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Highlights-Reviews

From Histology to High-Resolution Mapping: The Rise of Spatial Omics in Immunology

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ABSTRACT

The immune system is deeply shaped by its anatomical context, with spatial organization emerging as a fundamental principle of immune regulation. Recent advances in spatial omics technologies—encompassing transcriptomics, proteomics, metabolomics, lipidomics, and phosphoproteomics—have revolutionized our ability to study immune processes within intact tissue environments. By preserving spatial coordinates while capturing high-dimensional molecular data, these technologies offer unprecedented insight into how immune cell states and functions are governed by local cues and tissue architecture. In this review, we provide an overview of the major spatial omics platforms, emphasizing methodologies that have gained traction within the immunology community and in our own research. We then illustrate how these tools have begun to elucidate the logic of immune compartmentalization across anatomically complex tissues. While not exhaustive, we highlight selected examples from the intestine, secondary lymphoid organs, and liver to show how spatial omics has uncovered region-specific immune programs, microenvironmental niches, and context-dependent signaling pathways. Together, these studies demonstrate how spatial omics technologies are redefining immunological inquiry—shifting the focus from isolated cell types to their spatially embedded roles in tissue physiology and pathology.

1 | Introduction

The quest to unravel the intricate relationship between the spatial organization of anatomical systems, including the immune system, and their functions—referred to here as "spatial immunobiology"—has a long and rich history. Herophilos of Chalcedon (335–280 BCE), a pioneering anatomist working in Alexandria, performed systematic dissections of human cadavers, meticulously documenting the spatial arrangement of organs and tissues [1]. His efforts laid the groundwork for understanding how the spatial organization of anatomical structures dictates their functional roles. This turned out to be particularly relevant for the immune system, as highlighted by the work of Rudolf Virchow (1821–1902 CE), a German physician who emphasized that cellular arrangements within tissues are critical to understanding immune responses, setting the stage for modern pathology [2]. More recent groundbreaking studies confirmed this concept by showing how immune cells migrating within and between different tissue niches adapt their functions to their spatial context, and how this is relevant

during disease [3–19]. Thus, reaching a deeper understanding of how immune cell function is spatially regulated within tissues is critical for the development of next-generation immunotherapies.

Over the past two decades, there has been a determined effort to unravel how immune cells are spatially regulated within tissues in health and disease. This growing focus has catalyzed the development of innovative methodologies collectively referred to as "spatial omics" technologies [20–23]. These cutting-edge approaches enable detailed characterization of the transcriptome, proteome, metabolome, or lipidome of individual cells while preserving their precise spatial coordinates within their tissue context. The advent of spatial omics, encompassing both imaging and sequencing-based techniques, has fundamentally reshaped our ability to study immunological processes in their native spatial environments [23, 24].

In this review, we provide an overview of the main strategies currently available for spatial multi-omics, including transcriptomics, proteomics, metabolomics, lipidomics, and phosphoproteomics approaches. We focus on the techniques most widely adopted by the scientific community, including in our own research, and that have recently proven particularly effective in revealing new principles of immune compartmentalization across different anatomical niches. We then discuss how these technologies are transforming our understanding of the immune system, and—on a parallel front—how they are being used to dissect the functional logic of tissue organization in anatomically complex organs such as the liver, intestine, and secondary lymphoid organs, where spatial compartmentalization plays a key role in shaping metabolic and immunological processes. For a more comprehensive and in-depth comparative analysis of the technical strengths, limitations, and challenges of spatial omics technologies, we refer readers to recently published reviews [21, 25, 26].

1.1 | Spatial Omics Overview

Spatial omics refers to a class of technologies that integrate molecular profiling with spatially resolved imaging, enabling the study of biological systems within their native tissue architecture. Unlike traditional single-cell or bulk omics approaches—which require tissue dissociation and thereby disrupt spatial context—spatial omics seeks to preserve anatomical organization while capturing high-dimensional data on gene expression, protein abundance, metabolite distribution, and posttranslational modifications. This spatial layer of information is essential for understanding how cell states are regulated by local microenvironments, anatomical gradients, particularly in complex tissues such as immune organs or the liver.

Depending on the molecular layer being profiled, spatial omics technologies vary widely in terms of resolution, throughput, coverage, and methodological approach. In this review, we organize the methodological overview into four major domains: transcriptomics, proteomics, metabolomics/lipidomics, and posttranslational modifications. Within each domain, distinct technological strategies have emerged [21].

Spatial transcriptomics (ST) includes both sequencing-based and imaging-based approaches. Sequencing-based platforms rely on spatially barcoded capture probes to link RNA molecules to their tissue coordinates, followed by next-generation sequencing [20, 27]. These methods provide broad transcriptome coverage but are typically limited to single-cell resolution. In contrast, imaging-based platforms, such as combinatorial hybridization strategies, can visualize hundreds to thousands of RNA species directly in situ, reaching subcellular resolution but with constrained transcriptome breadth [28, 29].

Spatial proteomics can likewise be divided into antibody-based and label-free approaches. Antibody-based methods use panels of barcoded antibodies [30–33] or detection of rare metal isotope-conjugated antibodies [34] to localize dozens of proteins at single-cell resolution, enabling quantitative mapping of immune cell types and interactions. Alternatively, label-free workflows based on laser microdissection and mass spectrometry offer unbiased proteome-scale coverage across defined tissue regions, though at lower spatial resolution and throughput [35–37].

Spatial metabolomics and lipidomics primarily rely on mass spectrometry imaging (MSI) technologies [38–40]. These approaches detect small molecules directly from cryosectioned tissue without labeling but are limited by ionization efficiency and analyte volatility. Nevertheless, recent advances in cryogenic MSI and multimodal integration have greatly improved resolution and chemical coverage, allowing detailed mapping of metabolic zonation and lipid remodeling [41].

Finally, we need to consider that the function of many proteins is not primarily regulated by their mRNA or protein abundance, but by signaling mechanisms controlled through posttranslational modifications (PTMs). Yet, among all layers of molecular regulation, spatial profiling of PTM remains one of the most technically challenging frontiers in spatial biology. This is particularly evident for protein phosphorylation, where the low abundance and transient nature of phosphorylated peptides necessitate enrichment protocols and high sample input, drastically increasing the complexity of the workflow and data analysis. Nonetheless, new approaches are beginning to resolve signaling gradients within intact tissues, even in anatomically defined vascular beds [42, 43].

1.2 | Spatial Transcriptomics

Spatial transcriptomics (ST) has emerged as a groundbreaking sequence-based technology that maps gene expression within tissue sections while preserving spatial context. First introduced in 2016, early ST methods relied on barcoded capture primers printed in fixed arrays on glass slides, featuring large 100 μm spots capable of capturing RNA from multiple cells [20]. While these methods provided broad transcriptome coverage and high throughput, their relatively low spatial resolution limits single-cell-level precision. To address this limitation, newer approaches like Slide-seq [44] and high-definition spatial transcriptomics (HDST) [45] were developed. Slide-seq uses beads coated with spatially barcoded oligonucleotides, randomly distributed on glass slides to form a dense layer, achieving a resolution of 10 μm—approaching single-cell dimensions. HDST takes this

further by employing magnetic bead arrays in 2 μm wells, with a much higher image resolution. However, these high-resolution methods often involve lower RNA capture efficiency, complex workflows, and higher sequencing costs.

Visium [27, 46] represents a significant advancement in ST by offering improved sensitivity and throughput while maintaining a user-friendly workflow. Featuring 55 μm spots, Visium strikes a balance between spatial resolution and RNA capture efficiency, with up to 5000 spatial spots per capture area. The platform integrates histological imaging to align gene expression data with tissue architecture and supports both RNA and protein detection through multiomic assays. Tools like Visium CytAssist and the Visium HD spatial gene expression assay [45] further expand sample compatibility and enhance resolution. Compared with Slide-seq and HDST, Visium provides a streamlined, commercially available platform that is versatile and accessible, with broad applications in fields such as cancer, neuroscience, and developmental biology. However, ongoing innovations in both Visium and Slide-seq continue to address challenges in resolution, efficiency, and scalability, solidifying ST as a rapidly evolving field with transformative implications for single-cell biology.

Despite significant advancements, Slide-seq and Visium remain constrained to capturing gene expression in thin tissue sections, providing only a two-dimensional view and leaving the intricate spatial architecture of three-dimensional (3D) tissue structures largely unexplored. To overcome some of these limitations, a pioneer sequence-based protocol has been developed for analyzing the transcriptome of spatially defined 3D tissue niches, named NICHE-seq [22]. This technique combines photoactivatable fluorescent markers, 2-photon laser excitation, and single-cell RNA sequencing (scRNA-seq) to map the molecular and cellular composition of specific tissue regions with remarkable spatial precision and single-cell resolution. Using transgenic mice that ubiquitously express photoactivatable GFP [47, 48], cells within visually defined microenvironments—such as in vivo labeled immune niches in lymph nodes and spleens—can be selectively photoconverted by 2-photon laser excitation to uncage GFP fluorescence. Photoconverted organs are then processed to single-cell suspensions, and the photoconverted GFP^+ cells are sorted for scRNA-seq [22]. This approach preserves single-cell resolution and spatial origin of the cells, enabling the identification of rare, niche-specific immune subpopulations and their corresponding gene expression profiles with whole-transcriptome coverage [22]. By bridging the gap between spatial context and transcriptional data, NICHE-seq provides a powerful framework for understanding how the 3D spatial organization of cells governs immune function and pathology. Nevertheless, NICHE-seq has several limitations. These include reduced photoconversion efficiency in certain organs such as the liver, its current applicability only to transgenic murine models expressing PA-GFP, and the low cell yield obtained when targeting small tissue niches (100–200 mm^3). This technique also requires advanced two-photon imaging expertise to achieve rapid and precise photoconversion, which is essential for preserving RNA integrity. An additional constraint is that, since only one photoactivatable fluorophore (PA-GFP) has been validated to date, NICHE-seq currently allows the photoconversion of only one niche type at a time per organ, limiting multiplexed spatial sampling. Future developments should aim to enable photoconversion in human tissues—potentially through the use of photoconvertible dyes—and to improve protocols for maximizing the recovery of photoconverted cells. In parallel, the engineering of new photoactivatable proteins with distinct spectral properties would open the possibility of simultaneously tagging multiple niche types within the same organ, greatly expanding the spatial resolution and throughput of the approach.

In parallel with sequence-based methods, imaging-based ST approaches have also been developed to achieve sequencing-independent analysis at subcellular resolution. One main example is MERFISH (Multiplexed Error-Robust Fluorescence In Situ Hybridization, MERSCOPE platform from Vizgen), which allows direct visualization of RNA molecules within thin tissue slices without the need for sequencing [28]. Derived from single-molecule FISH (smFISH), MERFISH enhances the original method by employing combinatorial barcoding and sequential imaging cycles, allowing the simultaneous detection of multiple RNA targets. Briefly, each transcript is assigned a unique binary barcode—a pattern of ones and zeros—decoded across multiple imaging rounds to precisely identify RNA molecules at nanometer-scale resolution. MERFISH's error-robust barcoding system ensures high accuracy by correcting readout errors, mapping transcripts reliably within complex tissue architectures with high resolution [28]. While this approach excels in spatial precision and can reveal subcellular localization of RNA, it remains limited in transcriptome coverage, as it can interrogate a maximum of about 1000 transcripts in a 1×1 cm area within a single experiment.

Additional imaging-based platforms, such as Xenium (10x Genomics) and CosMx (NanoString), expand this coverage substantially. Indeed, Xenium can profile up to ∼5000 transcripts within a capture area of approximately 1×2.5 cm, while CosMx offers comparable high-plex capacity with subcellular resolution [49–52]. Both platforms maintain high sensitivity, enabling broader transcriptome interrogation than MERFISH while preserving fine spatial detail [29, 49]. In a recent benchmarking study by the Satija group, MERFISH was shown to outperform Xenium in balancing sensitivity with specificity, producing lower background noise from nonspecific fluorescent dot detection at matched sensitivity levels [49]. Nevertheless, for studies prioritizing transcriptome breadth and larger capture areas, Xenium and CosMx offer distinct advantages over MERFISH, making them particularly suited for comprehensive spatial atlasing of complex tissues.

1.3 | Spatial Proteomics

Compared with transcriptomics, proteomics is intrinsically limited by the amount of material required and is characterized by lower coverage. Nonetheless, spatial proteomics provides a powerful complement by enabling the in situ mapping of protein abundance and localization, capturing posttranscriptional regulation and functionally active protein states.

Recent advances in sample preparation, mass spectrometry sensitivity, and tissue imaging have enabled the development of workflows capable of profiling thousands of proteins with spatial

resolution approaching the single-cell level. A recent example of this progress is the integration of high-resolution immunofluorescence imaging with laser microdissection and multiplexed mass spectrometry, allowing quantitative proteomic reconstruction across defined tissue coordinates. In this approach, cryosections are stained for anatomical markers and imaged at high resolution to enable segmentation of individual cells and their spatial assignment. Laser capture microdissection is then used to isolate cells or small groups of cells from specific microanatomical regions, preserving native architecture. Protein extraction, digestion, and isobaric labeling (e.g., TMTpro) are optimized for low-input material, and samples are analyzed using high-resolution LC-MS/MS platforms coupled with ion mobility separation (e.g., FAIMS). These improvements yield high proteome coverage with minimal material input and allow the generation of spatially resolved proteomic maps of the liver zonation [36, 53]. By reconstructing the spatial proteome of hepatocytes along the porto-central axis, the study identified coordinated gradients of metabolic and structural proteins, revealing unexpected zonated programs not predicted by transcriptomics alone. The approach enables spatial proteomics to go beyond antibody-based imaging methods, offering deeper molecular coverage and unbiased protein discovery.

Despite the advances in label-free proteomics, the detection of low-abundance targets and subcellular localization remains challenging for comprehensive and unbiased protein analysis. In this context, antibody-based spatial proteomics offers the ability to detect specific molecules with higher sensitivity and spatial resolution. Recent developments now allow the co-localization of hundreds of protein targets within the same sample, effectively extending classical immunofluorescence into a high-dimensional proteomic platform. These approaches represent a valuable complement to mass spectrometry-based workflows.

CODEX (CO-Detection by Indexing, PhenoCycler platform from Akoya Biosciences) is a multiplexed imaging platform designed for high-resolution spatial proteomics, enabling the detection of up to 100 protein markers within intact tissue sections while preserving spatial context [30, 31]. Traditional confocal and epifluorescent microscopy are usually limited to detecting 4–8 markers due to spectral overlap, but CODEX largely overcomes this limitation using oligonucleotide-barcoded antibodies and iterative cycles of staining, imaging, and dye removal. This approach provides a map of protein expression at single-cell resolution, revealing how diverse immune cells are distributed and interact within tissues [30, 31]. By preserving tissue architecture, CODEX allows quantitative analysis of protein expression within specific spatial niches, revealing dynamic changes during disease progression and uncovering rare immune cell subsets. However, the iterative imaging process can be time-intensive and requires careful handling to prevent sample degradation over multiple cycles. Additionally, unlike transcriptomic approaches that can provide genome-wide data, CODEX is limited to the proteins targeted by available antibodies [30, 31]. Despite these constraints, the platform's ability to profile dozens of protein markers across entire tissue sections with high spatial resolution has made it an indispensable tool in spatial biology, offering insights that bridge the gap between molecular profiling and functional tissue analysis.

Additional antibody-based proteomics methods have been developed. For instance, COMET, a spatial proteomics approach from Lunaphore, uses microfluidics-based sequential immunofluorescence (seqIF) with off-the-shelf, unconjugated antibodies, enabling easier customization compared with CODEX and scalability across standard slides [32, 54, 55]. It supports up to 40-plex panels in a fully automated workflow, offering high throughput with reproducible staining and preserved tissue integrity. Unlike CODEX, COMET avoids the need for custom conjugation, lowering both cost and development time. However, as with other cyclic methods, total imaging time remains significant, and the number of markers that can be analyzed in parallel is lower compared with CODEX.

Another leading antibody-based spatial proteomics platform, MACSima (Miltenyi Biotec), operating via MICS (MACSima Imaging Cyclic Staining), offers a much deeper multiplex capability—reportedly up to ~300 protein markers on a single tissue sample—while preserving tissue morphology and subcellular resolution [32, 33, 56, 57]. It features a turnkey workflow including validated antibody libraries (>600 targets), fully automated staining and dye erasure cycles, and integrated analysis via MACS iQ View software. MACSima's strength lies in its ultrahigh marker throughput and suitability for discovery applications [33]. The platform also supports same-section multi-omics, combining protein profiling with RNAsky probes to capture dozens of RNA targets alongside hundreds of protein markers. However, increased cycle numbers may increase imaging time and risk sample photobleaching across hundreds of rounds.

1.4 | Spatial Lipidomics and Metabolomics

While ST and proteomics have significantly advanced our understanding of cell behavior in situ, the spatial mapping of lipids and metabolites has lagged, largely due to technical constraints. Unlike RNA and proteins, metabolites and lipids are small, chemically diverse, and highly mobile. Their volatility and susceptibility to degradation complicate both sample preparation and imaging, making it difficult to preserve and map their spatial distribution within intact tissue [38–40].

Recent advances are beginning to bridge this gap. Tian et al. describe a cryogenic multimodal mass spectrometry imaging (MSI) pipeline capable of simultaneously detecting metabolites, lipids, and proteins at subcellular resolution within cryopreserved sections of human and murine liver [41]. This workflow integrates desorption electrospray ionization (DESI), gas cluster ion beam SIMS (GCIB-SIMS), and C_{60}-SIMS. DESI provides broader tissue maps (~40 μm resolution), while GCIB-SIMS achieves ~3 μm resolution in frozen-hydrated sections, and C_{60}-SIMS detects metal-conjugated antibodies for proteomic overlay. By avoiding chemical fixation or matrix application, the method preserves native distributions of over 200 metabolites and lipids and reconstructs spatial coherence by aligning low-resolution DESI atlases to high-resolution SIMS data. One of the core innovations of this workflow is the use of frozen-hydrated tissue sections without chemical fixation or matrix application. This maintains the native spatial distribution of labile metabolites and lipids while preserving tissue integrity. To enable spatial reconstruction, low-resolution DESI-MS maps are used to register

high-resolution SIMS data across anatomical zones, allowing detailed visualization of zonated metabolic states across the liver lobule.

Beyond this, innovations like matrix-assisted laser desorption ionization (MALDI)-2 for enhanced ionization [58] and molecular probabilistic mapping frameworks [40, 59] have improved resolution, sensitivity, and interpretability of spatial metabolomic datasets. Together, these approaches enable integration with ST and proteomic data, offering unprecedented insight into how localized metabolic microenvironments influence immune regulation in liver homeostasis and disease.

1.5 | Spatial Phosphoproteomics

Among spatial omics technologies, phosphoproteomics remains one of the most technically demanding. The transient nature and low abundance of phosphorylated peptides require both extensive sample input and optimized enrichment strategies. As a result, phosphoproteomic profiling is still far from single-cell resolution and currently limited to bulk or regionally pooled samples. In contrast to transcriptomic and antibody-based proteomic methods, spatially resolved phosphosite analysis remains in an exploratory phase, constrained by material demands and analytical sensitivity.

Recently, a spatial FACS sorting of liver endothelial cells was performed to generate the first label-free phosphoproteomic atlas of a vascular bed [42]. Specifically, surface markers with defined spatial expression have been exploited to isolate and pool endothelial cells from four concentric layers of the liver lobule: portal, periportal, pericentral, and central. This strategy enabled the collection of sufficient material for comparative transcriptomic, proteomic, and phosphoproteomic analyses while retaining spatial information. For phosphoproteomics, phosphopeptides were enriched using titanium dioxide (TiO_2) and analyzed by high-resolution mass spectrometry, yielding a comprehensive map of 22,626 phosphosites across 5132 proteins. Although this required large amounts of input material, the approach revealed robust spatial gradients in phosphosite abundance across the liver lobule, offering novel insights into position-dependent signaling dynamics.

A more recent advance is represented by the nanoPhos strategy [43]. This platform adapts phosphoproteomics to cryosectioned tissue by combining several innovations aimed at maximizing sensitivity from minimal material. Tissue sections are cryopreserved and microdissected into defined circular areas—without fixation or staining. Proteins are extracted using a chaotropic buffer under denaturing conditions, followed by microscale trypsin digestion and phosphopeptide enrichment using Ti^{4+} immobilized metal affinity chromatography (IMAC). The enriched samples are then analyzed by ultrasensitive LC-MS/MS optimized for low input. In test applications, including mouse liver, the method yielded reproducible phosphosite profiles from spatially defined tissue regions, although the number of detected phosphosites per spot remains relatively low compared with bulk analyses. Nonetheless, nanoPhos demonstrates that tissue-intrinsic signaling gradients can be resolved at cellular resolution without prior dissociation or labeling.

Together, these studies highlight both the current limitations and emerging capabilities of spatial phosphoproteomics, underscoring its potential to illuminate how intracellular signaling is shaped by anatomical and microenvironmental context.

1.6 | Recent Advancements from Spatial Omics

1.6.1 | From Mapping to Mechanism: Spatial Omics and the Logic of Immune Organization

Spatial omics technologies are transforming immunology by providing unprecedented resolution into how cellular behaviors are orchestrated within the physical contours of tissues. Moving beyond static maps, these tools are now uncovering dynamic regulatory principles—how local signals drive immune adaptation, how niches form and collapse, and how these processes are perturbed in disease. In this section, we examine how spatial omics has illuminated the immune logic embedded within three distinct organs: the intestine, secondary lymphoid organs (SLOs), and the liver. We have deliberately focused on these organs as each serves as a unique model system where spatial context governs immune function, and where spatial omics technologies have unveiled new dimensions of immunological regulation. Moreover, these are tissues in which we have developed substantial expertise, allowing us to critically interpret recent findings and their broader implications. While not exhaustive, the examples highlighted here are meant to illustrate key conceptual advances and should be viewed as representative snapshots within a rapidly evolving and expansive body of literature

1.6.2 | Spatially Resolved Immunity in the Gut: From Homeostatic Architecture to Inflammatory Rewiring

The intestine is not a homogeneous conduit, but a patchwork of microenvironments shaped by segmental anatomy, microbial gradients, and epithelial diversity [60–62]. To adapt to these varying conditions, different intestinal regions have evolved specialized defence mechanisms, ensuring optimal protection and homeostasis throughout the gut [60, 62–64]. Thus, it is not surprising that spatial omics has enabled an unprecedented view of this landscape, revealing how immune surveillance is tailored to region-specific demands. For instance, by leveraging Visium and CODEX, Harnik et al. [65] have recently constructed a spatially resolved expression atlas of the human proximal small intestine, revealing significant zonation in gene expression and immune cell distribution along the crypt–villus axis (Figure 1). Their findings uncovered a previously unrecognized immunogenic role of villus tip enterocytes in humans, which actively recruit $\gamma\delta$ T cells and macrophages—challenging prior assumptions based on murine models. In contrast, $CD4^+$ T cells and conventional dendritic cells (cDCs) were found to be enriched at the villus bottom (Figure 1). This spatially distinct organization highlights species-specific intestinal adaptations that may influence host defence and inflammation [65]. Complementing this work, Hickey et al. [66] used a combination of Visium, CODEX, and single-nucleus transcriptomics to generate a comprehensive single-cell resolution map of the human intestine, revealing distinct immune microenvironments across different intestinal segments. Their study identified adaptive immune-enriched crypt niches in the

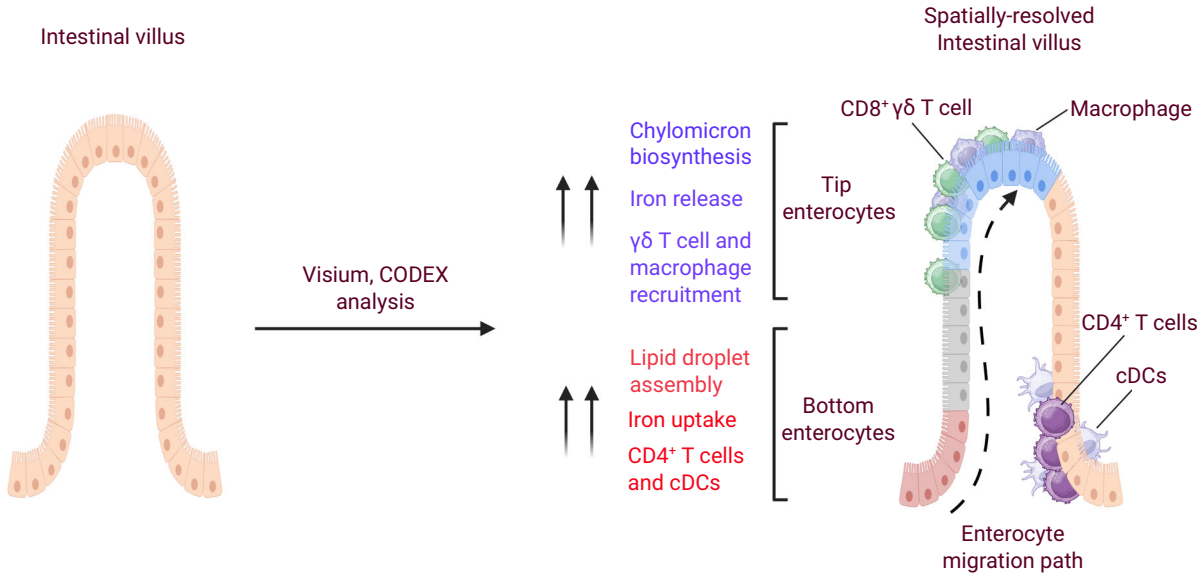

FIGURE 1 | Spatially resolved immune zonation along the human intestinal crypt–villus axis. Spatial transcriptomic (Visium) and proteomic (CODEX) profiling of the human proximal small intestine reveals distinct compartmentalization of immune cell populations. $\gamma\delta$ T cells and macrophages preferentially localize at the villus tip, where enterocytes exhibit an immunogenic transcriptional signature, while $CD4^+$ T cells and conventional dendritic cells (cDCs) are enriched at the villus base. Similarly, while lipid droplet assembly and iron uptake are enriched at the villus base, iron release and chylomicron biosynthesis are enhanced at the villus tip. This spatially organized immune landscape highlights functional specialization along the crypt–villus axis and reveals human-specific adaptations not captured in murine models [65].

small intestine, plasma cell-enriched neighborhoods in the colon, and regionally specialized macrophage populations linked to metabolic regulation and potential disease susceptibility. Collectively, these studies uncovered how gene regulatory programs orchestrate epithelial differentiation and immune surveillance within the intestine, providing useful atlases for understanding the immunological basis of conditions like Crohn's disease and celiac disease.

Beyond providing static maps of cellular organization, spatial omics technologies such as NICHE-seq and MERFISH have started to uncover dynamic immune interactions that shape both homeostasis and disease progression. For instance, using NICHE-seq, a recent study on intestinal immune regulation revealed how distinct niches orchestrate regulatory T cell (Treg) function in response to commensal pathobionts like *Helicobacter hepaticus* [67]. While Tregs are induced in mesenteric lymph nodes, their immunosuppressive function peaks in the lamina propria (LP), where $CD206^+$ macrophages interact with them via CCR2–CCL8 signaling to maintain tolerance. During colitis, this spatial compartmentalization collapses, leading to infiltration of proinflammatory dendritic cells and disruption of tolerance mechanisms, providing novel insights into inflammatory bowel disease (IBD) pathogenesis [67].

Along the same line, a recent study that applied MERFISH-based analysis of colitis progression identified specialized inflammation-associated fibroblast populations that emerge in response to tissue damage, forming inflammatory niches that remodel the gut microenvironment [68]. By charting the temporal evolution of fibroblast-driven immune responses, this study revealed how cellular interactions drive disease resolution or progression, offering a novel framework for targeted therapies in IBD.

1.7 | Programming Fate in Place: Niche-Imprinting in Secondary and Tertiary Lymphoid Structures

Understanding immunity regulation in secondary lymphoid organs during infections is essential for developing novel vaccination and therapeutic strategies [15, 64, 69, 70]. Spatial omics has reshaped our understanding of immune differentiation in secondary lymphoid organs, revealing that microanatomical context imprints T cell fate in ways that were previously invisible to bulk or single-cell approaches. For instance, using NICHE-seq in combination with two-photon intravital imaging, our group recently demonstrated that the timing and location of type I interferon (IFN) signaling within draining lymph nodes determines the polarization of antiviral $CD4^+$ T cells (Figure 2A,B) [71]. Early IFN exposure in the antigen-specific T cell priming niche triggered IL-6 production by dendritic cells, promoting follicular helper T cell (TFH) differentiation and humoral immunity (Figure 2A). In contrast, delayed or absent IFN signaling favored type 1 helper T cell (TH1) differentiation, supporting cellular immunity (Figure 2B) [71]. This spatial and temporal bifurcation of cytokine signaling exemplifies a broader principle of cytokine spatiotemporal pleiotropy: the notion that a single cytokine can exert divergent effects depending on when and where it is encountered. Thus, spatial omics enabled not only the mapping of immune cell distribution but also the decoding of contextual logic that governs immune fate decisions—an insight with direct implications for vaccine design and immunotherapy.

Beyond classical secondary lymphoid organs, spatial omics has begun to illuminate the structure, function, and therapeutic relevance of tertiary lymphoid structures (TLSs)—ectopic immune aggregates that arise in chronic inflammation and cancer. For instance, in nasopharyngeal carcinoma, Liu et al.

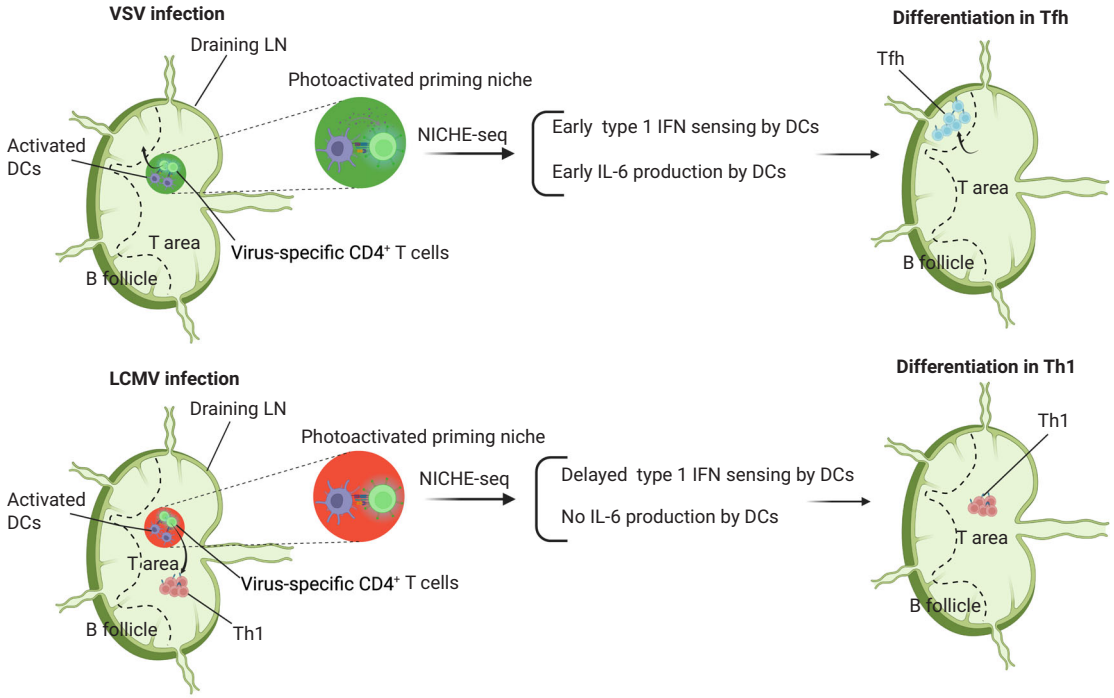

FIGURE 2 | Spatiotemporal control of CD4⁺ T cell fate by type I interferon signaling in lymph nodes. NICHE-seq combined with two-photon intravital imaging reveals that the timing and spatial location of type I interferon (IFN) signaling within draining lymph nodes orchestrates distinct CD4⁺ T cell differentiation pathways. Early IFN signaling in the antigen-specific T cell priming niche induces IL-6 production by dendritic cells, promoting differentiation into follicular helper T cells (TFH) and supporting humoral immunity. In contrast, delayed or absent IFN exposure skews CD4⁺ T cell fate toward type 1 helper T cells (TH1), fostering cellular immunity [71].

combined single-cell and Visium to uncover the cellular architecture and dynamics of TLSs in the tumor microenvironment [72]. Their work revealed that mature TLSs harbor CXCL13⁺ cancer-associated fibroblasts, TFH and B cells, and stem-like CXCL13⁺CD8⁺ T cells. These organized immune niches support germinal center reactions, give rise to antibody-producing plasma cells, and promote apoptosis of EBV-driven tumor cells. Spatial proximity of TLSs to tumor aggregates correlated with favorable responses to PD-1 blockade, and TLS-associated gene signatures predicted both prognosis and immunotherapy efficacy [72]. Complementing this, Tang et al. [73] used ST to dissect TLS heterogeneity in hepatocellular carcinoma and identified two distinct immature TLS states: conforming and deviating. While conforming TLSs resemble mature TLSs and support antitumor immunity, deviating TLSs were associated with dysfunctional immune responses and immunotherapy resistance. Their analysis pinpointed tumor-driven tryptophan metabolism as a key determinant of TLS deviation. Notably, pharmacological inhibition of tryptophan catabolism promoted TLS maturation and synergized with anti-PD-1 therapy to enhance tumor control [73]. These findings underscore how metabolic cues from the tumor microenvironment influence TLS fate and highlight the therapeutic potential of promoting TLS maturation as a strategy to improve immunotherapy efficacy.

Together, these studies demonstrate how spatial omics technologies are revealing the contextual logic of lymphoid structure formation, the cellular players involved, and the metabolic and molecular barriers to effective immune activation in both classical and ectopic niches. They also highlight TLSs as actionable immunological hubs with the potential to be leveraged or reprogrammed for therapeutic benefit.

1.8 | Metabolic and Immune Zonation in the Liver

The liver is a marvel of functional complexity, where hepatocytes, endothelial cells, and nonparenchymal immune populations distribute along the porto-central axis, forming concentric microenvironments with distinct metabolic, signaling, and immunological characteristics. Its complex lobular architecture—characterized by gradients of oxygen, nutrients, hormones, and signaling molecules—has revealed fundamental principles of metabolic compartmentalization, cellular plasticity, and immune regulation.

The advent of spatial omics technologies has profoundly expanded this framework. Early single-cell studies redefined the concept of liver zonation by showing that hepatocytes display distinct gene expression programs depending on their position along the lobule. This metabolic zonation is not random but follows precise anatomical gradients, with periportal hepatocytes enriched in gluconeogenesis and urea cycle enzymes, while pericentral hepatocytes specialize in glycolysis, xenobiotic metabolism, and lipogenesis [74]. Subsequent investigations revealed that hepatocyte zonation is not cell-autonomous, but actively shaped by spatially organized cues originating from the surrounding microenvironment. In particular, liver sinusoidal endothelial cells (LSECs) have emerged as critical regulators of

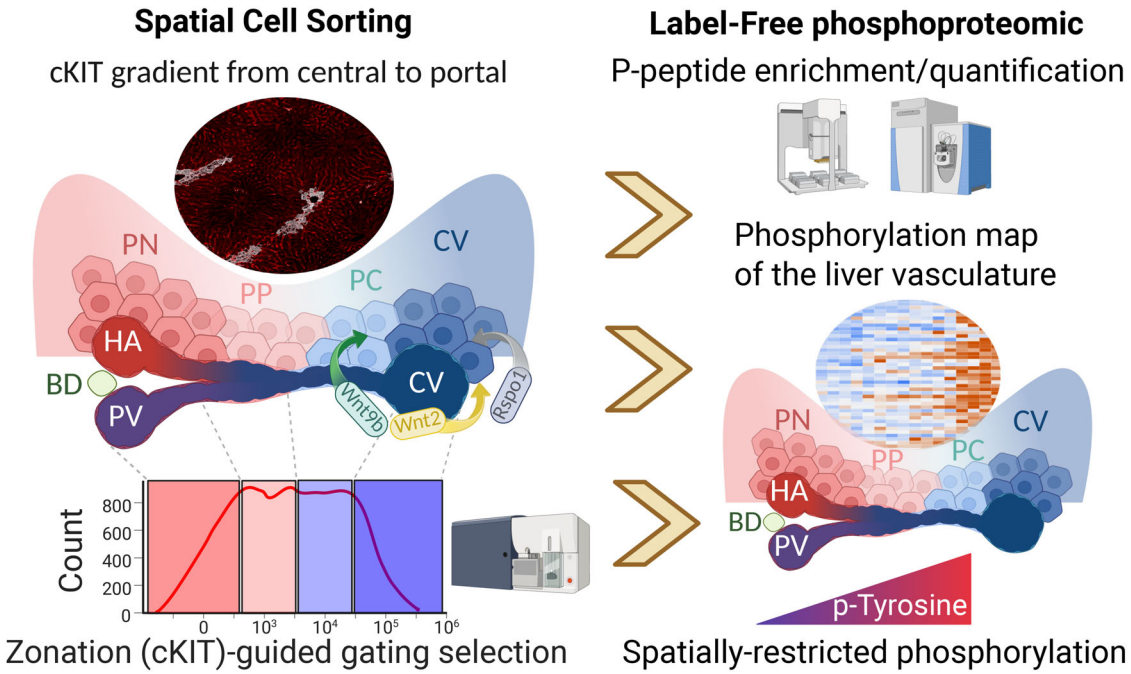

FIGURE 3 | **Spatially resolved phosphoproteomics of the hepatic vasculature**. The zonated expression of cKIT (red), which gradually decreases from the central vein (CV), was used as a surface marker to perform spatial sorting of endothelial cells (ECs) according to their anatomical position along the liver capillary network. Four consecutive EC populations—central vein (CV), pericentral (PC), periportal (PP), and portal vein (PV) regions—were sorted and pooled for phosphopeptide enrichment using a label-free proteomics approach. This strategy enabled a comprehensive and spatially resolved analysis of ECs despite the limited protein content of this rare cell population, allowing identification of distinct phosphorylation signatures characterizing different zones of the liver lobule [42]. BD: bile duct; HA: hepatic artery; PV: portal vein; CV: central vein.

hepatic patterning. Positioned strategically along the sinusoidal axis, LSECs produce graded levels of Wnt ligands, angiocrine signals, and other morphogens that imprint hepatocyte identity in a zone-specific manner, coordinating metabolic functions with vascular architecture [75].

Building on these insights, spatially resolved multi-omic profiling has deepened our understanding of endothelial specialization along the porto-central axis. This approach has uncovered discrete molecular identities for endothelial subpopulations, revealing that vascular zonation extends beyond transcriptional programs to include spatially patterned protein expression and phospho-signaling. Among the key findings, tyrosine phosphorylation emerged as a central spatially regulated signaling event in the murine liver, enriched in pericentral endothelial cells, precisely where Wnt ligands are also concentrated (Figure 3) [42]. This suggests that localized vascular phosphorylation may act as a sensor or amplifier of zone-specific cues, directly linking endothelial positional identity to the regulation of hepatocyte function. These results position liver endothelium not merely as a passive conduit, but as an instructive compartment that integrates spatial signals to organize tissue function (Figure 3).

Beyond its metabolic functions, the liver hosts a highly organized immune system shaped by its anatomical and vascular structure. Immune cells are not randomly distributed but display clear zonation patterns along the porto-central axis. Kupffer cells are enriched in periportal areas, where they intercept gut-derived antigens and promote tolerance through anti-inflammatory cytokines such as IL-10 and TGF-β [76, 77]. In contrast, innate lymphoid cells, NKT cells, and dendritic cells are more abundant in midlobular and pericentral zones, where they respond to metabolic stress and tissue damage [61, 76]. These insights highlight how the liver integrates positional cues to coordinate immune surveillance and tolerance within its complex microenvironment.

In this context, the advent of spatial multi-omics, offering the possibility of combining molecular biology and morphological information at single-cell resolution, is pushing the classical concept of anatomy and histology to the molecular scale.

A prominent example is the application of deep visual proteomics (DVP), a technique combining high-resolution imaging, laser microdissection, and mass spectrometry-based proteomics, recently employed to generate a spatial proteomic atlas of the mouse liver. This approach enabled the delineation of hepatocyte zonation with unprecedented depth, uncovering hundreds of differentially expressed proteins distributed along the porto-central axis. These included well-known metabolic enzymes involved in amino acid catabolism, detoxification, and lipid metabolism, but also regulatory proteins and transporters whose zonated distribution had not been previously appreciated. Importantly, the label-free nature of DVP allowed for unbiased coverage without relying on antibodies or genetic reporters, capturing the proteomic landscape of hepatocytes in their native microenvironment [36]. This work highlights the power of spatial proteomics to complement transcriptomic analyses and to resolve post-transcriptional regulation within complex tissues such as the liver.

Parallel efforts have applied spatial metabolomics and lipidomics to further dissect the functional architecture of the hepatic lobule. A recent multimodal study combined MALDI imaging with other MSI-based modalities to map the distribution of lipids and small metabolites in mouse and human livers. This spatial metabolomic analysis revealed striking zonation of key lipid species, including phosphatidylcholines, bile acids, and triglycerides, many of which showed sharp boundaries between periportal and pericentral zones. Moreover, by correlating metabolite distribution with histological and molecular markers, the study demonstrated how local lipid environments align with hepatocyte function and immune cell niches [41]. These findings establish the liver as a paradigm for spatial metabolite patterning and underscore the importance of integrating metabolomic and proteomic layers to fully resolve tissue physiology.

Finally, although still limited in the number of phosphosites detected per region, nanoPhos enables the detection of local signaling patterns in a label-free manner, without the need for cell dissociation or staining. This demonstrates that spatial phosphoproteomics, while still developing, is uniquely positioned to uncover how intracellular signaling adapts to microenvironmental and positional cues in complex tissues [43].

Collectively, these studies show how spatial omics technologies—each with distinct strengths in resolution, molecular depth, and spatial fidelity—are transforming our understanding of liver biology. By moving beyond traditional bulk or dissociated cell analyses, these methods are enabling an integrated view of how anatomy, metabolism, and signaling converge to organize tissue function.

1.9 | Final Remarks

Spatial omics technologies are transforming immunology by anchoring molecular insights within their native anatomical context. These tools have not only refined our understanding of where immune cells reside but also how spatially organized signals shape their behavior, differentiation, and interactions with surrounding cells and tissue structures. From static maps to dynamic regulatory principles, spatial omics now allows us to decode how immune functions are locally instructed, spatially constrained, and disrupted in disease.

In this review, we have highlighted a selection of applications across the intestine, lymph nodes, and liver—three tissues where spatial compartmentalization plays an outsized role in shaping immune regulation. These examples are not exhaustive, but they illustrate how spatial omics technologies are revealing new levels of organization, from zonated gene expression and metabolite gradients to niche-specific signaling cascades. Each molecular layer—transcriptome, proteome, metabolome, phosphoproteome—contributes a distinct perspective, and their integration offers a more holistic understanding of immune regulation in situ.

As the field advances, key challenges remain. Spatial phosphoproteomics is still in its infancy, and multimodal data integration across scales and tissues is complex. Moreover, most spatial omics approaches provide static snapshots, necessitating the development of spatiotemporal methods to capture dynamic immune processes. Despite these hurdles, spatial omics technologies are already reshaping the conceptual framework of immunology. By grounding molecular information in anatomical context, they are bridging histology and systems biology—unlocking a spatially resolved view of immunity that holds great promise for discovery and therapeutic innovation.

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Conflicts of Interest

M. I. participates in advisory boards/consultantship for or receives funding from BioNTech, BlueJay Therapeutics, Curie.Bio, Gilead Sciences, and GSK. The remaining authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

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