



Dynamic Virtual Tumor Cells Powered by Spatial Omics for Translational Oncology

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Abstract

Cancer remains a major global health challenge, with treatment resistance and variability in patient outcomes posing significant challenges in oncology. The tumor microenvironment (TME) plays a critical role in tumor development, treatment response, and spatial heterogeneity. These characteristics vary across cancer types, patients, and tumor regions. Traditional oncology approaches have been unable to fully capture the complexity of these interactions because they often lack spatial context. In recent years, the development of spatial omics has made significant progress. This technology enables the simultaneous analysis of gene activity, protein expression, and cell-to-cell communication within tissues, providing a deeper understanding of the TME. This review explores how spatial omics technologies can help connect molecular profiling with clinical applications. Particular emphasis is placed on the concept of a “virtual tumor ecosystem.” The discussion highlights how spatial omics data can be integrated with computational models to develop dynamic, patient-specific digital simulations. These virtual tumor organs can recapitulate tumor behavior and predict treatment responses. The use of patient-specific spatial data to simulate therapeutic interventions and anticipate drug resistance represents a significant advancement in precision oncology. The virtual tumor ecosystem can integrate spatial omics data with patient treatment outcomes and is expected to advance the level of personalized cancer therapy. This review summarizes recent advances in the application of spatial omics technologies for the development of cancer models. The main challenges associated with this approach and its future development prospects are also discussed. The ultimate goal is to facilitate the clinical translation of related research findings and support the development of more effective treatment strategies for individual patients.

Keywords:

spatial omics; tumor microenvironment (TME); virtual tumor; digital twins; personalized cancer therapy; precision oncology; computational oncology

1. Introduction

Despite advances in cancer diagnosis and treatment, therapeutic resistance and substantial interpatient variability in treatment outcomes continue to complicate effective cancer management. These factors complicate disease management. Tumors exist within a specialized biological environment known as the tumor microenvironment (TME), which includes immune cells, stromal cells, vascu-

lar components, extracellular matrix elements, and cancer cells. These cellular and non-cellular components interact with cancer cells across both central and peripheral tumor regions. The composition and characteristics of the TME vary depending on the type of cancer. The TME also varies among individual patients. Even within a single patient, different tumors may exhibit distinct TME compositions. Furthermore, tumors possess inherent intratumoral heterogeneity. This heterogeneity arises from

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two primary factors. First, cancer cells acquire distinct genetic alterations as they grow and proliferate. Second, cancer cells interact with immune cells and other stromal components within the surrounding tissue. Together, these processes contribute to the diverse characteristics observed across different regions of a single tumor [1,2]. An increasing number of studies have demonstrated that the spatial organization of the TME plays a critical role in determining treatment efficacy and contributes to therapeutic resistance. Tumors are often classified according to these immune features as “immune hot,” “immune cold,” or “immune-excluded.” These designations indicate that localized cellular habitats and their three-dimensional microenvironmental niches are not merely descriptive labels; rather, they are directly associated with therapeutic efficacy [3,4].

The use of two-dimensional tissue samples cannot fully reconstruct the three-dimensional architecture of tumor growth or accurately represent the spatial distribution of immune cells. Conventional bulk and single-cell approach often lose spatial information because cells are analyzed outside their original tissue context. During sample preparation, tissues are dissociated into individual cells, resulting in the loss of information regarding the original spatial locations of those cells within the tissue. Even the most advanced genomic and proteomic assays provide only a snapshot of a single time point. Consequently, such data cannot capture how therapeutic interventions dynamically reshape the tumor microenvironment over time. Furthermore, these approaches cannot adequately explain the substantial variability observed among patients. This missing dimension of information, referred to as the spatial–temporal–individualization (STI) divide, represents a major obstacle to the advancement of precision oncology [5].

In recent years, spatial omics has rapidly emerged as a technological framework capable of quantitatively assessing RNA and protein states within intact tissues. Advances in high-throughput sequencing and imaging technologies have enabled the identification and characterization of these aspects of heterogeneity. As a result, spatial omics has become a pivotal tool for understanding tumor heterogeneity and interactions within the TME [5–10]. These technologies enable the systematic study of molecular phenotypes across millions of cells within their tissue context, thereby bridging key limitations of both histology and single-cell omics. Platforms such as Visium and CosMx provide spatial transcriptomic profiling, while techniques such as CODEX and multiplex immunofluorescence enable high-dimensional spatial protein mapping [11]. By integrating tissue-clearing and three-dimensional imaging workflows, these technologies

have extended spatial omics into the realm of 3D analysis [12]. Compared with traditional bulk and single-cell omics approaches, spatial technologies enable the simultaneous analysis of cell types, neighborhood relationships, and functional pathways in situ within tissues, while increasingly supporting the integration of multimodal measurements [13,14].

Relying solely on descriptive spatial maps limits their clinical utility. To enable effective clinical application, spatial information must be translated into functional models that can capture dynamic biological processes and elucidate the mechanisms underlying disease progression and treatment response. These models must also be capable of adapting and evolving in a patient-specific manner. This requirement has led to the concept of the virtual tumor cell (VTC), a computational and continuously updatable digital framework that integrates spatial omics evidence with patient-specific biological data [15,16]. Building on VTCs, we assemble virtual tumor organs (VTOs). Tumor-specific virtual organs are designed to facilitate translational medical applications. By integrating multiscale data through computational frameworks such as graph networks and Bayesian models, they enable the simulation of drug and immunotherapy interventions within a virtual environment. As new patient samples are input, these models continuously refresh, offering the potential to truly close the loop of “evidence→VTC→decision” [17].

The construction of virtual cells and, subsequently, virtual organs, supported by spatial omics evidence and continuously refined through patient-level data, provides an actionable framework for translating biological observations into clinical decision-making. First, spatial evidence can more directly link the structure of the microenvironment with therapeutic outcomes [18,19]. Secondly, virtual representations enable the simulation of dynamic processes, such as clonal competition, immune infiltration, and drug diffusion, which are not directly observable. In this way, they extend beyond static descriptions to provide insights into the underlying biological dynamics [20,21]. Thirdly, patient-level updates transform the model from a research tool into a clinical instrument, capable of supporting biopsy site selection, treatment regimen prioritization, and resistance risk prediction [22]. These principles collectively constitute the virtual tumor ecosystem (VTE) (see **Box 1** for formal definitions and the hierarchical framework).

This review proposes that integrating spatial omics with VTCs and VTOs represents a natural progression in the advancement of precision oncology. By addressing the STI divide, constructing VTEs, and integrating

them into clinical decision-making, this approach offers substantial translational potential. It may enhance the clinical utility of spatial data, accelerate the translation of molecular findings into therapeutic strategies, and pro-

vide novel opportunities to overcome treatment resistance while enabling truly personalized cancer therapy (Figure 1).

Box 1: Terminology and Hierarchy of the Virtual Tumor Framework.

Virtual Tumor Cell (VTC)—the microscopic unit. The fundamental computational unit representing the molecular state and behavior of an individual cell. VTCs are initialized by high-dimensional spatial omics data and can simulate cellular responses to perturbations using generative foundation models.

Virtual Tumor Organ (VTO)—the mesoscopic assembly. A spatially resolved, multicellular assembly of VTCs that recapitulates tissue architecture, cell-cell interactions, and microenvironmental gradients in silico. VTOs simulate emergent properties of the tumor mass within a specific tissue context.

Virtual Tumor Ecosystem (VTE)—the systemic integration. The overarching, patient-specific digital twin framework that integrates VTOs with multi-scale clinical data streams, such as longitudinal imaging, treatment history). It represents the translational horizon aiming to enable dynamic simulation and real-time therapeutic decision-making.

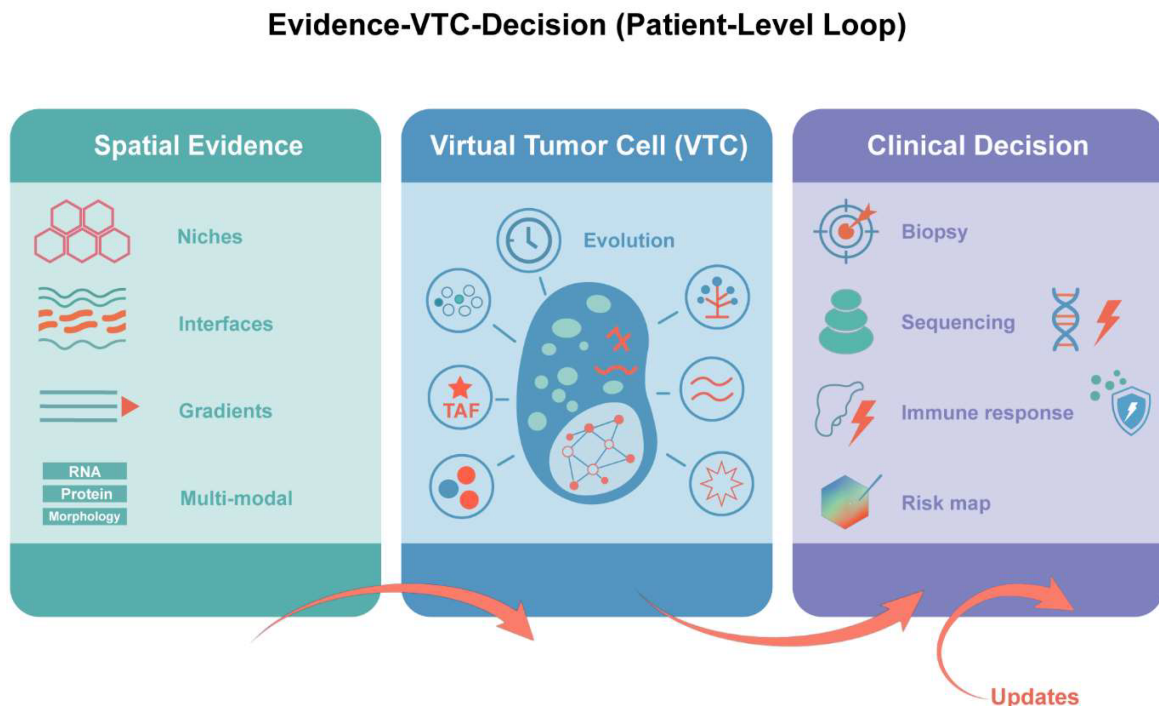


Figure 1: Evidence-VTC-Decision (Patient-Level Loop). This figure illustrates the iterative process from spatial evidence collection, through the creation of a VTC, to clinical decision-making, with continuous patient-level updates driving personalized treatment strategies.

2. Spatial Omics Technology Supports the Spatial Tumor Ecosystem Framework

2.1. Spatial Omics Technology

Spatial omics technologies have evolved from traditional molecular-level analyses to the characterization of spatiotemporal dynamics within tissue microenvironments.

In recent years, with advancements in high-resolution imaging and computational algorithms, the field of spatial omics has rapidly developed, progressing toward higher throughput, lower noise, and stronger integration capabilities [13]. In oncology, these technologies have revealed key mechanisms underlying cellular heterogeneity and spatial interactions, facilitating the transition from descriptive studies to predictive models. Representative spatial omics technologies are summarized in Table 1.

Table 1: Comparative Landscape of Spatial Omics Technologies.

Technology Category	Core Technology Principle	Representative Platforms/ Assays (Year)	Key Features	Resolution	Throughput	Panel Size/Plex	Sample Compatibility	Application Scenarios	References
Spatial transcriptomics	Multiplexed ISH	seqFISH (2014)/seq-FISH+ (2019)	Ultra-high resolution enabling organelle-level transcript localization.	Subcellular/ Single molecule	Low (limited by imaging time)	10,000+ genes	FFPE (partial) & FF	Basic research: chromatin structure, nuclear RNA localization, single-cell gene expression heterogeneity.	[23–26]
		Xenium (2023) /Xenium Prime (2024)	High sensitivity; Prime version significantly improves imaging speed and whole-slide scanning.	Subcellular, ~200 nm	High	5000+ genes/18,000+ WTA	FFPE & FF	Detailed TME mapping, rare cell type localization, and gene signature validation in translational research.	
		MERFISH (2015)/MER-SCOPE Ultra (2024)	High detection efficiency and molecular localization precision; Ultra version supports larger imaging area.	Subcellular, ~100 nm	Medium–high (Ultra)	500–1000 genes, expandable	FFPE & FF	Brain atlas construction, detailed cell atlas, and subcellular RNA distribution studies.	
	In situ sequencing	Fluorescent In Situ Sequencing (2014)	Visualizes individual RNA transcripts.	Subcellular	Low	Targeted, ~5000 genes/Whole transcriptome	Fixed Cells & Tissues	Subcellular RNA localization, analysis of intracellular gene expression heterogeneity.	[26–30]
		STARmap (2018)	Enables 3D imaging. Preserves spatial context in intact tissue.	Single cell and Subcellular	Medium–high	Highly targeted (hundreds to thousands of genes)	Intact Tissues	3D single-cell transcriptomics in intact tissues, brain structure mapping.	
		ExSeq (2021)	Uses physical/chemical tissue expansion (hydrogel) for sequencing within the enlarged space.	Nanoscale/ Super Sequencing	Low	Targeted/ Untargeted	Intact biological systems	Nanoscale spatial transcriptomics, subcellular structure analysis.	
	In situ capture	Slide-seq V2 (2021)	High sensitivity; improved capture efficiency vs. V1.	~10 μ m (near-single-cell)	High	Whole Transcriptome	FF	Retrospective analysis, high-resolution tissue mapping.	[26,31,32]
		seq-scope (2021)	Enables microscope-level examination of spatial transcriptomes.	0.5 μ m spot-to-spot resolution	High	Whole Transcriptome	FF	Study of transcriptome dynamics at the organelle level.	
		Stereo-seq V2(2022)/Stereo-seq V3(2025)	Combines large imaging area with high resolution.	Subcellular, ~500 nm center-to-center	Very high	Whole Transcriptome	FFPE & FF	Multi-organ tumor metastasis studies, large-sample mapping.	
		Visium-HD (2024)	~25x higher resolution than standard Visium (55 μ m), with no data gaps.	Subcellular, 2 μ m squares	Medium–high	Whole Transcriptome	FFPE & FF	Fine-grained TME analysis, retrospective clinical pathology studies, and gene expression mapping in fine anatomical structures.	
	Slide-tags	slide-tags (2023)	Spatial barcoding for single nuclei, automatic cell segmentation.	less than 10 μ m	High	Whole Transcriptome	FF	Immune microenvironment and ligand-receptor interactions, 3D digital reconstruction.	[33]

Table 1: *Cont.*

Technology Category	Core Technology Principle	Representative Platforms/ Assays (Year)	Key Features	Resolution	Throughput	Panel Size/Plex	Sample Compatibility	Application Scenarios	References
Spatial proteomics	Mass-spectrometry imaging cytology	IMC (2014)/IMC Hyperion Xti (2023)	Uses metal tags instead of fluorophores, minimal signal crosstalk, and very low background noise.	Subcellular	Low-high	30–50 plex	FFPE& FF	Immune checkpoint analysis, TIME neighborhood subtype classification.	[34,35]
		MIBI-TOF (2014)	High sensitivity and wide dynamic range.	Subcellular	Low-Medium	40–50 plex	FFPE	Structured immune microenvironment analysis.	
	Cyclic fluorescence multiplexing	CODEX (2018)/PhenoCycler-Fusion	Relies on cyclic hybridization and imaging.	Subcellular	Medium–high	100+ plex	FFPE & FF	Large cohort studies and single-cell protein interaction network construction.	[36,37]
		COMET (2023)	High automation and very short cycle times.	Subcellular	Very High	100+ plex	FFPE	Intra-tumoral clonal structure, spatial genetic heterogeneity, and cancer evolution.	
Spatial genomics	Microdissection-based	slide-DNA-seq (2021)	Built on Slide-seq; maps clonal evolution within tumors	10 μ m	Medium–high	Whole genome/Targeted Panel	FFPE	Intra-tumoral clonal structure, spatial genetic heterogeneity, and cancer evolution.	[38–41]
		LCM-WGBS (2018)	Performs whole-genome bisulfite sequencing on micro dissected samples.	ROI-level	Low	Whole Genome Methylation	FFPE & FF	Methylation profiling of specific tissue regions.	
		LCM-RNA-seq (2013)	Traditional sequencing post-microdissection; highly customizable region selection.	ROI-level	Low	Whole transcriptome/Targeted	FFPE & FF	Validation of region-specific gene expression from spatial transcriptomics discoveries.	
		Geo-seq (2016)	High precision, suitable for trace amounts of tissue.	Single to few-cell level	Medium–high	Whole transcriptome	FF	Early embryo development, spatial transcriptomics of trace amounts of rare cells.	
Spatial epigenomics	In situ chromatin profiling	scSpatial-ATAC (2021)	Combines ATAC-seq with spatial barcoding for in situ chromatin accessibility profiling.	Single-cell, $\sim$ 10 μ m	High	Genome-wide open chromatin regions	FF	Spatial mapping of regulatory elements in development/disease, identification of cell-type-specific enhancers.	[42–44]
		Spatial-CUT&Tag (2021)	Combines CUT&Tag with spatial technology for in situ detection of histone modifications or TF binding.	10–55 μ m (platform-dependent)	Medium	Targeted epigenetic marks	FF	Spatial epigenetic state analysis, epigenetic regulation of cell fate decisions.	
		sciMAP-ATAC (2022)	Microfluidics-based combinatorial indexing; retains partial spatial information.	Cell-cluster level	High	Genome-wide open chromatin regions	Fresh nuclei	Medium-throughput spatial epigenomic atlas construction.	

Table 1: *Cont.*

Technology Category	Core Technology Principle	Representative Platforms/ Assays (Year)	Key Features	Resolution	Throughput	Panel Size/Plex	Sample Compatibility	Application Scenarios	References
Spatial metabolomics/ lipidomics	Mass spectrometry imaging	MALDI-MSI (2000s/2021)	Most established spatial metabolomics technique; label-free, directly detects metabolites/lipids.	5–100 μm /Sub-micron, <5 μm (with OTCD)	Medium	Untargeted, hundreds to thousands of metabolites	FFPE (partial) & FF	Tumor metabolic reprogramming, spatial distribution of drugs and their metabolites, and neuroscience.	[45–47]
		DESI-MSI (2000s)	Operates under ambient conditions, requires no matrix, and is suitable for real-time analysis.	50–200 μm	Medium	Untargeted, hundreds of metabolites	Untreated tissue sections	Intraoperative real-time tissue analysis, spatial lipid-metabolism profiling.	
		ToF-SIMS (1980s)	Extremely high surface sensitivity; detects elements and small inorganic/organic molecules.	<1 μm	Low	Elements, small molecules, lipid fragments	Various solid samples	Subcellular elemental distribution, drug-penetration studies, and material-surface analysis.	
Combination/ Multimodal integration	Sequential multimodal	Visium + IHC/IF	H&E/IHC/IF imaging first, followed by Visium sequencing on the same tissue region.	ROI-level/near single-cell	Medium	RNA + Protein (~10–40 markers)	FFPE & FF	Direct correlation of gene expression with key protein markers.	[48–50]
		Xenium + IF/H&E	High-plex RNA in situ detection and protein/morphology imaging on the same slide.	Subcellular	High	RNA (5000+) + Protein (~4–10 markers)	FFPE & FF	High-precision multi-omics co-localization.	
		CODEX $\times$ RNAscope	Multiplexed protein imaging followed by targeted RNA in situ hybridization.	Subcellular	Medium-High	Protein (100+) + RNA (a few targets)	FFPE	Deep profiling of cellular functional states (protein) and key gene expression.	
	Simultaneous multimodal	DBiT-seq (2020)	Simultaneously captures RNA and protein via microfluidic barcoding (oligonucleotide-tagged antibodies).	10–25 μm	Medium	RNA + Protein (a few to tens of markers)	FF	Matched transcriptome and proteome data from the same cells.	[26,51,52]
		GeoMx (2019)	Non-destructive; tissue section can be used for subsequent staining.	Multicellular, ROI-level	High sample-throughput	Whole transcriptome (18,000+ RNA) + High-plex protein (100+)	FFPE & FF	Clinical biomarker discovery, regional analysis of tumor/immune microenvironment, retrospective pathology studies.	
		CosMx SMI 2.0 (2024)	Dual RNA and protein detection, version 2.0, significantly increases RNA coverage.	Single cell and subcellular, ~0.5–1.2 μm	High-plex	6000+ RNA (up to whole-transcriptome ~18 k plex) + 64-plex protein	FFPE & FF	Studying complex ligand–receptor interactions within tissues.	
		G4X (2025)	Enables in situ multi-omics detection, allowing for the simultaneous measurement of RNA and proteins in a single run.	Single molecule	High	RNA: ~300–500 genes (Targeted). Protein: ~15–18 markers.	FFPE	Supports 3D spatial multi-omics analysis through serial sections.	

2.1.1. Spatial Transcriptomics

Spatial transcriptomics reveals gene expression patterns and cellular behaviors within the TME by mapping gene expression profiles onto tissue sections and correlating these data with their spatial coordinates [8,53]. Early technologies relied on array-based transcript capture; however, their spatial resolution was limited. Modern high-resolution techniques combine imaging and sequencing methods, including in situ sequencing and probe-based approaches, enabling transcript localization at subcellular resolution while preserving spatial information [54,55]. By registering consecutive tissue slices to reconstruct 3D transcriptional spatial information and integrating temporal data, dynamic spatial transcriptomics has emerged as an extension of spatial omics [56]. Recent deep learning models have contributed to the technical optimization of these technologies. Advances in AI-assisted real-time transcriptome reconstruction, combined with neural network-based simulations of treatment-induced transcriptional remodeling, have enhanced the capacity to dynamically monitor transcriptional changes from baseline through the development of treatment resistance [57].

2.1.2. Spatial Proteomics

Spatial proteomics addresses the limitations of spatial transcriptomics by directly providing information on the spatial distribution of functional proteins. Through mass spectrometry imaging (MSI) and cyclic labeling strategies, spatial proteomics enables the simultaneous quantification of dozens to hundreds of proteins, overcoming the multiplexing limitations of traditional immunofluorescence techniques [58]. In dynamic spatial proteomics, advanced high-resolution imaging techniques enable tracking of protein reprogramming throughout treatment [59]. Xu et al. employed Panoramic Spatial Enhanced Resolution Proteomics to monitor spatial gradients in key proteins, such as epidermal growth factor receptor, within tumors, thereby supporting the prediction of drug-resistant clone expansion [60].

2.1.3. Other Spatial Omics Technologies

Other omics layers, such as metabolomics and epigenomics, are expanding the research dimensions of spatial omics [61]. Spatial epigenomics investigates the spatial distribution of epigenetic modifications, including DNA methylation and histone modifications, providing insights into how these regulatory mechanisms function across different tissues and cellular environments [62].

Spatial metabolomics employs imaging mass spectrometry to characterize spatial metabolite gradients, such

as lactate accumulation in hypoxic regions, thereby providing insights into the spatial heterogeneity of tumor metabolic reprogramming [45,63]. The incorporation of Matrix-assisted laser desorption ionization MSI and ion-mobility spectrometry has markedly broadened the detectable repertoire of small molecules and lipids, with substantial gains in sensitivity and resolving power [64,65]. Extensions of spatial metabolomics, such as SpaceM, enable high-resolution characterization of subcellular spatial features [66].

2.1.4. Spatial Multi-Omics and the Integration of Multimodal Data

Spatial multi-omics technologies capture the distribution of multiple omics layers within tissue space, offering a multidimensional view of cellular behavior and spatial information. By integrating spatial transcriptomics with spatial proteomics and other omics layers, researchers can characterize functional states and regulatory networks of tumor-infiltrating immune cells. The integration of spatial multi-omics is further advanced through data fusion algorithms, such as soScope, developed by Li et al., and Multi-modal Spatial Omics, developed by Coleman et al., which enhance the efficiency and utilization of spatial omics data [67,68].

Current state-of-the-art technologies emphasize cross-modal integration, including AI-driven alignment algorithms that enable pretraining across diverse omics datasets while incorporating spatial information for comprehensive molecular characterization of cells [69]. For instance, the integration of single-cell and spatially resolved multimodal approaches enables a multidimensional and comprehensive understanding of molecular information across epigenomics, transcriptomics, and proteomics [70]. These technologies have provided insights into the complex, multilayered interactions underlying cancer biology.

2.2. Spatial Omics--Derived Spatial Signatures and Clinical Oncologic Links

A spatial signature refers to the characteristic spatial pattern or sets of features defined by the relationships among cell types, molecular expression profiles, and spatial localization within tissues or the TME. By leveraging spatial signatures, multidimensional information encompassing gene expression, spatial localization, and intercellular interactions can be interpreted and, when necessary, integrated through three-dimensional serial-section registration and cross-modal alignment (Figure 2).

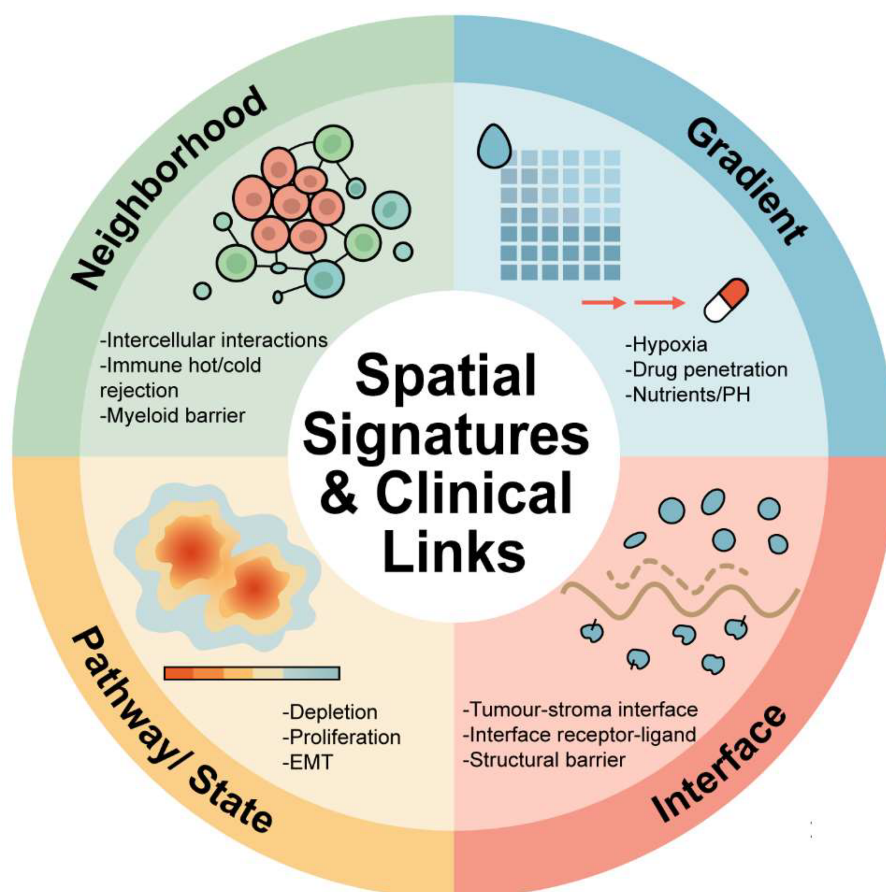


Figure 2: Spatial Signatures and Clinical Links. This figure summarizes the major spatial signatures and their clinical relevance. These spatial features, such as how cells interact, how molecules spread, and the boundaries between tumor and supporting tissue, are key to understanding why tumors differ and how they respond to treatment. Spatial signatures may inform clinical decision-making by providing a dynamic and integrated view of the TME and its functional organization.

2.2.1. Spatial Molecular Gradient

Gradient-related phenomena within the TME, including hypoxia, drug penetration, nutrient availability, and pH variation, contribute to a complex landscape of spatial heterogeneity. Gradient signatures map these spatial features onto tissue architecture, enabling the characterization of the relationships between continuous spatial variations and cellular state trajectories, clonal landscapes, and patterns of immune infiltration [71]. The tumor center and periphery exhibit reproducible pathway stratification, with the center tending toward metabolic and high-energy states, while the periphery is enriched in inflammation and innate immunity [72]. Greenwald et al. developed a five-layer gradient model that integrates hypoxia, blood supply, and cellular states in glioblastoma using a cross-modal framework that combines Visium and CODEX technologies. This study also validated the consistency of internal and external tumor gradients across multiple spatial scales [73].

By integrating spatial risk maps, gradient signatures can identify features such as drug penetration barriers, resistant ecological niches, and immune exclusion. These insights can inform clinical strategies for drug optimization, dose stratification, and the sequencing of therapeutic interventions. Research conducted by Natalie Landon-Brace et al. demonstrated that oxygen gradients can increase the proportion of basal-like cells, which is associated with resistance to gemcitabine [74]. Terrassoux et al. recreated the spatial heterogeneity of treatment responses by engineering radial gradients of hypoxia and acidification within an extracellular matrix (ECM)-composite microenvironment. Their work successfully characterized the spatial origins of radioresistance and established a controllable platform for investigating the relationship between microenvironmental gradients and the emergence of treatment resistance [75]. Recent advancements in the controllable modeling and visualization of gradients in the TME have provided actionable toolchains to push mechanisms toward clinical applicability. Auxillos et al.

developed an integrated microfluidic system capable of generating and monitoring pH gradients in a 3D collagen matrix in real time. This system enables the simultaneous integration of live-cell imaging and spatial transcriptomic sequencing, thereby providing a continuous framework for mapping the progression from microenvironmental gradient perturbations to spatially resolved transcriptional responses [76]. Meanwhile, MSI directly images the tissue distribution of drugs or metabolites, identifying pharmacokinetic resistance and optimizing dosage and administration paths, while photoacoustic chemical imaging noninvasively and quantitatively monitors pH to predict chemotherapy efficacy [77–79]. Thus, gradient signatures not only quantify spatial phenomena but also serve as spatial biomarkers for neoadjuvant and perioperative treatment stratification, enabling the early identification of poorly permeable and therapy-resistant ecological niches and guiding the optimization of sequential treatment strategies.

2.2.2. Cellular Neighborhood

Cellular neighborhood signatures characterize the spatial organization of cells by identifying which cell types are adjacent and the interactions between them. Cellular neighborhood analysis further partitions tissues into distinct spatial microenvironments, or niches, thereby revealing factors that influence tumor progression and therapeutic response.

The hot/cold/excluded framework has become a common paradigm for immunotherapy stratification. In this context, immune-hot, immune-cold, and immune-excluded phenotypes are not merely distinguished by differences in immune cell abundance; rather, they are defined by the spatial architecture of immune cells and their functional states within the tumor microenvironment [5, 80]. In a 3D context, spatial scoring of immune hot/cold neighborhoods surrounding spatial subclones, based on distance- and neighborhood-related relationships, can more accurately predict patient outcomes and therapeutic responses [81,82]. The spatial neighborhoods and functional interactions among tumor cells, myeloid cells, and T cells further define the fundamental distinction between immune-hot and immune-excluded phenotypes, providing mechanistic insights into the heterogeneity of responses to immunotherapy [83]. In the multimodal breast cancer atlas, recurrent immune-stroma-tumor niches and a PD-L1/PD-L2-positive macrophage population associated with clinical outcomes were identified, providing a cellular basis for the immune-hot/immune-excluded classification [84]. Compared to total immune quantity, the combined effects of “location × composition” provide a

more robust explanation of prognosis. Zhang et al. confirmed that immune hotspots enriched with effector T cells are located more frequently at accessible tumor peripheries/interfaces rather than deep within the tumor core. Notably, the study demonstrated that the spatial localization of these hotspots is a stronger predictor of clinical outcomes than effector T-cell abundance alone [85]. BANKSY, developed by Vipul Singhal, is a machine-learning framework that integrates spatial information through a biologically informed approach, enabling the clustering of cells into distinct cell types or tissue domains. It encodes the cellular microenvironment using spatial kernels, thereby quantifying gene expression gradients surrounding each cell [14,86].

Cellular neighborhood signatures provide incremental information that extends beyond conventional approaches based on total cell abundance or single biomarkers. Large cohort studies using imaging mass cytometry have revealed that the spatial organization of cellular neighborhoods and multicellular ecological patterns is not only closely associated with molecular subtypes and somatic alterations but also correlates with prognosis and adverse clinical outcomes. This suggests that the coupling of genomics to ecology can directly manifest as clinical risk phenotypes through the structure of cellular neighborhoods [87,88].

2.2.3. Inter-Domain Interfaces

Inter-domain interface signatures focus on the organizational boundaries and material exchange between two ecological or clonal domains. This includes the location, width, and curvature of the interface; alterations in cellular composition and pathway activity on either side of the interface; and the enrichment of specific receptor–ligand interactions at the interface. By combining morphological and molecular segmentation to delineate the tumor invasive frontier and tumor–stroma boundary, a symbolic distance field can be constructed to quantify layering indices, surface roughness, curvature, and cross-boundary interaction flux. These are further assessed for 3D continuity and curvature through nonlinear registration of multiple slices.

Ordered and permeable interfaces are often associated with greater immune infiltration and improved clinical outcomes. In contrast, serrated or highly curved interfaces, accompanied by strong stromal responses and reduced T cell functionality, suggest a synergistic relationship between structural barriers and immune suppression. According to the tumor–stroma interface band framework proposed by Feng et al. in colorectal cancer, disorganized and accessible tumor–stroma interfaces are associated with enhanced immune infiltration and im-

proved responsiveness to immune checkpoint blockade. Conversely, ordered, dense barrier interfaces formed by cancer-associated fibroblast-ECM (CAF-ECM) are associated with T cell exclusion and a lack of response [89]. Keren et al.'s study using Multiplexed Ion Beam Imaging in triple-negative breast cancer further demonstrated that the organized stratification of the tumor-immune boundary and the enrichment of inhibitory signaling axes are significantly associated with patient prognosis [19].

The ordered stratification of interface structures and the enrichment of immune/suppressive axes are associated with clinical outcomes and therapeutic responses. In cutaneous squamous cell carcinoma, Andrew L. Ji et al. demonstrated that the tumor frontier is characterized by tumor-specific keratin enrichment and is located adjacent to the CAF-endothelial fibrous-vascular ecological niche, accompanied by Treg-CD8 co-localization and CD8 exclusion immune patterns. This suggests that the coupling between interface architecture and immune suppression may represent a critical determinant of variability in response and resistance to immune checkpoint inhibitors [90]. The immune microenvironment at the interface and CAF-mediated signaling axes frequently constitute druggable targets. At the invasive frontier of basal cell carcinoma, tumor-derived Activin A, encoded by inhibin β A, drives ECM remodeling through activin receptor-like kinase 4 on CAFs, highlighting the therapeutic potential of targeting these tumor-stroma interface pathways [91]. This interface pattern is also recapitulated at the spatial protein level across large cohorts and highlights the involvement of the TGF- β /VEGF axis [92]. A similar matrix-receptor-pathway-transcription factor cascade has been validated across multiple cohorts in consensus molecular subtype 2-dominated colorectal cancer, further supporting the stratification and therapeutic potential of interface signatures [93]. Therefore, interface geometry and interactions serve not only as descriptive morphological features but also as predictive indicators of neoadjuvant and perioperative treatment responses, as well as spatially defined targets for combination therapies.

2.2.4. Functional State Spatialization

Pathway and state signatures characterize the functional states of cells, including exhaustion, proliferation, epithelial-mesenchymal transition, and stemness, as well as their associated pathway activities within spatial coordinates. These signatures focus on the spatial layout and co-occurrence/exclusion relationships of these states within the microenvironment. Pathway/State signatures translate specific functional states into spatial hotspots or band-like enrichment patterns, together with their connectivity

and self-correlation characteristics. A common analytical pipeline begins with the calculation of state scores using single-sample gene set enrichment analysis, gene set activity scoring methods, or protein composite markers. This is followed by generating spatial heatmaps and quantifying Moran's I/Geary's C, hotspot area, and connectivity. Finally, these spatial features are integrated with neighborhood and interface characteristics to determine whether they represent structural hotspots [94].

Zhang et al. found that immune hotspots within tumors are not necessarily high-functioning immune activation zones. Instead, these regions are associated with increased B-cell enrichment signals, a decreased CD8+/Treg ratio, and reduced T-B interaction diversity, reflecting an immunosuppressive state profile. This pattern suggests that the spatial position of the hotspot drives the functional state, rather than the functional state determining the spatial position of the hotspot [85]. Combined analyses of multiplex imaging and spatial transcriptomics have further demonstrated that exhaustion hotspots are frequently located on the tumor side of the tumor-immune interface and co-occur with immunosuppressive signaling axes [19]. Therefore, evaluating immune functionality requires more than assessing immune cell abundance or the presence of hotspots; it also necessitates the integrated evaluation of state scores together with hotspot location and type. Ma et al. introduced CalicoST, providing a critical framework for tracing expression signals back to their genetic drivers, thereby closing the loop between spatial evidence, underlying mechanisms, and clinical decision-making [95].

3. AI-Driven Multiscale Data Integration and Virtual Cell Construction

Cells exist and interact in 3D space, and 3D tissue reconstruction enhances our understanding of disease mechanisms [8,12,24]. The key advantage of spatial omics is its spatial sensitivity—the ability to analyze molecular profiles of cells in their original tissue context. This enables the capture of critical features such as immune infiltration, cell-cell interactions, and clonal organization, all of which serve as fundamental building blocks for the development of computable virtual organs [96–98]. Complementary high-dimensional inputs, such as spectral cytometry data profiling immune cell subsets, further refine the cellular representation, particularly for modeling the complex immune landscape relevant to cytotoxic therapies [99,100].

Once spatial omics has revealed the complexity of tumor ecology, the next challenge is to transform these high-dimensional, heterogeneous, and spatiotemporally

distributed data into computable biological units. Addressing this challenge is essential for translating spatial insights into practical clinical applications in diagnosis and treatment. Systematic differences across platforms in resolution, throughput, measurable molecular dimensions, and sampling intervals make information alignment and cross-validation more difficult [49,96]. AI, particularly graph neural networks (GNNs), generative models, and Bayesian temporal models, offer new avenues for cross-modal integration and dynamic simulation, enabling spatial remodeling and providing a novel bridge for translating basic research into clinical practice [56,101–104].

3.1. Unified Representation Specific to Tumors

Tumors can be regarded as heterogeneous cellular systems composed of diverse microenvironments that continuously exchange information, thereby driving dynamic changes. A unified representation for tumors (UR-Tumor) has been introduced as a tumor-specific framework that provides a coherent digital representation at the cellular and microenvironmental levels [17,105]. Virtual cells achieve an operable and shareable state by mapping biological data into the UR space. Through the reconstruction of cellular activity, spatial omics data transform static spatial images into dynamic, continuously updatable virtual cellular frameworks, with the two approaches serving complementary roles [96].

In virtual cell modeling, an ideal strategy is to construct models across three hierarchical levels: molecular entities, single cells, and multicellular systems [106]. When modeling a single molecular species, protein language models may be more suitable for dynamic modeling. However, such approaches may lose fine-grained structural information compared with atomic-level modeling methods [107,108]. When building the state of individual cells, molecular interactions and signal networks are used, and molecular- or imaging-specific representations are employed to construct units [109–111]. Swat et al. used CompuCell3D to develop a multicellular virtual tissue model. In this model, external factors, such as nutrient and oxygen gradients and boundary conditions, were incorporated as constraints, whereas inheritable internal physical parameters of biological entities, including cell adhesion forces and survival thresholds, were treated as adjustable variables [112]. It is essential to obtain stable functional expressions for these units to ensure accurate predictions while maintaining mechanistic interpretability and system scalability. In contrast, constructing multicellular models places greater demands on spatial information. Spatial characteristics in two-dimensional tissue

slices and three-dimensional tissue volumes, such as spatial signatures, can be obtained by simulating with graph learning techniques, for example, GNNs and equivariant neural networks [113–115]. Jeong et al. investigated lung adenocarcinoma and used this model to characterize the exhaustion patterns of CD8⁺ T cells at the tumor interface, supporting the prediction of responses to immune checkpoint blockade [116].

The main task of UR-Tumor is to achieve cross-modal alignment via multi-modal representation. Cross-modal alignment is achieved through the joint training of transcriptomic, proteomic, and morphological data to construct a shared embedding space during the learning process. Contemporary multimodal deep-learning frameworks build a shared latent space. In spatial omics, methods such as Tangram and DestVI are particularly appropriate [103,117]. For instance, combining spatial transcriptomics with multiplex immunofluorescence protein maps via a shared embedding reduces batch effects and inter-slice variations [118–120]. Uncertainty estimation is essential; Bayesian neural networks or Monte Carlo dropout can be used to quantify uncertainty, and when uncertainty exceeds a predefined threshold, data reacquisition and model reevaluation can be initiated, although this may increase the computational burden of the analytical pipeline [121,122]. Spatial batch-effect correction in spatial omics data can be achieved using Harmony or Seurat-based approaches, while spatial regularization can be applied to preserve geometric structure. Such cross-modal alignment also facilitates three-dimensional reconstruction, enabling the inference of tissue-level architecture from a limited number of tissue sections [123–126].

Static models are unable to account for treatment-induced changes and deformation. In contrast, multilevel virtual cell models can incorporate temporal dynamics and achieve long-term information integration through Bayesian filtering or neural differential equation-based approaches. The newly obtained spatial omics data can be used to update the cellular state-transition matrix through particle filtering, thereby predicting the evolution of drug-resistant clones under multiple cycles of chemotherapy [127–129]. In principle, this framework is expected to incorporate new spatial omics data after each treatment cycle, and UR-Tumor will be upgraded from a static substrate to a dynamic representation [130].

AI can combine sequencing and imaging data to create dynamic virtual-cell models for research and clinical applications. In addition to generating animations, this tool offers several additional functionalities. It enables visualization of changes in the expression of numerous

genes across developmental stages and under various conditions throughout an organism's life cycle. These capabilities facilitate the investigation of the tumor microenvironment across multiple spatial and biological scales.

3.2. Oncology-Specific Virtual Instrument

Multimodal foundation models integrating RNA, protein, and DNA data can be assembled into virtual cells and subsequently expanded into patient-specific VTOs [69,131]. Based on UR-Tumor, virtual instrumentation for therapy (VI-Therapy) has been proposed as a specialized virtual instrument that operates on UR-Tumor embeddings to perform a range of patient-level functions and tasks, including therapeutic simulation and clinical decision support. By perturbing the URs of molecules, cells, and tissues as input, the decoder can encode biological entities and predict one or more specific attributes, including physical structure, cell type or state, adaptability, expression patterns, and drug responses [17]. Wenckstern et al. developed VirTues as a representative virtual tissue foundation model capable of learning unified cross-scale representations from spatial proteomics data. Key design features include protein language model embeddings for marker identity encoding, factorized attention mechanisms for processing high-dimensional data, and hierarchical summary tokens that facilitate multi-scale analysis. As demonstrated in the triple-negative breast cancer case in Keren et al.'s study, virtual biomarkers derived from patients' pre-treatment biopsy samples can be used to predict the likelihood of response to anti-PD-L1 therapy. Furthermore, when integrated with additional patient data, these biomarkers enable more accurate assessment of survival outcomes than conventional approaches. Models capable of generating such high-quality tissue representations provide important technical support for VI-Therapy [132].

Virtual biopsy may be implemented as a potential function of VI-Therapy. Through the use of UR-Tumor to generate spatial risk maps highlighting high-risk areas, such as simulating sample collection along vascular pathways in glioblastoma to predict drug infiltration and the dispersion patterns of stem-like tumor cells, these processes can be achieved without additional invasive procedures [133]. In prostate cancer, Sandy Figiel et al. analyzed the gene expression profiles of different prognostic features within a virtual biopsy framework to further investigate tumor heterogeneity [134]. This capability is based on generative adversarial networks or diffusion models, which convert stochastic noise into structured outputs, such as images or text, thereby enabling the imputation of unobserved regions while maintaining the realism of the simulation [135,136].

Virtual tumor models can be evaluated using multi-feature integrative classifiers and performance metrics to enhance virtual diagnosis and treatment planning [137,138].

Therapeutic what-if simulations expand the scope of VI-Therapy, enabling the modeling of local-to-systemic effects associated with dosing, treatment sequencing, and combination regimens. Ji GW et al. developed an immune machine learning model based on gradient boosting machines, demonstrating that dense, reactive stroma restricts immune infiltration and, together with PD-1/PD-L1-mediated functional suppression, defines a high-risk group, thereby revealing an "immune infiltration gradient." [139]. In immunotherapy, such simulations can also be expanded to evaluate the reversal of interface-level exhaustion by PD-1 blockade and to simulate the dynamics of T-cell infiltration [140]. The prioritization of combination strategies can be optimized using Monte Carlo Tree Search or reinforcement learning, thereby improving dose selection, the sequencing of radiotherapy, chemotherapy, and immunotherapy, as well as the design of combination regimens, providing an implementable pathway toward individualized cancer treatment [141–143].

By using AI to transform spatial omics data into a dynamic virtual cell framework, an essential step has been taken toward the precise and scalable translation of basic research findings into clinical oncology applications. UR-Tumor provides a unified representation of cancer-related spatial omics, while VI-Therapy enables clinically oriented simulation; together, they support the construction of VTE.

4. From VTCs to VTOs and Patient-Level Applications

Single-time-point spatial data are difficult to translate directly into practical clinical tools. However, when developing a tumor-specific VTE that integrates AI technologies and spans from virtual cells to VTOs, the use of multiscale modeling is feasible. In addition, by integrating pre- and post-treatment information, a patient-specific feedback loop can be established to help ensure therapeutic effectiveness [144,145]. Compared with previous models, this approach emphasizes online updating, while uncertainty is handled more flexibly to maintain clinical practicality. In this way, clinicians can use the model to optimize treatment strategies based on the specific characteristics and needs of individual patients, while also enabling earlier identification of potential drug resistance [146,147].

4.1. VTE Assembling

The assembly of a VTE can be conceptualized as a multilayered reconstruction process based on spatial data, beginning with the generation of virtual cells and progressively expanding into a three-dimensional, multicellular, multiclonal model that replicates the complex interactions within the TME. Throughout this progression, cellular spatial signatures, such as signaling mechanisms driven by cytokine gradients and receptor activation, molecular gradients shaped by the distribution of oxygen, nutrients, and metabolites, and interface dynamics arising from cell-cell interactions, serve as core components. These elements are implemented through the instantiation of agents, cellular automata, and related modeling frameworks [148,149]. For example, in agent-based models, the state-transition probabilities of agents are adjusted to simulate processes such as clonal expansion and immune evasion [150].

To enhance the realism of VTE, the finite element method can be used in assembly to simulate diffusion

fields and obtain permeability and uneven distributions under drug gradients [151]. State switching, clonal expansion, and immune escape are regulated according to distinct rule sets that govern their interactions under different conditions, thereby enabling the simulation of these processes [15,152].

To ensure the robustness of the model in VTE assembly, uncertainty management is required. By introducing Monte Carlo sampling or Bayesian inference at each stage of the simulation, the model can quantify parametric uncertainty. If this uncertainty exceeds a predefined threshold, it should be determined whether the model parameters require readjustment or whether additional data needs to be collected [153]. This robustness makes VTE particularly applicable to highly complex tumors, such as glioblastoma [154]. VTE, when integrated with digital twin ecosystems, can be extended to the whole-body scale to model metastatic spread and systemic responses [152] (Figure 3).

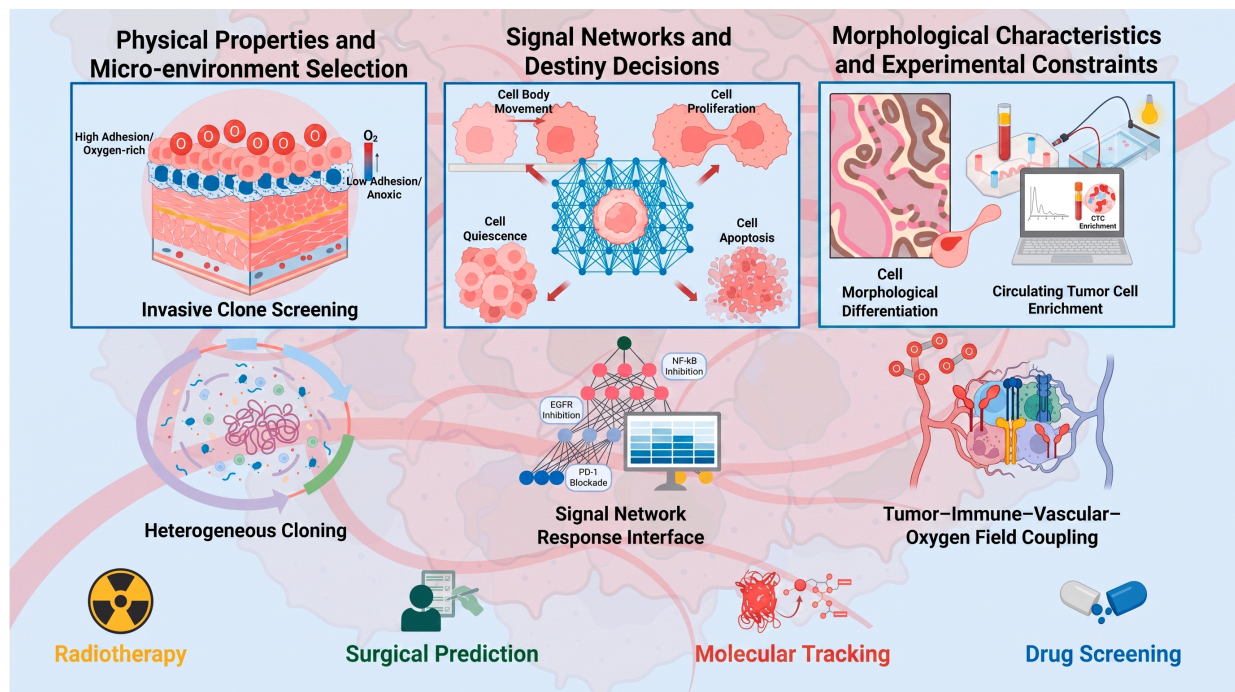


Figure 3: Schematic diagram illustrating the cross-scale role of VTCs in advancing toward patient-level clinical applications. Reproduced with permission from Bai and Su [155].

4.2. Patient-Level Digital Update Pipeline

VTE construction requires the integration of data from multiple sources, including electronic health records, genomic information, imaging examinations, and laboratory

indicators; otherwise, it cannot provide a robust foundation for digitalization. In terms of clinical application, VTE needs to be integrated into a system-level digital update framework and, through a closed-loop feedback mechanism, continuously optimize the pathway from signal acquisition to decision-making output. During data

acquisition, sparse sampling of key molecular features and spatial compositional analysis are combined with AI-driven image–morphology registration to establish an initial VTE evaluation model. Here, scalable and robust spatial clustering algorithms are required to rapidly identify discontinuous tissue domains and microenvironmental niches from sparse clinical inputs, ensuring timely model recalibration [156]. Over time, new data will be introduced into the system. Tools such as Kalman filters can be used to update model states promptly when new observations become available within the UR-Tumor framework. It also uses the area under the receiver operating characteristic curve and other performance indicators to monitor its own performance [152,157]. To ensure that these updates reflect the evolution of living organisms rather than discrepancies arising from different operating conditions, robust computational alignment is required [158].

Batch effects must be eliminated while preserving genuine biological differences [159]. Out-of-distribution detectors can be incorporated into the VTE during monitoring. These tools have been implemented using a variety of models and training datasets and generally operate effectively without significant complications. These tools can measure uncertainty in the monitored data stream. When confidence falls below a certain threshold, the system may recommend a repeat biopsy or additional imaging [160]. Human verification remains essential. Experts must ensure that tumor representations within the digital twin environment accurately reflect biological reality. They must verify that model-generated predictions are consistent with clinical judgment and laboratory findings. Pharmaceutical companies and research institutes have begun exploring the clinical applications of digital twins. The development of a comprehensive digital twin for each patient remains a major objective of future medicine [152].

To enable broader clinical implementation and enable its use by doctors, several key challenges need to be addressed [161]. For clinical implementation, the system needs to integrate with existing clinical workflows. It should request new data or updates only at points when doctors typically make decisions. Alternatively, the system can be continuously monitored, and an update can be triggered when uncertainty is detected; the threshold for “uncertainty” is determined by the level of risk the patient can tolerate. The model must also provide answers that clinicians can apply in practice. These predictions must be validated in real-world clinical settings to determine whether the anticipated outcomes, such as tumor reduction and prolonged recurrence-free survival, can be achieved. Performance metrics such as the area under the curve and

the C-index can be used to evaluate these tools. When presenting information to clinicians, the model should display results through a clear interface with informative visualizations, such as risk maps, side-by-side comparisons of treatment options, and a ranked list of interventions by predicted effectiveness [162].

4.3. The Current State of Digital Twin Development

At the cellular level, researchers have integrated six omics modalities across cell lines and human tissue samples from ten tumor types to construct a database of protein and peptide targets associated with multiple therapeutic pathways, enabling the simulation of drug–cell interactions and the development of resistance [146,163]. At the tissue-organ level, virtual tumor platforms such as Oncosimulator integrate genomics, proteomics, metabolomics, and imaging data to reconstruct spatiotemporal evolution at the supracellular and tissue levels. Wang et al. calibrated data from mouse bladder cancer studies to construct virtual tumors containing high- and low-antigenicity tumor cells and incorporating different cytotoxic T-lymphocyte killing mechanisms. By comparing agent-based models with ordinary differential equation models, it was found that the spatial complexity of the TME significantly influences the efficacy of PD-1/PD-L1 immune checkpoint inhibitors [164]. Within the European CHIC project, this platform has been applied to preoperative chemotherapy planning for Wilms tumor and to radiotherapy response prediction in non-small cell lung cancer, demonstrating a high degree of concordance with actual clinical outcomes [145,165]. At the patient level, the GLIOMATH study achieved clinical validation of an individualized biomechanical model for glioblastoma, which utilizes patient magnetic resonance imaging data to reconstruct a biochemically and mechanically coupled virtual TME. The model achieved 99% agreement with the observed tumor growth pattern in a 55-year-old patient over an eight-month period. Therefore, it became the first system to enable postoperative tumor digital twin monitoring and treatment planning [166]. Analogous systems, including CPDT and the DITTO visual digital twin platform, have also developed individualized cancer evolution models and interactive virtual patient frameworks based on multimodal data for breast cancer and head-and-neck malignancies, respectively [167,168].

By integrating diverse data modalities, VTE bridges fundamental biology and translational medicine. This will help promote the development of precision oncology. Cancer research is increasingly applying the concept of digital twins to help scientists and doctors address the com-

plexity and challenges associated with drug development in laboratory studies and clinical trials. This provides a foundation for tailoring treatment adjustments to individual patients in clinical practice.

5. Limitations and Challenges

Despite the considerable clinical potential of combining spatial omics with VTOs, the field still faces substantial challenges. First, cross-modal alignment and integration remain technically immature. Integrating spatial transcriptomic, proteomic, and epigenomic data into a unified representational format is prone to failure modes. Although AI can assist with unification by leveraging large-scale data, both the construction of faithful virtual cell models and the realization of patient-updatable VTEs impose stringent demands on cross-modal data quality and analytic pipelines [169,170]. In the near term, efforts to integrate data across institutions are likely to face challenges stemming from batch effects and interoperability issues arising from differences in spatial omics platforms and protocols, further compromising model robustness and generalizability.

At the same time, current approaches for constructing virtual cells and organ remain immature. Although methodological advances have been achieved in this emerging field, validation evidence and real-world clinical applications remain limited [171]. Progress is constrained by the limited availability of measured data and the lack of standardized approaches for model development.

Second, the application of deep learning in this context involves inherent methodological risks that must be recognized [172]. One challenge is that deep neural networks often operate as black boxes, making it difficult for clinicians to understand the rationale underlying model-generated predictions and decisions. If the rationale for establishing virtual models remains unclear, clinicians may be reluctant to trust these models when planning critical treatments. Generative AI models used to create virtual cells may also produce biologically plausible but unsupported outputs, often described as hallucinations [173,174]. If model outputs remain insufficiently interpretable, their adoption as clinical decision-support tools will be limited. In addition, generative AI systems may produce fabricated content, generating biologically plausible details that appear realistic despite not being supported by the underlying data [162,175].

In addition, spatial analysis based on biopsy samples is limited by the representativeness of the biopsy, and factors such as tissue subtypes, tumor grades, and clonal compositions may all influence expression patterns, thereby

affecting the credibility of virtual-organ predictions [134]. Although spatial omics workflows can be naturally integrated into routine histopathological processes and complement virtual cell construction, their clinical translation still requires standardized procedures and robust validation frameworks [15]. Particularly when these models are used to support clinical decision-making, their outputs should be transparent and verifiable. As reported by Sandy Figiel et al., although virtual tumor biopsies revealed substantial differences in gene expression among distinct grade groups and tumor clones at the bulk level, such analyses may still lead to inaccurate characterization of gene signatures [134].

In addition, a fundamental challenge lies in the data's scale and complexity. Although the volume of biological sequence data is enormous, the capacity of mainstream AI training corpora remains limited. Systematic research on the scaling laws governing model performance is required, along with robust strategies for filtering and accurately discarding redundant data [176]. In addition, the construction and continuous updating of patient-specific digital twins require substantial computing power; large-scale spatial omics data processing and complex AI model training need significant graphics processing unit resources and scalable cloud infrastructure [172].

In addition to the technical and logistical challenges involved, the use of patient data to construct clinical VTE and digital twin models raises significant concerns about data privacy, project governance, and ethical oversight. How to leverage existing AI ethics frameworks and integrate them with medical regulation to build an ethical framework for patient digital models remains a pressing and unresolved issue [172,177].

6. Outlook

As foundation models capable of joint pretraining across RNA, protein, and epigenetic modalities continue to advance, the transition from “virtual cells” to truly patient-specific “VTOs” may become achievable. These systems could be used to perform virtual biopsies at each treatment cycle and to simulate and evaluate therapeutic regimens, thereby converting spatial omics evidence from tumor cells into calibrated, continuously adaptable personalized clinical decisions. Finally, through VTC-based VTOs, multi-institutional data sharing, standardized platforms, and cross-disciplinary collaboration, VTOs may become a core infrastructure of precision oncology, accelerating the digital transformation of cancer care [178].

7. Conclusions

Spatial omics technologies are transforming the understanding of tumor ecology by revealing the molecular, cellular, and spatial organization of the TME. When integrated with VTEs, the data summarized above have the potential to be translated into practical applications in precision oncology. By constructing UR-Tumor and VI-Therapy, dynamic VTCs can serve as a computational foundation for simulating tumor development, predicting drug responses, and developing personalized treatment plans.

Future research should focus on standardizing data integration pipelines, improving model transparency, strengthening multi-institutional data sharing, and enabling the development of clinically validated, continuously updatable digital twins. Finally, dynamic VTEs have the potential to bridge the gap between molecular evidence and clinical application in cancer treatment, thereby advancing the digitalization of oncology care.

List of Abbreviations

AI	Artificial Intelligence
ATAC	Assay for Transposase-Accessible Chromatin
ATAC-seq	Assay for Transposase-Accessible Chromatin Sequencing
CAF	Cancer-Associated Fibroblast
CNV	Copy-Number Variation
CUT&Tag	Cleavage Under Targets and Tagmentation
DBiT-seq	Deterministic Barcoding in Tissue Sequencing
DESI-MSI	Desorption Electrospray Ionization Mass Spectrometry Imaging
ECM	Extracellular Matrix
EMT	Epithelial-Mesenchymal Transition
ExSeq	Expansion Sequencing
FF	Fresh Frozen
FFPE	Formalin-Fixed, Paraffin-Embedded
Geo-seq	Geographical Positioning Sequencing
GNNs	Graph Neural Networks
H&E	Hematoxylin and Eosin
IF	Immunofluorescence
IHC	Immunohistochemistry
IMC	Imaging Mass Cytometry
LCM	Laser Capture Microdissection
MALDI-MSI	Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging
MERFISH	Multiplexed Error-Robust Fluorescence in Situ Hybridization

MIBI-TOF	Multiplexed Ion Beam Imaging by Time Of Flight
MSI	Mass Spectrometry Imaging
ROI	Region Of Interest
sciMAP-ATAC	Single-Cell Combinatorial Indexing with Spatial Mapping
seqFISH (+)	Sequential Fluorescence in Situ Hybridization (plus)
STARmap	Spatially Resolved Transcript Amplicon Readout Mapping
STI	Spatial-Temporal-Individualization
TME	Tumor Microenvironment
ToF-SIMS	Time-of-Flight Secondary Ion Mass Spectrometry
UR-Tumor	Unified Representation for Tumor
VI-Therapy	Virtual Instrumentation for Therapy
VTCs	Virtual Tumor Cells
VTE	Virtual Tumor Ecosystem
VTOs	Virtual Tumor Organs
WGBS	Whole-Genome Bisulfite Sequencing
WTA	Whole Transcriptome Atlas

Author Contributions

Conceptualization: C.L. and K.L.; Investigation and formal analysis: K.L.; Visualization: K.L. and R.Z.; Writing—original draft preparation: K.L.; Writing—review and editing: C.L., K.L., Y.L., G.H. and R.Z.; Supervision: C.L. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

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AI Declaration

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References

- [1] Galon, J.; Bruni, D. Approaches to Treat Immune Hot, Altered and Cold Tumors with Combination Immunotherapies. *Nat. Rev. Drug Discov.* **2019**, *18*, 197–218. [[CrossRef](#)] [[PubMed](#)]

- [2] Janiszewska, M. The Microcosmos of Intratumor Heterogeneity: The Space-Time of Cancer Evolution. *Oncogene* **2019**, *39*, 2031–2039. [[CrossRef](#)] [[PubMed](#)]
- [3] Fan, J.; Slowikowski, K.; Zhang, F. Single-Cell Transcriptomics in Cancer: Computational Challenges and Opportunities. *Exp. Mol. Med.* **2020**, *52*, 1452–1465. [[CrossRef](#)] [[PubMed](#)]
- [4] McGranahan, N.; Swanton, C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell* **2017**, *168*, 613–628. [[CrossRef](#)] [[PubMed](#)]
- [5] Mo, C.-K.; Liu, J.; Chen, S.; Storrs, E.; Targino da Costa, A.L.N.; Houston, A.; Wendl, M.C.; Jayasinghe, R.G.; Iglesia, M.D.; Ma, C.; et al. Tumor Evolution and Microenvironment Interactions in 2D and 3D Space. *Nature* **2024**, *634*, 1178–1186. [[CrossRef](#)] [[PubMed](#)]
- [6] Qu, Y.; Dou, B.; Tan, H.; Feng, Y.; Wang, N.; Wang, D. Tumor Microenvironment-Driven Non-Cell-Autonomous Resistance to Antineoplastic Treatment. *Mol. Cancer* **2019**, *18*, 69. [[CrossRef](#)] [[PubMed](#)]
- [7] Schott, M.; León-Periñán, D.; Splendiani, E.; Strenger, L.; Licha, J.R.; Pentimalli, T.M.; Schallenberg, S.; Alles, J.; Samut Tagliaferro, S.; Boltengagen, A.; et al. Open-ST: High-Resolution Spatial Transcriptomics in 3D. *Cell* **2024**, *187*, 3953–3972. [[CrossRef](#)] [[PubMed](#)]
- [8] Pentimalli, T.M.; Karaikos, N.; Rajewsky, N. Challenges and Opportunities in the Clinical Translation of High-Resolution Spatial Transcriptomics. *Annu. Rev. Pathol. Mech. Dis.* **2025**, *20*, 405–432. [[CrossRef](#)] [[PubMed](#)]
- [9] Gulati, G.S.; D’Silva, J.P.; Liu, Y.; Wang, L.; Newman, A.M. Profiling Cell Identity and Tissue Architecture with Single-Cell and Spatial Transcriptomics. *Nat. Rev. Mol. Cell Biol.* **2025**, *26*, 11–31. [[CrossRef](#)] [[PubMed](#)]
- [10] Lewis, S.M.; Asselin-Labat, M.-L.; Nguyen, Q.; Berthelet, J.; Tan, X.; Wimmer, V.C.; Merino, D.; Rogers, K.L.; Naik, S.H. Spatial Omics and Multiplexed Imaging to Explore Cancer Biology. *Nat. Methods* **2021**, *18*, 997–1012. [[CrossRef](#)] [[PubMed](#)]
- [11] Cervilla, S.; Grases, D.; Perez, E.; Real, F.X.; Musulen, E.; Aprea, J.; Esteller, M.; Porta-Pardo, E. Benchmarking of Spatial Transcriptomics Platforms across Six Cancer Types. *bioRxiv* **2025**. [[CrossRef](#)]
- [12] Xu, X.; Su, J.; Zhu, R.; Li, K.; Zhao, X.; Fan, J.; Mao, F. From Morphology to Single-Cell Molecules: High-Resolution 3D Histology in Biomedicine. *Mol. Cancer* **2025**, *24*, 63. [[CrossRef](#)] [[PubMed](#)]
- [13] Hui, T.; Zhou, J.; Yao, M.; Xie, Y.; Zeng, H. Advances in Spatial Omics Technologies. *Small Methods* **2025**, *9*, e2401171. [[CrossRef](#)] [[PubMed](#)]
- [14] Singhal, V.; Chou, N.; Lee, J.; Yue, Y.; Liu, J.; Chock, W.K.; Lin, L.; Chang, Y.-C.; Teo, E.M.L.; Aow, J.; et al. BANKSY Unifies Cell Typing and Tissue Domain Segmentation for Scalable Spatial Omics Data Analysis. *Nat. Genet.* **2024**, *56*, 431–441. [[CrossRef](#)] [[PubMed](#)]
- [15] De Domenico, M.; Allegri, L.; Caldarelli, G.; d’Andrea, V.; Di Camillo, B.; Rocha, L.M.; Rozum, J.; Sbarbati, R.; Zambelli, F. Challenges and Opportunities for Digital Twins in Precision Medicine from a Complex Systems Perspective. *npj Digit. Med.* **2025**, *8*, 37. [[CrossRef](#)] [[PubMed](#)]
- [16] Mollica, L.; Leli, C.; Sottotetti, F.; Quaglini, S.; Locati, L.D.; Marcegaglia, S. Digital Twins: A New Paradigm in Oncology in the Era of Big Data. *ESMO Real World Data Digit. Oncol.* **2024**, *5*, 100056. [[CrossRef](#)] [[PubMed](#)]
- [17] Bunne, C.; Roohani, Y.; Rosen, Y.; Gupta, A.; Zhang, X.; Roed, M.; Alexandrov, T.; AlQuraishi, M.; Brennan, P.; Burkhardt, D.B.; et al. How to Build the Virtual Cell with Artificial Intelligence: Priorities and Opportunities. *Cell* **2024**, *187*, 7045–7063. [[CrossRef](#)] [[PubMed](#)]
- [18] Williams, H.L.; Frei, A.L.; Koessler, T.; Berger, M.D.; Dawson, H.; Michielin, O.; Zlobec, I. The Current Landscape of Spatial Biomarkers for Prediction of Response to Immune Checkpoint Inhibition. *npj Precis. Oncol.* **2024**, *8*, 178. [[CrossRef](#)] [[PubMed](#)]
- [19] Keren, L.; Bosse, M.; Marquez, D.; Angoshtari, R.; Jain, S.; Varma, S.; Yang, S.-R.; Kurian, A.; Van Valen, D.; West, R.; et al. A Structured Tumor-Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Ion Beam Imaging. *Cell* **2018**, *174*, 1373–1387.e19. [[CrossRef](#)] [[PubMed](#)]
- [20] Ciepla, J.; Smolarczyk, R. Tumor Hypoxia Unveiled: Insights into Microenvironment, Detection Tools and Emerging Therapies. *Clin. Exp. Med.* **2024**, *24*, 235. [[CrossRef](#)] [[PubMed](#)]
- [21] Shen, X.; Pan, D.; Gong, Q.; Gu, Z.; Luo, K. Enhancing Drug Penetration in Solid Tumors via Nanomedicine: Evaluation Models, Strategies and Perspectives. *Bioact. Mater.* **2024**, *32*, 445–472. [[CrossRef](#)] [[PubMed](#)]
- [22] Wang, H.; Arulraj, T.; Ippolito, A.; Popel, A.S. From Virtual Patients to Digital Twins in Immunology: Lessons Learned from Mechanistic Quantitative Systems Pharmacology Modeling. *npj Digit. Med.* **2024**, *7*, 189. [[CrossRef](#)] [[PubMed](#)]
- [23] Eng, C.-H.L.; Lawson, M.; Zhu, Q.; Dries, R.; Koulina, N.; Takei, Y.; Yun, J.; Cronin, C.; Karp, C.; Yuan, G.-C.; et al. Transcriptome-Scale Super-Resolved Imaging in Tissues by RNA seqFISH. *Nature* **2019**, *568*, 235–239. [[CrossRef](#)] [[PubMed](#)]
- [24] Cadinu, P.; Sivanathan, K.N.; Misra, A.; Xu, R.J.; Mangani, D.; Yang, E.; Rone, J.M.; Tooley, K.; Kye, Y.-C.; Bod, L.; et al. Charting the Cellular Biogeography in Colitis Reveals Fibroblast Trajectories and Coordinated Spatial Remodeling. *Cell* **2024**, *187*, 2010–2028. [[CrossRef](#)] [[PubMed](#)]
- [25] Marco Salas, S.; Kuemmerle, L.B.; Mattsson-Langseth, C.; Tismeyer, S.; Avenel, C.; Hu, T.; Rehman, H.; Grillo, M.; Czarnewski, P.; Helgadottir, S.; et al. Optimizing Xenium In Situ Data Utility by Quality Assessment and Best-Practice Anal-

- p>
ysis Workflows.
- Nat. Methods*
- 2025**
- ,
- 22*
- , 813–823. [
- [CrossRef](#)
-] [
- [PubMed](#)
-]
- [26] Liu, Y.; Dai, Y.; Wang, L. Spatial Omics at the Forefront: Emerging Technologies, Analytical Innovations, and Clinical Applications. *Cancer Cell* **2026**, *44*, 24–49. [[CrossRef](#)] [[PubMed](#)]
- [27] Alon, S.; Goodwin, D.R.; Sinha, A.; Wassie, A.T.; Chen, F.; Daugharthy, E.R.; Bando, Y.; Kajita, A.; Xue, A.G.; Marrett, K.; et al. Expansion Sequencing: Spatially Precise In Situ Transcriptomics in Intact Biological Systems. *Science* **2021**, *371*, eaax2656. [[CrossRef](#)] [[PubMed](#)]
- [28] To, K.; Fei, L.; Pett, J.P.; Roberts, K.; Blain, R.; Polański, K.; Li, T.; Yayon, N.; He, P.; Xu, C.; et al. A Multi-Omic Atlas of Human Embryonic Skeletal Development. *Nature* **2024**, *635*, 657–667. [[CrossRef](#)] [[PubMed](#)]
- [29] Shi, H.; He, Y.; Zhou, Y.; Huang, J.; Maher, K.; Wang, B.; Tang, Z.; Luo, S.; Tan, P.; Wu, M.; et al. Spatial Atlas of the Mouse Central Nervous System at Molecular Resolution. *Nature* **2023**, *622*, 552–561. [[CrossRef](#)] [[PubMed](#)]
- [30] Lee, J.H.; Daugharthy, E.R.; Scheiman, J.; Kalhor, R.; Yang, J.L.; Ferrante, T.C.; Terry, R.; Jeanty, S.S.F.; Li, C.; Amamoto, R.; et al. Highly Multiplexed Subcellular RNA Sequencing In Situ. *Science* **2014**, *343*, 1360–1363. [[CrossRef](#)] [[PubMed](#)]
- [31] Zhao, Y.; Li, Y.; He, Y.; Wu, J.; Liu, Y.; Li, X.; Li, Z.; Yuan, Q.; Li, J.; Zhang, X.; et al. Stereo-seq V2: Spatial Mapping of Total RNA on FFPE Sections with High Resolution. *Cell* **2025**, *188*, 6554–6571. [[CrossRef](#)] [[PubMed](#)]
- [32] Cable, D.M.; Murray, E.; Shanmugam, V.; Zhang, S.; Zou, L.S.; Diao, M.; Chen, H.; Macosko, E.Z.; Irizarry, R.A.; Chen, F. Cell Type-Specific Inference of Differential Expression in Spatial Transcriptomics. *Nat. Methods* **2022**, *19*, 1076–1087. [[CrossRef](#)] [[PubMed](#)]
- [33] Russell, A.J.C.; Weir, J.A.; Nadaf, N.M.; Shabet, M.; Kumar, V.; Kambhampati, S.; Raichur, R.; Marrero, G.J.; Liu, S.; Balderrama, K.S.; et al. Slide-Tags Enables Single-Nucleus Barcoding for Multimodal Spatial Genomics. *Nature* **2023**, *625*, 101–109. [[CrossRef](#)] [[PubMed](#)]
- [34] Zhu, M.; Li, N.; Fan, L.; Wu, R.; Cao, L.; Ren, Y.; Lu, C.; Zhang, L.; Cai, Y.; Shi, Y.; et al. Single-Cell Transcriptomic and Spatial Analysis Reveal the Immunosuppressive Microenvironment in Relapsed/Refractory Angioimmunoblastic T-Cell Lymphoma. *Blood Cancer J.* **2024**, *14*, 218. [[CrossRef](#)] [[PubMed](#)]
- [35] McCaffrey, E.F.; Donato, M.; Keren, L.; Chen, Z.; Delmastro, A.; Fitzpatrick, M.B.; Gupta, S.; Greenwald, N.F.; Baranski, A.; Graf, W.; et al. The Immunoregulatory Landscape of Human Tuberculosis Granulomas. *Nat. Immunol.* **2022**, *23*, 318–329. [[CrossRef](#)] [[PubMed](#)]
- [36] Monasterio, G.; Morales, R.A.; Bejarano, D.A.; Abalo, X.M.; Fransson, J.; Larsson, L.; Schlitzer, A.; Lundeberg, J.; Das, S.; Villablanca, E.J. A Versatile Tissue-Rolling Technique for Spatial-Omics Analyses of the Entire Murine Gastrointestinal Tract. *Nat. Protoc.* **2024**, *19*, 3085–3137. [[CrossRef](#)] [[PubMed](#)]
- [37] Millian, D.E.; Saldarriaga, O.A.; Wanninger, T.; Burks, J.K.; Rafati, Y.N.; Gosnell, J.; Stevenson, H.L. Cutting-Edge Platforms for Analysis of Immune Cells in the Hepatic Microenvironment-Focus on Tumor-Associated Macrophages in Hepatocellular Carcinoma. *Cancers* **2022**, *14*, 1861. [[CrossRef](#)] [[PubMed](#)]
- [38] Zhao, T.; Chiang, Z.D.; Morriss, J.W.; LaFave, L.M.; Murray, E.M.; Del Priore, I.; Meli, K.; Lareau, C.A.; Nadaf, N.M.; Li, J.; et al. Spatial Genomics Enables Multi-Modal Study of Clonal Heterogeneity in Tissues. *Nature* **2021**, *601*, 85–91. [[CrossRef](#)] [[PubMed](#)]
- [39] Zhao, L.; Wu, X.; Zheng, J.; Dong, D. DNA Methylation Profiling of Circulating Tumor Cells in Lung Cancer at Single Base-Pair Resolution. *Oncogene* **2021**, *40*, 1884–1895. [[CrossRef](#)] [[PubMed](#)]
- [40] Tang, L.P.; Zhai, L.M.; Li, J.; Gao, Y.; Ma, Q.L.; Li, R.; Liu, Q.F.; Zhang, W.J.; Yao, W.J.; Mu, B.; et al. Time-Resolved Reprogramming of Single Somatic Cells into Totipotent States during Plant Regeneration. *Cell* **2025**, *188*, 6923–6938. [[CrossRef](#)] [[PubMed](#)]
- [41] Chen, J.; Suo, S.; Tam, P.P.; Han, J.-D.J.; Peng, G.; Jing, N. Spatial Transcriptomic Analysis of Cryosectioned Tissue Samples with Geo-Seq. *Nat. Protoc.* **2017**, *12*, 566–580. [[CrossRef](#)]
- [42] Thornton, C.A.; Mulqueen, R.M.; Torkenczy, K.A.; Nishida, A.; Lowenstein, E.G.; Fields, A.J.; Steemers, F.J.; Zhang, W.; McConnell, H.L.; Wolter, R.L.; et al. Spatially Mapped Single-Cell Chromatin Accessibility. *Nat. Commun.* **2021**, *12*, 1274. [[CrossRef](#)] [[PubMed](#)]
- [43] Deng, Y.; Bartosovic, M.; Kukanja, P.; Zhang, D.; Liu, Y.; Su, G.; Enniful, A.; Bai, Z.; Castelo-Branco, G.; Fan, R. Spatial-CUT&Tag: Spatially Resolved Chromatin Modification Profiling at the Cellular Level. *Science* **2022**, *375*, 681–686. [[CrossRef](#)] [[PubMed](#)]
- [44] Deng, Y.; Bartosovic, M.; Ma, S.; Zhang, D.; Kukanja, P.; Xiao, Y.; Su, G.; Liu, Y.; Qin, X.; Rosoklija, G.B.; et al. Spatial Profiling of Chromatin Accessibility in Mouse and Human Tissues. *Nature* **2022**, *609*, 375–383. [[CrossRef](#)] [[PubMed](#)]
- [45] Planque, M.; Igelmann, S.; Ferreira Campos, A.M.; Fendt, S.-M. Spatial Metabolomics Principles and Application to Cancer Research. *Curr. Opin. Chem. Biol.* **2023**, *76*, 102362. [[CrossRef](#)] [[PubMed](#)]
- [46] Kumar, B.S. Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) in Disease Diagnosis: An Overview. *Anal. Methods* **2023**, *15*, 3768–3784. [[CrossRef](#)] [[PubMed](#)]
- [47] Yoon, S.; Lee, T.G. Biological Tissue Sample Preparation for Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) Imaging. *Nano Conver.* **2018**, *5*, 24. [[CrossRef](#)] [[PubMed](#)]

- [48] Cheng, Y.; Burrack, R.K.; Li, Q. Spatially Resolved and Highly Multiplexed Protein and RNA In Situ Detection by Combining CODEX with RNAscope In Situ Hybridization. *J. Histochem. Cytochem.* **2022**, *70*, 571–581. [\[CrossRef\]](#) [\[PubMed\]](#)
- [49] Marconato, L.; Palla, G.; Yamauchi, K.A.; Virshup, I.; Heidari, E.; Treis, T.; Vierdag, W.-M.; Toth, M.; Stockhaus, S.; Shrestha, R.B.; et al. SpatialData: An Open and Universal Data Framework for Spatial Omics. *Nat. Methods* **2024**, *22*, 58–62. [\[CrossRef\]](#) [\[PubMed\]](#)
- [50] Vicari, M.; Mirzazadeh, R.; Nilsson, A.; Shariatgorji, R.; Bjärterot, P.; Larsson, L.; Lee, H.; Nilsson, M.; Foyer, J.; Ekvall, M.; et al. Spatial Multimodal Analysis of Transcriptomes and Metabolomes in Tissues. *Nat. Biotechnol.* **2023**, *42*, 1046–1050. [\[CrossRef\]](#) [\[PubMed\]](#)
- [51] Liu, Y.; Yang, M.; Deng, Y.; Su, G.; Enniful, A.; Guo, C.C.; Tebaldi, T.; Zhang, D.; Kim, D.; Bai, Z.; et al. High-Spatial-Resolution Multi-Omics Sequencing via Deterministic Barcoding in Tissue. *Cell* **2020**, *183*, 1665–1681. [\[CrossRef\]](#) [\[PubMed\]](#)
- [52] Galeano Niño, J.L.; Wu, H.; LaCourse, K.D.; Kempchinsky, A.G.; Baryames, A.; Barber, B.; Futran, N.; Houlton, J.; Sather, C.; Sicinska, E.; et al. Effect of the Intratumoral Microbiota on Spatial and Cellular Heterogeneity in Cancer. *Nature* **2022**, *611*, 810–817. [\[CrossRef\]](#) [\[PubMed\]](#)
- [53] Rao, A.; Barkley, D.; França, G.S.; Yanai, I. Exploring Tissue Architecture Using Spatial Transcriptomics. *Nature* **2021**, *596*, 211–220. [\[CrossRef\]](#) [\[PubMed\]](#)
- [54] Gyllborg, D.; Langseth, C.M.; Qian, X.; Choi, E.; Salas, S.M.; Hilscher, M.M.; Lein, E.S.; Nilsson, M. Hybridization-Based In Situ Sequencing (HybISS) for Spatially Resolved Transcriptomics in Human and Mouse Brain Tissue. *Nucleic Acids Res.* **2020**, *48*, e112. [\[CrossRef\]](#) [\[PubMed\]](#)
- [55] Rodrigues, S.G.; Stickels, R.R.; Goeva, A.; Martin, C.A.; Murray, E.; Vanderburg, C.R.; Welch, J.; Lin, J.Q.; Chen, F.; Zhuang, X. Slide-Seq: A Scalable Technology for Measuring Genome-Wide Expression at High Spatial Resolution. *Science* **2019**, *363*, 1463–1467. [\[CrossRef\]](#) [\[PubMed\]](#)
- [56] Zhang, Z.; Sun, Y.; Peng, Q.; Li, T.; Zhou, P. Integrating Dynamical Systems Modeling with Spatiotemporal scRNA-Seq Data Analysis. *Entropy* **2025**, *27*, 453. [\[CrossRef\]](#) [\[PubMed\]](#)
- [57] Zhang, J.; Hou, L.; Ma, L.; Cai, Z.; Ye, S.; Liu, Y.; Ji, P.; Zuo, Z.; Zhao, F. Real-Time and Programmable Transcriptome Sequencing with PROFIT-Seq. *Nat. Cell Biol.* **2024**, *26*, 2183–2194. [\[CrossRef\]](#) [\[PubMed\]](#)
- [58] Kip, A.M.; Valverde, J.M.; Altelaar, M.; Heeren, R.M.A.; Hundscheid, I.H.R.; Dejong, C.H.C.; Olde Damink, S.W.M.; Balluff, B.; Lenaerts, K. Combined Quantitative (Phospho)proteomics and Mass Spectrometry Imaging Reveal Temporal and Spatial Protein Changes in Human Intestinal Ischemia-Reperfusion. *J. Proteome Res.* **2022**, *21*, 49–66. [\[CrossRef\]](#) [\[PubMed\]](#)
- [59] Goltsev, Y.; Samusik, N.; Kennedy-Darling, J.; Bhate, S.; Hale, M.; Vazquez, G.; Black, S.; Nolan, G.P. Deep Profiling of Mouse Splenic Architecture with CODEX Multiplexed Imaging. *Cell* **2018**, *174*, 968–981.e15. [\[CrossRef\]](#) [\[PubMed\]](#)
- [60] Xu, Z.; Wang, Y.; Xie, T.; Luo, R.; Ni, H.-L.; Xi-ang, H.; Tang, S.; Tan, S.; Fang, R.; Ran, P.; et al. Panoramic Spatial Enhanced Resolution Proteomics (PSERP) Reveals Tumor Architecture and Heterogeneity in Gliomas. *J. Hematol. Oncol.* **2025**, *18*, 58. [\[CrossRef\]](#) [\[PubMed\]](#)
- [61] Bressan, D.; Battistoni, G.; Hannon, G.J. The Dawn of Spatial Omics. *Science* **2023**, *381*, eabq4964. [\[CrossRef\]](#) [\[PubMed\]](#)
- [62] Zhang, D.; Deng, Y.; Kukanja, P.; Agirre, E.; Bartosovic, M.; Dong, M.; Ma, C.; Ma, S.; Su, G.; Bao, S.; et al. Spatial Epigenome-Transcriptome Co-Profiling of Mammalian Tissues. *Nature* **2023**, *616*, 113–122. [\[CrossRef\]](#) [\[PubMed\]](#)
- [63] Zhang, H.; Lu, K.H.; Ebbini, M.; Huang, P.; Lu, H.; Li, L. Mass Spectrometry Imaging for Spatially Resolved Multi-Omics Molecular Mapping. *npj Imaging* **2024**, *2*, 20. [\[CrossRef\]](#) [\[PubMed\]](#)
- [64] Spangenberg, P.; Bessler, S.; Widera, L.; Bottek, J.; Richter, M.; Thiebes, S.; Siemes, D.; Krauß, S.D.; Migas, L.G.; Kasarla, S.S.; et al. msiFlow: Automated Workflows for Reproducible and Scalable Multimodal Mass Spectrometry Imaging and Microscopy Data Analysis. *Nat. Commun.* **2025**, *16*, 1065. [\[CrossRef\]](#) [\[PubMed\]](#)
- [65] Spraggins, J.M.; Djambazova, K.V.; Rivera, E.S.; Migas, L.G.; Neumann, E.K.; Fuetterer, A.; Suetting, J.; Goedecke, N.; Ly, A.; Van de Plas, R.; et al. High-Performance Molecular Imaging with MALDI Trapped Ion-Mobility Time-of-Flight (tim-TOF) Mass Spectrometry. *Anal. Chem.* **2019**, *91*, 14552–14560. [\[CrossRef\]](#) [\[PubMed\]](#)
- [66] Rappez, L.; Stadler, M.; Triana, S.; Gathungu, R.M.; Ovchinnikova, K.; Phapale, P.; Heikenwalder, M.; Alexandrov, T. SpaceM Reveals Metabolic States of Single Cells. *Nat. Methods* **2021**, *18*, 799–805. [\[CrossRef\]](#) [\[PubMed\]](#)
- [67] Li, B.; Bao, F.; Hou, Y.; Li, F.; Li, H.; Deng, Y.; Dai, Q. Tissue Characterization at an Enhanced Resolution across Spatial Omics Platforms with Deep Generative Model. *Nat. Commun.* **2024**, *15*, 6541. [\[CrossRef\]](#) [\[PubMed\]](#)
- [68] Coleman, K.; Schroeder, A.; Loth, M.; Zhang, D.; Park, J.H.; Sung, J.-Y.; Blank, N.; Cowan, A.J.; Qian, X.; Chen, J.; et al. Resolving Tissue Complexity by Multimodal Spatial Omics Modeling with MISO. *Nat. Methods* **2025**, *22*, 530–538. [\[CrossRef\]](#) [\[PubMed\]](#)
- [69] Cui, H.; Tejada-Lapueta, A.; Brbić, M.; Saez-Rodriguez, J.; Cristea, S.; Goodarzi, H.; Lotfollahi, M.; Theis, F.J.; Wang, B. Towards Multimodal Foundation Models in Molecular Cell Biology. *Nature* **2025**, *640*, 623–633. [\[CrossRef\]](#) [\[PubMed\]](#)

- [70] Vandereyken, K.; Sifrim, A.; Thienpont, B.; Voet, T. Methods and Applications for Single-Cell and Spatial Multi-Omics. *Nat. Rev. Genet.* **2023**, *24*, 494–515. [[CrossRef](#)] [[PubMed](#)]
- [71] Kueckelhaus, J.; Frerich, S.; Kada-Benotmane, J.; Koupourtidou, C.; Ninkovic, J.; Dichgans, M.; Beck, J.; Schnell, O.; Heiland, D.H. Inferring Histology-Associated Gene Expression Gradients in Spatial Transcriptomic Studies. *Nat. Commun.* **2024**, *15*, 7280. [[CrossRef](#)] [[PubMed](#)]
- [72] Berglund, E.; Maaskola, J.; Schultz, N.; Friedrich, S.; Marklund, M.; Bergenstr hle, J.; Tarish, F.; Tanoglid , A.; Vickovic, S.; Larsson, L.; et al. Spatial Maps of Prostate Cancer Transcriptomes Reveal an Unexplored Landscape of Heterogeneity. *Nat. Commun.* **2018**, *9*, 2419. [[CrossRef](#)] [[PubMed](#)]
- [73] Greenwald, A.C.; Darnell, N.G.; Hoefflin, R.; Simkin, D.; Mount, C.W.; Gonzalez Castro, L.N.; Harnik, Y.; Dumont, S.; Hirsch, D.; Nomura, M.; et al. Integrative Spatial Analysis Reveals a Multi-Layered Organization of Glioblastoma. *Cell* **2024**, *187*, 2485–2501.e26. [[CrossRef](#)] [[PubMed](#)]
- [74] Landon-Brace, N.; Latour, S.; Innes, B.T.; Nurse, M.; Cadavid, J.L.; Co, I.L.; Tan, C.M.; Nowlan, F.; Drissler, S.; Notta, F.; et al. Analysis of an Engineered Organoid Model of Pancreatic Cancer Identifies Hypoxia as a Contributing Factor in Determining Transcriptional Subtypes. *Sci. Rep.* **2025**, *15*, 23610. [[CrossRef](#)] [[PubMed](#)]
- [75] Terrassoux, L.; Hazard, C.; Laratte, A.; Balsamelli, J.; Arcicasa, M.; Fong, E.; Baland, L.; Soncin, F.; Cappello, G.; Maurage, C.-A.; et al. Novel Diffuse Midline Glioma-on-Chip Recapitulating Tumor Biophysical Microenvironment to Assess the Heterogeneity of Response to Therapies. *Small* **2025**, *21*, e05343. [[CrossRef](#)] [[PubMed](#)]
- [76] Auxillos, J.; Crouigneau, R.; Li, Y.-F.; Dai, Y.; Stigliani, A.; Tavernaro, I.; Resch-Genger, U.; Sandelin, A.; Marie, R.; Pedersen, S.F. Spatially Resolved Analysis of Microenvironmental Gradient Impact on Cancer Cell Phenotypes. *Sci. Adv.* **2024**, *10*, eadn3448. [[CrossRef](#)] [[PubMed](#)]
- [77] Duncan, K.D.; P tro sov , H.; Lum, J.J.; Goodlett, D.R. Mass Spectrometry Imaging Methods for Visualizing Tumor Heterogeneity. *Curr. Opin. Biotechnol.* **2024**, *86*, 103068. [[CrossRef](#)] [[PubMed](#)]
- [78] Folz, J.; Jo, J.; Eido, A.; Gonzalez, M.E.; Caruso, R.; Klee, C.G.; Wang, X. Photoacoustic Chemical Imaging of Tumor Microenvironment pH for Prediction of Chemotherapeutic Efficacy in Breast Cancer. *Nano Lett.* **2025**, *25*, 14808–14816. [[CrossRef](#)] [[PubMed](#)]
- [79] Jove, M.; Spencer, J.; Clench, M.; Loadman, P.M.; Twelves, C. Precision Pharmacology: Mass Spectrometry Imaging and Pharmacokinetic Drug Resistance. *Crit. Rev. Oncol./Hematol.* **2019**, *141*, 153–162. [[CrossRef](#)] [[PubMed](#)]
- [80] Sch rch, C.M.; Bhate, S.S.; Barlow, G.L.; Phillips, D.J.; Noti, L.; Zlobec, I.; Chu, P.; Black, S.; Demeter, J.; McIlwain, D.R.; et al. Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front. *Cell* **2020**, *182*, 1341–1359.e19. [[CrossRef](#)] [[PubMed](#)]
- [81] Dakal, T.C.; George, N.; Xu, C.; Suravajhala, P.; Kumar, A. Predictive and Prognostic Relevance of Tumor-Infiltrating Immune Cells: Tailoring Personalized Treatments against Different Cancer Types. *Cancers* **2024**, *16*, 1626. [[CrossRef](#)] [[PubMed](#)]
- [82] Allam, M.; Hu, T.; Lee, J.; Aldrich, J.; Badve, S.S.; G kmen-Polar, Y.; Bhawe, M.; Ramalingam, S.S.; Schneider, F.; Coskun, A.F. Spatially Variant Immune Infiltration Scoring in Human Cancer Tissues. *npj Precis. Oncol.* **2022**, *6*, 60. [[CrossRef](#)] [[PubMed](#)]
- [83] Griffin, G.K.; Weirather, J.L.; Roemer, M.G.M.; Lipschitz, M.; Kelley, A.; Chen, P.-H.; Gusenleitner, D.; Jeter, E.; Pak, C.; Gjini, E.; et al. Spatial Signatures Identify Immune Escape via PD-1 as a Defining Feature of T-Cell/Histiocyte-Rich Large B-Cell Lymphoma. *Blood* **2021**, *137*, 1353–1364. [[CrossRef](#)] [[PubMed](#)]
- [84] Wu, S.Z.; Al-Eryani, G.; Roden, D.L.; Junankar, S.; Harvey, K.; Andersson, A.; Thennavan, A.; Wang, C.; Torpy, J.R.; Bartonicek, N.; et al. A Single-Cell and Spatially Resolved Atlas of Human Breast Cancers. *Nat. Genet.* **2021**, *53*, 1334–1347. [[CrossRef](#)] [[PubMed](#)]
- [85] Zhang, H.; AbdulJabbar, K.; Moore, D.A.; Akarca, A.; Enfield, K.S.S.; Jamal-Hanjani, M.; Raza, S.E.A.; Veeriah, S.; Salgado, R.; McGranahan, N.; et al. Spatial Positioning of Immune Hotspots Reflects the Interplay between B and T Cells in Lung Squamous Cell Carcinoma. *Cancer Res.* **2023**, *83*, 1410–1425. [[CrossRef](#)] [[PubMed](#)]
- [86] Singhal, V.; Chou, N. BANKSY: Scalable Cell Typing and Domain Segmentation for Spatial Omics. *Nat. Rev. Genet.* **2024**, *25*, 527–528. [[CrossRef](#)] [[PubMed](#)]
- [87] Danenberg, E.; Bardwell, H.; Zanotelli, V.R.T.; Provenzano, E.; Chin, S.-F.; Rueda, O.M.; Green, A.; Rakha, E.; Aparicio, S.; Ellis, I.O.; et al. Breast Tumor Microenvironment Structures Are Associated with Genomic Features and Clinical Outcome. *Nat. Genet.* **2022**, *54*, 660–669. [[CrossRef](#)] [[PubMed](#)]
- [88] Ali, H.R.; Jackson, H.W.; Zanotelli, V.R.T.; Danenberg, E.; Fischer, J.R.; Bardwell, H.; Provenzano, E.; Rueda, O.M.; Chin, S.-F.; Aparicio, S.; et al. Imaging Mass Cytometry and Multiplatform Genomics Define the Phenogenomic Landscape of Breast Cancer. *Nat. Cancer* **2020**, *1*, 163–175. [[CrossRef](#)] [[PubMed](#)]
- [89] Feng, Y.; Ma, W.; Zang, Y.; Guo, Y.; Li, Y.; Zhang, Y.; Dong, X.; Liu, Y.; Zhan, X.; Pan, Z.; et al. Spatially Organized Tumor-Stroma Boundary Determines the Efficacy of Immunotherapy in Colorectal Cancer Patients. *Nat. Commun.* **2024**, *15*, 10259. [[CrossRef](#)] [[PubMed](#)]
- [90] Ji, A.L.; Rubin, A.J.; Thrane, K.; Jiang, S.; Reynolds, D.L.; Meyers, R.M.; Guo, M.G.; George, B.M.; Mollbrink, A.; Bergenstr hle, J.; et al. Multimodal Analysis of Composition and Spatial Architecture in Hu-

- p>man Squamous Cell Carcinoma.
- Cell*
- 2020**
- ,
- 182*
- , 497–514.e22. [
- [CrossRef](#)
-] [
- [PubMed](#)
-]
- [91] Yerly, L.; Pich-Bavastro, C.; Di Domizio, J.; Wyss, T.; Tissot-Renaud, S.; Cangkrama, M.; Gilliet, M.; Werner, S.; Kuonen, F. Integrated Multi-Omics Reveals Cellular and Molecular Interactions Governing the Invasive Niche of Basal Cell Carcinoma. *Nat. Commun.* **2022**, *13*, 4897. [[CrossRef](#)] [[PubMed](#)]
- [92] Cords, L.; Engler, S.; Haberecker, M.; Rüschoff, J.H.; Moch, H.; de Souza, N.; Bodenmiller, B. Cancer-Associated Fibroblast Phenotypes Are Associated with Patient Outcome in Non-Small Cell Lung Cancer. *Cancer Cell* **2024**, *42*, 396–412.e5. [[CrossRef](#)] [[PubMed](#)]
- [93] Valdeolivas, A.; Amberg, B.; Giroud, N.; Richardson, M.; Gálvez, E.J.C.; Badillo, S.; Julien-Laferrière, A.; Túrós, D.; Voith von Voithenberg, L.; Wells, I.; et al. Profiling the Heterogeneity of Colorectal Cancer Consensus Molecular Subtypes Using Spatial Transcriptomics. *npj Precis. Oncol.* **2024**, *8*, 10. [[CrossRef](#)] [[PubMed](#)]
- [94] Jiang, R.; Li, Z.; Jia, Y.; Li, S.; Chen, S. SINFONIA: Scalable Identification of Spatially Variable Genes for Deciphering Spatial Domains. *Cells* **2023**, *12*, 604. [[CrossRef](#)] [[PubMed](#)]
- [95] Ma, C.; Balaban, M.; Liu, J.; Chen, S.; Wilson, M.J.; Sun, C.H.; Ding, L.; Raphael, B.J. Inferring Allele-Specific Copy Number Aberrations and Tumor Phylogeography from Spatially Resolved Transcriptomics. *Nat. Methods* **2024**, *21*, 2239–2247. [[CrossRef](#)] [[PubMed](#)]
- [96] Du, J.; Yang, Y.C.; An, Z.J.; Zhang, M.H.; Fu, X.H.; Huang, Z.F.; Yuan, Y.; Hou, J. Advances in Spatial Transcriptomics and Related Data Analysis Strategies. *J. Transl. Med.* **2023**, *21*, 330. [[CrossRef](#)] [[PubMed](#)]
- [97] Jing, S.-Y.; Wang, H.-Q.; Lin, P.; Yuan, J.; Tang, Z.-X.; Li, H. Quantifying and Interpreting Biologically Meaningful Spatial Signatures within Tumor Microenvironments. *npj Precis. Oncol.* **2025**, *9*, 68. [[CrossRef](#)] [[PubMed](#)]
- [98] Lee, R.Y.; Ng, C.W.; Rajapakse, M.P.; Ang, N.; Yeong, J.P.S.; Lau, M.C. The Promise and Challenge of Spatial Omics in Dissecting Tumor Microenvironment and the Role of AI. *Front. Oncol.* **2023**, *13*, 1172314. [[CrossRef](#)] [[PubMed](#)]
- [99] Gupta, P.; Dang, M.; Oberai, S.; Migliozi, S.; Trivedi, R.; Kumar, G.; Peshoff, M.; Milam, N.; Ahmed, A.; Bojja, K.; et al. Immune Landscape of Isocitrate Dehydrogenase-Stratified Primary and Recurrent Human Gliomas. *Neuro-Oncol.* **2024**, *26*, 2239–2255. [[CrossRef](#)] [[PubMed](#)]
- [100] Cordes, M.; Pike-Overzet, K.; Van Den Akker, E.B.; Staal, F.J.T.; Canté-Barrett, K. Multi-Omic Analyses in Immune Cell Development with Lessons Learned from T Cell Development. *Front. Cell Dev. Biol.* **2023**, *11*, 1163529. [[CrossRef](#)] [[PubMed](#)]
- [101] Hu, J.; Li, X.; Coleman, K.; Schroeder, A.; Ma, N.; Irwin, D.J.; Lee, E.B.; Shinohara, R.T.; Li, M. SpaGCN: Integrating Gene Expression, Spatial Location and Histology to Identify Spatial Domains and Spatially Variable Genes by Graph Convolutional Network. *Nat. Methods* **2021**, *18*, 1342–1351. [[CrossRef](#)] [[PubMed](#)]
- [102] Long, Y.; Ang, K.S.; Li, M.; Chong, K.L.K.; Sethi, R.; Zhong, C.; Xu, H.; Ong, Z.; Sachaphibulkij, K.; Chen, A.; et al. Spatially Informed Clustering, Integration, and Deconvolution of Spatial Transcriptomics with GraphST. *Nat. Commun.* **2023**, *14*, 1155. [[CrossRef](#)] [[PubMed](#)]
- [103] Lopez, R.; Li, B.; Keren-Shaul, H.; Boyeau, P.; Kedmi, M.; Pilzer, D.; Jelinski, A.; Yofe, I.; David, E.; Wagner, A.; et al. DestVI Identifies Continuums of Cell Types in Spatial Transcriptomics Data. *Nat. Biotechnol.* **2022**, *40*, 1360–1369. [[CrossRef](#)] [[PubMed](#)]
- [104] Sha, Y.; Qiu, Y.; Zhou, P.; Nie, Q. Reconstructing Growth and Dynamic Trajectories from Single-Cell Transcriptomics Data. *Nat. Mach. Intell.* **2024**, *6*, 25–39. [[CrossRef](#)] [[PubMed](#)]
- [105] Jafari Nivlouei, S.; Soltani, M.; Shirani, E.; Salimpour, M.R.; Travasso, R.; Carvalho, J. A Multiscale Cell-Based Model of Tumor Growth for Chemotherapy Assessment and Tumor-Targeted Therapy through a 3D Computational Approach. *Cell Prolif.* **2022**, *55*, e13187. [[CrossRef](#)] [[PubMed](#)]
- [106] Metzcar, J.; Wang, Y.; Heiland, R.; Macklin, P. A Review of Cell-Based Computational Modeling in Cancer Biology. *JCO Clin. Cancer Inform.* **2019**, *3*, 1–13. [[CrossRef](#)] [[PubMed](#)]
- [107] Peng, F.Z.; Wang, C.; Chen, T.; Schussheim, B.; Vincoff, S.; Chatterjee, P. PTM-Mamba: A PTM-Aware Protein Language Model with Bidirectional Gated Mamba Blocks. *Nat. Methods* **2025**, *22*, 945–949. [[CrossRef](#)] [[PubMed](#)]
- [108] Dai, B.; Mattox, D.E.; Bailey-Kellogg, C. Attention Please: Modeling Global and Local Context in Glycan Structure-Function Relationships. *bioRxiv* **2021**. [[CrossRef](#)]
- [109] Saar, K.L.; Scrutton, R.M.; Blozelyte, K.; Morgunov, A.S.; Good, L.L.; Lee, A.A.; Teichmann, S.A.; Knowles, T.P.J. Protein Condensate Atlas from Predictive Models of Heteromolecular Condensate Composition. *Nat. Commun.* **2024**, *15*, 5418. [[CrossRef](#)] [[PubMed](#)]
- [110] Theodoris, C.V.; Xiao, L.; Chopra, A.; Chaffin, M.D.; Al Sayed, Z.R.; Hill, M.C.; Mantineo, H.; Brydon, E.M.; Zeng, Z.; Liu, X.S.; et al. Transfer Learning Enables Predictions in Network Biology. *Nature* **2023**, *618*, 616–624. [[CrossRef](#)] [[PubMed](#)]
- [111] Kha, Q.-H.; Ho, Q.-T.; Le, N.Q.K. Identifying SNARE Proteins Using an Alignment-Free Method Based on Multiscan Convolutional Neural Network and PSSM Profiles. *J. Chem. Inf. Model.* **2022**, *62*, 4820–4826. [[CrossRef](#)] [[PubMed](#)]
- [112] Swat, M.H.; Thomas, G.L.; Shirinifard, A.; Clendenon, S.G.; Glazier, J.A. Emergent Stratification in Solid Tumors Selects for Reduced Cohesion of Tumor Cells: A Multi-Cell, Virtual-Tissue Model of

- Tumor Evolution Using CompuCell3D. *PLoS ONE* **2015**, *10*, e0127972. [CrossRef] [PubMed]
- [113] Zhu, H.; Ma, M.; Ma, W.; Jiao, L.; Hong, S.; Shen, J.; Hou, B. A Spatial-Channel Progressive Fusion ResNet for Remote Sensing Classification. *Inf. Fusion* **2021**, *70*, 72–87. [CrossRef]
- [114] Zhang, Z.; Cui, P.; Zhu, W. Deep Learning on Graphs: A Survey. *IEEE Trans. Knowl. Data Eng.* **2022**, *34*, 249–270. [CrossRef]
- [115] Chen, Y.; Chang, H.; Meng, J.; Zhang, D. Ensemble Neural Networks (ENN): A Gradient-Free Stochastic Method. *Neural Netw.* **2019**, *110*, 170–185. [CrossRef] [PubMed]
- [116] Jeong, H.; Oh, J.; Choi, Y.-L. GraphTME: A Framework for Predicting Response to Immune Checkpoint Inhibitors by Interpreting Cell-Cell Interactions in the Tumor Microenvironment Using Spatial Transcriptomics of Tumor Tissue. *bioRxiv* **2025**. [CrossRef]
- [117] Biancalani, T.; Scalia, G.; Buffoni, L.; Avasthi, R.; Lu, Z.; Sanger, A.; Tokcan, N.; Vanderburg, C.R.; Segerstolpe, Å.; Zhang, M.; et al. Deep Learning and Alignment of Spatially Resolved Single-Cell Transcriptomes with Tangram. *Nat. Methods* **2021**, *18*, 1352–1362. [CrossRef] [PubMed]
- [118] Xu, H.; Fu, H.; Long, Y.; Ang, K.S.; Sethi, R.; Chong, K.; Li, M.; Uddamvathanak, R.; Lee, H.K.; Ling, J.; et al. Unsupervised Spatially Embedded Deep Representation of Spatial Transcriptomics. *Genome Med.* **2024**, *16*, 12. [CrossRef] [PubMed]
- [119] Wang, Y.; Cao, G.; Zeng, H.; Zhi, Y.; Xu, M.; Wang, Y.; Liu, M.; Ruan, Y.; Tse, K.Y.; Zhang, Q.; et al. Multi-Omics Analysis Reveals the Transforming Growth Factor- β Signaling-Driven Multicellular Interactions with Prognostic Relevance in Cervical Cancer Progression. *J. Cancer* **2025**, *16*, 2857–2876. [CrossRef] [PubMed]
- [120] Guo, W.; Zhou, B.; Dou, L.; Guo, L.; Li, Y.; Qin, J.; Wang, Z.; Huai, Q.; Xue, X.; Li, Y.; et al. Single-Cell RNA Sequencing and Spatial Transcriptomics of Esophageal Squamous Cell Carcinoma with Lymph Node Metastases. *Exp. Mol. Med.* **2025**, *57*, 59–71. [CrossRef] [PubMed]
- [121] LeCun, Y.; Bengio, Y. Convolutional Networks for Images, Speech, and Time Series. In *Handbook of Brain Theory and Neural Networks*; Arbib, M., Ed.; MIT Press: Cambridge, MA, USA, 1995. Available online: <https://www.researchgate.net/publication/216792820>.
- [122] Baião, A.R.; Cai, Z.; Poulos, R.C.; Robinson, P.J.; Reddel, R.R.; Zhong, Q.; Vinga, S.; Gonçalves, E. A Technical Review of Multi-Omics Data Integration Methods: From Classical Statistical to Deep Generative Approaches. *Brief. Bioinf.* **2025**, *26*, bbaf355. [CrossRef] [PubMed]
- [123] Korsunsky, I.; Millard, N.; Fan, J.; Slowikowski, K.; Zhang, F.; Wei, K.; Baglaenko, Y.; Brenner, M.; Loh, P.-r.; Raychaudhuri, S. Fast, Sensitive and Accurate Integration of Single-Cell Data with Harmony. *Nat. Methods* **2019**, *16*, 1289–1296. [CrossRef] [PubMed]
- [124] Stuart, T.; Butler, A.; Hoffman, P.; Hafemeister, C.; Papalexi, E.; Mauck, W.M.; Hao, Y.; Stoeckius, M.; Smibert, P.; Satija, R. Comprehensive Integration of Single-Cell Data. *Cell* **2019**, *177*, 1888–1902.e21. [CrossRef] [PubMed]
- [125] Fang, Z.; Krusen, K.; Priest, H.; Wang, M.; Kim, S.; Sriram, A.; Yellanki, A.; Singh, A.; Horwitz, E.; Coskun, A.F. Graph-Based 3-Dimensional Spatial Gene Neighborhood Networks of Single Cells in Gels and Tissues. *BME Front.* **2025**, *6*, 0110. [CrossRef] [PubMed]
- [126] Heitz, M.; Ma, Y.; Kubal, S.; Schiebinger, G. Spatial Transcriptomics Brings New Challenges and Opportunities for Trajectory Inference. *Annu. Rev. Biomed. Data Sci.* **2024**, *8*, 1–19. [CrossRef] [PubMed]
- [127] Chen, R.T.Q.; Rubanova, Y.; Bettencourt, J.; Duvenaud, D.K. Neural Ordinary Differential Equations. *arXiv* **2018**, arXiv:1806.07366. [CrossRef]
- [128] Tong, A.; Huang, J.; Wolf, G.; van Dijk, D.; Krishnaswamy, S. TrajectoryNet: A Dynamic Optimal Transport Network for Modeling Cellular Dynamics. *Proc. Mach. Learn. Res.* **2020**, *119*, 9526–9536. [CrossRef]
- [129] Hess, K.; Melnychuk, V.; Frauen, D.; Feuerriegel, S. Bayesian Neural Controlled Differential Equations for Treatment Effect Estimation. *arXiv* **2023**, arXiv:2310.17463. [CrossRef]
- [130] Chen, Y.; Wang, D.; Li, Y.; Qi, L.; Si, W.; Bo, Y.; Chen, X.; Ye, Z.; Fan, H.; Liu, B.; et al. Spatiotemporal Single-Cell Analysis Decodes Cellular Dynamics Underlying Different Responses to Immunotherapy in Colorectal Cancer. *Cancer Cell* **2024**, *42*, 1268–1285.e7. [CrossRef] [PubMed]
- [131] Moor, M.; Banerjee, O.; Abad, Z.S.H.; Krumholz, H.M.; Leskovec, J.; Topol, E.J.; Rajpurkar, P. Foundation Models for Generalist Medical Artificial Intelligence. *Nature* **2023**, *616*, 259–265. [CrossRef] [PubMed]
- [132] Wenckstern, J.; Jain, E.; Vasilev, K.; Pariset, M.; Wicki, A.; Gut, G.; Bunne, C. AI-Powered Virtual Tissues from Spatial Proteomics for Clinical Diagnostics and Biomedical Discovery. *arXiv* **2025**, arXiv:2501.06039. [CrossRef]
- [133] Park, J.E.; Eun, D.; Kim, H.S.; Lee, D.H.; Jang, R.W.; Kim, N. Generative Adversarial Network for Glioblastoma Ensures Morphologic Variations and Improves Diagnostic Model for Isocitrate Dehydrogenase Mutant Type. *Sci. Rep.* **2021**, *11*, 9912. [CrossRef] [PubMed]
- [134] Figiel, S.; Yin, W.; Doultinos, D.; Erickson, A.; Poulou, N.; Singh, R.; Magnussen, A.; Anbarasan, T.; Teague, R.; He, M.; et al. Spatial Transcriptomic Analysis of Virtual Prostate Biopsy Reveals Confounding Effect of Tissue Heterogeneity on Genomic Signatures. *Mol. Cancer* **2023**, *22*, 162. [CrossRef] [PubMed]
- [135] Ho, J.; Jain, A.; Abbeel, P. Denoising Diffusion Probabilistic Models. *Adv. Neural Inf. Process. Syst.* **2020**, *33*, 6840–6851. [CrossRef]

- [136] Mirza, M.; Osindero, S. Conditional Generative Adversarial Nets. *arXiv* **2014**, arXiv:1411.1784. [[Cross-Ref](#)]
- [137] Chowell, D.; Yoo, S.-K.; Valero, C.; Pastore, A.; Krishna, C.; Lee, M.; Hoen, D.; Shi, H.; Kelly, D.W.; Patel, N.; et al. Improved Prediction of Immune Checkpoint Blockade Efficacy across Multiple Cancer Types. *Nat. Biotechnol.* **2022**, *40*, 499–506. [[CrossRef](#)] [[PubMed](#)]
- [138] Tang, J.; Fan, W.; Ruan, Y.; Liu, X.; Qiu, F.; Feng, J.; Huang, G.; Yan, M.; Wang, H.; Mu, Q.; et al. Protein-Based Classification Reveals an Immune-Hot Subtype in IDH Mutant Astrocytoma with Worse Prognosis. *Cancer Cell* **2025**, *43*, 2136–2155.e14. [[CrossRef](#)] [[PubMed](#)]
- [139] Ji, G.-W.; Wang, K.; Xia, Y.-X.; Wang, J.-S.; Wang, X.-H.; Li, X.-C. Integrating Machine Learning and Tumor Immune Signature to Predict Oncologic Outcomes in Resected Biliary Tract Cancer. *Ann. Surg. Oncol.* **2020**, *28*, 4018–4029. [[CrossRef](#)] [[PubMed](#)]
- [140] Zinselmeyer, B.H.; Heydari, S.; Sacristán, C.; Nayak, D.; Cammer, M.; Herz, J.; Cheng, X.; Davis, S.J.; Dustin, M.L.; McGavern, D.B. PD-1 Promotes Immune Exhaustion by Inducing Antiviral T Cell Motility Paralysis. *J. Exp. Med.* **2013**, *210*, 757–774. [[CrossRef](#)] [[PubMed](#)]
- [141] Lin, Y.; Ma, J.; Yuan, H.; Chen, Z.; Xu, X.; Jiang, M.; Zhu, J.; Meng, W.; Qiu, W.; Liu, Y. Integrating Reinforcement Learning and Monte Carlo Tree Search for Enhanced Neoantigen Vaccine Design. *Brief. Bioinf.* **2024**, *25*, bbae247. [[CrossRef](#)] [[PubMed](#)]
- [142] Schmucker, R.; Farina, G.; Faeder, J.; Fröhlich, F.; Saglam, A.S.; Sandholm, T. Combination Treatment Optimization Using a Pan-Cancer Pathway Model. *PLoS Comput. Biol.* **2021**, *17*, e1009689. [[Cross-Ref](#)] [[PubMed](#)]
- [143] Eastman, B.; Przedborski, M.; Kohandel, M. Reinforcement Learning Derived Chemotherapeutic Schedules for Robust Patient-Specific Therapy. *Sci. Rep.* **2021**, *11*, 17882. [[CrossRef](#)] [[PubMed](#)]
- [144] Bordukova, M.; Makarov, N.; Rodriguez-Esteban, R.; Schmich, F.; Menden, M.P. Generative Artificial Intelligence Empowers Digital Twins in Drug Discovery and Clinical Trials. *Expert Opin. Drug Discov.* **2024**, *19*, 33–42. [[CrossRef](#)] [[PubMed](#)]
- [145] D’Amico, S.; Dall’Olio, D.; Sala, C.; Dall’Olio, L.; Sauta, E.; Zampini, M.; Asti, G.; Lanino, L.; Maggioni, G.; Campagna, A.; et al. Synthetic Data Generation by Artificial Intelligence to Accelerate Research and Precision Medicine in Hematology. *JCO Clin. Cancer Inform.* **2023**, *7*, e2300021. [[CrossRef](#)] [[PubMed](#)]
- [146] Shen, S.; Qi, W.; Liu, X.; Zeng, J.; Li, S.; Zhu, X.; Dong, C.; Wang, B.; Shi, Y.; Yao, J.; et al. From Virtual to Reality: Innovative Practices of Digital Twins in Tumor Therapy. *J. Transl. Med.* **2025**, *23*, 348. [[CrossRef](#)] [[PubMed](#)]
- [147] Țefăniță, S.A.; Cordoș, A.A.; Ivascu, T.; Feier, C.V.I.; Muntean, C.; Stupinean, C.V.; Călinici, T.; Aluaș, M.; Bolboacă, S.D. Advancing Precision Oncology with Digital and Virtual Twins: A Scoping Review. *Cancers* **2024**, *16*, 3817. [[CrossRef](#)] [[PubMed](#)]
- [148] Reinoso-Burrows, J.C.; Toro, N.; Cortés-Carmona, M.; Pineda, F.; Henriquez, M.; Galleguillos Madrid, F.M. Cellular Automata Modeling as a Tool in Corrosion Management. *Materials* **2023**, *16*, 6051. [[Cross-Ref](#)] [[PubMed](#)]
- [149] Sadhukhan, S.; Mishra, P.K.; Basu, S.K.; Mandal, J.K. A Multi-Scale Agent-Based Model for Avascular Tumor Growth. *BioSystems* **2021**, *206*, 104450. [[CrossRef](#)] [[PubMed](#)]
- [150] Stephan, S.; Galland, S.; Labbani Narsis, O.; Shoji, K.; Vachenc, S.; Gerart, S.; Nicolle, C. Agent-Based Approaches for Biological Modeling in Oncology: A Literature Review. *Artif. Intell. Med.* **2024**, *152*, 102884. [[CrossRef](#)] [[PubMed](#)]
- [151] Thurber, G.M.; Reiner, T.; Yang, K.S.; Kohler, R.H.; Weissleder, R. Effect of Small-Molecule Modification on Single-Cell Pharmacokinetics of PARP Inhibitors. *Mol. Cancer Ther.* **2014**, *13*, 986–995. [[CrossRef](#)] [[PubMed](#)]
- [152] Bouriga, R.; Bailleux, C.; Gal, J.; Chamorey, E.; Mograbi, B.; Hannoun-Levi, J.-M.; Milano, G. Advances and Critical Aspects in Cancer Treatment Development Using Digital Twins. *Brief. Bioinf.* **2025**, *26*, bbaf237. [[CrossRef](#)] [[PubMed](#)]
- [153] Jørgensen, A.C.S.; Ghosh, A.; Sturrock, M.; Shahrezaei, V. Efficient Bayesian Inference for Stochastic Agent-Based Models. *PLoS Comput. Biol.* **2022**, *18*, e1009508. [[CrossRef](#)] [[PubMed](#)]
- [154] Jørgensen, A.C.S.; Hill, C.S.; Sturrock, M.; Tang, W.; Karamched, S.R.; Gorup, D.; Lythgoe, M.F.; Parrinello, S.; Marguerat, S.; Shahrezaei, V. Data-Driven Spatio-Temporal Modelling of Glioblastoma. *R. Soc. Open Sci.* **2023**, *10*, 221444. [[CrossRef](#)] [[PubMed](#)]
- [155] Bai, L.; Su, J. Artificial Intelligence Virtual Organoids (AIVOs). *Bioact. Mater.* **2026**, *59*, 45–68. [[CrossRef](#)] [[PubMed](#)]
- [156] Yuan, Z.; Zhao, F.; Lin, S.; Zhao, Y.; Yao, J.; Cui, Y.; Zhang, X.-Y.; Zhao, Y. Benchmarking Spatial Clustering Methods with Spatially Resolved Transcriptomics Data. *Nat. Methods* **2024**, *21*, 712–722. [[CrossRef](#)] [[PubMed](#)]
- [157] Knapp, A.; Cruz, D.A.; Mehrad, B.; Laubenbacher, R.C. Personalizing Computational Models to Construct Medical Digital Twins. *J. R. Soc. Interface* **2025**, *22*, 20250055. [[CrossRef](#)] [[PubMed](#)]
- [158] Luecken, M.D.; Büttner, M.; Chaichoompu, K.; Danese, A.; Interlandi, M.; Mueller, M.F.; Strobl, D.C.; Zappia, L.; Dugas, M.; Colomé-Tatché, M.; et al. Benchmarking Atlas-Level Data Integration in Single-Cell Genomics. *Nat. Methods* **2022**, *19*, 41–50. [[CrossRef](#)] [[PubMed](#)]
- [159] Karaman, I.; Sebin, B. From Data-Driven Cities to Data-Driven Tumors: Dynamic Digital Twins for Adaptive Oncology. *Front. Artif. Intell.* **2025**, *8*, 1624877. [[CrossRef](#)] [[PubMed](#)]

- [160] Kim, B.C.; Kim, B.; Hyun, Y. Investigation of Out-of-Distribution Detection across Various Models and Training Methodologies. *Neural Netw.* **2024**, *175*, 106288. [[CrossRef](#)] [[PubMed](#)]
- [161] Wang, X.; Lv, X.; Wang, J.; Zou, L.; Chen, Z.; Zhao, R.; Zhao, L.; Zhao, M.; Zhang, X.; Zhang, B.; et al. A Multimodal Uncertainty-Aware AI System Optimizes Ovarian Cancer Risk Assessment Workflow. *npj Digit. Med.* **2025**, *8*, 614. [[CrossRef](#)] [[PubMed](#)]
- [162] Bartusik-Aebisher, D.; Justin Raj, D.R.; Aebisher, D. Artificial Intelligence in Medical Diagnostics: Foundations, Clinical Applications, and Future Directions. *Appl. Sci.* **2026**, *16*, 728. [[CrossRef](#)]
- [163] Savage, S.R.; Yi, X.; Lei, J.T.; Wen, B.; Zhao, H.; Liao, Y.; Jaehnig, E.J.; Somes, L.K.; Shafer, P.W.; Lee, T.D.; et al. Pan-Cancer Proteogenomics Expands the Landscape of Therapeutic Targets. *Cell* **2024**, *187*, 4389–4407.e15. [[CrossRef](#)] [[PubMed](#)]
- [164] Wang, Y.; Bergman, D.R.; Trujillo, E.; Fernald, A.A.; Li, L.; Pearson, A.T.; Sweis, R.F.; Jackson, T.L. Agent-Based Modeling of Virtual Tumors Reveals the Critical Influence of Microenvironmental Complexity on Immunotherapy Efficacy. *Cancers* **2024**, *16*, 2942. [[CrossRef](#)] [[PubMed](#)]
- [165] Kolokotroni, E.; Abler, D.; Ghosh, A.; Tzamali, E.; Grogan, J.; Georgiadi, E.; Büchler, P.; Radhakrishnan, R.; Byrne, H.; Sakalis, V.; et al. A Multidisciplinary Hyper-Modeling Scheme in Personalized In Silico Oncology: Coupling Cell Kinetics with Metabolism, Signaling Networks, and Biomechanics as Plug-In Component Models of a Cancer Digital Twin. *J. Pers. Med.* **2024**, *14*, 475. [[CrossRef](#)] [[PubMed](#)]
- [166] Park, S.-Y.; Kim, S.-J.; Park, C.-H.; Kim, J.; Lee, D.-Y. Data-Driven Prediction Models for Forecasting Multistep Ahead Profiles of Mammalian Cell Culture toward Bioprocess Digital Twins. *Biotechnol. Bioeng.* **2023**, *120*, 2494–2508. [[CrossRef](#)] [[PubMed](#)]
- [167] Stahlberg, E.A.; Abdel-Rahman, M.; Aguilar, B.; Asadpoure, A.; Beckman, R.A.; Borkon, L.L.; Bryan, J.N.; Cebulla, C.M.; Chang, Y.H.; Chatterjee, A.; et al. Exploring Approaches for Predictive Cancer Patient Digital Twins: Opportunities for Collaboration and Innovation. *Front. Digit. Health* **2022**, *4*, 1007784. [[CrossRef](#)] [[PubMed](#)]
- [168] Wentzel, A.; Attia, S.; Zhang, X.; Canahuat, G.; Fuller, C.D.; Marai, G.E. DITTO: A Visual Digital Twin for Interventions and Temporal Treatment Outcomes in Head and Neck Cancer. *IEEE Trans. Vis. Comput. Graph.* **2025**, *31*, 65–75. [[CrossRef](#)] [[PubMed](#)]
- [169] Chen, S.; Zhu, B.; Huang, S.; Hickey, J.W.; Lin, K.Z.; Snyder, M.; Greenleaf, W.J.; Nolan, G.P.; Zhang, N.R.; Ma, Z. Integration of Spatial and Single-Cell Data across Modalities with Weakly Linked Features. *Nat. Biotechnol.* **2024**, *42*, 1096–1106. [[CrossRef](#)] [[PubMed](#)]
- [170] Wang, G.; Zhao, J.; Lin, Y.; Liu, T.; Zhao, Y.; Zhao, H. scMODAL: A General Deep Learning Framework for Comprehensive Single-Cell Multi-Omics Data Alignment with Feature Links. *Nat. Commun.* **2025**, *16*, 4994. [[CrossRef](#)] [[PubMed](#)]
- [171] Görtz, M.; Brandl, C.; Nitschke, A.; Riediger, A.; Stromer, D.; Byczkowski, M.; Heuveline, V.; Weidemüller, M. Digital Twins for Personalized Treatment in Uro-Oncology in the Era of Artificial Intelligence. *Nat. Rev. Urol.* **2026**, *23*, 29–39. [[CrossRef](#)] [[PubMed](#)]
- [172] Bhardwaj, S.; Kumar, G.; Yang, H.; Bhardwaj, S.; Wang, Q.; Shen, M.; Wang, Y.; Souza, C.M.D. Virtual Cells: From Conceptual Frameworks to Biomedical Applications. *arXiv* **2025**, arXiv:2509.18220. [[CrossRef](#)]
- [173] Chen, F.; Li, Y.; Chen, Y.; Bian, Z.; Duo, L.; Zhou, Q.; Zhang, L.; Group, A.W. Strategies for the Analysis and Elimination of Hallucinations in Artificial Intelligence Generated Medical Knowledge. *J. Evid. - Based Med.* **2025**, *18*, e70075. [[CrossRef](#)] [[PubMed](#)]
- [174] Arunraju, C. Explainable AI (XAI) for Trustworthy and Transparent Decision-Making: A Theoretical Framework for AI Interpretability. *World J. Adv. Eng. Technol. Sci.* **2025**, *14*, 170–207. [[CrossRef](#)]
- [175] Adepoju, D.A.; Adepoju, A.G. Establishing Ethical Frameworks for Scalable Data Engineering and Governance in AI-Driven Healthcare Systems. *Int. J. Res. Publ. Rev.* **2025**, *6*, 8710–8726. [[CrossRef](#)]
- [176] Yang, W.; Wei, Q.; Ma, C.; Tan, W.; Yan, B. Scaling Laws for Data-Efficient Visual Transfer Learning. *arXiv* **2025**, arXiv:2504.13219. [[CrossRef](#)]
- [177] Pan, R.; Sun, H.; Chen, X.; Pedrielli, G.; Huang, J. Human Digital Twin: Data, Models, Applications, and Challenges. *arXiv* **2025**, arXiv:2508.13138. [[CrossRef](#)]
- [178] Hernandez-Boussard, T.; Macklin, P.; Greenspan, E.J.; Gryshuk, A.L.; Stahlberg, E.; Syeda-Mahmood, T.; Shmulevich, I. Digital Twins for Predictive Oncology Will Be a Paradigm Shift for Precision Cancer Care. *Nat. Med.* **2021**, *27*, 2065–2066. [[CrossRef](#)] [[PubMed](#)]

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