Easydecon: A Lightweight Workflow for Cell Type Analysis in High-Definition Spatial Transcriptomics Assays

Sinan U. Umu[1,2*+] , Victoria T. Karlsen[1,2+], Espen Sønderaal Bækkevold[2,3], Frode Lars Jahnsen[1,2] and Diana Domanska[2,4]

1 Department of Pathology, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
2 Department of Pathology, Oslo University Hospital-Rikshospitalet, Oslo, Norway
3 Institute of Oral Biology, University of Oslo, Oslo, Norway
4 Department of Microbiology, University of Oslo, Oslo, Norway

*corresponding author: sinanuu@uio.no

Abstract
Spatial transcriptomics enables mapping gene expression within tissue structures. VisiumHD by 10x Genomics advances this further, offering near-single-cell resolution across tissue sections. Here we introduce Easydecon, a lightweight bioinformatics workflow leveraging similarity algorithms and regression-based cell type proportion estimation to transfer cell types and deconvolute spatial bins. Easydecon streamlines visualization and spatial deconvolution by utilizing differentially expressed genes identified from single-cell RNA sequencing datasets.

Main
Biological tissues are composed of various cell types and extracellular elements, and their functionality is determined by both these components and their spatial arrangement within the tissue (Ståhl et al. 2016). Novel imaging and molecular profiling tools allow us to study these tissues in more detail than before, giving us a better understanding of their composition and organization. Spatial transcriptomics (ST) uses RNA sequencing (RNA-seq) to investigate cells and their gene expression patterns within their spatial context (Marx 2021). It has revolutionized research in areas such as developmental biology, cancer, and neuroscience by enabling integration of molecular data with spatial organization, overcoming limitations of scRNA-seq alone (Kim et al. 2023). In recent years, we have seen major developments in ST technologies such as resolution, multiplexing capacity, compatibility with clinical samples, enabling more detailed, accurate, and scalable analysis, which allow us to better understand spatial patterns among various diseases and tissues (You et al. 2024; Tadross et al. 2025; Androvic et al. 2023; Cilento et al. 2024).

The Visium platform from 10x Genomics has been one of the most popular among the ST platforms. This allowed spatial resolution of target tissue resolved in 55 µm bins. The platform has already enabled better understanding of the heterogeneous nature of cancer and tumor microenvironment (TME) (Peng et al. 2022; Ozato et al. 2023). However, the analyses and single-cell data integration of ST usually require computationally expensive deconvolutional tools for seamless integration with other modalities and for fine-grained spatial resolution (Li et al. 2022). The new version of the 10x Visium platform (VisiumHD) achieves near single-cell resolution with smaller and gapless spots i.e. bins. This partly solves the problem of the old Visium technology, where the same spots contain multiple cells, and may ease the requirement of complex deconvolutional algorithms. The new

platform does this by offering different bin sizes with the default 8 µm resolution having more than half a million squared bins on a single slide. It is also possible to use 2 µm (offering millions of squared 2x2 µm spots) or 16 µm sized bins, which is a tremendous improvement compared to the previous version (Oliveira et al. 2024).

However, the efficient analysis of VisiumHD data is not straightforward as most of the tools have been developed considering the previous version or other ST platforms. Many deconvolutional tools are also not working efficiently on very large VisiumHD objects. This poses challenges for researchers who want to do multimodal cell type analysis using data coming from various scRNA-seq platforms. Fortunately, some of the frameworks like SpatialData (Marconato et al. 2024) operate effectively on different ST technologies including VisiumHD which creates a strong basis for analysis. There are also recent solutions for analysis like Bin2cell (Polański et al. 2024), which works on 2 µm bin sizes to identify cells on the H&E image, or Enact workflow, which allows segmentation followed by multiple cell type annotation methods (Kamel et al. 2025). Yet, cell type analysis is still not streamlined to utilize any scRNA-seq data and requires additional bioinformatics solutions.

In this study, we describe Easydecon, a novel workflow to analyze high definition spatial datasets. Since visualization of distinct cell types and markers are still one of the major challenges, Easydecon utilizes lightweight algorithms and regression-based estimation for marker visualization, single-cell cluster transfer and deconvolution. Easydecon uses the latest bioinformatics packages, compatible with VisiumHD, and enables fast analyses of high-definition ST datasets. It is a Python package with minimal dependencies and any scRNA-seq dataset coming from different single-cell technologies (i.e. 10x Genomics, Parse biosciences etc.) can be used. We tested Easydecon on publicly available colorectal cancer (CRC) datasets and standard annotations as a showcase and suggested how it can be used effectively and easily.

Results

Easydecon workflow allows fast ST analysis

Easydecon is designed to operate on the VisiumHD platform optimally for 8 µm resolution bins while the platform offers 2, 8 and 16 µm resolutions. Easydecon utilizes permutation tests, similarity metrics and bin decomposition modelling to transfer cell type labels from scRNA-seq datasets to high resolution ST data or among different ST samples. The analysis workflow starts from tables representing DE markers, in CSV or Excel format. By relying on pre-computed DE results, Easydecon ensures efficient and reliable label transfer while maintaining high computational performance. Furthermore, it supports the use of selected marker genes for different cell types, which can serve as pre-filtering markers or can be directly mapped onto spatial locations for basic visualization.

This design allows researchers to easily combine any publicly available or own scRNA-seq data with their high definition ST datasets, making it straightforward to identify distinct cell types and spatial gene expression patterns without relying on complex, computationally expensive deconvolution algorithms (Fig. 1).

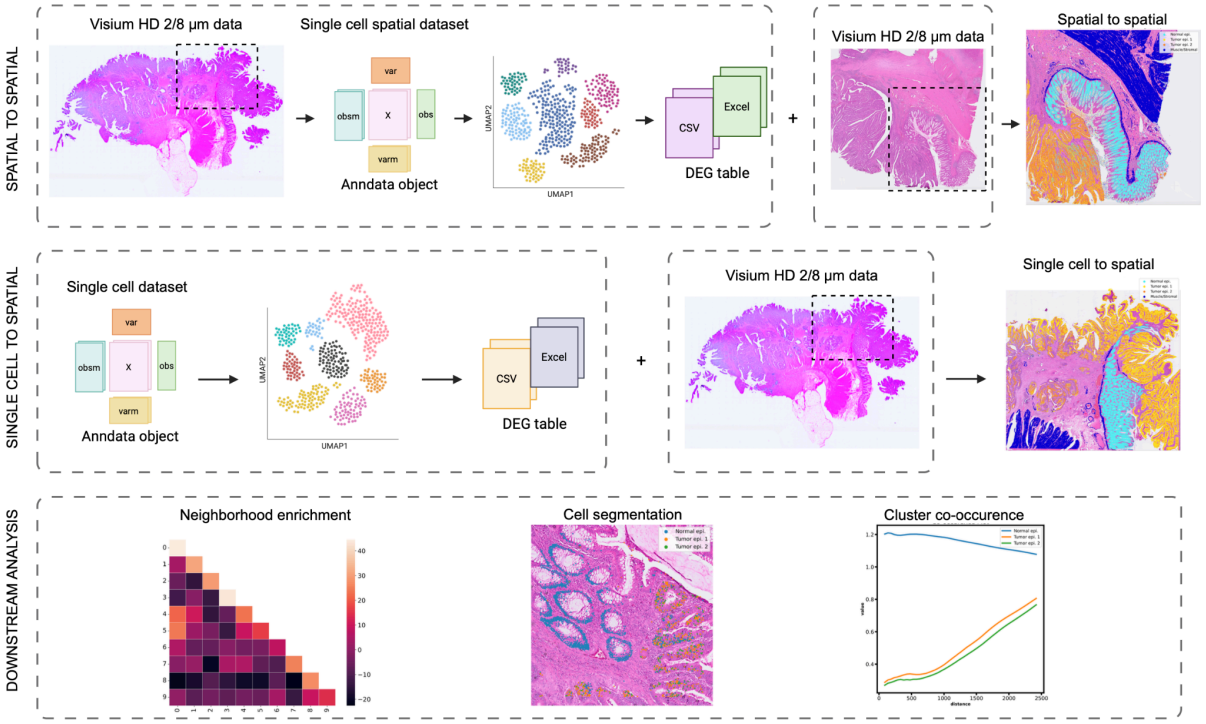

Figure 1. Overview of the Easydecon workflow shows the use cases. Easydecon supports spatial-to-spatial and single-cell-to-spatial mapping, utilizing Anndata or Seurat objects and DE tables. It also allows for cell segmentation using third party algorithms like Bin2cell. Afterwards, it is followed by downstream analyses based on bin or cells such as neighborhood enrichment, counts or co-occurence.

Easydecon enables flexible cell type transfer from single-cell datasets using DE markers

As a general and robust test case for Easydecon, we acquired a publicly available single-cell CRC atlas dataset (Pelka et al. 2021) and a VisiumHD CRC sample provided by 10X Genomics (Sample P2). Easydecon is designed to leverage scRNA-seq data without requiring the exact single-cell object. Easydecon employs a two-step approach.

In the first step, global markers (e.g. major cell lineages markers or top-level markers) are used to detect expression hotspots, meaning the most likely positions of target cells among bins. These global markers can be manually selected based on prior knowledge and existing literature or directly extracted from the DE tables. In our test case, we used seven top-level clusters: epithelial (epi) cells, stromal (strom) cells, T-NK-ILC (TNKILC) cells, plasma cells, mast cells, B cells, and myeloid cells from the acquired CRC atlas dataset (Pelka et al. 2021). From each cluster, we extracted only the best scored 60 markers (Fig. 2a shows top 5 of these). At this low-granularity phase, bins with the highest expression levels are identified as the most probable locations of top-level clusters (Fig. 2b). Easydecon filters out bins with low or no expression for these clusters.

In the second step, the left-over bins are used to refine granularity. A similarity algorithm is applied to assign specific cell types to these bins. Since the first step removes low-expressing bins, the second step operates with reduced noise, improving efficiency. Using the CRC dataset, we demonstrated this approach with myeloid cells, which comprise

four main subtypes: dendritic cells (DC), granulocytes (granulo), macrophages (macro), and monocytes (mono). We increased the granularity by transferring these to myeloid cell bins (Fig. 2c,d) on a region of interest (ROI) containing a CRC tumor. To refine myeloid hotspots on this ROI (Fig. 2c), we did another filtering and refined these hotspots on this section. In this example, the majority of bins are assigned to macrophages. It is also possible to infer cell type proportion. We found that the myeloid associated bins mostly contain macrophages (Fig. 2e).

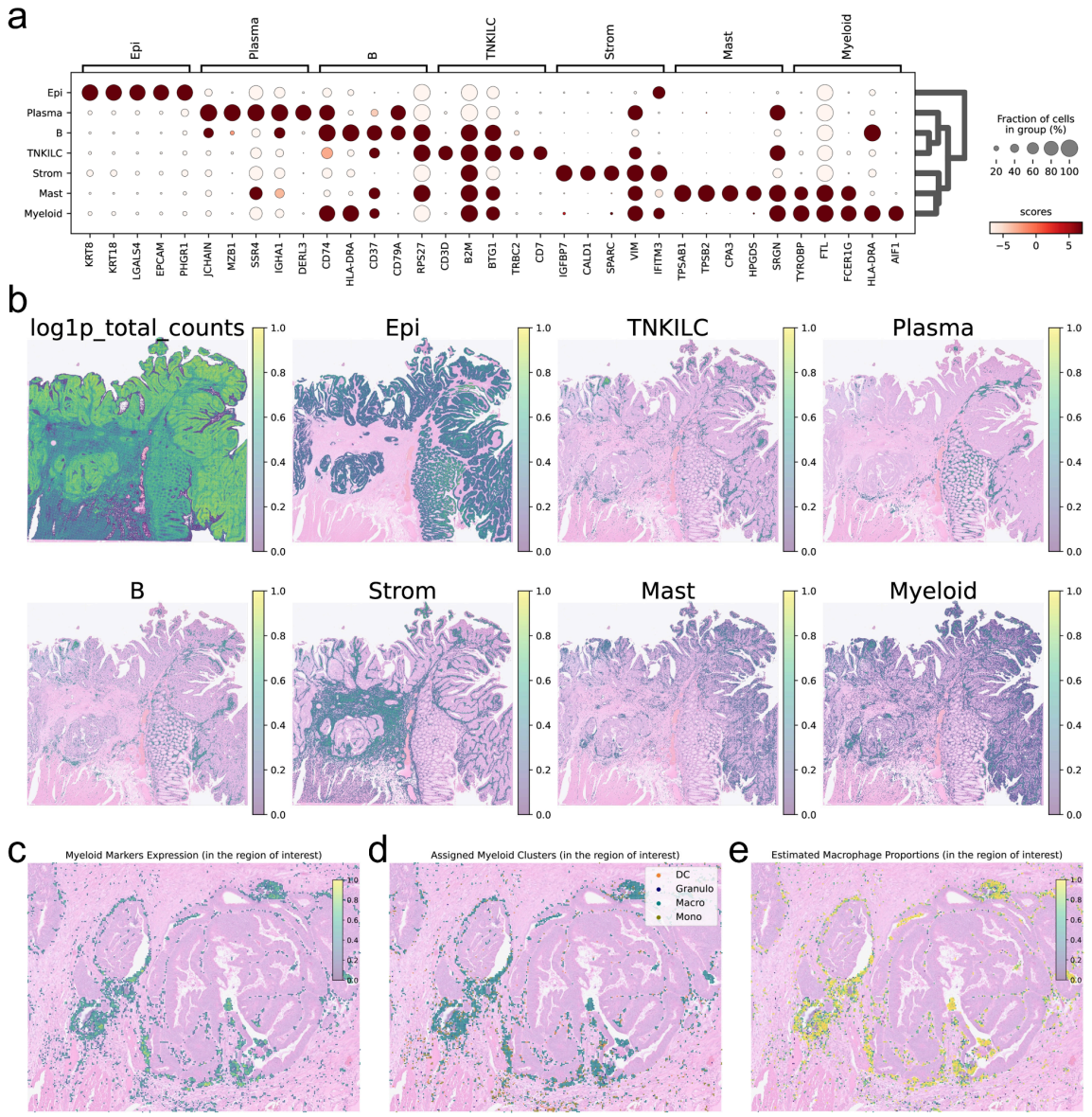

Figure 2. Eeasydecon uses global and local strategies to enable robust label transfer. **a,** Here the top 5 markers for each top-level cluster are visible from the publicly available CRC dataset and we used 60 best markers overlapping with the ST object for the analyses. **b,** The top level cluster markers are visualized in each panel with the H&E tissue image at the background. The first panel shows the normalized total counts. We summed normalized expressions of the markers to aggregate them for each bin. Expression hotspots signal most likely locations of the clusters ($p < 0.0001$). **c,** Here a ROI is focused for myeloid cell markers

and it shows the myeloid marker expression at this region. **d,** We assigned mid-level markers to myeloid hotspots showing macrophages are the most common cell types. **e,** Cell type proportion estimates are also confirming this.

Easydecon can transfer cell types between multiple VisiumHD samples

Easydecon also allows the transfer of cluster information across multiple Visium HD samples. This workflow is similar to single-cell to spatial mapping. The “donor” spatial dataset (Sample P2) can be used to generate a DE table using standard single-cell pipelines such as Scanpy (Wolf et al. 2018) or Seurat (Satija et al. 2015). This table can be then transferred to another compatible spatial sample, enabling rapid analysis.

We tested this approach on two publicly available VisiumHD datasets from colon tissue, one CRC sample (Sample P5) and one normal tissue sample (Sample P3). Tissue clusters were taken from the official 10x Genomics object (Sample P2). After generating the DE table using these putative clusters, we transferred the cluster information to the other two samples: Sample P5 and Sample P3. In this example, we applied only the similarity approach (i.e. phase two or high granularity step) and assigned each spot to the most similar cluster.

All available clusters were included in the analysis with focus on visualizing two tumor-associated clusters, the normal epithelial and muscle/stromal clusters (Fig. 3a). In the colon tissue sample containing both tumor and normal regions (tumor adjacent), these areas are clearly separated (Fig. 3b). The normal tissue sample showed only normal epithelial and muscle annotations (Fig. 3c). These results demonstrate that both CRC tumor and normal tissue sections were successfully mapped to their correct spatial locations in other samples and a single DE table from the donor ST is sufficient.

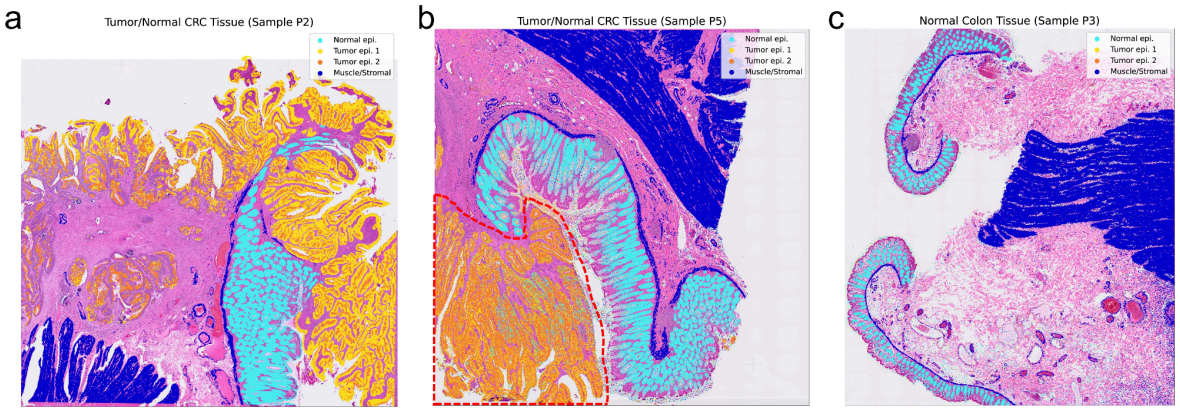

Figure 3. Spatial clusters are transferred to samples containing tumor and normal tissue. a, Here we highlighted clusters from both tumor and normal epithelial regions. We also included muscle/stromal clusters, displaying at the bottom left. **b,** The sample tissue containing both tumor (yellow/orange) (inside the red region) and normal epithelial (cyan) clusters are visible. The large muscle tissue is located at the top and below the normal epithelial region, similar to the donor sample on the left panel. **c,** Normal tissue only contains the normal epithelial cluster and the muscle tissue. Even though we included tumor clusters, only the normal tissue layer was spatially transferred as expected.

Easydecon can be used with cell segmentation

The H&E tissue images of VisiumHD samples can be used for cell segmentation. Later, the segmented cells can be used with Easydecon for cell type transfer or deconvolution. Bin2cell is a computational method designed to reconstruct biologically meaningful single-cell transcriptomic profiles from high-resolution spatial transcriptomics data (Polański et al. 2024). The tool aggregates transcriptomic signals from 2 µm bins into cellular units using morphological segmentation with the StarDist algorithm (Weigert & Schmidt 2022). This bin-to-cell transformation enhances the accuracy of cell type annotation, provides higher per-cell gene detection and significantly improves spatial localization of cell populations compared to default analysis approaches using 8 µm bins. Bin2cell achieves these improvements efficiently, without the need for GPU computing resources which makes it an ideal tool to use with lightweight Easydecon.

To evaluate Bin2cell in conjunction with Easydecon, we selected another ROI in the Sample P5 spanning both tumor and normal tissue (Fig. 5a). Bin2cell aggregates 2 µm bins into segmented cells (Fig. 5b), which we then annotated using Easydecon and the same spatial clusters (Fig. 5c). Both the segmented and non-segmented analyses yielded consistent cell-type assignments, accurately separating normal and tumor regions (Fig. 5d). We also estimated cell-type proportions within the segmented cells using three different algorithms (Figs. 6a-c), and these results were highly concordant with the bin-based cell-type assignments.

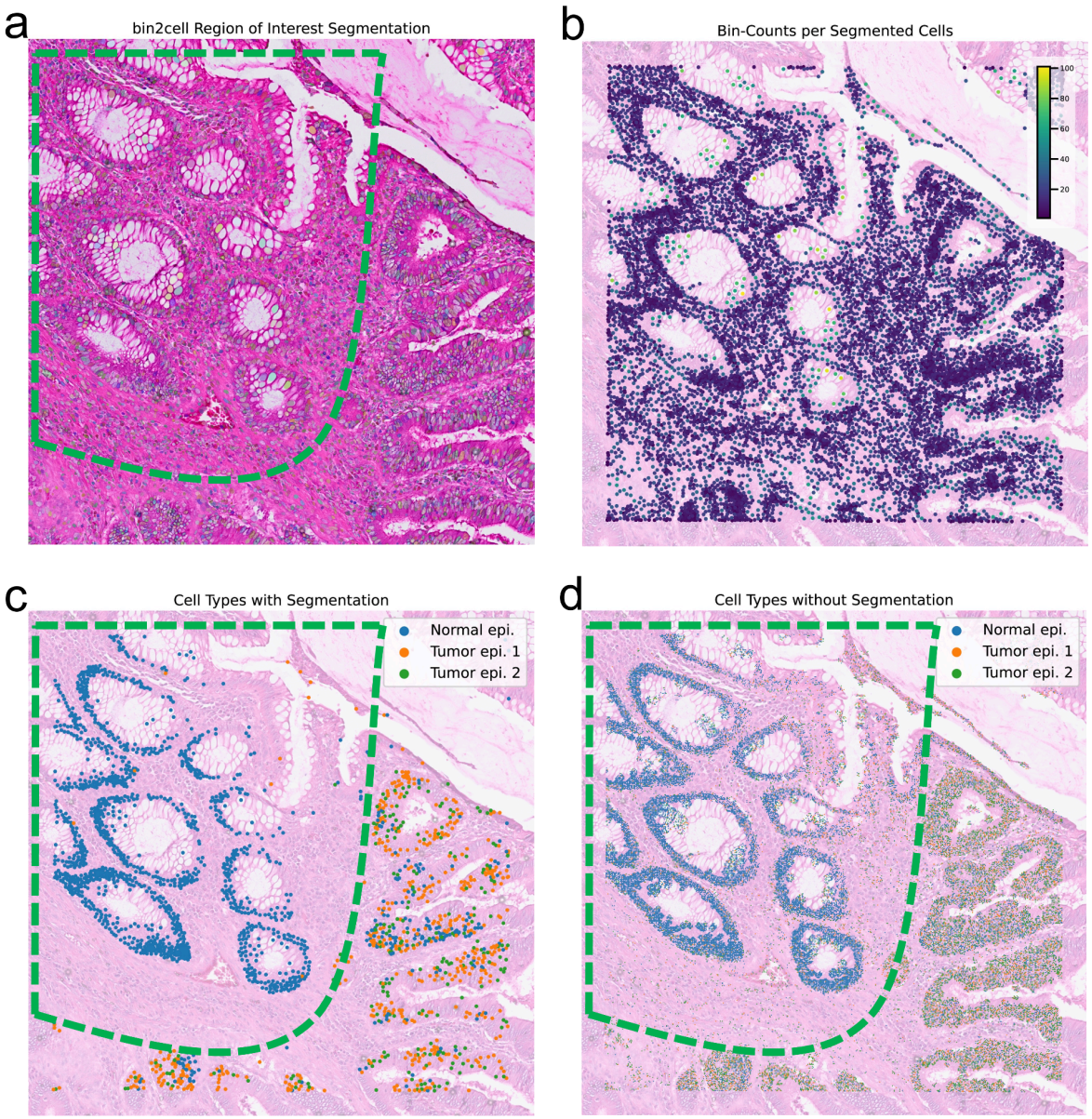

Figure 4. Easydecon can annotate Bin2cell segmentation on human colon tissue. a, The H&E-stained bright-field image shows the selected ROI in Sample P5, encompassing both normal and tumor regions (the dashed outline indicates the tumor-adjacent area). Segmentation within this ROI was performed using the Bin2cell tool. **b,** Segmented cells are color-coded according to the total number of 2 µm bins they encompass, with higher values indicating more bins per segment. **c,** Easydecon assigns cell types to the segmented cells via similarity-based label transfer, generating a precise spatial distribution of identified cell populations and clearly distinguishing normal from tumor tissue. **d,** The ROI annotations without segmentation largely mirror the segmented results, underscoring Easydecon's consistency and accuracy in both approaches.

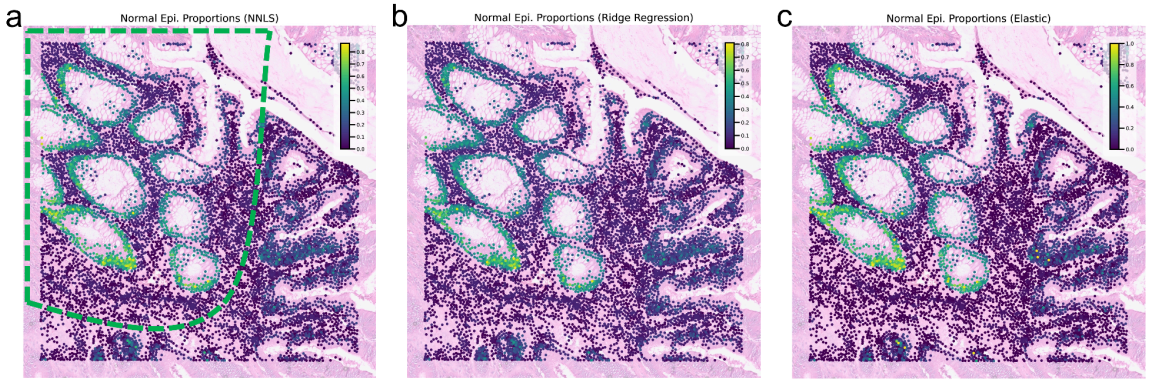

Figure 5. Cell type proportion estimates of segmented cells shows separation between normal and tumor tissues. a, Each spot represents a cell segmented by Bin2cell, with normal epithelial cluster proportions estimated via NNLS. The highlighted region corresponds to tumor-adjacent normal tissue. **b,** Ridge regression produces a similar distribution of proportions. **c,** Elastic-net regression likewise reflects the same pattern, providing sharper estimates for certain bins.

Easydecon helps to differentiate between regional macrophage clusters

Easydecon can be applied to identify the spatial distribution of specific cell types. As a case study, we compiled single-cell macrophage datasets from multiple studies focused on tumor-adjacent regions (Pelka et al. 2021; Uhlitz et al. 2021; Liu et al. 2022; Qian et al. 2020; Joanito et al. 2022) into one object (Fig. 6a). To provide anatomical context, we included background information from two distinct regions: the mucosa and the muscularis (Domanska et al. 2022).

We clustered this combined dataset and produced a DE table for 10 macrophage clusters. Adopting Easydecon's two-phase approach, we initially identified macrophage expression hotspots (Fig. 6b), which mostly occurred in the mucosal region. In the second phase, we employed similarity metrics to assign these clusters to the hotspots (Fig. 6c,d). Neighborhood enrichment and co-occurrence analyses indicated that cluster 5 macrophages were spatially distinct and minimally interacted with the remaining clusters (Fig. 6e,f). Cluster 5 cells primarily inhabited the muscularis layer, while the other clusters were enriched within the mucosa.

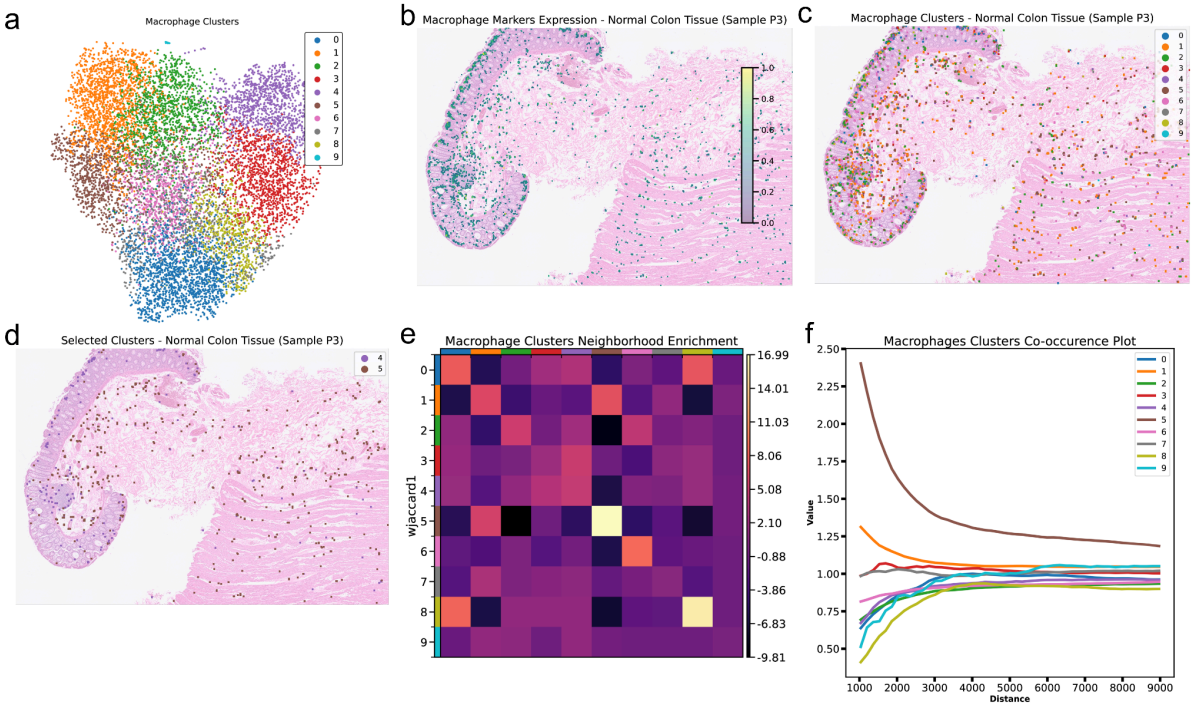

Figure 6. Easydecon can analyze spatial location specific macrophages. a, A UMAP plot displaying 10 macrophage clusters. **b,** Macrophage expression hotspots, primarily located in the mucosal region. The scaling has been magnified for clarity. **c,** Easydecon transfers the UMAP-identified clusters to the ROI. **d,** Two specific clusters are highlighted: cluster 4, which predominantly localizes to the mucosa, and cluster 5, which is mainly found in the muscularis. **e,** The distance matrix shows that cluster 5 macrophages are significantly more distant from the other, mostly mucosal, clusters. **f,** The co-occurance plot confirms that cluster 5 macrophages remain distinct from other clusters, which are predominantly situated in the mucosal region.

Discussion

There has been growing interest in scRNA-seq and ST due to their combined ability to reveal cellular identities and their spatial contexts within tissues (Kim et al. 2023; Peng et al. 2022). Although various computational methods exist for integrating these modalities (Wolf et al. 2018; Satija et al. 2015; Palla et al. 2022; Kamel et al. 2025; Marconato et al. 2024), the recent advancements introduced by VisiumHD require efficient and tailored analytical workflows. Here, we introduce Easydecon, a lightweight and user-friendly bioinformatics workflow specifically developed for rapid and reliable analysis of high-resolution VisiumHD spatial datasets using diverse scRNA-seq data sources.

A significant advantage of Easydecon is its foundational assumption that the 8 µm spatial bins provided by VisiumHD represent near-single-cell resolution and are thus homogeneous. This assumption effectively bypasses the need to handle complex scenarios involving doublets or triplets, common in earlier versions of Visium technology. Consequently, this simplifies downstream computational steps, enhancing analysis speed and reliability without compromising accuracy.

Easydecon addresses multiple critical aspects of spatial analysis, including marker visualization, cell type transfer, and spatial bin deconvolution. By employing robust similarity algorithms and permutation-based statistical approaches, Easydecon ensures precise identification of spatial expression hotspots. The flexible use of global and local markers allows researchers to integrate existing knowledge from literature or DE results from single-cell datasets seamlessly. However, the users do not need to employ the two phase approach but they can simply choose a similarity or proportion estimation for cell type transfer and deconvolution.

It is also possible to use Easydecon with available single-cell and spatial frameworks such as SpatialData (Marconato et al. 2024), Squdipy (Palla et al. 2022) and Scanpy (Wolf et al. 2018). R language workflows like Seurat (Satija et al. 2015) are also supported via a DE table. Furthermore, Easydecon supports cell segmentation workflows, as demonstrated by our successful integration with Bin2cell (Polański et al. 2024). This also does not break our foundational assumption (Fig. 4 and Fig. 5). By aggregating high-resolution bins into biologically meaningful cellular units, Easydecon not only improves the spatial localization and accuracy of cell-type annotations but also provides compatibility with downstream spatial analyses such as co-occurrence and neighborhood enrichment (Palla et al. 2022).

EasST allows detection of spatially relevant cell clusters. We tested Easydecon on a macrophage single-cell dataset.

One limitation of Easydecon is its dependency on high-quality marker gene selection. Future work could explore automated and data-driven approaches for marker selection, potentially improving its accuracy and applicability across different biological contexts. It can be hard to decide a cut-off for a total number of markers to be used. This can also differ between phase one, expression hotspot detection, and phase two, granularity improving or deconvolution. To overcome this, we recommend trying different parameters or methods that we include. For example, using different permutation parameters or testing different weight penalties in methods like weighted Jaccard could be a good starting strategy.

In addition, while Easydecon is optimized for the 8 µm bins commonly used in VisiumHD, extending its functionality to the higher-resolution 2 µm bins could further enhance its analytical power. If a smaller ROI is selected, it can benefit from increased accuracy while keeping the runtime relatively small. Selection of a ROI also helps with further parameter optimization. Easydecon can also benefit from a second filtering step depending on the cell types especially on crowded sections (Fig. 2c).

In summary, Easydecon offers a streamlined, efficient solution for analyzing complex spatial transcriptomics datasets, delivering rapid insights into both tissue biology and disease pathology. Its design circumvents the need for specialized hardware, such as GPUs or high-performance computing environments, making it accessible to a wider range of laboratories and researchers with limited computational resources. By leveraging a two-phase approach and robust similarity algorithms, Easydecon seamlessly integrates single-cell and spatial data, enabling more precise and user-friendly analyses.

Materials and Methods

Easydecon Analytical Framework

Easydecon follows a two-step approach. The first step focuses on identifying spatially informative marker gene expression patterns based on differential expression analyses from scRNA-seq or from another ST dataset. These markers are then assessed within the VisiumHD dataset by aggregating expression levels in spatial bins using selected aggregation methods (e.g., sum, mean, median). To identify significant marker gene expression spots, Easydecon uses filtering algorithms like quantile-based or permutation-based approaches to separate meaningful expression signals from background noise. This helps refine marker-gene expression profiles for each spatial bin. Next, Easydecon assigns cell type labels to these spatial bins by calculating similarity scores between spatial bin expression profiles and predefined marker gene profiles from scRNA-seq data. It does this by calculating similarity scores using different metrics, such as correlation, cosine similarity or weighted Jaccard. This step ensures that spatial bins are efficiently matched to distinct cell types or clusters, effectively integrating scRNA-seq data into spatial analysis. The results can be merged back into the VisiumHD dataset, enabling streamlined visualization, downstream analysis and interpretation.

Detection of Expression Hotspots

Easydecon implements two filtering strategies: parametric thresholding and empirical thresholding. Parametric thresholding (gamma or exponential distributions) is used to model the null distribution generated by permutation sampling. Model parameters are fitted to the empirical null distribution, and a significance threshold is determined via the inverse cumulative distribution function based on a predefined significance level (α). An empirical quantile approach directly estimates the threshold by selecting the (1 - α) quantile from the permutation-generated null distribution without assuming an underlying parametric distribution. Additionally, filtering can be performed using quantiles, providing an alternative approach for refining expression hotspots.

Similarity Algorithms

Weighted Jaccard Similarity

This extends the classic Jaccard similarity by incorporating weights to reflect gene importance or expression levels. This method is particularly suitable for comparing expression profiles between spatial bins and reference marker gene sets.

The weighted jaccard similarity J_w between two gene sets A (reference markers) and B (spatial bin expression) is defined as follows:

$$J_w(A,B) = \frac{\sum_{i \in A \cup B} \min(a_i, b_i)}{\sum_{i \in A \cup B} \max(a_i, b_i)}$$

where:

- $A \cup B$ denotes the union of genes from both sets.
- a_i and b_i represent the weights associated with the gene i in the reference marker set and the spatial bin, respectively.

Weights for genes can be assigned using two distinct strategies:

- Pre-calculated Weights: If available, predefined weights derived from differential expression analyses (e.g., log fold-changes) are normalized and directly utilized as gene weights.
- Rank-based Exponential Decay: If predefined weights are unavailable, gene weights are assigned based on their rank positions within the marker gene list using an exponential decay function:

$$w_i = e^{-\lambda \cdot \mathrm{rank}(i)}$$

where $rank(i)$ denotes the rank position of gene i, and the parameter λ controls the decay rate, emphasizing genes ranked higher in significance. The resulting Weighted Jaccard index provides a robust measure of similarity that captures both the presence of genes and their relative importance or expression strength, enhancing the accuracy of spatial cluster assignments.

Other Similarity Metrics

We also utilize other similarity metrics. Szymkiewicz–Simpson similarity also known as overlap similarity. It evaluates similarity based on the intersection relative to the smaller set and emphasizes complete or nearly complete overlap of gene sets. Cosine similarity based on the cosine of the angle between two gene-expression vectors, emphasizing directional concordance. Here we use the normalized spatial expression and fold changes or scores. Jaccard similarity measures the overlap between two gene sets relative to their union. Since we measure similarity in each bin spot separately, similarity metrics are robust to enable a direct similarity comparison among cell clusters.

Cell Type Proportion Estimation

Easydecon includes a deconvolution method for estimating cell-type proportions per spatial bin using non-negative linear models. A reference matrix is built from marker genes and their fold-change values derived from single-cell RNA-seq data. This matrix is aligned with spatial gene expression data and normalized using one of the following methods: unit norm, L1 norm, or z-score normalization.

The estimation process supports multiple regression models:

- Non-negative Least Squares (NNLS) (default)
- Ridge Regression (with L2 penalty)
- Lasso Regression (with L1 penalty)
- ElasticNet Regression (both L1 and L2 penalty)

Each spatial bin is independently decomposed into a weighted mixture of cell-type profiles, with proportions constrained to be non-negative and optionally normalized to sum to one. The results are stored per bin and optionally added to the spatial data object for downstream visualization.

Cluster Assignment Strategies

Clusters are assigned to spatial bins based on calculated similarity metrics. Two primary approaches are supported:

Direct Maximum Similarity ("max"): Each bin is assigned to the cluster with the highest similarity or proportion score.

Hybrid Adaptive Assignment ("hybrid"): A softmax-based probabilistic approach is used, incorporating both similarity magnitude and relative distribution across clusters to robustly assign bins, especially handling ambiguous cases.

The cluster assignments are merged back into the dataset, enabling visualization and further spatial analyses.

Data availability

The CRC atlas dataset was downloaded using the accession SCP1162 (Pelka et al. 2021). We also used 3 Visium HD slides, 2 representing CRC patients and 1 representing normal colon. All are taken from 10xgenomics.com/products/visium-hd-spatial-gene-expression/dataset-human-crc. To perform region's differentiation we used single cell data available from (Pelka et al. 2021; Uhlitz et al. 2021; Liu et al. 2022; Qian et al. 2020; Joanito et al. 2022; Domanska et al. 2022). We shared the DE tables and codes at our GitHub.

Code availability

The source code of Easydecon is made available via GitHub, https://github.com/sinanugur/easydecon. All scripts used to reproduce the analyses included in this manuscript can be downloaded from the Easydecon notebook repository, https://github.com/sinanugur/easydecon/notebooks. Easydecon is also available at PyPi.

Androvic, P. et al., 2023. Spatial Transcriptomics-correlated Electron Microscopy maps transcriptional and ultrastructural responses to brain injury. *Nature communications*, 14(1), p.4115.

Cilento, M.A., Sweeney, C.J. & Butler, L.M., 2024. Spatial transcriptomics in cancer research and potential clinical impact: a narrative review. *Journal of cancer research and clinical oncology*, 150(6), p.296.

Domanska, D. et al., 2022. Single-cell transcriptomic analysis of human colonic macrophages reveals niche-specific subsets. *The Journal of experimental medicine*, 219(3). Available at: http://dx.doi.org/10.1084/jem.20211846.

Joanito, I. et al., 2022. Single-cell and bulk transcriptome sequencing identifies two epithelial tumor cell states and refines the consensus molecular classification of colorectal cancer. *Nature genetics*, 54(7), pp.963–975.

Kamel, M. et al., 2025. ENACT: End-to-end analysis of Visium High Definition (HD) data. *Bioinformatics*. Available at: http://dx.doi.org/10.1093/bioinformatics/btaf094.

Kim, Y.-S., Choi, J. & Lee, S.H., 2023. Single-cell and spatial sequencing application in pathology. *Journal of pathology and translational medicine*, 57(1), pp.43–51.

Li, B. et al., 2022. Benchmarking spatial and single-cell transcriptomics integration methods for transcript distribution prediction and cell type deconvolution. *Nature methods*, 19(6), pp.662–670.

Liu, Y. et al., 2022. Immune phenotypic linkage between colorectal cancer and liver metastasis. *Cancer cell*, 40(4), pp.424–437.e5.

Marconato, L. et al., 2024. SpatialData: an open and universal data framework for spatial omics. *Nature methods*. Available at: http://dx.doi.org/10.1038/s41592-024-02212-x.

Marx, V., 2021. Method of the Year: spatially resolved transcriptomics. *Nature methods*, 18(1), pp.9–14.

Oliveira, M.F. et al., 2024. Characterization of immune cell populations in the tumor microenvironment of colorectal cancer using high definition spatial profiling. *bioRxiv*. Available at: https://doi.org/10.1101/2024.06.04.597233.

Ozato, Y. et al., 2023. Spatial and single-cell transcriptomics decipher the cellular environment containing HLA-G+ cancer cells and SPP1+ macrophages in colorectal cancer. *Cell reports*, 42(1), p.111929.

Palla, G. et al., 2022. Squidpy: a scalable framework for spatial omics analysis. *Nature methods*, 19(2), pp.171–178.

Pelka, K. et al., 2021. Spatially organized multicellular immune hubs in human colorectal cancer. *Cell*, 184(18), pp.4734–4752.e20.

Peng, Z. et al., 2022. Spatial transcriptomics atlas reveals the crosstalk between cancer-associated fibroblasts and tumor microenvironment components in colorectal cancer. *Journal of translational medicine*, 20(1), p.302.

Polański, K. et al., 2024. Bin2cell reconstructs cells from high resolution Visium HD data. *Bioinformatics (Oxford, England)*, 40(9). Available at: http://dx.doi.org/10.1093/bioinformatics/btae546.

Qian, J. et al., 2020. A pan-cancer blueprint of the heterogeneous tumor microenvironment revealed by single-cell profiling. *Cell research*, 30(9), pp.745–762.

Satija, R. et al., 2015. Spatial reconstruction of single-cell gene expression data. *Nature biotechnology*, 33(5), pp.495–502.

Ståhl, P.L. et al., 2016. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science (New York, N.Y.)*, 353(6294), pp.78–82.

Tadross, J.A. et al., 2025. A comprehensive spatio-cellular map of the human hypothalamus. *Nature*, 639(8055), pp.708–716.

Uhlitz, F. et al., 2021. Mitogen-activated protein kinase activity drives cell trajectories in colorectal cancer. *EMBO molecular medicine*, 13(10), p.e14123.

Weigert, M. & Schmidt, U., 2022. Nuclei Instance Segmentation and Classification in Histopathology Images with Stardist. In *2022 IEEE International Symposium on Biomedical Imaging Challenges (ISBIC)*. 2022 IEEE International Symposium on Biomedical Imaging Challenges (ISBIC). IEEE. Available at: http://dx.doi.org/10.1109/isbic56247.2022.9854534.

Wolf, F.A., Angerer, P. & Theis, F.J., 2018. SCANPY: large-scale single-cell gene expression data analysis. *Genome biology*, 19(1), p.15.

You, Y. et al., 2024. Systematic comparison of sequencing-based spatial transcriptomic methods. *Nature methods*, 21(9), pp.1743–1754.