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Redefining clinical practice through spatial profiling: a revolution in tissue analysis

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ABSTRACT

Spatial biology, which combines molecular biology and advanced imaging, enhances our understanding of tissue cellular organisation. Despite its potential, spatial omics encounters challenges related to data complexity, computational requirements and standardisation of analysis. In clinical applications, spatial omics has the potential to revolutionise biomarker discovery, disease stratification and personalised treatments. It can identify disease-specific cell patterns, and could help risk stratify patients for clinical trials and disease-appropriate therapies. Although there are challenges in adopting it in clinical practice, spatial omics has the potential to significantly enhance patient outcomes. In this paper, we discuss the recent evolution of spatial biology, and its potential for improving our tissue level understanding and treatment of disease, to help advance precision and effectiveness in healthcare interventions.

KEYWORDS

spatial biology – spatial transcriptomics – multiplexed imaging – immunohistochemistry – image analysis

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Introduction

The rise of medical genomics has seen the application of increasingly sophisticated platforms to assess disease molecular phenotype, with advancing technologies permitting highly granular investigation at the level of a single cell. However, this focus on the study of particular cell types in isolation does not account for the cellular environment. Spatial biology is a rapidly evolving field that combines both molecular biology and spatial imaging tools to delineate cellular organisation in a tissue, bridging the gap between cells and their location.

This ability to increasingly capture information within the tissue context has great potential for biological discovery, allowing researchers to identify novel cell types/phenotypes,[1] delineate cell interactions[2,3] and determine spatial organisation rules for tissues.[4,5] Deeper understanding can be further unpacked through integration of genomic information with spatial data, allowing us to see how pathology remodels tissue landscapes, with diseases like cancer generating complex ecosystems that evolve in real time in the host tissue environment.[6,7]

The ability to study tissues at a spatial level also has significant benefits in clinical applications. Spatial omics can help identify biomarkers for diseases,[8] understand the mechanisms of drug resistance[9,10] and optimise treatment strategies.[11] It enables researchers and clinicians to move beyond bulk sample analysis, and capture the complexity and heterogeneity of tissues, leading to more precise diagnostics and personalised treatments. Integration of spatial information with molecular data provides a more comprehensive and contextual understanding of biological systems. Of course, each great opportunity is accompanied by a multitude of challenges. This review aims to explore the rapidly advancing technologies for spatial profiling, and the opportunities and challenges associated with their translation from bench to bedside.

Spatial profiling techniques

Techniques for characterising individual cells at the molecular level in their natural spatial context are advancing rapidly. Recently, conventional experimental procedures like DNA barcoding,[12] immunohistochemistry (IHC) for protein detection,[13] and fluorescent in situ hybridisation (FISH) for nucleic acid detection[14] have paved the way for spatial omics technologies to encompass a broader range of targets and/or regions. Spatial omics technologies capture a range of properties, with consideration for spatial resolution, coverage, scale, throughput and multiplexing capacity required

depending on the scientific question being asked.[15] Many advanced spatial omics methods, offering exceptional subcellular precision, are executed on slides and as of 2022, more than 50 distinct spatial mapping techniques exist.[15] Depending on the specific research goals, these profiling methods can be categorised into two groups: targeted or multiplexed approaches for protein or RNA detection and transcriptome profiling using next generation sequencing technology.

Technologies for spatial proteomic analysis

IHC is a technique that allows identification of proteins in a tissue and continues to be an extremely valuable clinical tool.[16,17] Single-protein IHC is a standard technique embedded in the majority of clinical pathology departments and is utilised extensively to help in routine pathological diagnosis. However, the requirement for increasing numbers of markers to aid in patient diagnosis and prognosis has increased the burden on clinical pathology services, and this is likely to continue to increase with the translation of advanced therapies.

For example, IHC is utilised clinically to identify and image T cells in tumours, which provide a better predictor of colorectal cancer patient outcomes, in a metric called the Immunoscore® (Veracyte, San Francisco, CA, US). Immunoscore® is a highly reproducible technique that uses IHC to simultaneously identify $CD3^+$ and $CD8^+$ T cells, generating a score based on cell densities in the tumour core and invasive edge. Greater scores generated from the Immunoscore® correlate with longer patient survival but it also has the potential to stratify patients into those who would or would not benefit from immunotherapy.[18,19]

Multiplexed IHC is a technique that allows multiple antibodies to be applied to tissue at once and visualised using separate fluorescent tags. This technique is applicable to paraffin-embedded tissue, reduces the requirement for a large number of tissue sections and thus vastly increases the discovery potential from small tissue biopsies. There is now a large number of platforms that boast different numbers of marker capabilities; examples include the PhenoImager® HT (formerly Vectra® Polaris™; Akoya Biosciences, Menlo Park, CA, US) with 8+ markers, the PhenoCycler® (formerly CODEX®; Akoya Biosciences) with 66 markers and Imaging Mass Cytometry™ (Standard BioTools, San Francisco, CA, US) with 40 markers.[20] These tools are currently only used in the research space but translation of the discovery work that these platforms have enabled is inevitable.

The first study to utilise the Vectra® Polaris™ platform was by Barua *et al*, who investigated the interaction between T cells and tumour epithelium in non-small cell lung cancer (n=120).[21] They found that regulatory T cell tumour infiltration and interaction with epithelium in the tumour core was associated with worse survival compared with interaction at the invasive edge. Additionally, interaction of regulatory T cells with cytotoxic T cells at the invasive edge was associated with overall better survival but not with interaction in the tumour core.

In the seminal paper by the Nolan laboratory at Stanford University, the authors explore the cellular landscape of colorectal tumours in 35 patients using the CODEX® platform with 56 markers.[22] They found that cells organised themselves into 'cellular neighbourhoods', and that the organisation of these differs for low and high-risk patients. Indeed, a correlation was found between local enrichment for PD-$1^+$$CD4^+$ T cells and better survival in high-risk patients.

Imaging Mass Cytometry™ is a mode of multiplexed imaging that utilises heavy metal tags to obtain multiplexed images with high resolution and clarity in a localised region of interest.[23] Danenberg *et al* employed this technology to study the microenvironment of breast tumours, and stained for 37 markers across 693 breast tumour samples with complementary genomic and clinical data.[24] A key finding in this study established the co-occurrence of regulatory T cells and dysfunctional T cells in sizable 'suppressed expansion' structures. These structures were characterised by proliferating cells and high cellular diversity with enrichment for *CASP8* and *BRCA1* mutations. Together, these cellular structures predicted poor prognosis in oestrogen receptor-positive diseased patients and so this shows potential in patient stratification based on microenvironmental functionality.

This work highlights the power of spatial biology to define the cellular rules of engagement in a disease context and provides the power to stratify patients based on cellular interactions. This will ultimately drive therapy selection (Figure 1).

Technologies for spatial transcriptomic analysis

In situ hybridisation is a technique that can circumvent the resolution challenges of many spatial transcriptomic technologies as it allows the visualisation of RNA molecules in tissue using probes formulated to be complementary to the transcripts they target. These probes can be used to detect one or many transcripts at any one time and give subcellular resolution, making this an excellent tool for determining cell type-specific expression, or to complement RNA sequencing data in order to spatially map the cells of interest.[25]

Chen *et al* introduced multiplexed error-robust FISH (MERFISH), a highly multiplexed, single-molecule FISH protocol that incorporates combinatorial labelling, sequential hybridisation imaging and error-robust encoding.[26] Efforts have advanced MERFISH to enable the simultaneous detection of RNA molecules, achieving up to 10,000 transcripts.[27] Additionally, MERFISH can be employed for high-throughput analysis of intercellular gene expression variations, concurrently revealing spatial distributions of multiple RNA transcripts. The MERFISH approach has been commercialised as Vizgen's MERSCOPE® (Cambridge, MA, US).[28]

One of the key RNA-focused technologies used in spatial biology is spatial transcriptomics, which enables the simultaneous mapping of gene expression levels

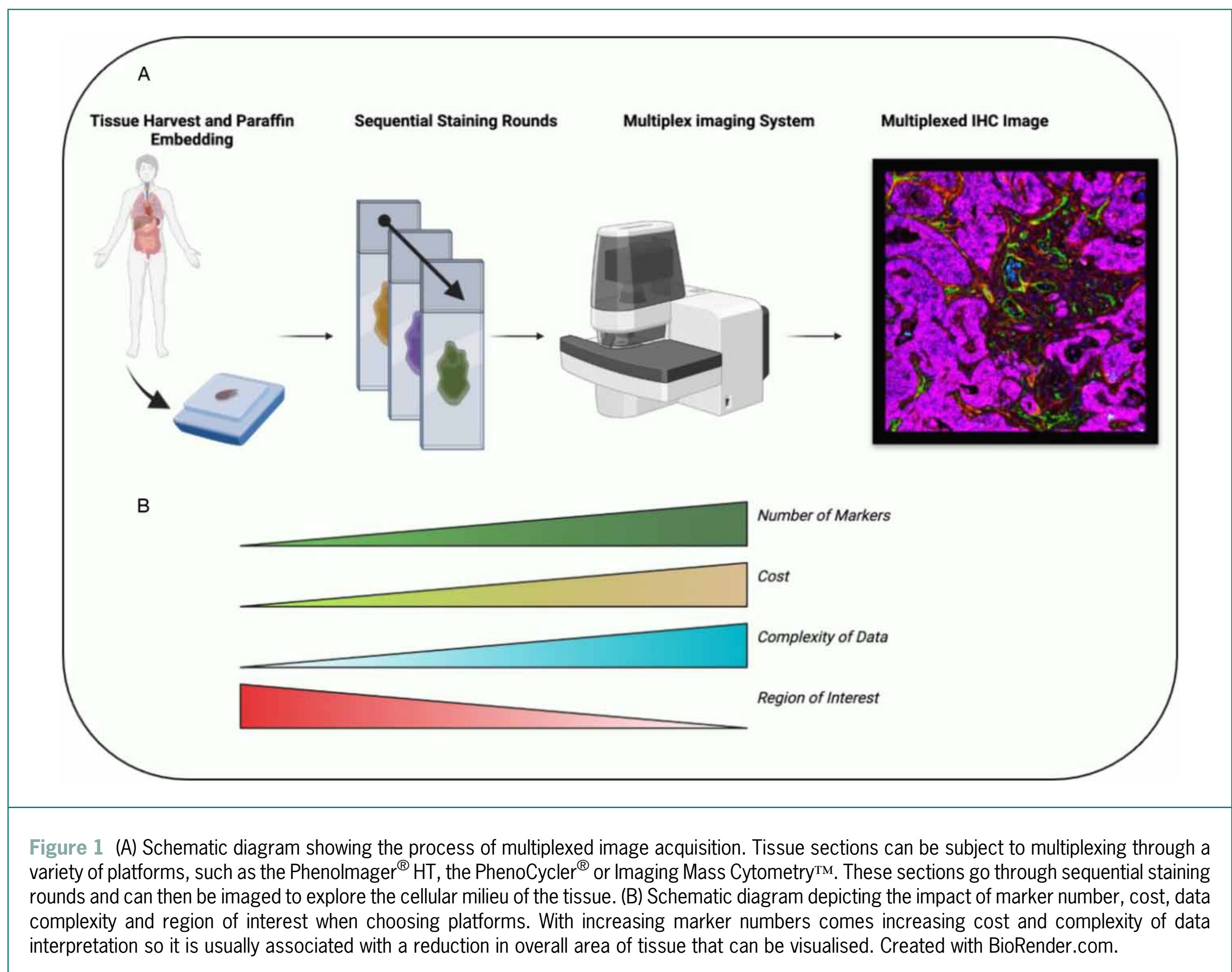

Figure 1 (A) Schematic diagram showing the process of multiplexed image acquisition. Tissue sections can be subject to multiplexing through a variety of platforms, such as the PhenoImager® HT, the PhenoCycler® or Imaging Mass Cytometry™. These sections go through sequential staining rounds and can then be imaged to explore the cellular milieu of the tissue. (B) Schematic diagram depicting the impact of marker number, cost, data complexity and region of interest when choosing platforms. With increasing marker numbers comes increasing cost and complexity of data interpretation so it is usually associated with a reduction in overall area of tissue that can be visualised. Created with BioRender.com.

across a tissue sample. This technique can capture and remove transcripts from within tissue for sequencing, which allows the identification of cell types present in a tissue and their corresponding gene expression profiles in their native spatial context. It provides a comprehensive view of the molecular landscape within a tissue, highlighting functional differences and interactions between different cell populations.

Although impressive, this technique currently has several drawbacks, mainly that the resolution is relatively low across most technologies[29] or that it is limited to a very localised region of interest.[30] This means that while we can say that cell types are present, we may not be able to spatially resolve them on a single-cell level or across a whole-tissue section, meaning that valuable cell interaction information may be lost.[29] Likewise, the depth of sequencing is often low and so key phenotypic changes associated with low-level transcription may not be identified.[31] In saying this, spatial transcriptomic analysis is an extremely useful research tool and has been applied to many types of tissues, such as human heart,[32] liver[33] and intestine tissues,[34] as well as a variety of cancers.[35,36]

Erickson *et al* used spatial transcriptomics (Visium; 10x Genomics, Pleasanton, CA, US) to assess the transition from benign to cancerous tissue in prostate samples.[37] They found that the transcriptomic technology could infer spatial copy number variation and map district clonal patterns in benign tissue, which may have links to early cancer development, and so this has promise in the early detection/prevention of cancer and the identification of dangerous emergent clones.

The ability to co-detect protein and RNA targets simultaneously is an emerging powerful biological tool. This technique enables researchers to concurrently investigate gene expression specific to cell types and pinpoint the cellular origins of secreted proteins with spatial precision.

For example, Cheng *et al* utilised for the first time the PhenoCycler® technology in combination with RNAscope

in situ hybridisation.[38] They developed a reproducible method for combining these two technologies that involved protein detection followed by RNA probing.

Schulz *et al* combined RNAscope-based metal in situ hybridisation with concurrent antibody detection of 16 proteins by Imaging Mass Cytometry™ and identified three distinct messenger RNA (mRNA) target species in a set of 70 breast cancer samples.[39] They found a moderate correlation between HER2 and CK19 mRNA and protein levels at the single-cell level. Nevertheless, it was observed that only HER2 exhibited a robust mRNA-to-protein correlation at the cell population level while CK19 did not display such a strong correlation. Studies like this help to bridge the analysis of spatial transcriptome and protein biology, which has great potential for drug development and clinical application.

The next generation of spatial transcriptomics tools is now emerging, promising single or subcellular tissue resolution across increasingly large regions of interest. The CosMx™ SMI system (NanoString, Seattle, WA, US) allows detection of up to 6,000 RNA and 64 protein analytes in intact tissue sections.[40] Using a multimodal approach, it provides cell segmentation that is superior to that of many spatial transcriptomic platforms, enabling cell typing, functional analysis and cell interaction studies from single images. CosMx™ SMI has been employed for a variety of tissues including normal and hepatocellular carcinoma liver formalin-fixed paraffin-embedded tissues, generating a subcellular expression map of 1,000 genes and a single-cell tissue atlas with 18 distinct cell types.[40,41] However, a major issue with the very rapid evolution of these increasingly sophisticated tools is that platform technology advancement is greatly outstripping our ability to analyse and interpret the vast datasets that are being produced.

The challenges and opportunities of spatial omics in the clinic

One of the major challenges facing spatial omics is the complexity of the data that are being generated. When tissues are subject to spatial omics, they generate large volumes of data relating to cells, their positions or the genes they express. The computational power and infrastructure required to handle and store these datasets is therefore challenging.[42–44] Together, all of the metadata generated from spatial image analysis need to be interpretable and reusable if it is to be a worthwhile resource, and so there is an urgent need to store these data under FAIR (Findable, Accessible, Interoperable and Reusable) standards.[45]

Schapiro *et al* highlight the clear and imminent need for metadata streamlining.[46] The upcoming introduction of tissue atlases, which amalgamate multichannel microscopy along with single-cell sequencing and various other omics data derived from both healthy and pathological specimens, underscores the pressing requirement for well-defined protocols concerning data and metadata. Such protocols would effectively steer processes related to data deposition, curation and eventual release. In this context, a MITI (Minimum Information about highly multiplexed Tissue Imaging) standard is proposed by the authors; for this, they draw on established optimal methodologies from genomics and microscopy domains to create a framework applicable to intricate tissue images, embracing both high multiplicity and conventional histological features.

Furthermore, the number of platforms now available to generate these images has grown exponentially over recent years but there is still a lack of standardisation in how to analyse the data being generated.[47] Spatial biology technologies assign an individual coordinate to a cell, rapidly converting a histological image to a dense point cloud. An entirely new suite of computational tools is urgently required to help mathematically test for non-random interactions between points in the point cloud (and thus between cells in the tissue) in order to permit full biological interpretation of disease-specific and shared cell associations.

The analysis of low-marker panels of tissue (<3 markers) has some well-established protocols for study including cell number counts and nearest neighbour analysis. Nearest neighbour analysis determines whether two cell types are closely located within tissue.[48] This technique could be useful to explore how cell dynamics change with time or treatments but can be hard to interpret. For example, Backman *et al* applied nearest neighbour analysis to multiplexed images of immune cells in samples of non-small cell lung cancers.[49] They found that depending on the ‘starting point’ cell, the nearest neighbour output changed (e.g. CD8 cell to regulatory T cell was different from regulatory T cell to CD8 cell).

As the number of cellular markers capable of deployment rises exponentially, there is an increasing need for more sophisticated mathematical analysis. Arnol *et al* used a technique called spatial variance component analysis, which is a computational tool that enables the quantification of various dimensions of spatial information and, most importantly, can measure the effect of cell-to-cell interaction on gene expression.[2]

A further example from Bull *et al* focuses on the enhancement of the cross-pair correlation function (cross-PCF), a specific spatial statistical technique used to discern both positive and negative spatial associations among cells across various length scales.[50] The cross-PCF has proved valuable but is encumbered by certain limitations that have restricted the widespread application in multiplexed histology. For instance, it can solely assess relationships between pairs of cells and relies on discrete categorical labels for cell classification, precluding the consideration of continuous labels like stain intensity.

Bull *et al* propose several novel extensions to the cross-PCF that effectively mitigate these limitations and enable a more comprehensive analysis of multiplexed images.[50] These include the neighbourhood correlation

function, designed to identify instances of co-localisation involving two or more cell types, as well as the weighted pair correlation function, which extends the cross-PCF to describe spatial correlations between points with continuous labels, thereby accommodating a broader range of label types.

Together, these examples show progress in the field of spatial analysis beyond cell pairs. With proper standardisation and statistical processes, we can hope to overcome the difficulties that comparing results from different studies and platforms can create.

Although faced with challenges, the opportunities that spatial omics offers are immense, and it has the power to unlock the potential of the pathology archives and give insight into tissues/diseases that otherwise would not be possible. For instance, spatial omics allows us to unpack the cellular and molecular heterogeneity in tissues with regard to cellular organisation, interactions and their functional inferences. Furthermore, it can unveil temporal dynamics within tissues so we can study how interactions change with time, providing insight into developmental processes, disease progression or even response to therapeutics.

Gatenbee *et al* investigated the cause of tumour initiation using multiplexed image analysis of carcinoma-in-adenoma samples, where regions of paired precursor and invasive tissue can be analysed together.[51] Their results suggested that recruitment of immunosuppressive cells was the primary factor driving the transition to malignancy. Cell ecology analysis supported this, showing that advanced adenomas were associated with immunosuppressive cells and cytokines while benign adenomas had a mixed immune landscape. Carcinomas all exhibited a similar immune environment lacking immune activity, which reduced selection against immunogenicity and high neoantigen levels. Surprisingly, there was little evidence to suggest that PD-L1 overexpression played a significant role in tumour initiation. These findings propose that altering the immunosuppressive environment may be an effective approach for immunotherapy in colorectal cancers (Figure 2).

With this also comes the opportunity to discover novel biomarkers in disease, which could have therapeutic or diagnostic purposes. In a meta-analysis, Lu *et al* pooled data from over 50 studies across more than 10 tumour types, all of which had had anti-PD-1/PD-L1 therapy.[52] They explored a range of modalities for predictive outcome measures including single-plexed IHC for PD-L1, mutation burden, gene expression profiling and multiplexed IHC. Multiplexed IHC outperformed all other categories in being able to predict patient response to immunotherapy. Consequently, if implemented clinically with better participant selection, this could increase the number of patients who have positive outcomes. It is predicated that the refining of patient selection based on these results could double response rates in clinical trials, with the potential to save up to $20 billion annually.[11]

Clinically, spatial omics will revolutionise several fields of study. One example is that spatial omics will facilitate biomarker discovery by allowing us to identify particular cell types or neighbourhoods of cells that exist in particular diseases or disease subtypes. This premise will also fit into other advantages such as more sophisticated disease stratification and gaining deeper understanding of response and/or resistance to treatments. This information can help guide a more personalised medicine approach in which particular drug combinations or surgical options are offered to patients.

For instance, Gouin *et al* used single-nuclei RNA sequencing, spatial transcriptomics and spatial proteomics to study bladder cancer.[53] They discovered that cadherin 12 (CDH12)-enriched tumours predict poor outcomes after surgery, with or without neoadjuvant chemotherapy, but show excellent response to immune checkpoint therapy. These CDH12-enhanced tumour epithelial cells were found to co-express PD-L1 and PD-L2, and co-localised with exhausted T cells. This study highlights a unique response to bladder cancer therapies and suggests a promising path for spatial biology-guided clinical trials.

Looking to the future

At present, large pathology institutes typically have >300 standardised immunohistochemical antibodies.[54] Using multiplexed IHC, this could be ideally condensed into several panels as opposed to individual stains. For this reason, the clinical introduction of highly multiplexed tissues could be a powerful diagnostic/prognostic tool that could define patient-to-patient and inter-tissue heterogeneity.

Work from the Levesque laboratory in Switzerland is exploring the integration of multiomic workflows for tumour profiling and clinical decision making.[55] The TuPro (Tumour Profiler) study was designed to be integrated into treatment recommendations and constructed on a set of ten multiomic profiling tools, including single-cell RNA sequencing, single-cell DNA sequencing and digital pathology. The tools are being applied to 240 tumour samples over three years, collected from three cancer types: metastatic epithelial ovarian cancer, metastatic melanoma and acute myeloid leukaemia. The goal of this study is to formulate a workflow for clinical integration of multiomic technologies with a relevant turnaround time to be viable.

Of course, pathologist assessment of tissue is considered the gold standard of clinical IHC assay verification and so introduction of multiplexed imaging systems into this workflow will need to accommodate this. However, it is unrealistic for a pathologist to be expected to score tissues based on multiple markers, especially with some platforms boasting 60+ IHC markers or >1,000 RNA markers. There is therefore a need for reliable and approved machine learning or artificial intelligence platforms that can generate outcomes that are easy to

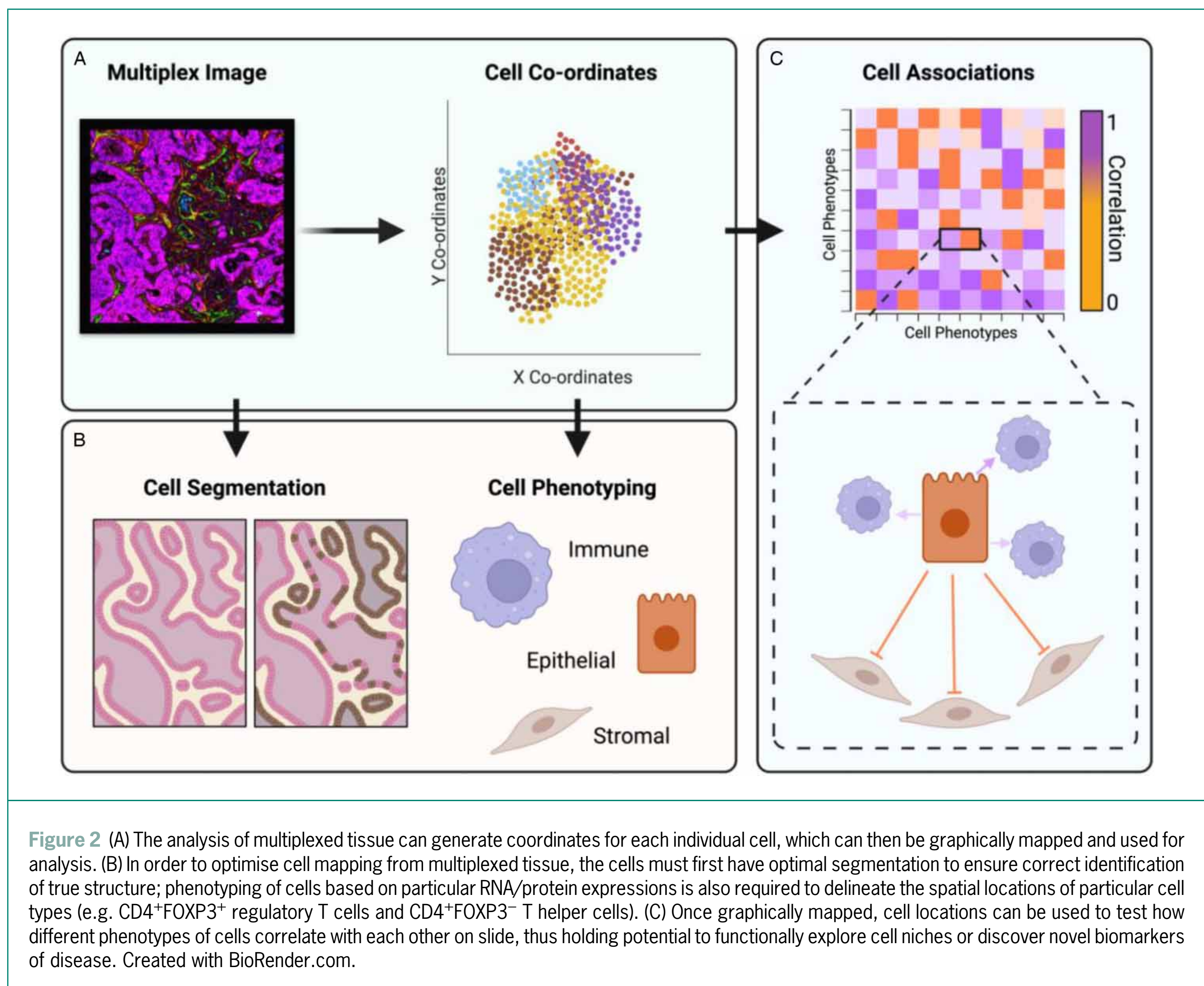

Figure 2 (A) The analysis of multiplexed tissue can generate coordinates for each individual cell, which can then be graphically mapped and used for analysis. (B) In order to optimise cell mapping from multiplexed tissue, the cells must first have optimal segmentation to ensure correct identification of true structure; phenotyping of cells based on particular RNA/protein expressions is also required to delineate the spatial locations of particular cell types (e.g. $CD4^{+}FOXP3^{+}$ regulatory T cells and $CD4^{+}FOXP3^{-}$ T helper cells). (C) Once graphically mapped, cell locations can be used to test how different phenotypes of cells correlate with each other on slide, thus holding potential to functionally explore cell niches or discover novel biomarkers of disease. Created with BioRender.com.

comprehend. Examples of such platforms include AstroPath (Johns Hopkins University, Baltimore, MD, US). AstroPath is a tool optimised using images generated from the PhenoImager® that converts validated predictive signatures into validated assay for clinical application. It delivers a reproducible pipeline that uses imaging data to classify cells in tissue environments and concurrently stores the data in an assessable way.[56]

Conclusions

Spatial biology represents a groundbreaking fusion of molecular biology and advanced imaging tools, revolutionising our understanding of cellular organisation and function in tissues. It offers unparalleled insights into genes, proteins, RNA and therapeutic targets within a tissue's context. The integration of spatial information with molecular data unveils new dimensions of biological systems, enabling personalised treatments, exploration of disease mechanisms and biomarker discovery.

Despite its immense potential, the translation of spatial omics from research to clinical environments faces considerable challenges related to data complexity, computational infrastructure, data standardisation, data analysis and platform cost. Distillation of research-based, high-granularity but low-throughput discovery biology into small, useable and scalable panels of key cell markers is an attractive option for successful translation in the near term. Initiatives like the MITI standard are also key to streamlining metadata handling.

In clinical applications, spatial omics promises to reshape biomarker discovery, disease stratification and treatment response assessment. It facilitates personalised medicine by identifying disease-specific cell patterns and guiding surgical planning. Although transitioning to

clinical practice presents cost and scalability challenges, it holds great promise for improving patient outcomes.

As the field continues to evolve, integrating multiomic workflows into clinical decision making (alongside machine learning and artificial intelligence-based tools) will be crucial for translating spatial biology's potential into real-world clinical impact. Spatial omics is currently an exciting, disruptive technology that stands poised to reshape how we perceive and treat diseases at a tissue level. Ultimately, clinical translation seems destined to follow in time. However, this will be subject to significant constraints including the development of the necessary computational tools and infrastructure required to interpret the datasets, apply them clinically and realise the potential of the technologies for patient benefit.

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Declaration of interests

SJL has received grant income from UCB Pharma. All other authors declare no competing interests.

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