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An analysis of deconvolution methods for spatial transcriptomics data in biopsies of patients with melanoma who received immune checkpoint blockade therapy

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Abstract

Spatial Transcriptomics is a cutting-edge molecular profiling method that can profile the transcriptome while preserving the morphological context. However, a limitation of the technology is that each spot (55 µm) may be derived from multiple cells. Fortunately, methods exist to resolve cellular heterogeneity by quantifying the relative contribution from cell types in every spot, termed deconvolution. Melanoma is characterized by a complex tumor microenvironment, thus, studying the cellular mechanisms spatially helps delineate progression and responses to immune checkpoint blockade (ICB) treatment of the malignancy. This study explores and compares several existing deconvolution methods on spatial transcriptome datasets from patients with melanoma who received ICB therapy. We analyzed the deconvolved results alongside the histopathologic annotation, which was used as a reference, to measure the performance of the deconvolution method. Our results reveal that RCTD and CARD performed superior to other methods in resolving cell type deconvolution at the spot level.

Background

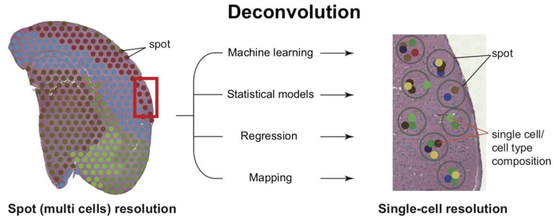

Figure 1. Visium Spatial Gene Expression is a sequencing-based spatial transcriptomics (ST) technology that maps the whole transcriptome with morphological context but lacks the single-cell resolution; each spot is derived from multiple overlapping cells. Deconvolution is a tool developed to dissect the single cell type profile composition of the Visium spots. [1]

Regression	Statistical Modeling	Machine Learning	Mapping	Reference-Free
SpatialDWLS **CARD** SpatialDecon NMFreg **SPOTlight**	**RCTD** Cell2location AdRoit DestVI Steroscope	STRIDE DSTG	Seurat V3 Tangram	**STdeconvolve**

A summary of various deconvolution methods. The methods explored in this project are bolded. These tools require Spatial Transcriptomics data (all) and scRNA-seq reference dataset (all except STdeconvolve). The algorithms underlying each method can be divided into 5 categories. Data from Zhang, Y., et al., *Comput. Struct. Biotechnol. J.*, 2023, 21, 176.

Methods

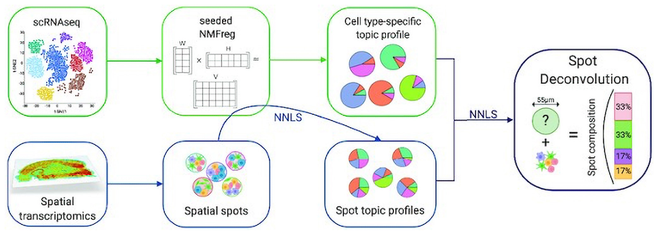

Figure 2. SPOTlight utilizes a seeded non-negative matrix factorization (NMF) regression with non-negative least squares (NNLS) to determine cell type composition per spots. Factorization is carried with using a non-smooth NMF which produces sparser results which promotes cell-type-specific topic profile and reduces overfitting. [2]

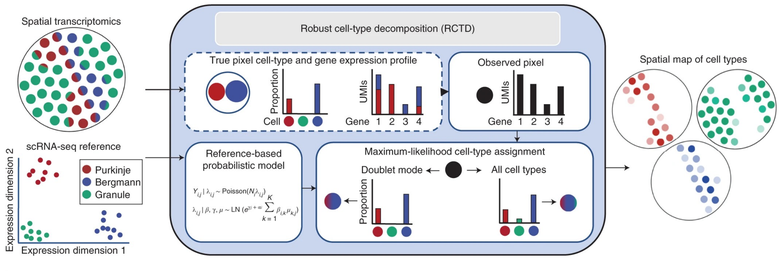

Figure 3. Robust cell-type decomposition (RCTD) utilizes statistical modeling to identify cell types proportions per spots. The expression of each cell type given a spot (the observed gene counts) is assumed to be Poisson distributed and is optimized using maximum-likelihood estimation (MLE). A notable asset of RCTD is that it explicitly addresses platform effects. [3]

Methods

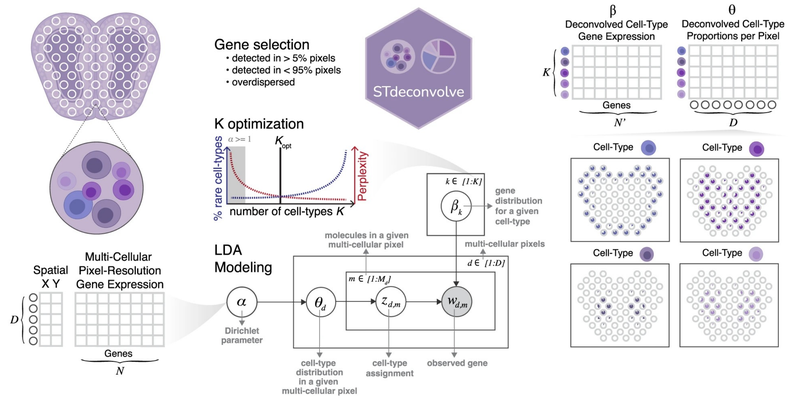

Figure 4. STdeconvolve is a scRNA-seq reference-free deconvolution method. This method utilizes Latent Dirichlet Allocation (LDA) to deconvolve latent cell types per spots. Each spot is defined as a multinomial distribution of cell type probabilities and each cell type is defined as a probability distribution over all the genes present in the spatial dataset. [4]

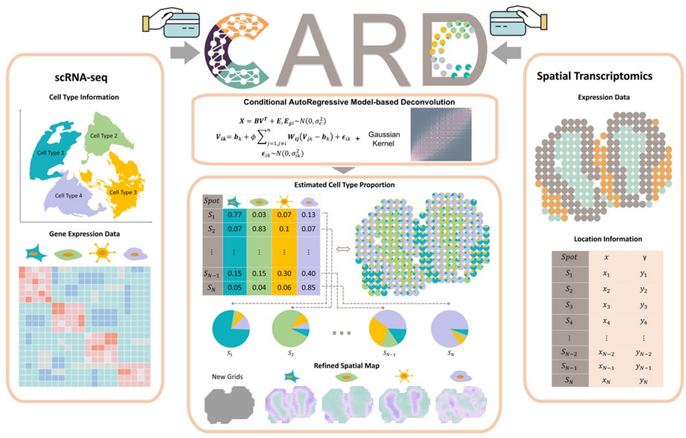

Figure 5. Conditional AutoRegressive Model-based Deconvolution (CARD) uses NMF in linking scRNAseq data, spatial spot composition, and residual error. Additionally, CARD takes advantage of the spatial correlation structure to enable accurate and robust deconvolution of ST data even in the presence of mismatched scRNA-seq references.

Results

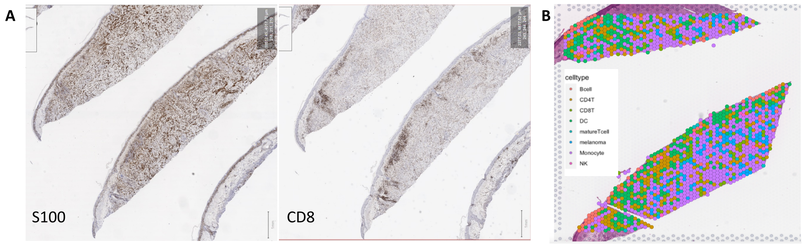

Figure 6. Histopathologic assessment for melanoma (S100) and CD8+ T-cells (CD8) A) and cell type clustering B) of a baseline biopsy from a patient with desmoplastic melanoma who responded to anti-PD-1 therapy shows slight co-localization of some melanoma areas (on the biopsy edges) with CD8 expression.

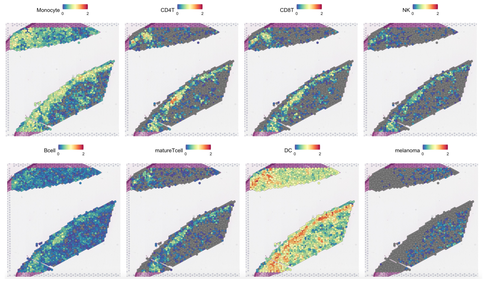

Figure 7. Expression levels of selected immune cell types using spatial feature plot show generally that most of the immune cell clusters are outside of the melanoma cell cluster, with varying expression.

Results

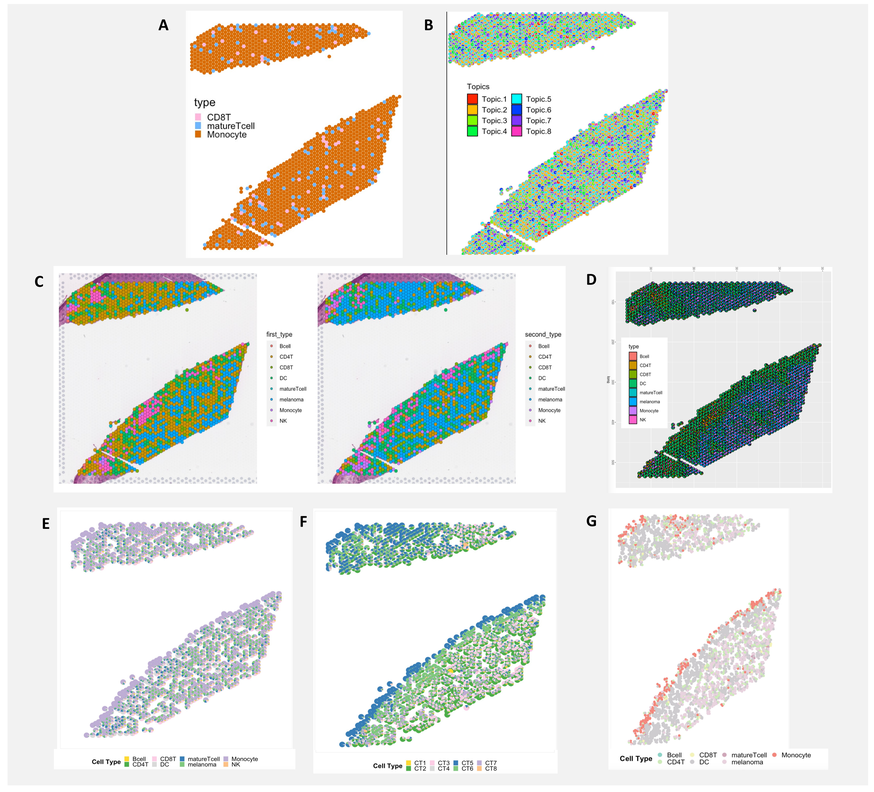

Figure 8. Spot level deconvolution of the selected immune cell clusters shows different performance using A) SPOTlight B) STdeconvolve C) RCTD Doublet Mode D) RCTD Full Mode E) CARD, F) CARDFree extension, G) CARD Single-Cell Resolution Mapping.

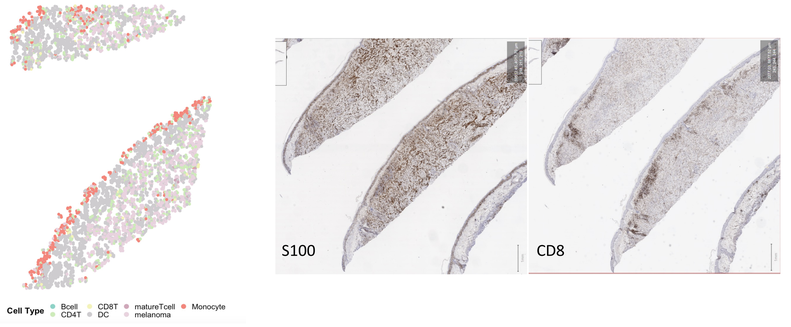

Figure 9. Deconvolution Results from CARD Single-Cell Resolution Mapping showed the best performance compared to histopathologic staining for the patient baseline biopsy.

Conclusion

Spatial transcriptome technologies are powerful tools in cancer research. However, current ST sequencing technologies are incapable of reaching single-cell resolution. Several deconvolution methods were developed to address this issue. Studying SPOTlight and STdeconvolve results shed light on two main issues with current deconvolutional methods:

1. Deconvolution is critically dependent on the availability and accuracy of scRNA-seq data
2. Technical variations between scRNA-seq and Spatial Transcriptomics data exist

Being aware of to these issues led to a more educated exploration of deconvolution methods including RCTD and CARD. Our results show that both methods have been successful in addressing at most one of the issues.

Acknowledgments

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References

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