

Combining spatial transcriptomics and ECM imaging in 3D for mapping cellular interactions in the tumor microenvironment

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Summary

Initial Submission: Received July 12, 2024
Preprint: N/A
Scientific editor: Bernadett Gaal, DPhil

First round of review: Number of reviewers: 2
2 confidential, 0 signed
Revision invited Oct 8, 2024
Minor changes anticipated
Revision received Dec 13, 2024

Second round of review: Number of reviewers: 1
1 original, 0 new
1 confidential, 0 signed
Accepted March 19, 2025

This Transparent Peer Review Record is not systematically proofread, type-set, or edited. Special characters, formatting, and equations may fail to render properly. Standard procedural text within the editor's letters has been deleted for the sake of brevity, but all official correspondence specific to the manuscript has been preserved.

Editorial decision letter with reviewers' comments, first round of review

Dear Nikolaus,

Thank you again for your patience while we waited for the reviews to come in on your manuscript. I have appended the reviews below. You'll see that the reviewers find the manuscript compelling and their comments are intended to strengthen an already strong piece of work. We're happy to invite a revision.

Please let me know if you have any questions or concerns about the revision, I'm always happy to talk either over email or over a call. More technical information and advice about resubmission can be found below my signature. Please read it carefully, as it can save substantial time and effort later.

I look forward to seeing your revised manuscript.

All the best,

Bernadett

Bernadett Gaál, DPhil
Editor-in-Chief, *Cell Systems*

Reviewers' comments:

Reviewer #1: In this study, Pentimalli et al. present a novel approach that combines spatial transcriptomics and extracellular matrix (ECM) imaging to create a detailed 3D map of cellular interactions within the tumour microenvironment (TME) of a clinical lung carcinoma. This integrative analysis has uncovered critical mechanisms of immune escape and tumour invasion, identifying several druggable targets that could be pivotal in developing personalized oncology treatments. This manuscript is well-structured, and the proof-of-concept pipeline presented in this study is likely to be highly valuable to the scientific community, particularly as it can be applied in various other settings. The computational techniques employed are state-of-the-art across all aspects, including segmentation tools, image classification, 3D alignment, and the incorporation of microscopy modalities that are not often seen in computational studies, such as multiphoton imaging. These elements make the manuscript particularly attractive and impactful.

Several comments on the manuscript:

1. The authors leverage a deep learning method to classify tumour, stroma, normal lung, and necrosis. However, the region of interest (ROI) presented in Figure 1b lacks the necrosis class. It would be beneficial to provide higher magnification images to demonstrate the accuracy of these predictions within the tested dataset.
2. The reviewer is unfamiliar with how the authors normalize hybridization from CosMx MSI, which contains only 960 genes, with single-cell RNA sequencing (scRNAseq) data from healthy lung tissue. This process needs clarification to ensure that the comparison is valid and meaningful.
3. Why was a 50 μm distance chosen for neighbourhood analysis? Did the authors explore other distances? Was this choice based on biological significance, computational limitations, or an estimation? A detailed explanation would help clarify the rationale behind this choice.
4. The naming of "3D neighborhood" in Figure 2b,c is confusing when compared with Figure 3, which

seems to involve different z-layers. If both are considered real 3D neighbourhoods, what is the actual difference between the neighbourhoods depicted in Figures 2 and 3? This needs to be clarified to avoid confusion.

5. The terms "tumor core" and "tumor surface" may be misleading. What is the actual percentage of tumour cells in the "tumor surface" space? When reviewing the maps in Figure 2e, there appears to be no overlap between the tumour surface labelled as tumor cells in the right map. The manuscript should include higher-resolution images that clearly show tumour cells in the "tumor surface" space.

6. The identification of dendritic cell niches in the 3D neighbourhood analysis is a significant claim, and this reviewer agrees with the importance of this finding. However, validating this discovery through consecutive slides and 3D reconstructions with staining for dendritic cells, T cells, and macrophages would greatly enhance the biological significance of this finding.

7. The inclusion of SHG imaging to study the ECM is novel and could provide new insights into how ECM changes affect neighbourhood interactions and tumour plasticity. However, the authors primarily use SHG to assess changes in ECM composition (elastin vs. collagen) rather than the actual ECM organization. SHG can offer valuable information on how the fibrillar structures of the matrix are organized. To validate the claims regarding elastin and collagen, the authors should include positive staining to confirm these areas' compositions. While label-free imaging with SHG is powerful, it could provide more information about abundance and structure than actual composition. Moreover, SHG has only been performed on one slide, whereas the rest of the study is conducted in a 3D manner.

Reviewer #2: In this study, the authors performed single-cell spatial transcriptomics profiling on serial sections from a lung tumor sample paired with extracellular matrix (ECM) imaging. The integrative analysis pinpointed known immune escape and tumor invasion mechanisms and revealed potentially druggable targets of tumor progression. This study represents in-depth profiling of one tumor sample with the high-resolution and high-cost CosMx technology. Also, the computational analysis performed on this dataset covered almost all possible methods that I could imagine. In general, I think this study presents a very timely and useful resource for the spatial transcriptomics field. I am OK if this study is published at Cell Systems as it is. I only have two minor suggestions for the authors to consider.

First, as this study performed many analyses and presented a complicated 3D imaging dataset, it will be great to tell readers how to explore the results and data through a public portal or freely available software. Most existing visualization frameworks only work for 2D data to my knowledge.

Second, please include some histograms of gene coverage rates across cells. In particular, for about 1000 genes included, do you always capture the same group of highly expressed genes across all cell types? Or do you capture different set of genes in distinct cell lineages.

Authors' response to the reviewers' first round comments

Attached.

Editorial decision letter with reviewers' comments, second round of review

Dear Nikolaus,

I'm very pleased to let you know that the reviews of your revised manuscript are back, the peer-review process is complete, and only a few minor, editorially-guided changes are needed to move forward towards publication.

I've made some suggestions about your manuscript within the "Editorial Notes" section below. Please review these notes along with the detailed formatting requirements listed in the [Final Files Checklist](#). We've also put together this [FAQ](#) (click the Final Formatting Checks tab) for your convenience. Please ask any questions you may have, make any necessary changes to your manuscript files, and then upload your final files into Editorial Manager. Once we receive your formatted files, we will go through our formatting checks and let you know if further changes are needed.

If this manuscript needs to be officially accepted by a particular date because of grant deadlines, applications, or because it will help your trainees, please let me know.

Introducing new referencing style

To standardize the referencing style across Cell Press journals, starting from October 2022, we ask that all in-text citations be formatted as superscripted numbers (e.g. "Multiple reports support this observation.^{1,2}"). Moving away from the Harvard referencing style (e.g. Smith *et al.*, 2020) will improve author and reader experiences. All manuscripts accepted from now on must use **the superscript numbered Cell Press referencing style**. Make sure to use this numbered referencing style for all new and revised submissions as well. Switching is easy. Just use the updated [CSL](#) and [EndNote](#) referencing styles for Cell Press articles.

Below my signature, you'll find specific information about what to expect next regarding formatting checks and working with our Production Department after acceptance. It's been a pleasure working with you, please feel free to contact our journal team with questions.

All the best,

Bernadett

Bernadett Gaál, DPhil
Editor-in-Chief, Cell Systems

Editorial Notes

Transparent Peer Review: Thank you for electing to make your manuscript's peer review process transparent. As part of our approach to Transparent Peer Review, we ask that you add the following

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Manuscript Text:

- House style disallows editorializing within the text (e.g. strikingly, surprisingly, importantly, etc.), especially the Results section. These terms are a distraction and they aren't needed—your excellent observations are certainly impactful enough to stand on their own. Please remove these words and others like them. "Notably" is suitably neutral to use once or twice if absolutely necessary.
- We don't allow "priority claims" (e.g. new, novel, etc.). For a discussion of why, read: <http://crosstalk.cell.com/blog/getting-priorities-right-with-novelty-claims>, <http://crosstalk.cell.com/blog/novel-insights-into-priority-claims>. When you are presenting something that you've created from scratch, a good workaround is to say that you're "introducing XXX," as opposed to "describing a novel XXX."

Figures and Legends:

Please look over your figures keeping the following in mind:

- When data visualization tools are used (e.g. UMAP, tSNE), please ensure that the dataset being visualized is named in the figure legend and, when applicable, its accession number is included.
- When color scales are used, please define them, noting units or indicating "arbitrary units," and specify whether the scale is linear or log.
- When figures include micrographs, please ensure that scale bars are included and defined within the legend, montages are made obvious, and any digital adjustments (e.g. brightness) have been applied equally across the entire image in a manner that does not obscure characteristics of the original image (e.g. no "blown out" contrast). **Note that all accepted papers are screened for image irregularities, and if this advice is not followed, your paper will be flagged.**
- Please ensure that all figures included in your point-by-point response to the reviewers' comments are present within the final version of the paper, either within the main text or within the Supplemental Information.

Resource Availability: Please note that Cell Press has recently changed the way it approaches "availability" statements for the sake of ease and clarity. Please revise your resource availability section as follows, noting that the examples used might not pertain to your study. Please note that the Resource Availability section should immediately follow the Discussion section in the main manuscript.

RESOURCE AVAILABILITY

Lead Contact: Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jane Doe (janedoe@qwerty.com).

Materials Availability: This study did not generate new materials. -OR- Plasmids generated in this study have been deposited at [Addgene, name and catalog number]. -OR- etc.

Data and Code Availability:

- **Source data statement** (described below)
- **Code statement** (described below)
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Data and Code Availability statements **have three parts and each part must be present. Each part should be listed as a bullet point, as indicated above.**

Instructions for section 1: Data. The statements below may be used in any number or combination, but at least one must be present. They can be edited to suit your circumstance. ***Please ensure that all datatypes (not only standardized datatypes) reported in your paper are represented in section 1.*** For more information, please consult [this list of standardized datatypes and repositories recommended by Cell Press](#).

- [Standardized datatype] data have been deposited at [datatype-specific repository] and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.
- [Adjective] data have been deposited at [general-purpose repository] and are publicly available as of the date of publication. DOIs are listed in the key resources table.
- [De-identified human/patient standardized datatype] data have been deposited at [datatype-specific repository]. They are publicly available as of the date of publication until [date or delete “until”]. Accession numbers are listed in the key resources table.
- This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table.
- [Adjective or all] data reported in this paper will be shared by the lead contact upon request.

Instructions for section 2: Code. The statements below may be used in any number or combination, but at least one must be present. They can be edited to suit your circumstance. ***If you are using GitHub, please follow [the instructions here](#) to archive a “version of record” of your GitHub repo at Zenodo, then report the resulting DOI. Additionally, please note that the Cell Systems strongly recommends that you also include an explicit reference to any scripts you may have used throughout your analysis or to generate your figures within section 2.***

- All original code has been deposited at [repository] and is publicly available as of the date of publication. DOIs are listed in the key resources table.

Instructions for section 3. Section 3 consists of the following statement: Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

STAR Methods:

Please ensure that original code has been archived in a [general purpose repository recommended by Cell Press](#) and that its DOI is provided in the Software and Algorithms section of the Key Resources Table. If you've chosen to use GitHub, please follow [the instructions here](#) to archive a "version of record" of your GitHub repo at Zenodo, complete with a DOI. Thank you!

Thank you!

Reviewer comments:

Reviewer #1: The authors addressed all my comments. The manuscript looks fantastic and the transparency of the datasets and analysis is exceptional. Congratulations for the study.

Dear Nikolaus,

Thank you again for your patience while we waited for the reviews to come in on your manuscript. I have appended the reviews below. You'll see that the reviewers find the manuscript compelling and their comments are intended to strengthen an already strong piece of work. We're happy to invite a revision.

Please let me know if you have any questions or concerns about the revision, I'm always happy to talk either over email or over a call. More technical information and advice about resubmission can be found below my signature. Please read it carefully, as it can save substantial time and effort later.

I look forward to seeing your revised manuscript.

All the best,

Bernadett

Bernadett Gaál, DPhil

Editor-in-Chief, Cell Systems

We would like to thank the reviewers for the positive evaluation of our work and their constructive feedback. In particular, we acknowledge the importance of validating our key findings regarding dendritic niches and ECM composition with orthogonal modalities and agree with the importance of an interactive browser for the readers to explore our data in 3D.

To validate our findings, we leveraged intervening sections and performed (1) the immunostaining (new **Supplementary Figure 3d**) of epithelial, macrophage and dendritic cell markers to compare the distribution of these cell types with the consecutive CosMx section (2) a van Gieson's staining (new **Supplementary Figure 5a**) to validate the patterns of collagen and elastin fibers highlighted by second harmonic imaging. In both cases, we are excited to report that these experiments strengthened our confidence in combining CosMx and SHG for the integrative study of ECM composition and multicellular niches. Furthermore, we developed a browser-based visualization tool modifying the recently published spateo-viewer (PMID: 39532097) to enable interactive exploration of gene expression and multicellular niches in 3D (<https://lung-3d-browser.mdc-berlin.de>).

Additionally, we now (1) provide a further interactive browser for the interactive comparison of high-resolution HE images and tissue segmentation results (<https://portal.aiagnostics.com/>), (2) systematically evaluate the impact of different radii on the unbiased identification of multicellular niches and (3) investigate gene detection rates across cell types following reviewer's suggestions. Some of the figures supporting the extended analysis are provided in this point-by-point response – if the referees or editor believe this information might be useful for the general readership, we are open to include them in the revised manuscript.

Reviewers' comments:

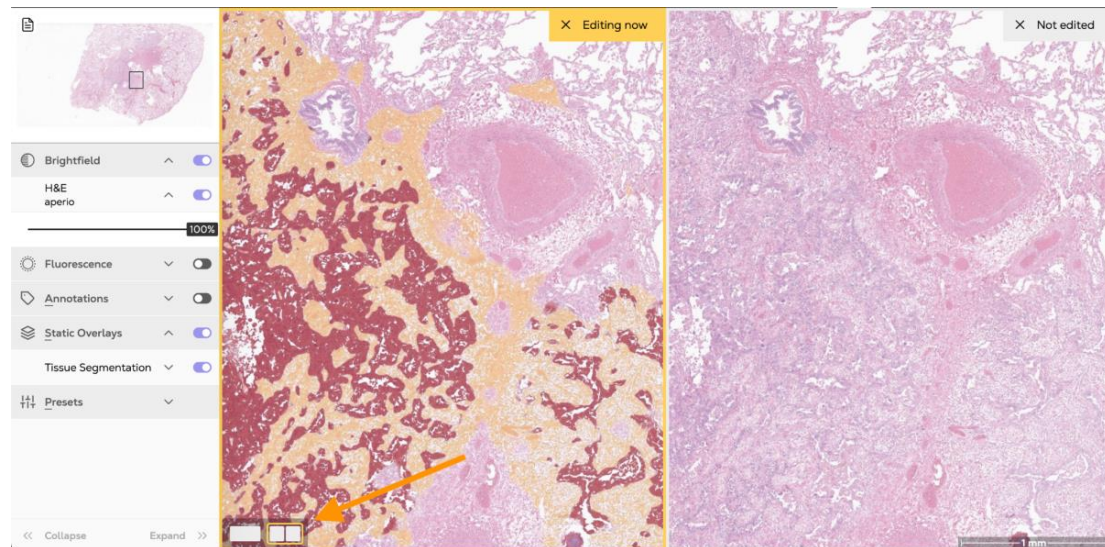
Reviewer #1: In this study, Pentimalli et al. present a novel approach that combines spatial transcriptomics and extracellular matrix (ECM) imaging to create a detailed 3D map of cellular interactions within the tumour microenvironment (TME) of a clinical lung carcinoma. This integrative analysis has uncovered critical mechanisms of immune escape and tumour invasion, identifying several druggable targets that could be pivotal in developing personalized oncology treatments.

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Several comments on the manuscript:

1. The authors leverage a deep learning method to classify tumour, stroma, normal lung, and necrosis. However, the region of interest (ROI) presented in Figure 1b lacks the necrosis class. It would be beneficial to provide higher magnification images to demonstrate the accuracy of these predictions within the tested dataset.

As the reviewer correctly points out, necrotic regions were not detected in the HE image analyzed by our deep learning model (**Figure 1b**), which was independently confirmed by two experienced clinical lung pathologists (S.S. and F.K). We agree with the reviewer and will make available the full-slide, high-resolution HE and model predictions on Zenodo. For the revision, the model predictions can be interactively explored on the Aignostic portal (<https://portal.aignostics.com/>, "Sign in with Google" username: cell.systems.d.24.00332@gmail.com, password: ssXw%T22Lc8Zlnf), both toggling the segmentation heatmap transparency and creating a side-by-side split view (**Revision plot 1**).



Revision plot 1. Interactive evaluation of model predictions and high-resolution tissue morphology. Screenshot of the Aignostic browser, which allows the comparison of 'Tissue Segmentation' model output (Left, red: Tumor, yellow: Stroma) with Hematoxylin-Eosin staining (Right) in a side-by-side split view (activated by clicking on the icon at the bottom left, arrow).

2. The reviewer is unfamiliar with how the authors normalize hybridization from CosMx MSI, which contains only 960 genes, with single-cell RNA sequencing (scRNAseq) data from healthy lung tissue. This process needs clarification to ensure that the comparison is valid and meaningful.

To integrate our CosMx SMI data with published scRNAseq atlases (healthy[17, 18] and NSCLC[19]), we leveraged the LabelTransfer pipeline[75] implemented in the Seurat package. This well-established approach (12'122 citations as of Dec 13th 2024) enables the comparison of single cell data across different sequencing technologies and modalities, including imaging-based spatial transcriptomics (as the authors demonstrated in Fig 5). For normalization, we opted for SCTransform[76]. While the same group developed this approach following their publication of the LabelTransfer pipeline, both the authors and the Nanostring company now recommend it for normalization of CosMx gene expression data (see 'Analysis of Image-based Spatial Data in Seurat' on the [Seurat website](#) and 'Tips when performing CosMx™ data analysis with AtoMx SIP' on the [Nanostring website](#)).

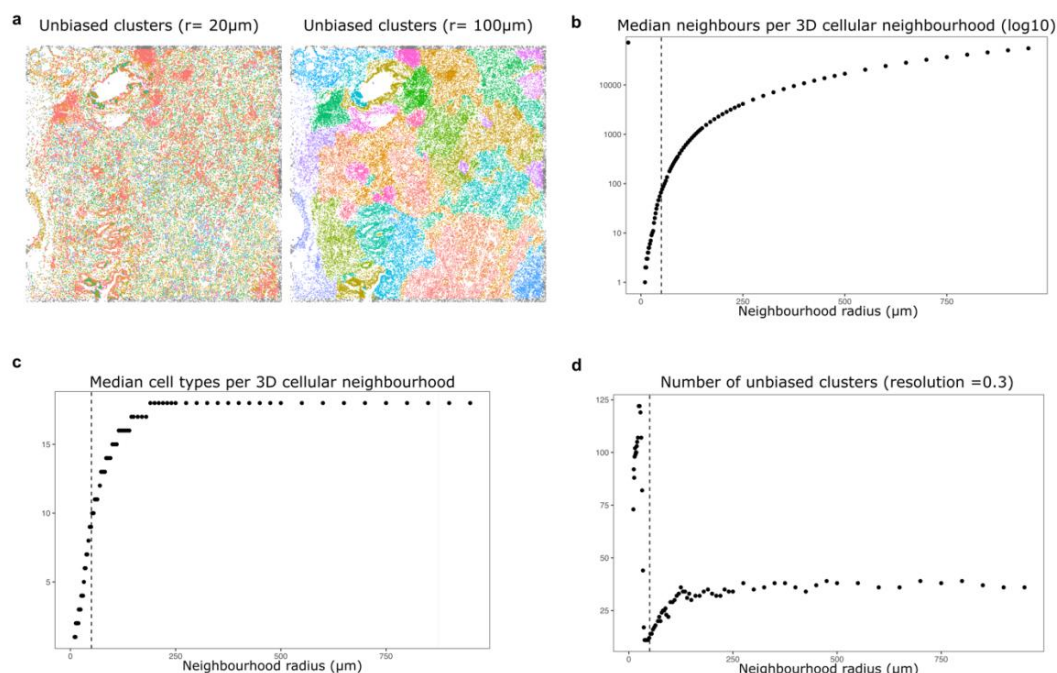
3. Why was a 50 µm distance chosen for neighbourhood analysis? Did the authors explore other distances? Was this choice based on biological significance, computational limitations, or an estimation? A detailed explanation would help clarify the rationale behind this choice.

We selected a 50µm radius as we reasoned that it would roughly correspond to ~3 cellular distances, which is commonly used to define cellular neighbourhoods in rank-based methods (for example CellCharter PMID: 38066188). In fact, median cell diameters range between ~11.5µm for non-malignant cells to 18µm for tumor cells in our dataset.

When selecting 50µm, we had also explored few other options and noticed that smaller radii (e.g. 20µm) resulted in overly fragmented niches, which we interpreted as noisy, while larger ones (e.g. 100µm) identified large tissue regions rather than multicellular niches, so that even the highly distinct airway niche was split into different clusters (**Revision figure 1.a**).

To systematically investigate the impact of neighborhood size, we now computed the median number of neighbours and cell types in each cellular neighborhood and the number of unbiased clusters identified as the radius increases over two orders of magnitude, from 10µm to 950µm. As expected, the median number of neighbours grows exponentially, from 1 at 10µm to more than 50'000 at 950µm (**Revision figure 1.b**). The median number of different cell types included in each neighbourhood, instead, reaches 15 already at 100µm and plateaus at the theoretical maximum of all 18 cell types at 190µm (**Revision figure 1.c**). Interestingly, the number of clusters (resolution=0.3) peaks at small radii (112 at 22µm), then rapidly stabilises between 10 and 20 clusters around 50µm (36-70µm range) and then progressively increases to plateau at ~30-35 clusters from 100µm onwards(**Revision figure 1.d**).

Overall, we consider 50µm radius to be a suitable choice to capture repeating multicellular niches in this dataset, mitigating the instability observed at smaller radii, while avoiding the 'local smoothing' at larger radii, as distal neighbors outweigh the signal from proximal ones. Nevertheless, the formal definition of cellular neighborhoods is still a matter of active research and best practices are still lacking in the field as the 'optimal radius' is likely to be tissue-, sample-, cell- and analysis-dependent (e.g. when studying short- vs long-range interactions). We have now added this statement to the Discussion (**lines 532-535**).



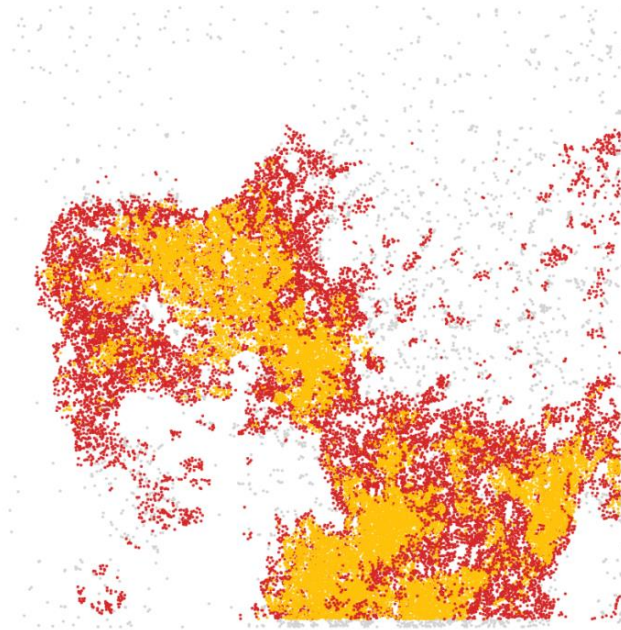
Revision figure 1. Neighborhood characteristics as a function of the radius. a) Spatial plots of unbiased clustered identified at small (left) and large (right) radii showing fragmented niches and large local patches, respectively. **b-d)** Scatter plots of the median number of neighbors (b), distinct cell types (c) per 3D cellular neighborhood and total unbiased clusters (d) computed over increasing neighborhood radii (10-950 μm).

4. The naming of "3D neighborhood" in Figure 2b,c is confusing when compared with Figure 3, which seems to involve different z-layers. If both are considered real 3D neighborhoods, what is the actual difference between the neighborhoods depicted in Figures 2 and 3? This needs to be clarified to avoid confusion.

Figure 2b and 3a represent 2D projections of the same 3D neighborhood from different angles. In Figure 3a, we opted for a 'side' view (like in Figure 2a) to visually highlight the differences between cellular neighbors in 2D (sitting in the same section of the center cell) and 3D (sitting in the sections above and below). We thank the reviewer for pointing this out and added xyz axes to improve the visual clarity of **Figures 2a, 2b and 3a**.

5. The terms "tumor core" and "tumor surface" may be misleading. What is the actual percentage of tumor cells in the "tumor surface" space? When reviewing the maps in Figure 2e, there appears to be no overlap between the tumor surface labeled as tumor cells in the right map. The manuscript should include higher-resolution images that clearly show tumor cells in the "tumor surface" space.

37.3% of tumor cells are found in the tumor surface, 38.7% in the tumor core and 24% in other niches (**Supplementary figure 6a**). We thank the reviewer for the suggestion and include below a spatial plot of all tumor cells in section 10 highlighting their abundance in the tumor core and surface (**Revision plot 2**), which can be compared with PanCK and CDH1 immunostainings in section 12 (**Supplementary Figure 6e**).



Revision plot 2. Tumor cells are abundant in the tumor core and surface. Spatial plot of tumor cells in section 10, colored by 3D niche assignment (yellow: tumor core, red: tumor surface, gray: other niches).

6. The identification of dendritic cell niches in the 3D neighborhood analysis is a significant claim, and this reviewer agrees with the importance of this finding. However, validating this discovery through consecutive slides and 3D reconstructions with staining for dendritic cells, T cells, and macrophages would greatly enhance the biological significance of this finding.

We thank the reviewer for this suggestion and performed the immunofluorescence (IF) staining for Epithelial (PanCK, magenta), Macrophage (CD68, cyan) and Dendritic cell (IDO1, green) markers plus DAPI (blue). Unfortunately a set of consecutive sections for 3D reconstruction was not available, therefore we focused on section 29. The IF patterns match the distribution of epithelial cells, macrophages and dendritic cells identified by CosMx in the consecutive section (**Supplementary Figure 3d**) and highlight foci of macrophages and dendritic cells at the tumor surface, compatible with macrophage and dendritic cell niches identified by our 3D neighborhood analysis.

7. The inclusion of SHG imaging to study the ECM is novel and could provide new insights into how ECM changes affect neighbourhood interactions and tumour plasticity. However, the authors primarily use SHG to assess changes in ECM composition (elastin vs. collagen) rather than the actual ECM organization. SHG can offer valuable information on how the fibrillar structures of the matrix are organized. To validate the claims regarding elastin and collagen, the authors should include positive staining to confirm these areas' compositions. While label-free imaging with SHG is powerful, it could provide more information about abundance and structure than actual composition. Moreover, SHG has only been performed on one slide, whereas the rest of the study is conducted in a 3D manner.

While studying ECM at the morphological level and in a 3D manner would be very interesting, it will not be possible in this study due to the limited resolution of the SHG

images and the aforementioned lack of consecutive slides. Nevertheless, we agree that a positive staining would be valuable to validate our SHG findings and thus performed Verhoef's Van Gieson staining in the next available slide (section 18). This staining confirmed the presence of abundant stromal collagen fibers (red) in the ECM compartment identified as desmoplastic by SHG imaging (new **Supplementary figure 5a**). On the other hand, collagen fibers are restricted to rings around airways and blood vessels in the degraded and homeostatic compartments, in line with SHG.

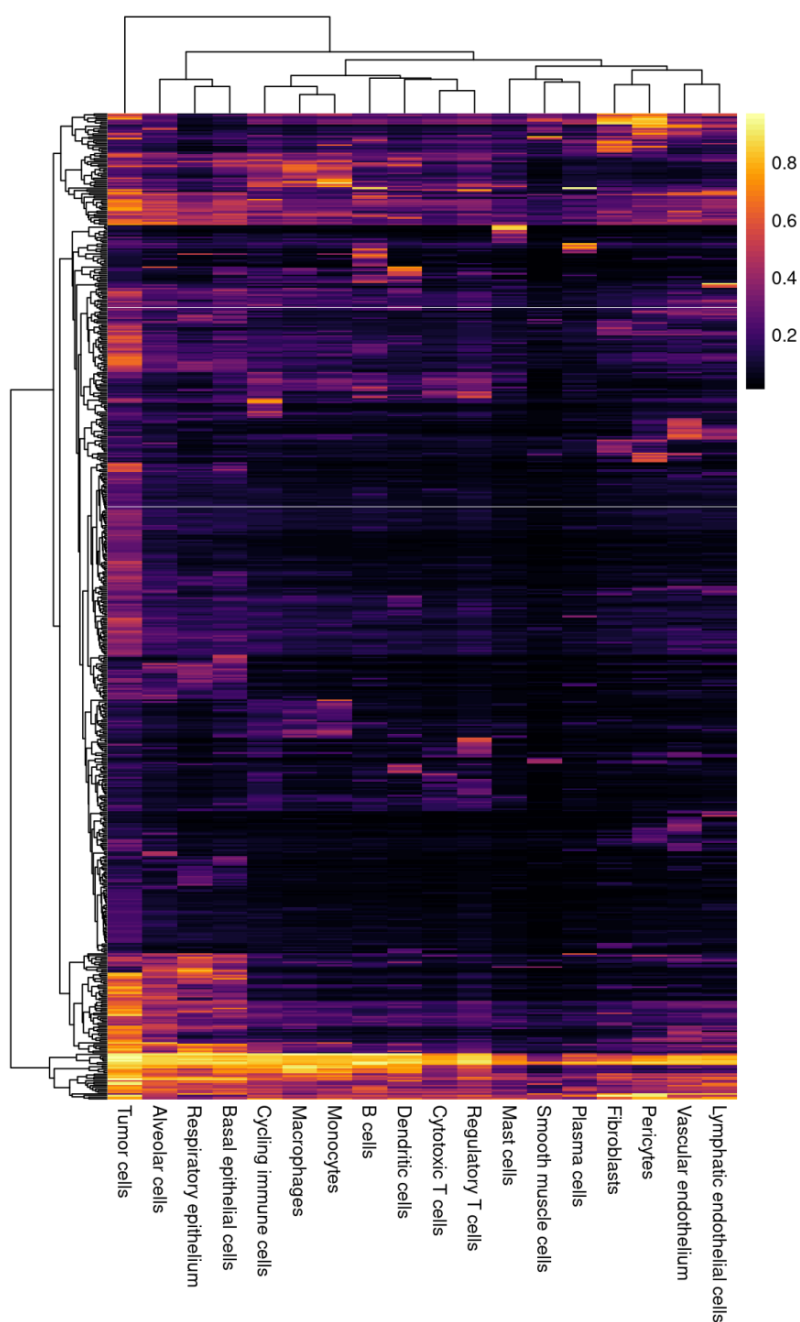
Reviewer #2: In this study, the authors performed single-cell spatial transcriptomics profiling on serial sections from a lung tumor sample paired with extracellular matrix (ECM) imaging. The integrative analysis pinpointed known immune escape and tumor invasion mechanisms and revealed potentially druggable targets of tumor progression. This study represents in-depth profiling of one tumor sample with the high-resolution and high-cost CosMx technology. Also, the computational analysis performed on this dataset covered almost all possible methods that I could imagine. In general, I think this study presents a very timely and useful resource for the spatial transcriptomics field. I am OK if this study is published at Cell Systems as it is. I only have two minor suggestions for the authors to consider.

First, as this study performed many analyses and presented a complicated 3D imaging dataset, it will be great to tell readers how to explore the results and data through a public portal or freely available software. Most existing visualization frameworks only work for 2D data to my knowledge.

We agree with the reviewer that it would be beneficial for the readers to explore our dataset in 3D. We thus developed a browser-based visualization tool (<https://lung-3d-browser.mdc-berlin.de>) built on the recently published spateo-viewer (PMID: 39532097) to enable interactive exploration of spatial single-cell expression data in three dimensions. The tool processes h5ad objects containing cell coordinates in both physical and UMAP space along with cell annotations and gene expression. Cell neighborhood represented as isosurfaces were converted to VTK format for rendering in spateo-viewer. We modified the original spateo-viewer code to support headless serving capabilities, customized isosurface coloring to maintain consistency with manuscript color schemes, and introduced several optimizations for improved performance and stability.

Second, please include some histograms of gene coverage rates across cells. In particular, for about 1000 genes included, do you always capture the same group of highly expressed genes across all cell types? Or do you capture different set of genes in distinct cell lineages.

To evaluate how gene detection rates across cell types, we quantified the percentage of cells from each cell type in which at least one transcript was detected. Among the 960 genes included in the panel, 599 are detected in at least 20% cells from at least one cell type. The detection rates for these genes are shown below (**Revision plot 3**). While some genes are detected at high rates across all cell types, the majority of genes show a lineage (e.g. epithelial) or cell type specific expression pattern.



Revision plot 3. CosMx genes show cell type-specific expression patterns. Heatmap of gene detection percentage across cell types. Hierarchical clustering groups cell types into lineages (e.g. Epithelial: Tumor, Alveolar, Respiratory and Basal epithelial cells) and genes into lineage-specific clusters.