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SPATIAL OMICS REWRITES THE COMPUTATIONAL MAP OF DRUG EXPOSURE, TARGET ENGAGEMENT, RESPONSE, AND RESISTANCE

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ABSTRACT

Pharmaceutical models commonly represent drug action through measurements aggregated across tissues, specimens, or patient populations. These averages can obscure the spatial separation between drug delivery, molecular target engagement, cellular response, adaptive resistance, and tissue toxicity. Spatial omics creates an opportunity to reconsider these relationships by preserving the locations of molecular states, cell populations, histologic structures, and pharmacologic signals. This Perspective develops a proposed Tissue-Aware Pharmacologic State Map that organizes drug action as four linked but non-equivalent spatial fields: local exposure, local target engagement, neighborhood-conditioned response, and resistance or toxicity. The framework treats tissue neighborhoods, anatomical barriers, vascular structures, stromal compartments, immune organization, and local metabolic states as components of the pharmacologic context rather than as secondary annotations. It also distinguishes directly measured spatial evidence from computationally reconstructed or predicted information. Under this formulation, a compound detected within tissue does not necessarily reach its intended molecular target; target expression does not establish target engagement; engagement does not guarantee a therapeutically meaningful response; and localized response does not establish organism-level efficacy or safety. The proposed construct therefore rejects the use of a single predictive or benchmarking measure as sufficient evidence of pharmaceutical usefulness. Instead, claims must be evaluated across assay validity, spatial registration, computational representation, perturbational consistency, external transportability, uncertainty, and decision-specific utility. The framework remains conceptual and requires empirical evaluation across drug classes, tissues, spatial platforms, and translational settings. Its principal implication is that computational pharmacology should model where drug action occurs, under which tissue conditions it occurs, and at which spatial and evidentiary scale a claim remains valid.

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Introduction

Spatial omics has expanded molecular analysis from determining which transcripts, proteins, metabolites, or cell states are present to determining where they occur and how their locations relate to tissue organization. Integrating spatial measurements with single-cell information makes it possible to examine intercellular relationships without fully separating molecular states from their tissue context, while spatial transcriptomic technologies provide increasingly diverse representations of tissue architecture, resolution, molecular coverage, and measurement chemistry [1–3]. For pharmaceutical science, this shift is consequential because drug action is not generated by an isolated molecular target. It unfolds within a tissue containing vascular routes, diffusion barriers, metabolic compartments, extracellular matrix, immune populations, stromal structures, and heterogeneous cell states.

The conventional computational map of pharmacologic action is frequently assembled from disconnected averages. Plasma or homogenized tissue concentration is used to characterize exposure; target abundance or a downstream biomarker is used to

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infer engagement; pooled molecular change is interpreted as response; and surviving cells or recurrent disease are labelled resistant. These measurements can each be scientifically useful, but their aggregation removes information about which cells produced a signal, whether the compound reached those cells, what neighboring structures shaped the response, and whether the sampled region represented the broader lesion or organ. Spatial integration can restore part of this missing context by aligning cellular states with their local environments and by examining how tissue organization contributes to observed molecular patterns [1].

The unresolved problem is therefore not merely insufficient measurement resolution. It is an interpretive mismatch between the spatial organization of pharmacologic processes and the non-spatial structure of many computational claims. A model may correctly predict a specimen-level response while assigning that response to the wrong cell population, anatomical compartment, or mechanism. Conversely, a spatial association may be highly reproducible yet remain non-causal, pharmacologically indirect, or irrelevant to the intended therapeutic decision. Tissue architecture must consequently be treated as a component of the modeled pharmacologic state rather than as a visualization layer placed beneath molecular predictions [2]. Platform diversity further means that spatial evidence cannot be interpreted independently of resolution, detection efficiency, tissue preparation, segmentation, deconvolution, and the distinction between measured and computationally reconstructed signals [3].

This Perspective proposes the Tissue-Aware Pharmacologic State Map, a conceptual framework for representing drug action through four linked but non-equivalent spatial fields: local exposure, local target engagement, neighborhood-conditioned response, and resistance or toxicity. These fields are embedded within a multiscale tissue representation spanning cells, multicellular neighborhoods, histologic regions, lesions, organs, and sampled individuals. The framework does not claim empirical validation or universal applicability. Its contribution is organizational: it specifies which relationships must remain distinct, how spatial omics can connect them, what uncertainties accompany the connections, and why pharmaceutical usefulness cannot be established through one performance measure. The resulting argument is that spatial omics rewrites the computational map of drug action only when location is integrated with pharmacologic meaning, evidentiary provenance, uncertainty, and bounded interpretation.

Why Bulk Measurements Misplace Drug Action

The phrase bulk measurements misplace drug action describes an interpretive error rather than a physical relocation of the drug. Bulk measurements combine signals across cells and tissue regions, thereby disconnecting molecular observations from the local conditions under which they were generated. Spatially resolved transcriptomics adds a necessary coordinate to genomic measurement by retaining the tissue location of molecular signals that would otherwise be pooled or dissociated [4]. In pharmacologic analysis, loss of this coordinate can lead investigators to infer that exposure, engagement, response, or resistance is uniformly distributed when the underlying process is restricted to a particular vascular zone, tumor boundary, stromal compartment, immune niche, or vulnerable organ region.

Spatial studies of human tumors demonstrate why this problem cannot be treated as a minor sampling issue. Prostate cancer tissues contain regional transcriptomic programs that can coexist within the same specimen; pancreatic ductal adenocarcinoma contains spatially organized malignant, stromal, and epithelial states; and squamous cell carcinoma contains structured cell-state niches and boundary-associated programs [5–7]. These findings support a general pharmaceutical proposition: a specimen-level average can be numerically accurate while being biologically misassigned. The average may reflect a dominant region, a rare but highly expressive cell population, or a mixture of opposing local responses. It cannot, by itself, identify where therapeutic action occurred or whether the sampled signal arose from the intended pharmacologic route.

Misplacement can occur at several levels. First, a tissue-average concentration can conceal regions with poor penetration or rapid local clearance. Second, pooled target abundance can combine target-positive and target-negative populations without revealing whether the compound reached either population. Third, an averaged downstream signature can merge responding cells with unaffected or compensating neighborhoods. Fourth, a resistance-associated signal can be attributed to the entire lesion when it originates from a spatially restricted sanctuary. Regional molecular heterogeneity in prostate cancer illustrates how distinct programs can disappear into a pooled representation [5]. Joint spatial and single-cell analysis in pancreatic cancer similarly shows that the biological meaning of a transcriptional state depends on the tissue compartment in which it is located [6]. Multimodal mapping of squamous cell carcinoma further indicates that composition, cellular state, and architecture must be interpreted together rather than as interchangeable measurements [7].

The proposed framework therefore separates the location of a signal from the pharmacologic state assigned to that location. Spatial localization establishes that a transcript, protein, metabolite, cell state, or compound-associated signal occurs within a defined region. It does not automatically establish direct drug exposure, target occupancy, causal response, therapeutic benefit, or toxicity. Spatial evidence can reduce one form of ambiguity while introducing others, including registration error, incomplete molecular capture, uncertain cell boundaries, inferred cell-type composition, and temporal mismatch between drug administration and tissue collection. The central purpose of the tissue-aware map is not to replace bulk measurements, which remain useful for systemic and specimen-level characterization, but to prevent those measurements from carrying spatial or mechanistic claims they cannot support. **Table 1** organizes the evidence, constructs, and interpretation boundaries needed to develop why bulk measurements misplace drug action within the article's central argument.

Table 1. Evidence domains, core questions, scientific requirements, and interpretation boundaries for Why bulk measurements misplace drug action

Construct or evidence domain	Core question	Scientific basis	Role in the article	Failure or overclaiming risk	Boundary statement
Specimen-level averaging	Which local signals are combined into the reported average?	Bulk measurements integrate molecular contributions across heterogeneous cells, compartments, and tissue regions.	Establishes how numerically valid averages can lose pharmacologically relevant location.	Treating a pooled value as representative of every region or cell population.	A specimen average describes the sampled mixture; it does not establish spatial uniformity.
Regional heterogeneity	Do distinct anatomical regions contain different molecular or cellular states?	Spatially resolved studies identify regional programs, boundaries, and microenvironments within individual tissues.	Demonstrates why local states should remain identifiable within the computational representation.	Assuming that one sampled region represents the entire lesion or organ.	Regional differences are context-specific and require replication and appropriate sampling.
Cellular source attribution	Which cells or compartments generated the observed molecular signal?	Spatial and single-cell integration can associate signals with candidate cell populations and tissue structures.	Connects molecular observations to plausible cellular sources.	Treating deconvolved or annotated sources as directly observed ground truth.	Cell assignment remains conditional on segmentation, markers, resolution, and model assumptions.
Local drug exposure	Was the compound or active metabolite present in the relevant tissue region?	Spatial chemical measurements can distinguish heterogeneous tissue distribution from organ-average concentration.	Separates administered dose and systemic exposure from local tissue availability.	Inferring target access or pharmacologic activity from drug presence alone.	Local compound detection does not establish intracellular availability or target engagement.
Target engagement	Was the intended molecular target occupied, inhibited, activated, or otherwise modulated locally?	Engagement requires direct occupancy evidence or a sufficiently specific proximal pharmacodynamic measurement.	Prevents target expression or downstream response from substituting for engagement.	Equating target abundance with target modulation.	Target expression is not target engagement; exposure is not engagement.
Neighborhood-conditioned response	Did local cell state, immune composition, stroma, matrix, vasculature, or metabolism condition the response?	Tissue neighborhoods can alter signaling, accessibility, adaptation, and cellular interactions.	Extends response modeling beyond isolated cell identity.	Interpreting spatial association as a causal neighborhood effect.	Contextual association requires perturbational evidence before a causal claim is made.
Resistance localization	Is persistence or therapeutic escape restricted to particular niches or regions?	Resistant states may arise through inadequate exposure, absent engagement, adaptive signaling, immune exclusion, or host-tissue support.	Organizes alternative spatial routes to treatment failure.	Labelling every surviving region as mechanistically resistant.	Persistence does not identify the route of resistance without additional evidence.
Spatial uncertainty	How reliable are location, alignment, assignment, and reconstructed resolution?	Spatial evidence depends on tissue quality, assay sensitivity, registration, segmentation, deconvolution, and sampling.	Places uncertainty inside the pharmacologic interpretation rather than outside it.	Presenting reconstructed or predicted spatial states as direct measurements.	Every spatial claim must retain its measurement status, scale, and uncertainty.

Spatial Determinants of Exposure, Target Engagement, and Response

Spatial pharmacology extends tissue analysis beyond molecular localization by asking how the location of a compound relates to the location of pharmacologic action. Mass-spectrometry imaging can map parent compounds, metabolites, lipids, and other endogenous molecules in tissue, while advanced pharmaceutical imaging approaches can contribute information about distribution and pharmacodynamic context. Single-cell spatial metabolomics can additionally connect cellular metabolic states with tissue position [8–10]. Together, these technologies support a layered interpretation of local pharmacology, but they do not collapse exposure, engagement, and response into one observable quantity. Each layer answers a different scientific question and carries distinct analytical limitations.

Local exposure is the spatial presence and relative or quantitative distribution of a parent drug, active metabolite, delivery vehicle, or other treatment-related material. It is conditioned by vascular access, perfusion, membrane transport, extracellular matrix, tissue pressure, binding, metabolism, sequestration, and clearance. A controlled xenograft study using mass-spectrometry imaging showed that the tissue distribution of erlotinib could differ when treatment was combined with bevacizumab, illustrating that administered dose and even whole-tissue concentration cannot be assumed to produce uniform local exposure [11]. Such observations are pharmacologically important because a poorly responding region may reflect inadequate delivery rather than target-independent resistance. However, detection of a compound within a region does not prove that the pharmacologically active species entered the relevant cells or reached the intended molecular target.

Local target engagement is a separate state. It refers to evidence that a drug has occupied, inhibited, activated, degraded, stabilized, or otherwise modulated its intended target within a defined tissue location. Spatial compound mapping can identify where a drug or metabolite is found, but mass-spectrometry imaging alone does not necessarily establish molecular occupancy

or functional modulation [8]. Target engagement may require activity-based probes, spatially resolved occupancy assays, conformational or phosphorylation measurements, target-proximal biomarkers, or orthogonal pharmacologic controls. Pharmaceutical imaging can therefore support engagement analysis when it is integrated with sufficiently specific molecular evidence, but the inferential chain must remain explicit [9]. Target expression should not be treated as engagement, and a downstream transcriptional response should not automatically be assigned to the intended target.

Neighborhood-conditioned response describes the local cellular or tissue change that follows exposure and possible engagement within a specific microenvironment. The relevant response may involve transcription, protein activity, metabolism, morphology, viability, differentiation, immune activation, matrix remodeling, or stress. Spatial metabolomic measurements can help reveal heterogeneous local metabolic states that would be obscured by pooled analysis [10]. Yet response remains conditional on timing, dose, pretreatment state, neighboring cells, anatomical barriers, and the scale of observation. The proposed Tissue-Aware Pharmacologic State Map therefore represents exposure, engagement, and response as adjacent but non-equivalent fields. A valid connection among them requires spatial alignment, temporal plausibility, assay specificity, and, where mechanistic claims are made, perturbational confirmation. The map is intended to show where evidence converges and where an inference remains unsupported; it is not a validated model of tissue pharmacokinetics, molecular causality, therapeutic efficacy, or clinical outcome.

Computational Representations of Tissue Neighborhoods and Microenvironments

The proposed Tissue-Aware Pharmacologic State Map requires a computational representation capable of preserving molecular identity, tissue location, anatomical scale, and evidentiary provenance. A useful starting point is a multiscale tissue graph in which nodes represent cells, assay spots, segmented microregions, histologic compartments, or anatomically defined regions. Edges may represent physical adjacency, distance, shared vasculature, diffusion barriers, ligand–receptor plausibility, morphological continuity, or membership within a broader tissue compartment. Squidpy demonstrates how spatial coordinates, molecular profiles, neighborhood relations, and image features can be analyzed within a scalable spatial framework [12]. In the proposed construct, however, a graph is not merely a convenient data structure. It is the substrate through which exposure, engagement, response, resistance, and toxicity are assigned to specific tissue units and connected across spatial scales.

Graph construction determines which biological relationships a model can express. SpaGCN integrates gene expression, spatial coordinates, and histologic information through graph convolution to identify spatial domains and spatially variable genes [13]. This type of representation is relevant to pharmaceutical modeling because a molecular response may be conditioned simultaneously by intrinsic cell state, proximity to a vessel, location at a tumor boundary, stromal density, and the states of neighboring immune cells. Nevertheless, the selected graph topology remains a modeling choice. A distance threshold, adjacency rule, or image-derived boundary can change which cells are considered neighbors and therefore which contextual effects become visible. Graph proximity should consequently be interpreted as a hypothesis about possible interaction or shared environment, not as direct evidence of molecular communication.

A second requirement is the ability to distinguish cell-intrinsic information from contextual influence. Explainable multiview modeling provides one approach by representing molecular information from several spatial perspectives rather than collapsing all context into a single neighborhood summary. MISTy, for example, separates local and broader spatial views and estimates their contributions to observed molecular variation [14]. Within the proposed pharmacologic map, such a decomposition may help distinguish a response associated with a cell's own molecular state from one associated with surrounding immune, stromal, vascular, or matrix conditions. This distinction is especially important when interpreting resistance. A persistent cell may survive because of an intrinsic alteration, insufficient local drug exposure, paracrine support, immune exclusion, or a combination of these factors. An explainable spatial representation can organize these alternatives, but it does not establish which alternative is causal without perturbational evidence.

Spatial resolution introduces a further layer of inference. BayesSpace illustrates how subspot structure can be estimated computationally from spot-based spatial transcriptomic data [15]. Such resolution enhancement may reveal candidate microdomains that are not visible at the native assay scale, but the resulting subspot map remains reconstructed rather than directly measured. The Tissue-Aware Pharmacologic State Map therefore requires every node and field to retain a provenance label indicating whether it was directly observed, segmented, deconvolved, imputed, predicted, or transferred from another modality. It also requires an uncertainty envelope around registration, cell assignment, graph construction, spatial interpolation, and cross-scale aggregation. These safeguards prevent improved visual granularity from being mistaken for improved pharmacologic certainty.

Integrating Spatial Omics with Imaging, Pathology, and Pharmacology

Spatial omics acquires greater pharmaceutical meaning when molecular measurements are aligned with tissue morphology, pathological interpretation, and direct pharmacologic evidence. Histology provides structural information about necrosis, fibrosis, tumor boundaries, vascular organization, immune infiltration, glandular architecture, and tissue damage that may not be recoverable from molecular profiles alone. Deep-learning integration of breast-tumor morphology with spatial gene expression has shown that histologic patterns can be computationally associated with local molecular programs [16]. In the proposed tissue-aware map, pathology is not treated as a decorative background. It helps define anatomical compartments, constrain spatial graphs, identify sampling artefacts, and determine whether a molecular signal occurs in a region relevant to exposure, engagement, response, or toxicity.

Image-derived molecular inference must nevertheless be separated from direct measurement. Whole-slide image models can predict aspects of tumor RNA expression from morphological features [17]. Such models may help prioritize regions for molecular analysis, identify candidate spatial patterns, or extend limited molecular measurements across larger tissue areas. They do not, however, convert routine histology into a direct assay of local drug concentration, target occupancy, pathway modulation, or therapeutic response. Predicted expression is a proxy conditioned on training data, image quality, specimen preparation, disease context, and model transportability. The proposed framework therefore records image-derived molecular information as inferred evidence and restricts the claims it can support until it is compared with spatially matched measurements.

Cross-modal data fusion can also produce spatial representations at a finer apparent resolution than the original molecular assay. Deep data fusion between histology and spatial transcriptomics has been used to generate super-resolved transcriptomic maps [18]. For tissue-aware pharmacology, this creates an opportunity to align molecular states more closely with microanatomical features such as vessels, invasive fronts, immune aggregates, or damaged tubules. It also creates a risk that registration error, histologic artefacts, or assumptions learned from one modality will be propagated into another. A super-resolved map should therefore indicate which features are measured, which are transferred from imaging, which are computationally reconstructed, and how sensitive the result is to alternative registration and fusion procedures.

Multiplexed tissue imaging provides another route to represent cell identity and spatial organization. Multiplexed ion-beam imaging of triple-negative breast cancer revealed structured tumor–immune microenvironments rather than randomly intermixed cell populations [19]. These patterns are relevant to pharmacology because therapeutic response can depend on whether immune cells are excluded, dispersed, activated, suppressed, or organized at specific tissue boundaries. Yet imaging, pathology, and spatial omics should not be combined merely to maximize dimensionality. Integration is justified when each modality addresses a defined pharmacologic question: chemical imaging for local exposure, engagement assays for molecular action, spatial omics for cellular response, pathology for tissue state, and longitudinal or perturbational evidence for resistance and toxicity. The value of multimodal integration lies in reducing interpretive ambiguity, not in generating the largest possible feature space.

Mapping Response, Resistance, and Toxicity at Multiple Resolutions

A spatial response map should identify not only whether a molecular or cellular change occurs, but where it occurs, in which cell state, under what neighborhood conditions, and at what time after treatment. Spatial transcriptomic analysis of macrophage infiltration in non-small-cell lung cancer has linked distinct macrophage-related tissue contexts with sensitivity and resistance to immune-checkpoint therapy [20]. This type of evidence supports the view that treatment response is partly a property of organized multicellular environments. It does not imply that a macrophage-associated spatial pattern is universally causal or transferable across tumors, treatments, and patients. Within the proposed map, response-associated neighborhoods are therefore represented as context-specific evidence layers whose mechanistic interpretation depends on orthogonal and perturbational support.

Spatial arrangements can also provide information beyond the abundance of individual cell types. Spatial predictors of immunotherapy response in triple-negative breast cancer demonstrate that multicellular organization may contribute to response stratification [21]. For computational pharmaceutical science, this means that a model based only on cell proportions or specimen-level expression can miss potentially relevant relationships among tumor cells, immune populations, stromal regions, and tissue boundaries. At the same time, predictive association must remain separate from mechanistic explanation. A spatial feature may improve discrimination because it is a surrogate for disease stage, tissue quality, prior treatment, or another unmeasured variable. Its usefulness must therefore be tested in external specimens and in the exact decision context for which it is proposed.

Resistance requires an explicitly multiscale interpretation because therapeutic escape can emerge from molecular adaptation within a cell, support from a local neighborhood, regional delivery failure, or broader organ-level physiology. Spatially resolved multi-omics in glioblastoma has demonstrated bidirectional interdependence between tumor and host states across tissue regions [22]. This evidence is consistent with a model in which resistance is not always a stable property of malignant cells considered in isolation. Instead, it may arise from reciprocal interactions among tumor cells, immune populations, neural or stromal elements, metabolic conditions, and regional treatment access. The Tissue-Aware Pharmacologic State Map consequently represents resistance as a spatial field with several possible routes: low exposure, absent engagement, adaptive response, bypass signaling, protected niche, immune exclusion, and host-supported persistence. These routes are proposed organizational categories rather than validated universal mechanisms.

Toxicity must be mapped with the same attention to scale. Spatial and single-cell transcriptomic analysis of drug-perturbed rat kidney has shown that treatment-associated molecular changes can differ among organ regions and cell populations [23]. Such studies can help identify candidate vulnerable compartments that would be diluted in an organ-average measurement. Their interpretation remains conditional on species, dose, collection time, tissue processing, and the relationship between transcriptional change and actual injury. A local stress signature does not by itself establish clinically meaningful toxicity, just as a local response signature does not establish therapeutic benefit. **Figure 1** presents the spatial drug-action tissue map, showing how the article's key components and boundaries are connected within mapping response, resistance, and toxicity at multiple resolutions.

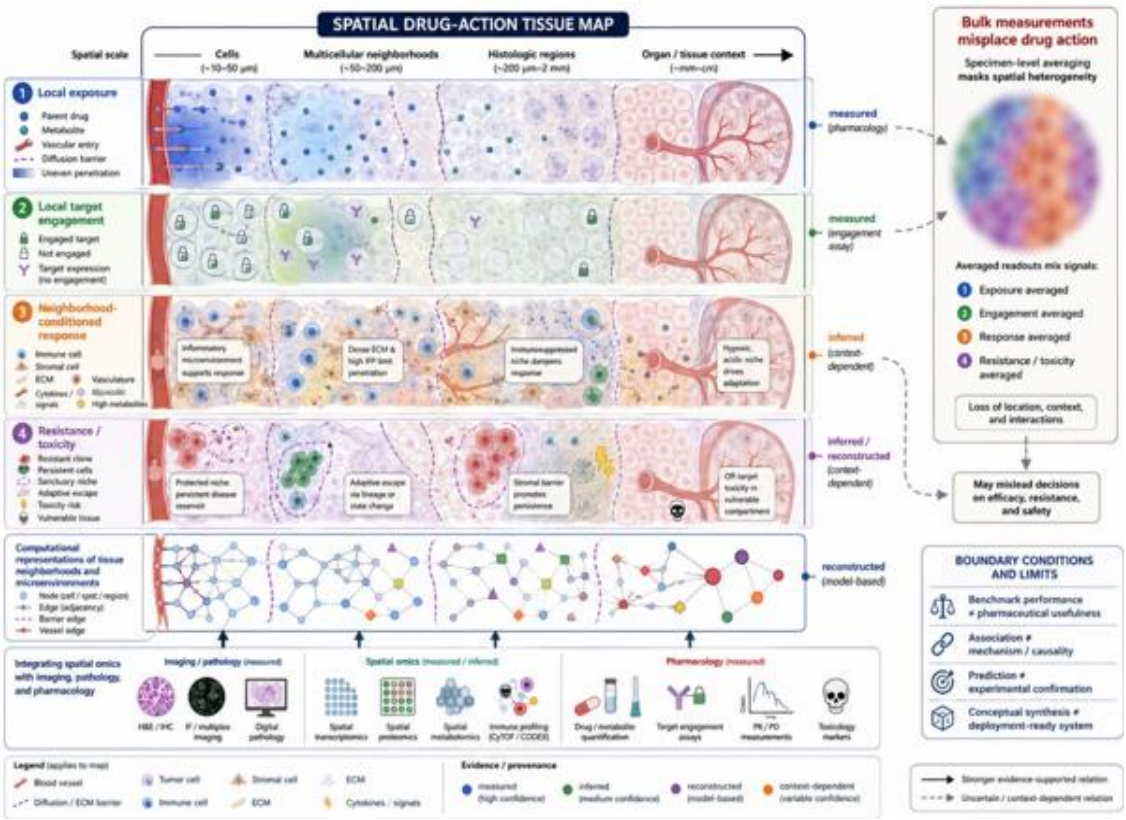

Figure 1. Spatial Drug-Action Tissue Map.

The figure is an original conceptual synthesis that organizes the article's central contribution across bulk measurements misplace drug action, spatial determinants of exposure, target engagement, and response, spatial determinants of exposure, target engagement, computational representations of tissue neighborhoods and microenvironments, integrating spatial omics with imaging, pathology, and pharmacology. Arrows and grouping indicate proposed or evidence-supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

Validation and Translational Implications for Tissue-Aware Therapeutics

Validation of a tissue-aware pharmacologic model cannot be reduced to one accuracy, clustering, reconstruction, or predictive score. Computational benchmarking guidance emphasizes that apparent method quality depends on benchmark design, comparator selection, data preprocessing, ground truth, parameter tuning, and the correspondence between the benchmark task and the intended use [24]. For spatial pharmacology, validation must therefore be component-specific. Analytical validity concerns whether each assay measures the stated analyte. Spatial validity concerns registration, segmentation, and assignment. Computational validity concerns representation, reconstruction, prediction, and uncertainty. Pharmacologic validity concerns whether exposure, engagement, response, resistance, and toxicity are connected by evidence appropriate to the claim.

Task-specific benchmarking is especially important when single-cell and spatial data are integrated. Comparative evaluation of transcript-distribution prediction and cell-type deconvolution methods has shown that performance can vary across objectives and data structures [25]. A method that performs well in deconvolution may not be the best method for spatial gene reconstruction, neighborhood identification, or response prediction. Internal benchmark success also does not establish that a model will support a target-selection, formulation, biomarker, combination-therapy, or toxicity decision. **Table 2** organizes the evidence, constructs, and interpretation boundaries needed to develop validation and translational implications for tissue-aware therapeutics within the article's central argument.

Platform choice creates an additional validation boundary. Systematic comparison of sequencing-based spatial transcriptomic methods has demonstrated differences in molecular sensitivity, spatial resolution, coverage, and other measurement characteristics [26]. These differences determine which biological structures can be observed and which computational reconstructions are required. A model developed on one platform may therefore fail when transferred to another because the feature space, missingness pattern, tissue resolution, or cell-assignment problem has changed. Platform transportability should be tested explicitly rather than assumed from nominally similar spatial data. The appropriate validation target is not generic method superiority but fitness for a defined pharmaceutical claim under specified specimen, assay, and computational conditions.

Translational value requires evidence beyond descriptive tissue mapping. Clinical and translational discussions of spatial transcriptomics emphasize the importance of specimen handling, pathology integration, workflow feasibility, interpretability,

and linkage to meaningful clinical or experimental questions [27]. For tissue-aware therapeutics, readiness should be staged from analytical validity through computational evaluation, perturbational consistency, external transportability, and decision-specific utility under human oversight.

Table 2. Evaluation, implementation, and research priorities arising from Spatial Omics Rewrites the Computational Map of Drug Exposure, Target Engagement, Response

Component or layer	Inputs	Core function	Expected output	Uncertainty or limitation	Validation requirement
Analytical measurement layer	Spatial transcriptomic, proteomic, metabolomic, chemical-imaging, engagement, and pathology measurements	Establish which analytes and tissue features were directly measured	Quality-controlled spatial signals with assay-specific provenance	Detection limits, molecular capture, ionization, antibody specificity, tissue degradation, batch effects	Reference materials, negative and positive controls, technical replicates, orthogonal confirmation
Registration and assignment layer	Tissue coordinates, images, fiducials, cell segmentation, spot geometry, anatomical landmarks	Align modalities and assign signals to cells, spots, or tissue regions	Registered multimodal tissue representation	Misregistration, section mismatch, segmentation error, signal leakage, uncertain cell identity	Landmark concordance, registration-error reporting, pathologist review, segmentation benchmarks
Local exposure layer	Parent-drug and metabolite maps, vasculature, perfusion, diffusion barriers, formulation information	Estimate where pharmacologically relevant material is present	Spatial exposure field	Relative rather than absolute quantification, sampling time, metabolite ambiguity, intracellular-access uncertainty	Quantitative calibration, orthogonal tissue concentration measurements, time-course assessment
Target-engagement layer	Occupancy assays, activity-based probes, target-proximal biomarkers, spatial protein or activity measurements	Determine whether the intended target is locally modulated	Spatial engagement field	Target expression may substitute incorrectly for engagement; off-target effects may confound interpretation	Specific controls, competing ligands, genetic or pharmacologic perturbation, proximal pharmacodynamic confirmation
Neighborhood-conditioned response layer	Spatial omics, cell states, histology, immune and stromal composition, matrix and vascular context	Link local molecular response to tissue neighborhood	Contextual response map	Association may be mistaken for mechanism; neighborhood definition may be arbitrary	Sensitivity to graph and scale choices, independent markers, dose and time consistency, perturbational testing
Resistance and escape layer	Paired or longitudinal tissues, residual-disease regions, adaptive pathways, exposure and engagement maps	Distinguish potential spatial routes to persistence	Candidate resistance or sanctuary map	Sparse sampling, temporal aliasing, clonal evolution, survival without proven resistance mechanism	Longitudinal sampling, reversal or rescue experiments, combination perturbation, external replication
Toxicity layer	Spatial exposure, injury markers, organ pathology, cell-state responses, non-target tissue measurements	Localize adverse response and vulnerable compartments	Candidate tissue-toxicity map	Species translation, surrogate endpoints, tissue-collection timing, incomplete systemic context	Histopathology, functional injury measures, cross-species assessment, human-relevant confirmation
Computational representation layer	Multimodal tissue graph, node and edge definitions, inferred domains, scale hierarchy	Integrate pharmacologic fields without erasing location or provenance	Tissue-Aware Pharmacologic State Map	Graph topology, imputation, deconvolution, model misspecification, domain shift	Component-wise benchmarks, ablation studies, calibration, alternative graph definitions, locked external testing
Uncertainty and provenance layer	Measurement error, posterior uncertainty, registration error, missingness, model confidence, data lineage	Identify where the map is reliable, ambiguous, or unsupported	Component-specific uncertainty and abstention indicators	Unmodeled error sources and miscalibrated confidence	Interval coverage, replicate consistency, calibration under shift, transparent measured-versus-inferred labels
Translational decision layer	Validated spatial features, intended use, comparator practice, error costs, human expertise	Evaluate whether the map improves a bounded pharmaceutical decision	Evidence for or against defined decision utility	Descriptive novelty may be mistaken for usefulness; utility may not transport across decisions	Prospective decision studies, external sites, comparison with current practice, multidisciplinary oversight

Limitations and Boundary Conditions

The Tissue-Aware Pharmacologic State Map is a proposed conceptual architecture, not an empirically validated model. Its four principal fields—local exposure, local target engagement, neighborhood-conditioned response, and resistance or toxicity—are scientifically distinguishable, but current datasets rarely measure all four in the same tissue, at the same spatial resolution, and at pharmacologically appropriate time points. Ground truth is especially limited for local target engagement and causal neighborhood effects. Consequently, many practical implementations would initially combine direct measurements with inferred or proxy variables. Such combinations may be useful for hypothesis generation, but their validity depends on transparent provenance and component-wise benchmarking rather than on the coherence of the final visualization alone [24].

The framework is also context-dependent. Spatial relationships observed in one tumor type, organ, treatment class, specimen workflow, or assay platform may not transport to another. Tissue architecture differs across diseases and stages, while

therapeutic modalities differ in diffusion, cellular uptake, intracellular localization, metabolism, target kinetics, and dependence on immune or stromal processes. Spatial platforms likewise vary in molecular coverage and effective resolution [26]. The framework therefore cannot specify a universal neighborhood size, graph topology, modality combination, or validation threshold. These choices must follow the pharmacologic mechanism, tissue anatomy, intended use, and measurement characteristics of the study.

Misuse could arise if spatial detail is treated as inherently more causal, more accurate, or more clinically meaningful than non-spatial evidence. A highly resolved map may remain analytically noisy, temporally mismatched, or biologically indirect. Predicted subcellular structure may be less trustworthy than a well-validated tissue-average measurement, and a reproducible spatial biomarker may add no value to a pharmaceutical decision. The proposed contribution cannot establish therapeutic efficacy, human safety, clinical utility, regulatory acceptability, or deployment readiness. Those conclusions require evidence specific to the intervention, population, specimen, workflow, and decision context [27].

Research Agenda and Implementation Priorities

The first research priority is to measure the missing links in the spatial pharmacologic chain. Studies should pair quantitative local exposure measurements with direct or proximal target-engagement evidence and spatially resolved downstream response. They should include pharmacologically informative dose and time designs rather than relying only on untreated-versus-treated snapshots. Longitudinal or paired specimens are particularly important for distinguishing transient stress, durable response, adaptive resistance, and pre-existing sanctuary regions. Spatial chemical imaging offers a foundation for local exposure analysis, but its greatest value will arise when compound distribution can be aligned with target-specific engagement and tissue response [8].

The second priority is to establish pharmacology-focused computational benchmarks. Existing spatial benchmarks often emphasize clustering, reconstruction, deconvolution, or domain identification. Tissue-aware therapeutic models additionally require evaluation of graph stability, scale sensitivity, multimodal registration, exposure–engagement concordance, perturbational consistency, uncertainty calibration, and transportability across laboratories and platforms. Explainable multiview approaches may help distinguish intrinsic and contextual contributions, but they should be tested against controlled perturbations and alternative neighborhood definitions rather than interpreted mechanistically from association alone [14]. Reporting standards should require explicit labels for measured, segmented, deconvolved, imputed, predicted, and transferred information.

The third priority is staged implementation around bounded research decisions. Early applications may include identifying poorly exposed tissue regions, prioritizing spatially restricted targets, selecting biopsy locations, generating combination-therapy hypotheses, or localizing candidate toxicity signals. Each application should specify the required inputs, comparator practice, human oversight, expected failure modes, and evidence needed before progression. Preclinical organ maps can guide further investigation of vulnerable compartments, but they should not be translated directly into human safety claims [23]. Infrastructure priorities include interoperable spatial data standards, pharmacology-aware metadata, validated reference tissues, image and assay registration controls, versioned analysis pipelines, and multidisciplinary interpretation involving computational scientists, pharmacologists, pathologists, toxicologists, and disease specialists.

Conclusion

Spatial omics changes the computational representation of drug action by restoring the tissue coordinates that bulk and dissociated measurements remove. The proposed Tissue-Aware Pharmacologic State Map organizes this opportunity through four connected but non-equivalent fields: local exposure, local target engagement, neighborhood-conditioned response, and resistance or toxicity. Its central contribution is not a new predictive score or a claim of validated therapeutic readiness. It is a disciplined structure for asking where a drug is present, where its target is modulated, where tissue response occurs, which microenvironments condition that response, and at which spatial and evidentiary scale resistance or toxicity can be inferred. The framework also makes explicit that spatial detail does not eliminate uncertainty, that association does not establish mechanism, that prediction does not substitute for experimental confirmation, and that benchmark performance does not establish pharmaceutical usefulness. Tissue-aware therapeutics will therefore depend not only on higher-resolution measurement but on better alignment among pharmacology, pathology, multimodal computation, perturbation, validation, and bounded decision use.

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