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Peer Review

Review of: "Non-Negative Blind Source Separation and the Identifiability of Cell-Type Deconvolution in Spatial Transcriptomics: A Mathematical Review"

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Summary

This manuscript offers a mathematical framing of spot-based spatial transcriptomics (ST) deconvolution as a non-negative blind source separation (BSS) problem. The author argues that the identifiability theory developed for independent component analysis (ICA), non-negative matrix factorisation (NMF), and hyperspectral unmixing transfers directly to ST because both domains share the same non-negative linear mixture model. The review structures the landscape around three conditions: (i) full column rank of the signature matrix (reference completeness), (ii) the condition number $\kappa(S)$ (noise amplification when sources are similar), and (iii) separability or minimum-volume conditions in unsupervised regimes. It also points out that the negative-binomial nature of count data makes squared-error objectives a high-count approximation, and concludes with guidance for algorithm selection and clinical/regulatory interpretation.

The manuscript is clearly written, conceptually coherent, and likely to be valuable to ST practitioners who lack a background in identifiability theory. However, from the perspective of chemometrics and NMF, the treatment of rotational ambiguity and uniqueness is too limited, and key developments—especially recent work on **partial identifiability**—are not integrated. The central claim that three conditions "organise the entire field" risks oversimplifying a richer body of identifiability theory. I recommend **major revision**.

Major Comments

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1. Rotational ambiguity and equivalence classes are underdeveloped

The manuscript recognises that in unsupervised regimes non-negativity alone does not guarantee uniqueness and that many factorizations $Y = AS$ exist. However, the core issue of *rotational ambiguity*—the continuum of equivalent solutions generated by admissible transformations—is only briefly mentioned.

In the noiseless model, if $Y = AS$ with $A \in \mathbb{R}_{\geq 0}^{n \times k}$ $S \in \mathbb{R}_{\geq 0}^{k \times g}$, any invertible matrix $T \in \mathbb{R}^{k \times k}$ that preserves the constraints yields an alternative decomposition $Y = (AT)(T^{-1}S)$. Chemometric work has extensively characterised such "rotation groups" and their implications for uniqueness and ambiguity in bilinear and multiway models (e.g. Bro 1997 on rotational ambiguity in PARAFAC (https://doi.org/10.1016/S0169-7439(97)00032-4); Rajkó 2005, 2009, 2017 on rotational ambiguity (https://doi.org/10.1002/cem.947, https://doi.org/10.1016/j.aca.2009.04.033) and local rank deficiency in multivariate curve resolution (https://doi.org/10.1021/acs.analchem.6b03134)).

I recommend that the author:

- Explicitly introduce the transform $A \mapsto AT$, $S \mapsto T^{-1}S$, and describe the associated solution manifold.
- Discuss which transforms are admissible under non-negativity and simplex constraints (cone-preserving maps) and when admissible transforms reduce to permutation–scaling, i.e. essential uniqueness.
- Distinguish explicitly between full non-uniqueness, essential uniqueness (uniqueness up to scaling and permutation), and *partial identifiability* (see below).

This would give the review a more rigorous mathematical backbone and connect it to well-established treatments of rotational ambiguity in chemometrics and NMF.

2. Partial identifiability in NMF is highly relevant and currently missing

Perhaps the most important omission is the absence of recent work on **partial identifiability** in NMF, notably the paper by Gillis & Rajkó, "Partial Identifiability for Nonnegative Matrix Factorization" (SIAM J. Matrix Anal. Appl., 2023 https://doi.org/10.1137/22M1507553) and the references in it. This work provides a formal framework for situations where a non-negative factorisation is neither fully unique nor fully ambiguous: some components (columns of A, rows of S) are uniquely determined, while others are structurally confounded.

This concept is directly relevant to spatial transcriptomics, where:

- Some cell-type signatures (e.g. distant lineages) may be geometrically well separated and therefore structurally identifiable.
- Others (e.g. closely related immune subtypes with highly similar expression programmes) may lie in regions where multiple factorisations are possible, making them intrinsically ambiguous irrespective of the algorithm used.

The current manuscript effectively treats identifiability as binary: either the decomposition is unique (under rank, conditioning, separability/minimum volume) or it "wobbles." Incorporating partial identifiability would allow the author to:

- Replace this binary picture with a componentwise view: which cell types are identifiable, which are partially identifiable, and which are structurally confounded.
- Clarify that some benchmark failures (e.g. for rare or transcriptionally similar cell types) reflect structural non-identifiability of specific components, not just algorithmic weakness.
- Provide more nuanced guidance to clinical users and regulators, distinguishing outputs that are structurally reliable from those that are inherently ambiguous.

I strongly recommend that Gillis & Rajkó's partial identifiability framework be cited and integrated into Sections 3.3 and 4, and into the Discussion, as it materially strengthens the identifiability narrative for ST deconvolution.

3. Chemometric uniqueness literature is under-integrated

The manuscript currently leans heavily on hyperspectral unmixing and signal-processing references, but under-represents the chemometric literature on uniqueness and identifiability in bilinear and multiway models, as well as classical NMF uniqueness work.

Important domains and results include:

- **Multiway (tensor) models and essential uniqueness.** Kruskal's uniqueness conditions for three-way arrays and their chemometric uptake (e.g. Kruskal 1977 https://doi.org/10.1016/0024-3795(77)90069-6; Bro 1997 https://doi.org/10.1016/S0169-7439(97)00032-4; Sidiropoulos & Bro 2000 https://doi.org/10.1002/1099-128X(200005/06)14:3%3C229::AID-CEM587%3E3.0.CO;2-N) show that multiway structure can remove rotational ambiguity present in two-way models, yielding essentially unique decompositions under Kruskal-rank conditions. This is relevant for ST when multiple samples, layers, or modalities are available.

- **MCR-ALS and constrained bilinear models.** Multivariate curve resolution (MCR-ALS) explicitly deals with rotational ambiguity, local rank deficiency, and soft selectivity in bilinear decompositions (Tauler 1995 https://doi.org/10.1002/cem.1180090105 and https://doi.org/10.1016/0169-7439(95)00047-X ; de Juan & Tauler 2006 https://doi.org/10.1080/10408340600970005; Rajkó 2005, 2009). Constraints such as non-negativity, unimodality, and local support are used to reduce ambiguity and achieve practically unique solutions.
- **NMF uniqueness beyond separability.** Donoho & Stodden's "When does NMF give a correct decomposition into parts?" is rightly cited, but subsequent work by Gillis and co-authors elaborates on geometric and sparsity-based conditions for uniqueness and partial identifiability, including minimum-volume criteria and near-separable settings.

The manuscript cites Deville (2019) on separability/identifiability in bilinear and linear-quadratic mixtures only in the reference list, without engaging with its content. Deville's work directly addresses identifiability properties of bilinear and mildly nonlinear mixing models and their relation to factorisation algorithms, which is highly relevant in an ST context where spillover and nonlinearities may be present.

For a "mathematical review," it is important that these chemometric and NMF uniqueness results be more fully integrated, not simply listed at the end. Doing so would position the ST deconvolution problem within a broader and more mature identifiability theory, rather than primarily within the narrower hyperspectral paradigm.

4. The "three conditions organise the field" claim should be softened

The author's claim that three conditions—reference completeness, $\kappa(S)$, and separability/minimum volume—"organise the entire field" is rhetorically effective but conceptually oversimplified.

Issues:

- These conditions are mainly **necessary** or **diagnostic**; they are not sufficient for uniqueness in general. Full rank and modest do not fully characterise the equivalence class of solutions, especially in the presence of additional structure (sparsity, approximate selectivity, multiway structure).
- They do not capture **partial identifiability**, where some components are unique and others are not (Gillis & Rajkó 2023), nor do they reflect the rich concept of soft selectivity in MCR-ALS (Tauler 1995; de Juan & Tauler 2006).

- They focus on two-way geometry and do not include multiway effects, which are known to dramatically alter identifiability in chemometric models.

I suggest reframing these three conditions as a **practical checklist** for ST practitioners—valuable pre-analysis diagnostics—rather than as a complete organising principle for identifiability. The manuscript could be strengthened by explicitly stating that these conditions are part of a larger landscape that includes partial identifiability, soft selectivity, and multiway uniqueness.

5. Supervised regime: beyond full rank and "plain regression"

In Section 3.1, the supervised regime is reduced to constrained regression per spot, with identifiability equivalent to $rank(S) = k$, and the benchmark observation that plain regression competes with dedicated methods is explained accordingly. This is a useful perspective but not sufficient as a full identifiability analysis.

Chemometric practice highlights several additional points:

- **Near collinearity and local rank deficiency.** Even when , near collinearity among signatures can produce local rank deficiency and practical non-identifiability; Rajkó's work on local rank deficiency and rotational ambiguity in MCR-ALS is instructive here.
- **Closure (simplex) and non-negativity.** Closure constraints (sum-to-one, which just reduce the subspace dimension by 1) do more than enforce biological plausibility; they combined with non-negativity and selectivity restrict the admissible rotations and can reduce ambiguity.
- **Residual diagnostics.** Residual structure and leverage/influence measures are standard tools for detecting missing components and mis-specified references in chemometric models. The manuscript briefly mentions residual structure for missing cell types, but a more systematic link to this diagnostic tradition would be useful.

Expanding the supervised-regime discussion to cover these aspects, with appropriate chemometric references, would provide a more complete picture of identifiability and ambiguity in reference-based ST deconvolution.

6. Unsupervised regime: separability vs realistic marker genes

Section 3.3 focuses on separability (anchor/marker genes) and minimum-volume assumptions in the unsupervised regime. While these concepts are central in NMF and hyperspectral unmixing, the

biological reality in ST seldom meets the idealised conditions of perfectly selective markers and exact separability.

Chemometric MCR-ALS work has long grappled with **soft selectivity** and approximate anchors: components can be identifiable even when no variable is strictly exclusive, provided there are regions where one component dominates sufficiently. Similarly, NMF work on near-separable and noisy anchors (e.g. Gillis & Vavasis) shows that approximate separability can still yield useful identifiability results.

I encourage the author to:

- Move from strict, exact markers to a discussion of approximate or soft selectivity, referencing MCR-ALS literature.
- Clarify the limitations of minimum-volume assumptions in biological settings, where batch effects, spatial correlation, and technical noise can distort the convex hull of the data.
- Link these ideas to partial identifiability: the existence of approximate anchors may guarantee uniqueness for some components but not others.

This would render the unsupervised identifiability discussion more realistic for ST and tie it more closely to existing work on constrained factor models.

7. Structural identifiability vs statistical optimality

The manuscript correctly notes that negative-binomial noise makes squared-error objectives a high-count approximation and that maximum-likelihood estimators should respect the NB likelihood. This is important for statistical optimality and robustness.

However, identifiability in the deterministic model $Y = AS$ under constraints (non-negativity, closure, separability, etc.) is a **structural** property that does not depend on the noise distribution. The NB vs Gaussian choice affects estimator performance, not the existence of a unique solution. Chemometric and statistical identifiability theory generally separates:

- **Model identifiability**: uniqueness of the parameterisation in the noiseless limit.
- **Estimator optimality and robustness**: how well algorithms recover parameters under specific noise models.

I recommend that the manuscript explicitly distinguish structural identifiability from statistical optimality. Doing so will prevent readers from conflating noise modelling issues with uniqueness, and

will clarify that the core identifiability conditions apply to the underlying factorisation independent of the chosen likelihood.

Recommendation

In its current form, the manuscript offers a valuable and accessible engineering perspective on ST deconvolution and introduces identifiability thinking into a domain that has largely focused on benchmarking and empirical performance. However, to qualify as a "mathematical review," it must engage more deeply with:

- Rotational ambiguity and equivalence classes of solutions.
- Partial identifiability in NMF (Gillis & Rajkó, 2023) and componentwise uniqueness.
- Chemometric uniqueness results in bilinear and multiway models (MCR-ALS, PARAFAC).
- A more nuanced framing of the proposed three conditions as diagnostics rather than a complete organising principle.
- The distinction between structural identifiability and statistical optimality.

I therefore recommend **major revision**, with explicit integration of the partial identifiability framework and the broader chemometric/NMF uniqueness literature. Doing so would significantly strengthen the manuscript's contribution and make it a more robust bridge between spatial transcriptomics and established identifiability theory.

Declarations

Potential competing interests: No potential competing interests to declare.