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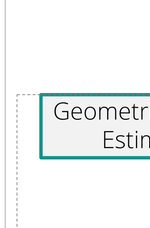
BENCHMARKING OF NEW AND PUBLISHED IMAGE ALIGNMENT METHODS FOR SPATIAL TRANSCRIPTOMICS DATA

Genomics of Gene Expression Lab



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Introduction

Spatial transcriptomics (ST) based on array mRNA capture is an innovative technique commercialized as Visium by 10x Genomics. This method enables the detection and quantification of gene expression in tissue sections while preserving the spatial organization of cells, which is essential for studying biological systems where tissue architecture drives function, such as tumour progression or tissue injury. However, comparing equivalent regions across different samples is challenging, as tissue size, shape or spatial layout often vary.

Current published alignment tools address this by creating common spatial references, but most rely on gene expression profiles to perform the alignment, which can introduce bias compromising biological independence between samples.

Here, we present a benchmark in which we tested three alignment approaches not originally design for ST data, which employ only the histological images guided by reference landmarks, These methods were implemented in an R package called STIMA (Spatial Transcriptomics Image-based Methods for Alignment). We compared them against two well-established alignment methods: PASTE2 and STalign.

Alignment tools

Visual Evaluation of Alignment

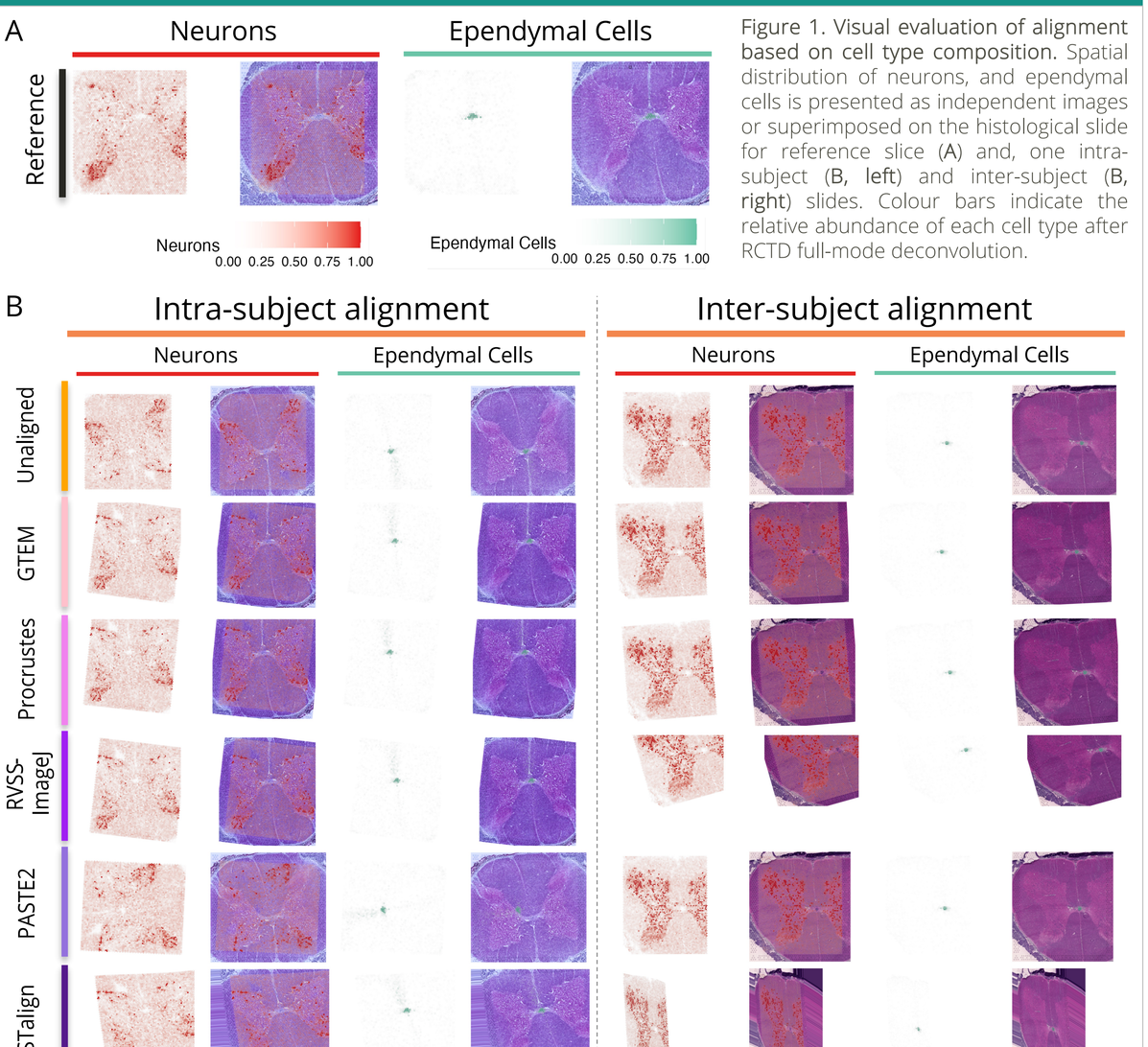

Figure 1. Visual evaluation of alignment based on cell type composition. Spatial distribution of neurons, and ependymal cells is presented as independent images or superimposed on the histological slide for reference slice (A) and, one intra-subject (B, left) and inter-subject (B, right) slides. Colour bars indicate the relative abundance of each cell type after RCTD full-mode deconvolution.

Evaluation of Alignment Similarity

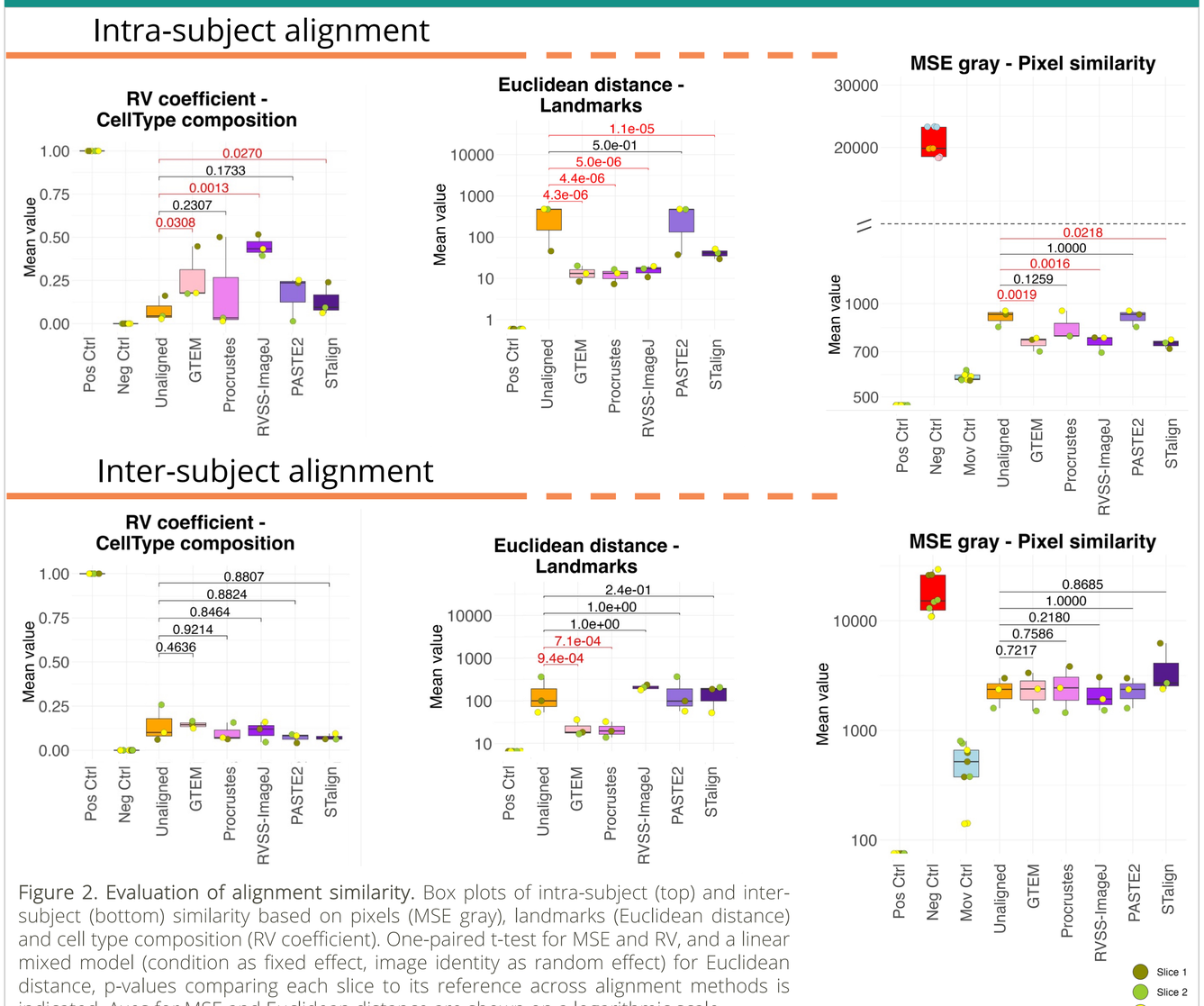

Figure 2. Evaluation of alignment similarity. Box plots of intra-subject (top) and inter-subject (bottom) similarity based on pixels (MSE gray), landmarks (Euclidean distance) and cell type composition (RV coefficient). One-paired t-test for MSE and RV, and a linear mixed model (condition as fixed effect, image identity as random effect) for Euclidean distance, p-values comparing each slice to its reference across alignment methods is indicated. Axes for MSE and Euclidean distance are shown on a logarithmic scale.

Conclusions

1. The image-only alignment methods implemented in STIMA achieve superior accuracy and precision while remaining independent of transcriptomic data, outperforming existing alignment tools such as PASTE2 or STalign.
2. Performance may vary depending on tissue architecture, staining quality, and biological variability.
3. STIMA R package is open-source, well-documented, and designed to support researchers seeking unbiased and anatomically consistent comparative analyses in ST across multiple samples.

Bibliography

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Acknowledgments

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