

REVIEW ARTICLE

In situ spatial profiling: Gaining molecular and cellular insights within content and contextShu-Ti Lin[†], Ian Molyneaux, and Chen Yeh^{†*} 

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Abstract

For years, valuable clinical samples preserved in formalin-fixed paraffin-embedded tissues were underutilized. However, with advanced spatial multiomics profiling tools, crucial information has become increasingly accessible. Integrating genomic data with spatial information has unveiled crucial insights into cellular activities, enhancing our comprehension of biology. Measuring cellular gene expression while capturing spatial context – including morphology and intercellular relationships – is vital for understanding both normal and diseased biological processes. To date, this approach has illuminated the mechanisms of complex diseases, such as cancer and has facilitated the discovery of biomarkers for early disease detection and new therapeutic targets, accelerating progress in cancer immunotherapies. Cutting-edge single-cell analysis tools are rapidly emerging as the gold standard for investigating intricate biological systems and medical specimens, fueling a multi-billion-dollar industry. Single-cell spatial research, in particular, is inherently cross-disciplinary and addresses questions that remain hidden when focusing solely on the genome or transcriptome of large cell populations. Leveraging advances in single-cell spatial profiling can offer insights into improving cancer immunotherapy and other modern medical treatments. This review will delve into the diverse applications of spatial profiling technology, showcasing examples that demonstrate its ability to provide a detailed picture of the underlying molecular and cellular mechanisms within cells. As a comprehensive reference, this review empowers researchers and industry leaders to harness single-cell and spatial omics for breakthroughs in biomedicine and translational science.

Keywords: Formalin-fixed paraffin-embedded tissues; Genomics; Single-cell analysis; Spatial profiling; Transcriptomics

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1. Introduction

In situ spatial profiling is a technique used to examine DNA or RNA within specific regions of interest in formalin-fixed paraffin-embedded (FFPE) or fresh, frozen tissue samples. The regions of interest for detailed study can be selected based on their location or distinct morphological features within the tissue. These regions can have any geometric shape – whether contoured, gridded, segmented, or specific to cell types. Typically, selected regions are up to 600 µm in diameter, and the high resolution of this technique makes single-cell analysis feasible.

Spatial profiling requires meticulous attention to detail. The quality of the tissue is paramount, especially when dealing with precious or limited samples. Every step of sample preparation can be challenging, from obtaining the right tissue sections to library preparation and sequencing. It involves a delicate balance between preserving the cell outline and visualizing its internal structural elements. Many decisions in spatial profiling experiments are influenced by the type of sample and the specific biological question being addressed. However, success also hinges on experience, careful planning, optimization, and a willingness to stay flexible and creative.

Single-cell spatial profiling technologies are becoming the de facto standard for studying complex biological and clinical samples, resulting in a multi-billion-dollar market.¹⁻⁴ Leading companies – including 10x Genomics, NanoString Technologies, and Vizgen – have gained widespread adoption of these technologies by offering robust assays, reliable technical support, and standardized data processing workflows. These innovative approaches are reshaping research paradigms – shifting the focus to fewer cells analyzed with deeper molecular coverage to better explore the underlying dysregulated pathways. Similarly, *in situ* spatial profiling technologies are undergoing a pivotal shift – once constrained by resolution, they now emphasize multiplexing capabilities. Several groups are pioneering novel methods, including hardware-free single-cell systems, which may complement existing platforms in the future.

The aim of this review is to track the translation of these technologies from academic laboratories to the commercial market, creating a valuable resource for the bioscience community. This includes two key objectives: (i) empowering potential adopters with clear, comparative insights into available tools, and (ii) accelerating the integration of cutting-edge academic discoveries into industrial workflows. The pace of advancement is striking – so rapid, in fact, that even dedicated researchers struggle to stay abreast of new technologies while balancing other scientific priorities.

2. An in-depth analysis of spatial genomics and transcriptomics

The multicellular layout of tissues has been meticulously explored through the use of microscopy, imaging, and a range of visualization techniques. Similarly, the intricate structure and configuration of genetic elements – such as DNA, RNA, and the chromatin network – within cells have been thoroughly examined. This wealth of information has been crucial for evaluating gene expression changes, cellular functions, and maintaining homeostasis, and it

has been linked to disease states and their progression. However, there has been a notable gap in understanding the spatial organization, differentiation, and localization at the genomic and transcriptomic levels both within individual cells and across tissues. Traditional tools have been limited in their ability to capture this data effectively, resulting in the loss of several critical insights into cellular mechanisms.

Spatial genomics and spatial transcriptomics are rapidly advancing fields aimed at addressing this knowledge gap. Recent technological breakthroughs have made it possible to achieve single-cell resolution.^{5,6} In recent years, the field has experienced significant growth with the introduction of multiplexed, high-throughput systems capable of analyzing tens of thousands of genes within a small tissue section. Examples of technologies that employ such an approach include NanoString Technologies' GeoMx Digital Spatial Profiler and 10x Genomics' Visium platform – both marking significant milestones in sequencing-based spatial omics methods. Spatial genomics has also seen substantial progress thanks to improvements in techniques, such as fluorescence *in situ* hybridization, live DNA imaging using microscopy, genome perturbation tools, massively parallel sequencing, and several other biochemical methods. This technological revolution enables researchers to dissect the biological architecture of both normal and diseased tissues while preserving the spatial relationships between cells. This approach provides valuable insights not only at the individual cell level but also at the tissue level. Researchers can now map whole-transcriptome spatial gene expression across multiple cells in complex tissue samples. This highly sensitive technique allows for the simultaneous analysis of hundreds of thousands of cells in a tissue section through total messenger RNA (mRNA) spatial gene expression analysis (Figure 1).⁷

2.1. Small details reveal the big picture

Despite being a new frontier in the genomics field, spatial genomics has demonstrated a broad range of impactful applications. This technology is agnostic to both tissue type and species, making it applicable to numerous studies involving both healthy and diseased tissues. It has been widely used to examine tumor heterogeneity, tissue morphology, mechanisms of tissue development, and responses to therapeutic interventions. Variations in the spatial organization of the genome have been leveraged as diagnostic biomarkers for identifying specific disease conditions. Recent research highlights the effectiveness of spatial technology in examining tumor-associated tertiary lymphoid structures – highly organized emergent immune compartments – to characterize molecular patterns associated with responses to checkpoint inhibitors in

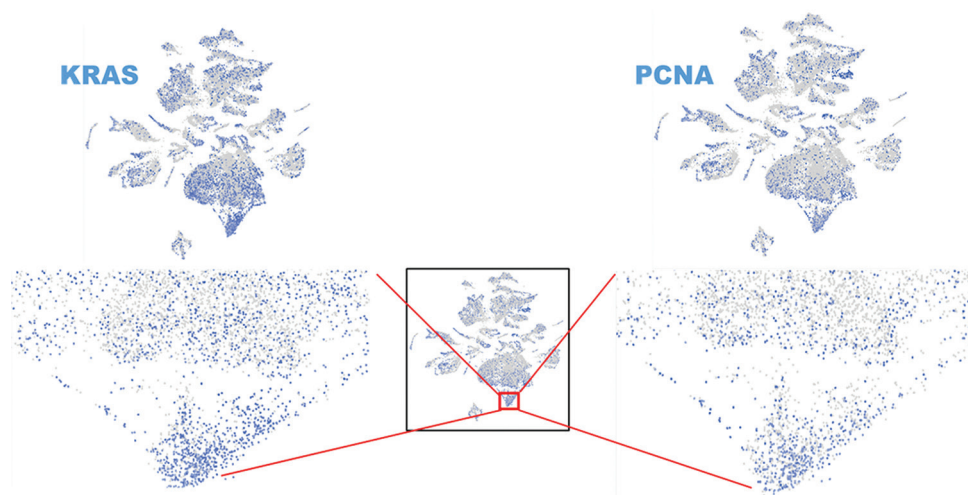


Figure 1. An illustrative example of spatial transcriptomic profiling performed on a lung cancer formalin-fixed paraffin-embedded sample to localize and quantify tumor cells coexpressing *KRAS* and *PCNA* genes. Image created by the authors.

advanced melanoma.⁸ Understanding the interaction between the immune system and tumor biology is central to comprehending disease progression and therapeutic responses to immuno-oncology agents. These studies are enhancing our understanding of patients' immune responses to checkpoint therapies and aiding in the development of biomarkers.

Spatial technologies have been utilized to gain a better understanding of the immune response on dementia disorders.⁹ Neuroscientists are using single-cell genomics and transcriptomics to study neural tissue architecture. The National Institutes of Health has launched the Brain Research through Advancing Innovative Neurotechnologies Initiative to leverage spatial transcriptomics for disease diagnosis. Recently, researchers have applied spatial technologies to investigate the mechanisms by which the SARS-CoV-2 virus infects patients and the subsequent immune responses in lung, brain, and cardiac tissues.¹⁰

In situ sequencing (ISS) enables the sequencing of hundreds of genes directly within tissue samples while preserving spatial information. This process involves generating and sequencing clonally amplified barcode sequences, which are introduced by ligating gene-specific probes at their original tissue locations. The newly developed *in situ* technology allows researchers to analyze fresh, fixed-frozen, or FFPE samples and rapidly generate single-cell gene expression maps for hundreds of genes (Figure 2). The ability to access high-plex information from FFPE tissue is particularly significant, as it provides access to millions of samples stored in biobanks worldwide and associated with clinical outcomes data. The introduction of the Cancer Transcriptome Atlas and Whole Transcriptome Atlas will offer researchers an unbiased approach to assess

spatial changes in RNA transcription in both fresh and FFPE tissues.¹¹

2.2. Spatial perspectives

Although spatial genomics and transcriptomics offer promising applications, there are still a few challenges that remain. Current advancements in spatial genomics and transcriptomics face several critical challenges – including validating disease-specific biomarkers, integrating multiomics sequencing with spatial imaging, resolving tissue phenotypes, and mapping cellular states within their microenvironmental niches. While these challenges persist, efforts to address them are actively underway. To support the field's rapid expansion, researchers must develop scalable, cost-effective assays and analytical pipelines that minimize bias while enhancing throughput. In addition, there is an urgent need to push technical boundaries by improving spatial resolution, expanding multiplexing capacity, and diversifying measurable molecular features. Widespread adoption will depend on standardized, reproducible protocols that ensure data consistency across laboratories, enabling robust cross-study comparisons and accelerating translation into clinical and research settings.

Next-generation spatial technologies will significantly advance clinical translational research by identifying gene signatures crucial for biomarker discovery, evaluating target antigen expression for engineered immune cell therapies, and enhancing stratification for companion diagnostics and clinical trials. Moreover, single-cell spatial analysis stands as one of the most vital technologies for deepening our biological understanding of the complexities within the approximately 40 trillion dynamic cells that make up the human body.

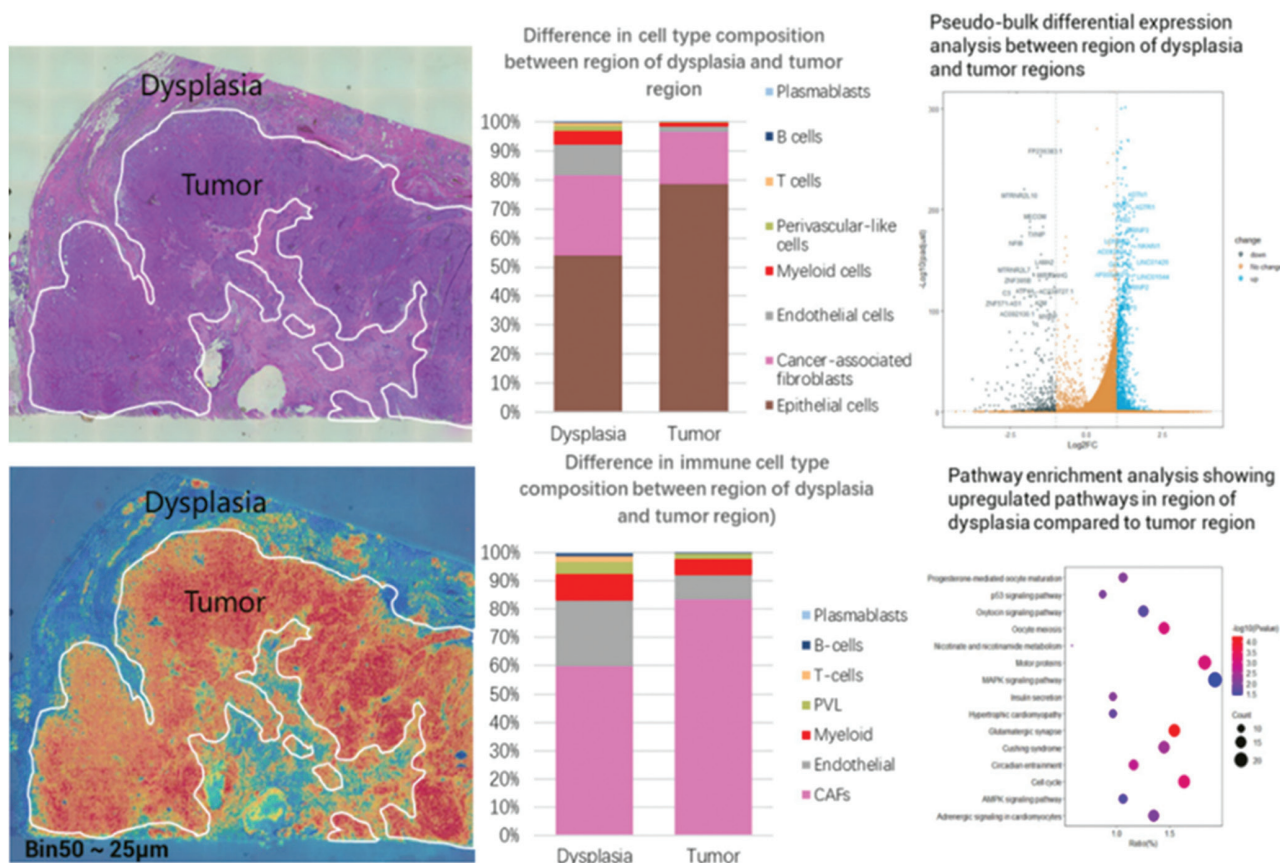


Figure 2. Spatial gene expression profiling of a breast cancer formalin-fixed paraffin-embedded sample using *in situ* sequencing. Image created by the authors.

Abbreviations: CAF: Cancer-associated fibroblasts; PVL: Perivascular-like cells.

3. Spatial profiling in drug discovery

Modern spatial genomics and transcriptomics technologies enable researchers to examine gene expression within tissue samples at a subcellular level. By profiling molecular phenotypes while preserving spatial context, these platforms are revealing novel interactions among signaling molecules and advancing our understanding of disease mechanisms.

Similar to humans, cells do not exist in isolation. Scientists are uncovering that cellular “neighborhoods” consist of diverse cell types that influence one another. Within the tumor microenvironment (TME), these neighborhoods form niches, where groups of cells interact to create functional hubs.¹² Profiling these tumors and their interactions at the neighborhood level provides new insights into how they can be disrupted, thereby making tumors less viable and aggressive. Extensive research has focused on mapping different cell types and their potential interactions in cancer, aiming to discover unknown drug targets and expand the use of cancer immunotherapies.¹³

Spatial profiling to investigate cell types and signals within organized cellular neighborhoods, such as the TME, is an emerging approach that is beginning to yield significant insights.¹⁴

Spatial biology platforms hold the potential to expedite the translational aspects of drug discovery by enabling the examination of entire samples – ranging from tens to thousands of tissue slides – with exceptional sensitivity and reproducibility. Researchers typically initiate this process with a drug candidate, searching for biomarker signatures – genomic or transcriptomic – that reveal the drug’s mechanism of action, among other insights.¹⁵ The challenge lies not only in identifying co-expression and spatial patterns but also in interpreting this information within the context of substantial heterogeneity both between and within patients. Typically, capturing a drug’s biological signature requires identifying approximately three to six biomarkers. While it is possible to include more, utilizing lower-plex panels simplifies the process of scanning entire slides.

In translational research, low levels of gene expression can also influence drug interactions and responses. Spatial phenotyping platforms, which incorporate an amplified workflow to enhance sensitivity, are particularly advantageous. Translational studies scan entire tissues, detecting and characterizing millions of cells. Thus, data-mining tools are crucial for identifying valuable spatial biology signatures.¹⁶ These identified patterns can be further applied to predict immunotherapy responses and to extract actionable measurements.

Pancreatic ductal adenocarcinoma ranks among the most aggressive gastrointestinal cancers, characterized by early metastatic spread and limited treatment options. Recent advances in whole-transcriptome digital spatial profiling have uncovered a treatment-resistant neural-like progenitor signature in tumor cells. These cells exhibit distinct receptor–ligand interactions that drive therapeutic evasion.¹⁷ The identification of this molecular program provides new avenues for targeted intervention, establishing it as a compelling candidate for next-generation therapies.

The field remains optimistic about the future of predictive biomarkers in drug combination trials. Biomarkers will facilitate the understanding of patient responses and help uncover the biological mechanisms underlying drug interactions. Advanced multiplex spatial biology platforms are now equipped to address the growing complexity of contemporary research questions. Multiplex spatial biology platforms are gaining increasing relevance, particularly as the focus shifts from directly targeting tumor cells to modulating cell–cell interactions.

4. Transforming biomarker discovery with spatial profiling

Discovering biomarkers in cancer is difficult due to the complexity of data, inherent tumor heterogeneity, and technological limitations. A major challenge is ensuring that potential biomarkers identified at the transcript level translate into functional proteins, as most current immunotherapies target proteins. Tumor heterogeneity further complicates biomarker discovery, as varying cell phenotypes and functions within the TME can influence disease progression and therapy response. In addition, logistical challenges – such as limited patient samples, high costs of large-scale validation, and the need for scalable technologies – play significant roles.

Revolutionary spatial biology approaches, with their ability to capture cell locations and interactions within the tissue microenvironment, can transform biomarker discovery by overcoming existing barriers and enhancing our understanding of disease pathogenesis and drug-resistance mechanisms. Specifically, spatial profiling offers

a game-changing solution, enabling researchers to study cancer biology *in situ*, identify biomarkers, and evaluate treatment responses with greater precision.

4.1. Visualizing hundreds of genes simultaneously

To identify quantifiable and reliable biomarkers associated with disease outcomes, rapid, sensitive, and targeted data generation is essential. Spatial profiling has the capability to measure hundreds of genes simultaneously, allowing for the visualization of all markers at once without cyclic detection. This facilitates the identification of meaningful signatures, prediction of responses, and elucidation of mechanisms in complex immunotherapies. This high-coverage approach is crucial for analyzing tumor reduction, immune cell infiltration, and the absence of checkpoints.¹⁸

In addition, this method enables the quantitative analysis necessary for targeted biomarker discovery from mass detection, thereby enhancing the ability to link biomarkers with outcomes and allowing for more detailed statistical analyses to explore on- and off-target effects. The field is progressing toward quantitative biomarkers that can elucidate mechanisms and support patient stratification, moving us closer to understanding true human *in vivo* biology as opposed to relying solely on genomics. These advantages allow researchers to identify predictive biomarkers by analyzing a broader spectrum of genes and visualizing more markers within their spatial context, leading to more precise biomarker predictions. Spatial profiling also boosts efficiency by simultaneously detecting all markers and revealing unique content through various imaging modes depending on study objectives.

4.2. Seamless integration from biomarker discovery to clinical applications

Spatial profiling solutions support the entire biomarker development continuum, from early discovery to translation and clinical applications. At the discovery stage, researchers can start with a defined set of content, such as an immune core panel, to phenotype the major immune cell subsets in tissue samples. The flexibility of spatial biology platforms allows scientists to seamlessly integrate newly identified targets from prior unbiased screens. Their high-plexing capability and ability to perform simultaneous imaging of multiple markers enable researchers to rigorously assess and validate the most critical targets. This approach provides relevant, meaningful, and actionable information for downstream targeted studies.

As studies progress into translational and clinical research, where the objective is to test and validate discoveries across numerous samples, cost and scalability become important considerations. Although novel

biomarkers may appear in a small cohort of patients or samples, for clinical application, they must be validated in a larger group of samples, tens to hundreds or even more patients. The seamless integration from discovery to clinical applications ensures consistent and reliable results across various research phases, providing a unique advantage in workflow scalability.

Spatial profiling brings together not only the “what” or the “who,” but also the crucial “where,” adding critical spatial context. This is significant because, in immunology, biomarkers are often not just a “yes” or “no” answer – such as cell density or the presence of different cell types or proteins – but also include information on how these elements are interconnected.

In this regard, the NanoString GeoMx Digital Spatial Profiling technology has demonstrated strong analytical performance, with validation supported by both regression analysis and clinical outcome correlation. Leveraging its high-plex capabilities, this platform has enabled the identification of multiple expression signatures linked to patient outcomes – most notably, the association between programmed death-ligand 1 expression in tumor-associated macrophages and therapeutic response.¹⁹

5. Advances in single-cell multiomics for cancer immunotherapy

Every human being is composed of approximately 30 trillion cells.²⁰ The functionality of each cell – and, by extension, the human body – relies on physical, signaling, and microenvironmental interactions with neighboring cells.²¹ Single-cell multiomics (SCMO) aims to understand these complex interactions by integrating various biomolecular hierarchies, such as the genome, transcriptome, and epigenome.²² For instance, SCMO has shown that the profile of immune cells in the TME can be crucial in determining clinical outcomes.²³ SCMO is increasingly recognized as a valuable tool in cancer immunotherapy and other medical applications. Continuous SCMO-related benchwork and computational tool development are essential for enhancing its utility in cancer immunotherapy.²⁴

Chimeric antigen receptor (CAR) T-cell immunotherapy utilizes a patient's genetically modified immune cells to target cancer.²⁵ Despite being the focus of hundreds of clinical trials, CAR T-cell therapy carries a significant risk of short-term relapse and severe side effects.²⁶ SCMO could potentially enhance the efficacy and safety of CAR T-cell therapy.^{27,28}

6. Single-cell spatial profiling delves into the TME

The TME – a dynamic ecosystem of malignant cells, immune infiltrates, stromal components, and extracellular matrix – functions as both a protective shield and a growth accelerator for tumors. By fostering immunosuppression and nutrient-rich niches, the TME enables tumors to evade immune detection, resist therapy, and drive metastatic spread or dormancy.²⁹ This intricate interplay positions the TME as a critical determinant of therapeutic outcomes, making its characterization essential for designing next-generation immunotherapies that dismantle tumor defenses.

Immunotherapies – including checkpoint inhibitors and targeted blockade therapies – leverage the body's immune defenses to target cancer; however, their efficacy remains limited to a minority of patients. Emerging spatial profiling technologies, which map the expression of immune and cancer cell biomarkers within the TME, are now illuminating mechanisms of treatment resistance. By revealing intricate cellular interactions and spatial heterogeneity, these tools are revolutionizing our understanding of the TME, offering actionable insights to enhance patient stratification and optimize immunotherapy design. This progress holds promise for expanding therapeutic responsiveness and delivering precision immunotherapies to a broader range of patient populations.

Single-cell spatial profiling platforms enable comprehensive mapping of tumor biopsies, producing detailed spatial phenotypic signatures that guide precision treatment selection. Researchers are leveraging this technology to dissect differences in the TME between patients who respond to therapies and those who develop resistance. In immunotherapy, the TME has emerged as a critical predictor of disease progression and therapeutic outcomes. Distinct spatial profiles – capturing tumor and stromal features, such as cellular density, transcriptional activity, and tissue architecture – enable clinicians to forecast treatment efficacy using spatially resolved biomarkers.³⁰ The growing ability to decode these spatially informed molecular patterns is unraveling the TME's complexities, from immune evasion mechanisms to stromal interactions. As this field advances, the integration of spatial profiling into clinical practice holds transformative potential, offering novel strategies to overcome therapy resistance and improve cancer care.

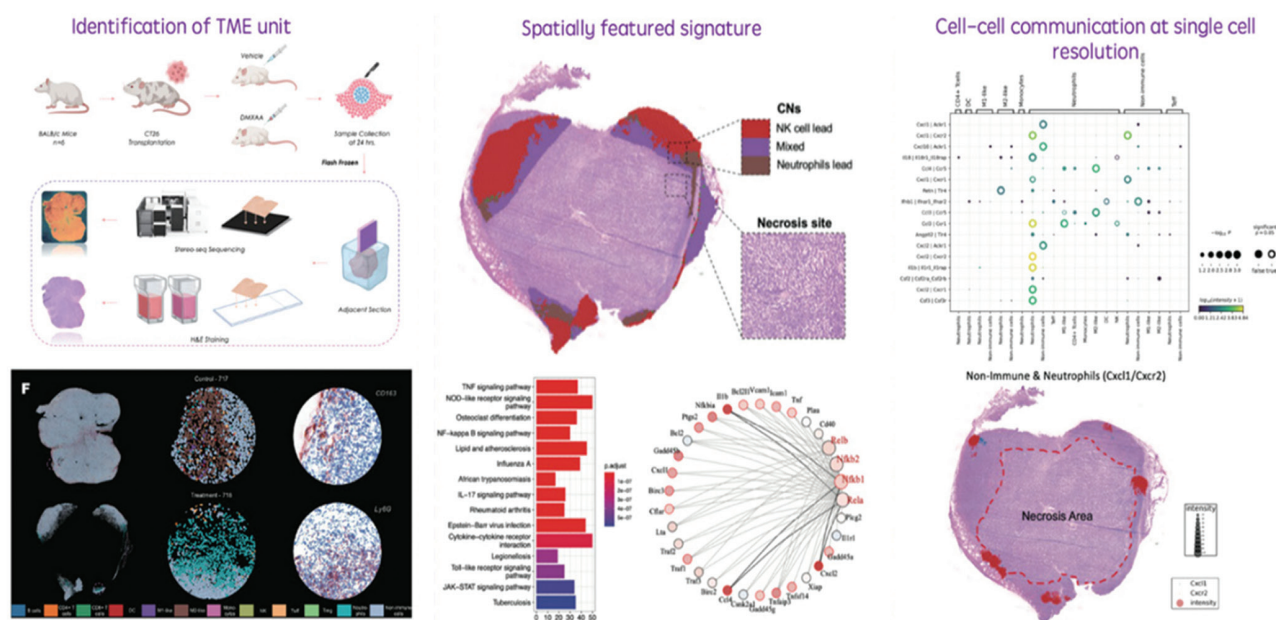


Figure 3. *In situ* spatial profiling for identifying druggable targets, cancer biomarkers, tumor microenvironment features, and enhancing immunotherapy. Single-cell spatial transcriptomics enables precise gene mapping and characterization of cellular function within native tissue architecture. Image created by the authors. Abbreviations: CN: Copy number; NK: Natural killer; TME: Tumor microenvironment.

7. Spatially resolved transcriptomics: advances and limitations

Spatial transcriptomics platforms are classified into two classes: (i) image-based methods, including *in situ* hybridization (ISH) and ISS, and (ii) sequencing-based methods that capture mRNA before sequencing. ISH detects mRNA using complementary probes but faces autofluorescence limitations. Multiplexed fluorescence ISH techniques have been widely used for direct imaging of individual RNA molecules within intact cells and tissues, with performance improving over time. ISS enables non-targeted, multiplexed gene detection beyond the capabilities of ISH. Commercial platforms now automate ISS-based spatial profiling at single-cell resolution, such as NanoString CosMx, Vizgen MERSCOPE, and 10× Genomics Xenium. These systems co-profile RNA and proteins (tens to thousands of targets), enhancing accessibility for tissue architecture studies.

Sequencing-based spatial transcriptomics captures localized gene expression by depositing tissue sections onto barcoded primer arrays. The standard platform with a barcoded oligonucleotide capture array typically achieves a 55 – 100 μm resolution. To further push subcellular resolution limits, several bead-based capture sequencing methods have been developed, including Slide-seq, High-Definition Spatial Transcriptomics, Slide-seqV2, Seq-Scope, and Stereo-seq.³¹ These technologies achieve subcellular

resolution ranging from 1 to 10 μm. Stereo-seq appears to be the most advanced, excelling with its DNA nanoball array technology, enabling simultaneous genome-wide transcript capture, single-cell resolution, high sensitivity, and a centimeter-scale field of view.³² However, there are limitations associated with these platforms, such as near-single-cell resolution, small analyzable areas, uncharacterized spaces between adjacent pixels (depending on channel distances), and the expertise required for fabricating and handling microfluidic chips for implementation.

8. Conclusion

The integration of *in situ* spatial profiling into modern precision and personalized medicine is widely recognized as transformative – particularly in fields, such as immunology, as illustrated in Figure 3 – but still faces challenges that slow its widespread adoption. Despite these challenges, the field is expected to see rapid advancements and become increasingly important in areas, such as precision therapeutics. As spatial profiling technology evolves, addressing these challenges will be a key to unlocking its full potential across various fields of the life sciences.

9. Future perspectives

Looking forward in spatial biology and the broader life sciences, fostering innovation and steering future research

may hinge on several key changes. First, integrating advanced technologies, such as machine learning and artificial intelligence with spatial profiling could enhance our ability to analyze complex diseases and tailor treatment options. This would be particularly impactful in cancer research, where precision is essential for understanding tumor diversity.

It is crucial to develop more cost-effective solutions to democratize access to spatial profiling tools, allowing for broader applications in research settings. Combining multiple technologies, such as single-cell analysis and multiomics approaches, could also provide holistic insights into disease progression and cellular function.

Overall, a shift toward affordability and accessibility, without compromising data quality, would significantly accelerate discoveries and interdisciplinary breakthroughs, positioning spatial profiling as a central player in personalized medicine and therapeutic innovation.

The future of personalized medicine is becoming increasingly intertwined with advancements in spatial biology. *In situ* spatial profiling could offer critical insights into individual patient profiles, especially in areas where personalized medicine is most effective, such as oncology and immunotherapy.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

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Consent for publication

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Availability of data

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