

Will Spatial Transcriptomics Transform Pathology?

Irá a Transcriptómica Espacial Transformar a Patologia?

Miguel Cristovão ¹, Maria Carmo-Fonseca ^{2,3}, Catarina Alves-Vale ^{2,3,4}

¹ Serviço de Anatomia Patológica, ULS São José, Lisboa, Portugal. ² Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

³ Gulbenkian Institute for Molecular Medicine, Lisboa, Portugal. ⁴ Department of Pathology, CUF Descobertas Hospital, Lisboa, Portugal

<https://doi.org/10.82582/thorac.75>

Autor Correspondente/Corresponding Author:

Miguel Cristovão

<https://orcid.org/0000-0002-0359-4778>

Serviço de Anatomia Patológica, ULS São José, Rua José António Serrano, 1150-199 Lisboa, Portugal

E-mail: miguelcristovao@gmail.com

Recebido/Received: October 8th, 2025

Aceite/Accepted: October 10th, 2025

Publicado online/Published online: October 31st, 2025

Publicado/Published: October 31st, 2025

© Author(s) (or their employer(s)) and THORAC 2025. Re-use permitted under CC BY-NC. No commercial re-use.

© Autor (es) (ou seu(s) empregador(es)) e THORAC 2025. Reutilização permitida de acordo com CC BY-NC. Nenhuma reutilização comercial.

ABSTRACT

Spatial transcriptomics (ST) is an emerging technology that maps gene expression directly onto tissue architecture. As it moves toward clinical adoption, ST is poised to become an integral tool in precision diagnostics. Here, we discuss its potential to complement and enhance traditional morphological assessment, reinforcing the pathologist's central role in the molecular era.

Keywords:

Gene Expression Profiling; Neoplasms/genetics; Neoplasms/pathology; Transcriptome

RESUMO

A transcriptómica espacial (TE) é uma tecnologia emergente que permite mapear a expressão génica diretamente na arquitetura do tecido. Com a sua progressiva integração na prática clínica, a TE está prestes a tornar-se uma ferramenta essencial no diagnóstico de precisão. Neste artigo, discutimos o seu potencial para complementar e aperfeiçoar a avaliação morfológica tradicional, reforçando o papel central do patologista na era molecular.

Palavras-chave:

Neoplasias/genética; Neoplasias/patologia; Perfil da Expressão Génica; Transcriptoma

INTRODUCTION

In Pathology, the adoption of technological advances into routine practice has typically occurred in a gradual, stepwise manner. Over the decades, the field has progressively integrated new methods, ranging from histochemistry and immunohistochemistry (IHC) to more recent molecular techniques such as *in situ* hybridization and next-generation sequencing (NGS), each contributing to greater diagnostic precision and a deeper understanding of disease biology.

Among the latest innovations, spatial transcriptomics (ST) is emerging as a particularly promising approach.¹ This technology allows gene expression to be mapped directly onto tissue, preserving spatial relationships. By adding a molecular layer to histological analysis, ST provides a more comprehensive view of tissue organization and cellular interactions, with far-reaching implications for both research and clinical oncology. As experts in tissue morphology and molecular pathology, pathologists are uniquely positioned to guide the adoption of ST into diagnostic workflows and to help demonstrate its potential in advancing precision medicine.

ST can be best appreciated in contrast to single-cell RNA sequencing (scRNA-seq), which provides detailed gene expression profiles at the individual cell level but relies on a cell suspension requiring prior tissue dissociation. This step disrupts the native tissue architecture and can introduce transcriptional artifacts, limiting the ability to interpret molecular data in its histological context. In contrast, ST preserves tissue landscape, enabling the specific localization of gene expression patterns within sections, either at near-single-cell resolution or across defined anatomical regions. This spatially resolved data provides critical insight into cellular organization, interactions, and the tissue microenvironment, making it particularly valuable in complex surgical specimens where context is key.

Consider the normal lung parenchyma: while alveolar epithelial cells, endothelial cells, fibroblasts, and immune populations can often be distinguished based on morphology, it remains challenging to resolve distinct cell states or functional subtypes, particularly within the immune and stromal compartments. IHC can assist in identifying some of these populations, but it is constrained by reliance on a relatively limited panel of predefined markers. ST addresses these limitations by enabling a comprehensive, unbiased transcriptomic profiling of cells within their native spatial context. This technology offers a level of molecular resolution that surpasses conventional methods, including NGS combined with laser capture microdissection. Importantly, it is compatible with formalin-fixed, paraffin-embedded (FFPE) sections, facilitating its integration into routine pathology workflows.

This article explores the potential of ST to enhance pathology practice, with a focus on its integration into existing diagnostic frameworks. Rather than replacing conventional histology or IHC, spatially resolved transcriptomic data can serve as a powerful complement by bridging morphology and molecular biology, opening new avenues for more accurate diagnostics and personalized therapeutic decision-making in oncology.

MAKING MOLECULAR DATA VISIBLE

Despite the increasing integration of molecular technologies into routine pathology, morphology remains the cornerstone of diagnosis. The pathologist's ability to interpret architectural patterns on H&E-stained slides underpins decisions about diagnosis, prognosis, and therapeutic stratification.

However, as molecular data become increasingly complex and central to oncology care, there is a growing disconnect: how often do molecular reports feel abstract or difficult to reconcile with the tissue architecture? In the current landscape of genomic data and targeted therapies, the morphological perspective risks being sidelined, yet it is precisely this spatial context that gives molecular findings their clinical relevance.

ST reinforces and elevates the pathologist's role by reuniting gene expression data with its morphological context. Unlike conventional molecular techniques that detach genetic information from tissue structure, ST maps transcriptomic profiles directly onto histological sections, preserving spatial architecture and enabling high-resolution molecular analysis of defined cell populations and regions. This spatially resolved approach is especially valuable in specimens with low tumor cellularity or dense inflammatory infiltrates, where traditional sequencing methods may be compromised.

In the context of lung cancer, where tumor heterogeneity, immune infiltration, and stromal interactions significantly influence disease progression and therapeutic response, ST provides a powerful means to map the tumor microenvironment *in situ*. By capturing both gene expression and spatial architecture, it allows detailed characterization of tumor subclones, immune cell niches, and cellular interactions, providing insights that are critical for refining diagnosis, risk stratification and prognostication.

Pathologists play a central role in enabling and shaping the implementation of ST in both research and clinical workflows. From proper tissue sampling, orientation, and preservation, to the expert cell segmentation and annotation of regions of interest, many critical steps in ST protocols rely on the pathologist's expertise. Furthermore, as intermediaries between clinical teams and molecular or bioinformatics specialists, pathologists are uniquely positioned to ensure that spatial data are interpreted in biologically and clinically meaningful ways.

Ultimately, ST offers a new lens through which to understand disease. As this technology advances, its integration into translational research and, eventually, routine diagnostics will require the active engagement of the pathology community.

DIAGNOSTIC APPLICATIONS OF SPATIAL TRANSCRIPTOMICS

Several real-world diagnostic challenges highlight the transformative potential of ST in both neoplastic and inflammatory pathology.²

1. Tumor Heterogeneity and Microenvironment. Malignant

neoplasms are intrinsically heterogeneous, composed of genetically and phenotypically diverse subclones with variable potential for invasion and metastasis. Pathologists routinely encounter tumors that appear well-differentiated under the microscope but behave aggressively. Although traditional prognostic features such as mitotic index, necrosis, and cytological atypia provide useful insights, they often fail to capture the full biological complexity of the tumor. ST enables high-resolution spatial mapping of gene expression across both neoplastic and stromal compartments, identifying aggressive subpopulations, immune evasion mechanisms, and microenvironmental cues. For example, a recent study combining ST and single-cell RNA-seq in squamous cell carcinoma uncovered unexpected spatial heterogeneity at the tumor periphery, revealing distinct tumor cell states and local immunosuppressive niches through detailed analysis of lymphocyte and stromal cell interactions.³

2. Assessment of Surgical Margins. Margin assessment is fundamental in oncologic pathology, yet uncertainty may persist in select cases. For instance, in breast resection specimens with cautery-induced artifacts, epithelial cells at the inked margin may appear suspicious, but it is often difficult to confidently determine their neoplastic nature in specific settings, such as post-neoadjuvant chemotherapy. ST enables transcriptomic profiling of these ambiguous cells *in situ*, potentially identifying malignant expression signatures that would otherwise go undetected. Such data could guide clinical decision-making, particularly regarding the need for re-excision or adjuvant therapy.

3. Diagnostic Precision in Undifferentiated Tumors. Undifferentiated or poorly differentiated tumors often present diagnostic dilemmas, particularly when morphology and IHC panels do not yield clear lineage information. Similarly, metastases of unknown primary origin frequently remain unclassified. ST offers a powerful tool for resolving these cases, as it can reveal lineage-specific transcriptional profiles. By integrating these data with computational approaches such as machine learning, pathologists can achieve more confident and precise tumor classification.

4. Pre-Neoplastic Conditions. A particularly exciting frontier for ST is the investigation of early molecular changes in morphologically normal or precursor lesions. ST makes it possible to resolve the transcriptomic landscape of "at-risk" epithelial cells, enabling lineage trajectory analysis and identification

of early molecular signatures associated with transformation. For example, a ST-based study revealed that UBE2C mediates the transformation of *in situ* adenocarcinoma to invasive adenocarcinoma, serving as a potential biomarker in the clinical setting to improve risk stratification and inform surveillance strategies in patients with pre-invasive lesions.⁴

5. Inflammatory Pathology. Inflammatory diseases, such as autoimmune conditions, transplant biopsies and chronic lung disease, frequently involve complex and heterogeneous immune infiltrates that defy precise characterization by conventional methods. Traditional IHC offers only limited resolution of immune cell phenotypes and activation states despite the advances provided by multiplexed IHC/immunofluorescence. ST overcomes these limitations by enabling a comprehensive and unbiased analysis of cell populations, their spatial distribution, and their interactions with surrounding tissue. For example, a recent study provided a spatially resolved characterization of the key cell types and molecular events involved in the progression of lung fibrosis.⁵

posing logistical and educational hurdles for many pathology departments.

Another significant barrier lies in the growing burden of technical knowledge required of today's pathologists. For ST to be successfully integrated into diagnostic workflows, targeted training in genomics, spatial data interpretation, and computational analysis will be essential. Without this, there is a risk that such powerful technologies remain confined to the realm of research, rather than translating into clinical benefit.

It is also important to acknowledge the limits of ST resolution and sensitivity in its current iterations. While the technology is evolving rapidly, not all platforms achieve single-cell resolution, and some gene expression signals may be diluted or missed in areas with low RNA content or high tissue autofluorescence. Standardization of protocols across laboratories remains a work in progress, and further studies are needed to define robust diagnostic and prognostic signatures that can be reliably used in patient care.

Despite these challenges, ST holds immense promise. As platforms improve, costs decrease, and interdisciplinary training becomes more widespread, ST has the potential to be integrated into routine clinical workflows, providing pathologists with deeper tools to interrogate disease processes. In this evolving landscape, the pathologist's role remains central. Morphological expertise will continue to guide meaningful interpretation of complex molecular data. Embracing technologies like ST not only enhances diagnostic precision but also reinforces the relevance of pathology in an increasingly molecular era.

CHALLENGES, LIMITATIONS, AND FUTURE PERSPECTIVES

Like any transformative innovation, ST comes with its own set of challenges that currently limit its widespread adoption in routine pathology. Chief among these is the high cost of instrumentation, reagents, and data storage, which restricts access to the technology outside of well-funded research and clinical institutions. In addition, the complexity of data analysis necessitates robust bioinformatics infrastructure and expertise,

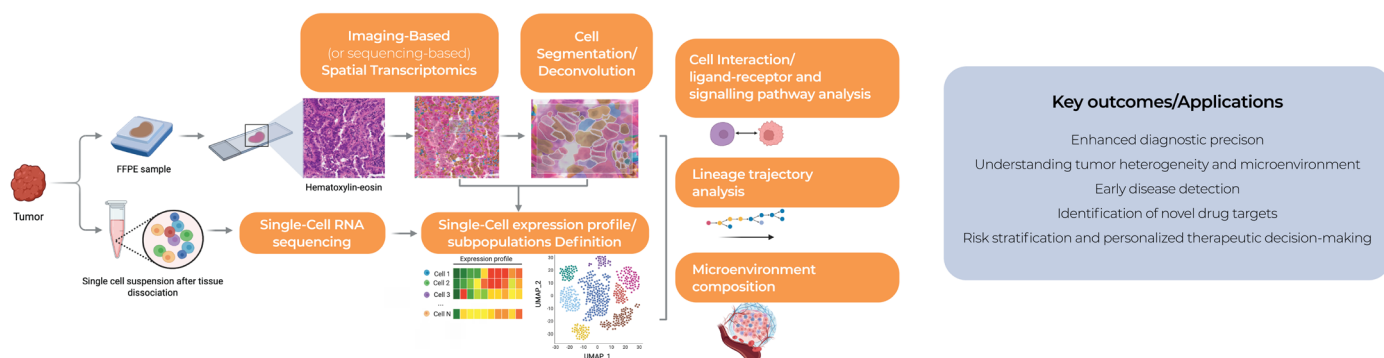


Figure 1

Spatial transcriptomics workflow (from tissue to data analysis) and potential clinical applications. An example of an imaging-based spatial transcriptomics method (*in situ* hybridization (ISH)-based) is depicted. Figure created with BioRender; images were adapted from 10x Genomics Xenium Explorer 4.0.0 (10x Genomics).

Contributorship Statement:

Conceptualization: MC-F and CA-V.
Manuscript Writing: MC and CA-V.
Final Approval: All authors have read and approved the final version of the manuscript.

Declaração de Contribuição:

Conceptualização: MC-F e CA-V.
Redação do Artigo: MC e CA-V.
Aprovação Final: Todos os autores leram e aprovaram a versão final do manuscrito.

Ethical Disclosures:

Conflicts of Interest: The authors have no conflicts of interest to declare.
Financing Support: This work has not received any contribution, grant or scholarship.
Provenance and Peer Review: Not commissioned; externally peer-reviewed.

Responsabilidades Éticas:

Conflitos de Interesse: Os autores declaram a inexistência de conflitos de interesse.
Apoio Financeiro: Este trabalho não recebeu qualquer subsídio, bolsa ou financiamento.
Proveniência e Revisão por Pares: Não solicitado; revisão externa por pares.

REFERENCES

1. Longo SK, Guo MG, Ji AL, Khavari PA. Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nat Rev Genet. 2021;22:627–44. doi:10.1038/s41576-021-00377-8.
2. Pang JM, Byrne DJ, Bergin ART, Caramia F, Loi S, Gorringe KL, et al. Spatial transcriptomics and the anatomical pathologist: Molecular meets morphology. Histopathology. 2024;84:577-86. doi: 10.1111/his.15093.
3. Ji AL, Rubin AJ, Thrane K, Jiang S, Reynolds DL, Meyers RM, et al. Multimodal analysis of composition and spatial architecture in human squamous cell carcinoma. Cell. 2020;182:497–514.e22. doi:10.1016/j.cell.2020.05.039.
4. Zhu J, Fan Y, Xiong Y, Wang W, Chen J, Xia Y, et al. Delineating the dynamic evolution from preneoplasia to invasive lung adenocarcinoma by integrating single-cell RNA sequencing and spatial transcriptomics. Exp Mol Med. 2022;54:2060-76. doi: 10.1038/s12276-022-00896-9.
5. Vannan A, Lyu R, Williams AL, Negretti NM, Mee ED, Hirsh J, et al. Spatial transcriptomics identifies molecular niche dysregulation associated with distal lung remodeling in pulmonary fibrosis. Nat Genet. 2025;57:647-58. doi: 10.1038/s41588-025-02080-x. 8ae6.

