \sum_{g \in V} \mathbb{E} \left[ w_g^4 \epsilon_i'^4 \epsilon_{i1} \right] \mathbb{E} \left[ \|\hat{\mu}_g\|_2^4 \right] + \sum_{g_1 \neq g_2 \in V} \mathbb{E} \left[ w_{g_1}^2 w_{g_2}^2 \epsilon_{i1}^2 \epsilon_{i2} \right] \mathbb{E} \left[ \|\hat{\mu}_1\|_2^2 \|\hat{\mu}_2\|_2^2 \right] + \sum_{g_1 \neq g_2 \neq g_3 \in V} \mathbb{E} \left[ w_{g_1}^2 w_{g_2} w_{g_3} \epsilon_{i1} \epsilon_{i2} \epsilon_{i3} \right] \mathbb{E} \left[ \|\hat{\mu}_1\|_2^2 \|\hat{\mu}_2\|_2^2 \|\hat{\mu}_3\|_2^2 \right] + \sum_{g_1 \neq g_2 \neq g_3 \neq g_4 \in V} \mathbb{E} \left[ w_{g_1} w_{g_2} w_{g_3} w_{g_4} \epsilon_{i1} \epsilon_{i2} \epsilon_{i3} \epsilon_{i4} \right] \mathbb{E} \left[ \|\hat{\mu}_1\|_2 \|\hat{\mu}_2\|_2 \|\hat{\mu}_3\|_2 \|\hat{\mu}_4\|_2 \right] = O(G) + O(G) + O(G^2) + O(G^2) = O(G^2)
\]
The last term has an order of $O(G^2)$ as every node needs to have an edge for $\mathbb{E} [\epsilon_{i1} \epsilon_{i2} \epsilon_{i3} \epsilon_{i4}] \neq 0$ and there are at most $O(G)$ edges. So $\max_i \mathbb{E} \left[ \left\| \hat{U}^T W \epsilon_i' \right\|_2^4 \right] = O(G^2)$. At the same time, we can also obtain $\max_i \mathbb{E} \left[ \left\| \hat{U}^T W \epsilon_i' \right\|_2^2 \right] = O(G)$ and $\max_i \mathbb{E} \left[ \left\| \hat{U}^T W \epsilon_i' \right\|_2^2 \right] = O(\sqrt{G})$. Then for any $\epsilon > 0$,
\[
P \left[ \frac{1}{G} \max_i \left\| \hat{U}^T W \epsilon_i' \right\|_2^2 > \epsilon \right] \leq \sum_{i=1}^{N} \frac{\mathbb{E} \left[ \left\| \hat{U}^T W \epsilon_i' \right\|_2^4 \right]}{G^4 \epsilon^4} = O \left( \frac{N}{G^2} \right) \to 0
\]
and for any $\Delta > 0$ and any $G$, there is a constant $\tilde{C}$ for when $N$ is sufficiently large

$$\Pr \left[ \frac{1}{\sqrt{GN}} \sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime \right\|_2 > \Delta \right] \leq \frac{\mathbb{E} \left[ \sum_{i} \left\| \hat{U}_i^T W e_i^\prime \right\|_2 \right]}{\sqrt{N} \sqrt{G}} \leq \frac{\max_{i} \mathbb{E} \left[ \left\| \hat{U}_i^T W e_i^\prime \right\|_2 \right]}{\sqrt{G}} \leq \frac{\tilde{C}}{\Delta}.
$$

$$\Pr \left[ \frac{1}{GN} \sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime \right\|_2^2 > \Delta \right] \leq \frac{\mathbb{E} \left[ \sum_{i} \left\| \hat{U}_i^T W e_i^\prime \right\|_2^2 \right]}{\sqrt{NG}} \leq \frac{\max_{i} \mathbb{E} \left[ \left\| \hat{U}_i^T W e_i^\prime \right\|_2^2 \right]}{\sqrt{G}} \leq \frac{\tilde{C}}{\Delta}.
$$

Thus, we have $\max_{i} \left\| \hat{U}_i^T W e_i^\prime \right\|_2 = o_p(G)$, $\sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime \right\|_2/N = O_p(\sqrt{G})$ and the relationship $\sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime \right\|_2^2/N = O_p(G)$.

Finally, consider the term $\hat{U}_i^T W e_i^\prime - \hat{U}_i^T W e_i^\prime$. Notice that,

$$\hat{U}_i^T W e_i^\prime - \hat{U}_i^T W e_i^\prime = \frac{1}{m} \sum_{j=1}^{m} \left( \frac{\gamma_j^i}{\tilde{\gamma}_j^i} - 1 \right) \sum_{g=1}^{G} w_{g} e_{ig}^i \frac{z_{jg}^i}{\tilde{\gamma}_j^i}.
$$

Similar to our previous argument, we also have

$$\max_{i} \left\| \sum_{g=1}^{G} w_{g} e_{ig}^i \frac{z_{jg}^i}{\tilde{\gamma}_j^i} \right\|_2 = o_p(G)
$$

and

$$\frac{1}{N} \sum_{i=1}^{N} \left\| \sum_{g=1}^{G} w_{g} e_{ig}^i \frac{z_{jg}^i}{\tilde{\gamma}_j^i} \right\|_2 = O_p(\sqrt{G}), \quad \frac{1}{N} \sum_{i=1}^{N} \left\| \sum_{g=1}^{G} w_{g} e_{ig}^i \frac{z_{jg}^i}{\tilde{\gamma}_j^i} \right\|_2^2 = O_p(G).
$$

As $\tilde{\gamma}_j^i - \gamma_j^i = O_p(1/\sqrt{G})$ and $M$ is fixed when $G \to \infty$, we obtain

$$\max_{i} \left\| \hat{U}_i^T W e_i^\prime - \hat{U}_i^T W e_i^\prime \right\|_2 = o_p(\sqrt{G}),$$

$$\frac{1}{N} \sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime - \hat{U}_i^T W e_i^\prime \right\|_2 = O_p(1), \quad \frac{1}{N} \sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime - \hat{U}_i^T W e_i^\prime \right\|_2^2 = O_p(1).
$$

Combining all above, we get $\sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime \right\|_2/N = O_p(\sqrt{G})$, $\sum_{i=1}^{N} \left\| \hat{U}_i^T W e_i^\prime \right\|_2^2/N = O_p(G)$ and $\max_{i} \left\| \hat{U}_i^T W e_i^\prime \right\|_2 = o_p(G)$, and we prove the lemma.

**Proof of Theorem 4.** First, recall that $\tilde{\beta}_i - \beta_i = \tilde{\Omega}^{-1} \phi(\beta_i)/G$. Also, notice that from Theorem 2,
\( \hat{\Omega}^{-1} \xrightarrow{p} \Omega^{-1} \), indicating \( \| \hat{\Omega}^{-1} \|_2 = O_p(1) \). Thus, using Lemma S7 we have

\[
\frac{1}{N} \sum_{i=1}^{N} \| \hat{\beta}_i - \beta_i \|_2 \leq \| \Omega^{-1} \|_2 \frac{1}{N} \sum_{i=1}^{N} \| \phi(\beta_i) \|_2 / G = O_p(G^{-1/2})
\]

and

\[
\max_i \| \hat{\beta}_i - \beta_i \|_2 \leq \| \Omega^{-1} \|_2 \max_i \| \phi(\beta_i) \|_2 / G = o_p(1).
\]

Then, using Lemma S6, we also have

\[
\frac{1}{N} \sum_{i=1}^{N} \| g(\hat{\beta}_i) - g(\beta_i) \|_2 \leq \frac{L}{N} \sum_{i=1}^{N} \| \hat{\beta}_i - \beta_i \|_2 = O_p(G^{-1/2})
\]

as Assumption 7c guarantees that \( \min_i \| \beta_i \|_1 = \min_i \gamma_i \geq C_2 \). In addition, for any \( \epsilon > 0 \),

\[
\mathbb{P} \left[ \frac{\sqrt{G}}{N} \sum_{i=1}^{N} \| g(\hat{\beta}_i^*) - g(\hat{\beta}_i) \|_2 > \epsilon \right] \\
\leq \mathbb{P} \left[ \frac{\sqrt{G}}{N} \sum_{i=1}^{N} \| g(\hat{\beta}_i^*) - g(\hat{\beta}_i) \|_2 \neq 0 \right] \\
= \mathbb{P} \left[ \cup_{i=1}^{N} \{ \hat{\beta}_i^* \neq \hat{\beta}_i \} \right] \\
= \mathbb{P} \left[ \cup_{i=1}^{N} \cup_{k=1}^{K} \{ \hat{\beta}_{ik} < 0 \} \right] \\
\leq \mathbb{P} \left[ \max_i \| \hat{\beta}_i - \beta_i \|_2 > C_2 \right] \\
\xrightarrow{G \to \infty} 0
\]

So \( \sum_{i=1}^{N} \| g(\hat{\beta}_i^*) - g(\hat{\beta}_i) \|_2 / N = o(G^{-1/2}) \) and thus,

\[
\frac{1}{N} \sum_{i=1}^{N} \| g(\hat{\beta}_i^*) - g(\beta_i) \|_2 = O_p(G^{-1/2}).
\]

By Assumption 7a,

\[
\| \hat{A} - A_N \|_F \leq \left\| (F^T F)^{-1} \right\|_F \left\| \sum_i f_i (\hat{p}_i^T - p_i^T) \right\|_F \\
\leq \left\| (F^T F)^{-1} \right\|_F \sum_i \| f_i \|_2 \| \hat{p}_i - p_i \|_2 \\
\leq c \left\| (F^T F)^{-1} \right\|_F \sum_i \| g(\hat{\beta}_i^*) - g(\beta_i) \|_2 = O_p(G^{-1/2})
\]

S28
Thus, we have $\|\hat{A} - A_N\|_F = O_p(1/\sqrt{G}) = o_p(1/\sqrt{N})$ when $N/G \to 0$.

If $N \to \infty$, as $\delta_i$ are i.i.d. under Assumption 6, then following classical linear regression theory, we have

$$\sqrt{N} \text{vec} (A_N - A) \xrightarrow{d} N (0, \Sigma \otimes (F^T F)^{-1}).$$

and $\sqrt{N}(\hat{A} - A)$ has the same limiting distribution. \hfill \square

### S2.4.6. Proof of Theorem 5

First, notice that our proof of $\|\hat{A} - A_N\|_F = O_p(1/\sqrt{G})$ in Theorem 4 above do not make use the assumption $N/G \to 0$, thus $\|\hat{A} - A_N\|_F = O_p(1/\sqrt{G})$ still holds when $N/G^2 \to \infty$.

Now we prove for the conclusion that $\|\hat{A} - A_N\|_F = O_p(1/\sqrt{NG}) + O_p(1/G)$ when $A = 0$.

Using Lemma S6,

$$\hat{A} - A_N = (F^T F)^{-1} \left[ \sum_i f_i (g(\hat{\beta}_i^T) - g(\beta_i^T)) \right]$$

$$= (F^T F)^{-1} \left[ \sum_i f_i (\hat{\beta}_i^* - \beta_i)^T \nabla g(\beta_i^T) \right] + O \left( \frac{1}{N} \sum_i ||\hat{\beta}_i^* - \beta_i||_2^2 \right)$$

Using Lemma S7, we have

$$\frac{1}{N} \sum_{i=1}^N ||\hat{\beta}_i - \beta_i||_2^2 \leq ||\hat{\Omega}^{-1}||_2 \left( \frac{1}{N} \sum_i ||\phi(\beta_i)||_2^2 / G^2 \right) = O_p(G^{-1})$$

and

$$\max_i ||\hat{\beta}_i - \beta_i||_2 \leq ||\hat{\Omega}^{-1}||_2 \max_i ||\phi(\beta_i)||_2 / G = o_p(1).$$

Also, same as in the proof of Theorem 4, for any $\epsilon > 0$,

$$\mathbb{P} \left[ \frac{G}{N} \sum_{i=1}^N ||\hat{\beta}_i^* - \hat{\beta}_i||_2^2 > \epsilon \right] \leq \mathbb{P} \left[ \bigcup_{i=1}^N \{ \hat{\beta}_i^* \neq \hat{\beta}_i \} \right] \leq \mathbb{P} \left[ \max_i ||\hat{\beta}_i - \beta_i||_2 > \delta \right] \xrightarrow{G \to \infty} 0$$

which indicates that $\frac{1}{N} \sum_i ||\hat{\beta}_i^* - \hat{\beta}_i||_2^2 = o(1/G)$. Thus, $\frac{1}{N} \sum_i ||\hat{\beta}_i^* - \beta_i||_2^2 = O_p(1/G)$.
Next, notice that

\[(F^T F)^{-1} \left[ \sum_i f_i (\hat{\beta}_i - \beta_i)^T \nabla g(\beta_i)^T \right] \]

\[= (F^T F)^{-1} \left[ \sum_i f_i (\hat{\beta}_i - \beta_i)^T \nabla g(\beta_i)^T \right] + (F^T F)^{-1} \left[ \sum_i f_i (\hat{\beta}_i - \beta_i)^T \nabla g(\beta_i)^T \right] \]

As \(\|\beta\|_1 \geq C_2, \|\nabla g(\beta_i)\|_2\) is uniformly bounded above. Under Assumption 7, we have

\[(F^T F)^{-1} \left[ \sum_i f_i (\hat{\beta}_i - \beta_i)^T \nabla g(\beta_i)^T \right] = O_p \left( \frac{1}{N} \sum_i \|\hat{\beta}_i - \beta_i\|_2 \right) = o_p(1/G). \]

Based on the above results, we simply (S7) and obtain

\[\hat{A} - A_N = (F^T F)^{-1} \left[ \sum_i f_i (\hat{\beta}_i - \beta_i)^T \nabla g(\beta_i)^T \right] + O_p(1/G). \]

Notice that \(\hat{\beta}_i - \beta_i = \hat{\Omega}^{-1} \phi(\beta_i)/G\), so

\[\frac{1}{N} \sum_i \nabla g(\beta_i) (\hat{\beta}_i - \beta_i) f_i^T \]

\[= \frac{1}{NG} \sum_i \nabla g(\beta_i) \hat{\Omega}^{-1} \phi(\beta_i) f_i^T \]

\[= \frac{1}{NG} \sum_i \nabla g(\beta_i) \Omega^{-1} \phi(\beta_i) f_i^T + \frac{1}{NG} \sum_i \nabla g(\beta_i) (\hat{\Omega}^{-1} - \Omega^{-1}) \phi(\beta_i) f_i^T \]

As both \(\nabla g(\beta_i)\) and \(f_i\) are uniformly bounded across \(i\), we have

\[\left\| \frac{1}{NG} \sum_i \nabla g(\beta_i) (\hat{\Omega}^{-1} - \Omega^{-1}) \phi(\beta_i) f_i^T \right\|_2 \]

\[= O_p \left( \frac{1}{NG} \|\hat{\Omega}^{-1} - \Omega^{-1}\|_2 \sum_i \|\phi(\beta_i)\|_2 \right) = O_p(1/G) \]

So finally, we simply (S7) to

\[\hat{A} - A_N = \frac{1}{G} (F^T F)^{-1} \left[ \sum_i f_i (\Omega^{-1} \phi(\beta_i))^T \nabla g(\beta_i)^T \right] + O_p(1/G) \] (S8)
and we will focus on proving that \( \frac{1}{NG} \sum_i \nabla g(\beta_i) \Omega^{-1} \phi(\beta_i) f_i^T = O_p(1/\sqrt{NG}) + O_p(1/G) \) when \( A = 0 \).

As \( \phi(\beta_i) = \hat{U}^T W \epsilon_i - H \beta_i \), we have

\[
\sum_i \nabla g(\beta_i) \Omega^{-1} \phi(\beta_i) f_i^T = \sum_i \nabla g(\beta_i) (\Omega^{-1} \hat{U}^T W \epsilon_i f_i^T - \Omega^{-1} H \beta_i f_i^T) \tag{S9}
\]

We prove for each of the two terms. For the first term, first notice that in the proof of Lemma S7, we have already shown that

\[
\frac{1}{N} \sum_{i=1}^N \| \hat{U}^T W \epsilon_i' - \hat{U}^{*T} W \epsilon_i' \|_2^2 = O_p(1).
\]

So given that \( \nabla g(\beta_i) \) and \( f_i \) are uniformly bounded across \( i \),

\[
\sum_i \nabla g(\beta_i) \Omega^{-1} \phi(\beta_i) f_i^T = \sum_i \nabla g(\beta_i) \Omega^{-1} \hat{U}^{*T} W \epsilon_i f_i^T + O_p(N).
\]

Because \( \hat{U}^*, g(\beta_i) \) and \( \epsilon_i' \) are mutually independent based on Assumption 1 and Assumption 6, and \( \mathbb{E} [\epsilon_i'] = 0 \) for each \( i \), we have for any \( i_1 \neq i_2 \), \( \text{Cov} \left[ \nabla g(\beta_i) \Omega^{-1} \hat{U}^* \epsilon_i', \nabla g(\beta_{i_2}) \Omega^{-1} \hat{U}^* \epsilon_{i_2}' \right] = 0 \). In the proof of Lemma S7, we have also shown that \( \max_i \mathbb{E} \left[ \| \hat{U}^T W \epsilon_i' \|_2^2 \right] = O(G) \). So,

\[
\text{Var} \left[ \sum_i \nabla g(\beta_i) \Omega^{-1} \hat{U}^* W \epsilon_i f_i^T \right] = \sum_i \text{Var} \left[ \nabla g(\beta_i) \Omega^{-1} \hat{U}^* W \epsilon_i f_i^T \right] = O(NG).
\]

This indicates that \( \sum_i \nabla g(\beta_i) \Omega^{-1} \hat{U}^* W \epsilon_i f_i^T = O_p(\sqrt{NG}) \), so the first term of (S9) is

\[
\sum_i \nabla g(\beta_i) \Omega^{-1} \hat{U}^T W \epsilon_i f_i^T = O_p(\sqrt{NG}) + O_p(N). \tag{S10}
\]

For the second term of (S9), note that \( \nabla g(\beta_i) = \frac{1}{\beta_i^T 1} \left( I - \frac{\beta_i 1^T}{\beta_i^T 1} \right) \), so

\[
\nabla g(\beta_i) \Omega^{-1} H \beta_i f_i^T = (I - p_i 1^T) \Omega^{-1} H p_i f_i^T \in \mathbb{R}^{K \times S}.
\]
Then the \((k, s)\) element of this matrix is

\[
(e_k^T - p_{ik} 1^T)\Omega^{-1} Hp_i f_{is} = \text{Tr} (f_{is} p_i (e_k^T - p_{ik} 1^T)\Omega^{-1} H) \\
= f_{is} \text{vec} (p_i (e_k^T - p_{ik} 1^T))^T \text{vec} (\Omega^{-1} H)
\]

Here \(e_k\) denote the unit vector that the \(k\)th element is 1 and \(f_{is}\) is the \(s\)th element of \(f_i\). Then

\[
\sum_i (e_k^T - p_{ik} 1^T)\Omega^{-1} Hp_i f_{is} = \text{vec} (\Omega^{-1} H)^T \sum_i f_{is} \text{vec} (p_i (e_k^T - p_{ik} 1^T)).
\]

Under the scenario of \(A = 0\), we have \(p_i = p_0 + e_i\), so \(p_i (e_k^T - p_{ik} 1^T)\) are i.i.d. across \(i\). Since W.L.O.G. we have already assumed that \(f_i\) are centered, which means that \(\sum_i f_{is} = 0\) for each \(s\), so we easily get \(\sum_i f_{is} \text{vec} (p_i (e_k^T - p_{ik} 1^T)) = O_p(\sqrt{N})\). Since \(H = O_p(\sqrt{G})\), we obtain

\[
\sum_i (e_k^T - p_{ik} 1^T)\Omega^{-1} Hp_i f_{is} = O_p(\sqrt{N\sqrt{G}}).
\]

This indicates that the second term

\[
\sum_i \nabla g(\beta_i)\Omega^{-1} H\beta_i f_i^T = O_p(\sqrt{NG})
\]  

(S11)

Combining (S8), (S9), (S10) and (S11), we have

\[
\hat{A} - A_N = O_p\left(\frac{1}{\sqrt{NG}}\right) + O_p\left(\frac{1}{G}\right) = o_p\left(\frac{1}{\sqrt{N}}\right).
\]

when \(N/G^2 \rightarrow 0\).

**Remark S3.** The condition that \(A = 0\) is only used to bound the second term (S11). So in the general case when the null \(A = 0\) does not hold, we have

\[
\hat{A} - A = -\frac{1}{NG} \sum_i (I - p_i 1^T)\Omega^{-1} Hp_i f_i^T + A_N - A + O_p\left(\frac{1}{\sqrt{NG}}\right) + O_p\left(\frac{1}{G}\right)
\]

where

\[
-\frac{1}{NG} \sum_i (I - p_i 1^T)\Omega^{-1} Hp_i f_i^T = O_p\left(\frac{1}{\sqrt{G}}\right).
\]

We can still establish the asymptotic normality of \(\hat{A} - A\) using our previous proof techniques, although estimating its asymptotic variance in practice will be very challenging.