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Mini-Review

Imaging-Guided Omics Technologies for Resolving Rare Cancer States and Advancing Nanomedicine

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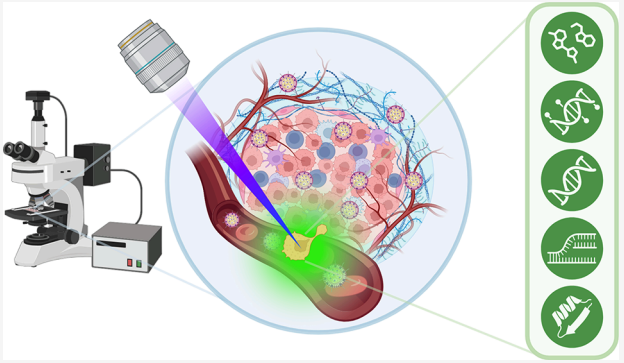
ABSTRACT: The ability to resolve rare and transient cellular states is critical for understanding metastasis, immune evasion, and therapy resistance in cancer, yet these dynamic processes often escape detection by conventional sequencing and imaging approaches. Recent advances at the interface of nanotechnology, high-resolution live-cell imaging, and single-cell/spatial multiomics methods have enabled functional profiling of cells with unprecedented precision within their native microenvironment. In this Mini-Review, we highlight emerging nanoscale platforms that couple real-time phenotypic imaging with molecular readouts, such as FUNseq and CIN-seq, to directly link functional heterogeneity to transcriptomic, proteomic, and epigenomic information. By integrating nanoscale optical imaging, microengineered perturbation tools, and AI-driven computational analysis, these technologies open up new avenues for dissecting rare metastatic, therapy-resistant, or immune-evasive subpopulations. We further discuss how these next-generation imaging-guided single-cell and spatial omics platforms not only advance fundamental cancer biology but also create opportunities to accelerate the development of nanomedicine applications.



KEYWORDS: *imaging-guided omics, rare cancer cell states, nanomedicine, functional profiling, spatial omics, tumor microenvironment*

THE CHALLENGE OF CAPTURING RARE AND TRANSIENT CANCER CELL PHENOTYPES AND STATES

Tumors consist of a highly heterogeneous collection of cells that differ in their molecular profiles and functional states. These states are dynamic rather than static, as cells are able to switch between phenotypes, a phenomenon known as cellular plasticity.[1] This intratumor heterogeneity is a key player in disease progression, metastasis, and therapy resistance, as certain subpopulations may act as hidden drivers.[1] Studying these subpopulations is crucial to better understanding cancer biology and could greatly improve therapy outcomes by targeting these specific, previously often ignored or undetected subpopulations.

Many conventional sequencing methods, such as bulk sequencing, fail to capture rare and transient cancer cell phenotypes and states due to the lack of single-cell resolution, thereby masking cellular heterogeneity. While single-cell omics techniques provide this resolution, they are typically static, rely on end point measurements, and lack microscopic phenotypic information, limiting their ability to capture dynamic cellular state transitions over time. As a result, critical information about cell state transitions, cell fate, lineage relationships, and interactions with the tumor microenvironment (TME) is often lost. Dynamic time-resolved approaches are therefore needed to observe these rare and transient phenotypes and states as they emerge, evolve, and influence disease progression, ultimately enabling the effective design of new (nano)medicine strategies and the improvement of existing ones.

To address the limitations of conventional omics methods, nanoscale imaging combined with functional profiling has emerged as a powerful approach to study critical cellular subpopulations in cancer. This strategy allows for visualizing subcellular structures and spatial organization of cells within their microenvironment while linking this imaging information to omics data. Such integrated studies reveal patterns of heterogeneity and interaction that remain invisible to conventional methods, improving our fundamental understanding of cancer biology. In this Mini-Review, we discuss the integration of imaging, nanotechnology, and single-cell/spatial multiomics (e.g., transcriptomics, epigenomics, metabolomics) for functional profiling of cells with dynamic states in the context of their microenvironment.

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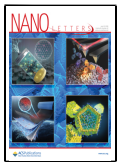

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NANOSCALE OPTICAL IMAGING AND NANOTECHNOLOGY FOR FUNCTIONAL CELL PROFILING

Profiling cells and measuring their characteristics did not start with and are not limited to omics-based approaches. A wide variety of platforms have been developed to investigate the biological, mechanical, or chemical properties of cells. Next to its application in targeted drug delivery with nanoparticles, nanotechnology has emerged as a key player in measuring, manipulating, and controlling cellular mechanisms.[2] Researchers have employed, among others, nanomaterials, perturbation tools, microfluidic platforms, and nanosensors to generate novel insights into cancer biology.[3,4] As these platforms function on the nanoscale, nanoscale optical imaging is often required to visualize and measure experimental outcomes.

Microscopy offers versatile, high-throughput, and sensitive measurements of multiple cellular characteristics. Over the past decades, high-resolution microscopy techniques (e.g., TIRF, STED, PALM) have been developed to enable visualization of subcellular structures, providing more detailed insights into molecular processes.[5] More recently, the integration of these imaging approaches with nanotechnology-based tools that generate an optical readout or require illumination input has allowed these processes to be monitored and manipulated in real time. Plasmonic nanorods,[6] quantum-dot-based biosensors,[7] optical tweezers,[8] optogenetic actuators,[9] organ-on-a-chip platforms,[10] and DNA-origami-enabled molecular scaffolds[11] exemplify the synergy between nanoscale engineering and optical interrogation in measuring, manipulating, and controlling biological systems (Box 1).

Nanotechnology combined with high-resolution microscopy is a powerful approach, but it is often highly specialized for probing a specific feature. Furthermore, it is most effective

Box 1

Plasmonic nanorods can be internalized by cells and used as nanoscale probes that report intracellular analytes through changes in their optical scattering signatures, enhancing ultrasensitive optical biosensing and probing specific biological processes.[6] Quantum-dot-based biosensors are fluorescent semiconductor nanocrystals with unique optical and electronic properties. They are tunable in their fluorescence and are functionalized to target specific biomolecules, supporting their use in a broad range of applications.[7] Optical tweezers can measure and manipulate single molecules, proteins, and cells by optically trapping them within a highly focused laser beam. Typical experiments include quantifying forces generated by molecular motors, visualizing a protein's folding pattern by pulling it apart, and probing mechanical properties of cells by stretching them.[8] In optogenetics, microscopy-based targeted illumination is utilized to switch specific genes or stimuli on or off in defined cells, enabling a better understanding of their downstream functions.[9] Organs-on-a-chip are specialized microfluidic platforms that mimic organ-level physiology in a controlled environment, enabling the investigation of tissue development and responses to specific stimuli. They are widely used to investigate disease models, evaluate therapeutic effects, and study how physical stress influences organ-level function.[10] Lastly, DNA origami, i.e., DNA or RNA sequences specifically designed to self-assemble into 2D or 3D structures with possible docking sites for other biomolecules,[11] are versatile nanotechnology platforms that can be engineered for a wide range of applications, including use as nanocarriers for drug delivery, biosensors in live-cell imaging, and scaffolds for other processes in tissue engineering.[11]

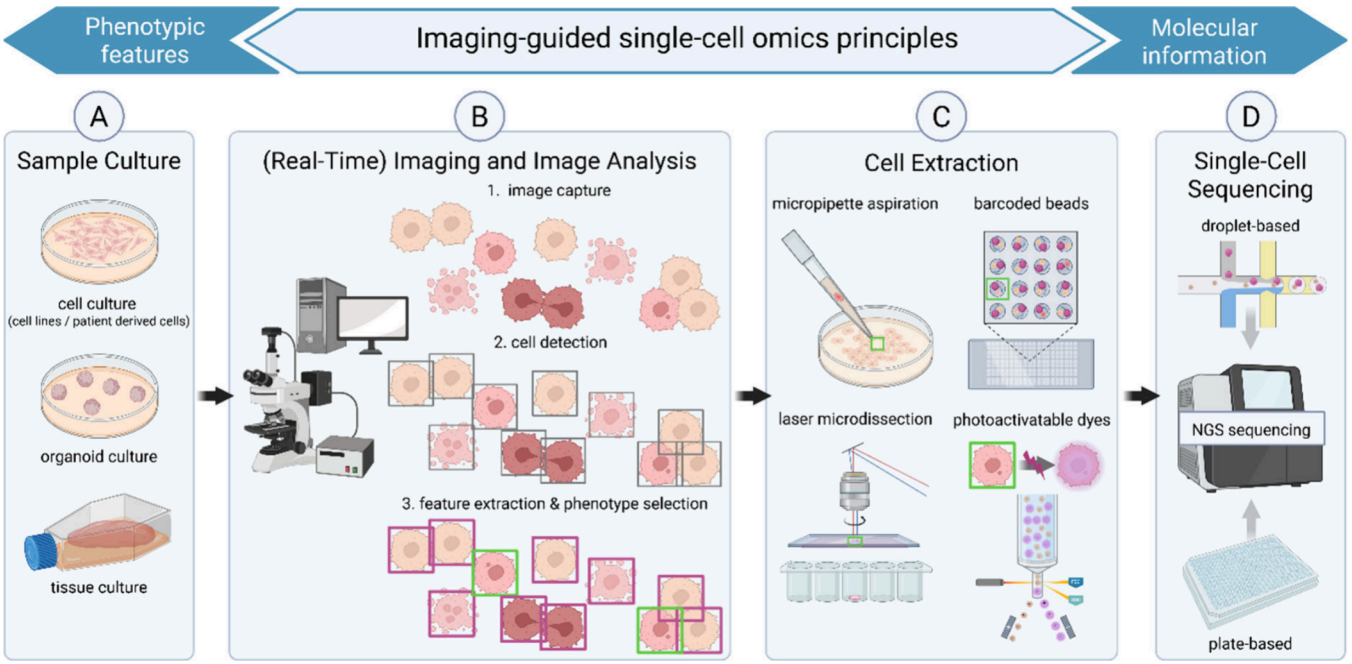

Figure 1. Imaging-guided single-cell omics principles. (A) Different sample types that imaging-guided single-cell omics can process, including cell culture, organoid culture, tissue samples, and more. (B) The sample is imaged, possibly in real time, and image analysis pipelines detect cells, extract features, and select phenotypes of interest. (C) Cells of interest are extracted from the sample via micropipette aspiration, barcoded bead identification, laser microdissection, or photoactivation of photoactivatable dyes in combination with fluorescence-activated cell sorting (FACS). (D) Finally, the extracted single cells are processed and sequenced using droplet-based or plate-based methods. Created with BioRender.

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Table 1. Strengths and Weaknesses of Single-Cell Imaging-Guided Omics and Spatial Transcriptomics, Including Imaging-Based, Sequencing-Based, and Imaging-Guided Technologies

isolation from	method category	example methods	advantages	limitations	refs
A. Single-Cell Imaging-Guided Omics					
2D culture		All methods	• Capturing single-cell phenotypes	• Loss of 3D organizational information	
	Well-based methods	SCOPE-seq	• Multiplexing capacity of phenotypes • Low loss of identified cells	• Basic phenotypes • Lack of capturing cell−cell interactions • No isolation of cells for downstream analyses	17, 19
	Photoactivation-based methods	MAGIC, FUNseq, CIN-seq	• Isolation of rare (dynamic) phenotypes for downstream analyses • Capturing cell−cell interactions	• Relatively high loss of cells because of sorting	12, 13, 16
Tissue		All methods	• Preserves spatial location	• Less suitable for single-cell behavior or phenotype studies	
	Micropipette-based methods	Image-seq	• Can be combined with intravital microscopy	• Low throughput • Sorting needed to obtain single-cell resolution	25
	Laser microdissection-based method	LCM-seq, TSCS, Geo-seq	• Single-cell resolution (TSCS)	• RNA damage possible due to laser • Possible contamination from neighboring cells/tissue	26−28
B. Spatial Transcriptomics					
Fixated cells or tissues	Imaging-based	FISH, MERFISH, SEQFISH+	• Single-molecule resolution • High RNA capture efficiency	• Potential target panel design bias • Extensive imaging setup and procedures	30−32
	Sequencing-based	Spatial transcriptomics, Slide-seq, DBiT-seq, spatial Mux-seq	• Unbiased whole transcriptome capture • High sample throughput	• Low RNA capture efficiency • Lack of true single-cell resolution • Contamination from neighboring cells	34−37
	Imaging-guided spatially resolved omics	TIVA, PIC, PSS, ZipSeq, Light-seq, spatial FUNseq	• Unbiased whole transcriptome capture • True single-cell resolution • Selective and enriched deep single-cell profiling of user-defined regions • Higher sequencing depth (compared to sequencing-based methods)	• Low throughput • Oligo barcode multiplex capacity	20, 39−43

when sufficient prior knowledge is available to guide the experimental design, which is not always the case. To further understand uncharted cellular processes, an unbiased, cell-wide pathway analysis is necessary. Conventional omics-based approaches can provide such unbiased insight but lack the ability to link these findings to cellular characteristics or phenotypic features. Imaging-guided omics platforms have been established to bridge this gap by linking omics information to the phenotypic traits, such as cell morphology and cellular dynamics, of individual cells.

IMAGING-GUIDED SINGLE-CELL OMICS PLATFORMS

Several imaging-guided single-cell omics platforms have been developed to study single-cell phenotypes. In these imaging-guided omics pipelines, cells, organoids, or tissues are cultured (Figure 1A) and subsequently imaged (Figure 1B) to extract phenotypic information from individual cells, which is then linked to their molecular profiles using different approaches (Figure 1C,D). Linking microscopically observed phenotypes to their underlying genetic programs is essential, given the still poorly understood phenotypic diversity observed in cancer cell populations. For example, using the imaging-guided single-cell omics method CIN-seq,[12] micronucleated cells (Figure 1B), a rare phenotype associated with chromosomal instability, can be specifically isolated for sequencing, allowing the characterization of heterogeneous abnormal division patterns across the cancer cell population. This research shows that phenotypic features, typically lost in traditional analyses, can reveal additional layers of cellular diversity, offering a more comprehensive view of the complexity underlying biological processes.

Methods to study these individual phenotypes or states (Figure 1) rely either on (1) the physical separation of cells of interest using photoactivatable dyes (e.g., CIN-seq,[12] FUNseq,[13] FUNpro,[14,15] MAGIC[16]) or (2) the analysis of cells that have already physically been separated, such as those confined to wells in SCOPE-seq[17] and in the flow cytometer approach described by Zhang et al.[18] To consistently detect phenotypes and increase the throughput, these methods use live-cell image analysis programs, with real-time performances in the cases of photoactivatable methods.[12−16] The phenotypes identified vary in a wide range of complexity, from static phenotypes, such as basic parameter extraction in SCOPE-seq2[19] and micronuclei detection in CIN-seq[12] and MAGIC,[16] to complex dynamic behaviors, including migration patterns in FUNseq[13] and tripolar mitoses in CIN-seq.[12]

A major limitation of first-generation single-cell phenotypic omics methods was the small number of cells that could be

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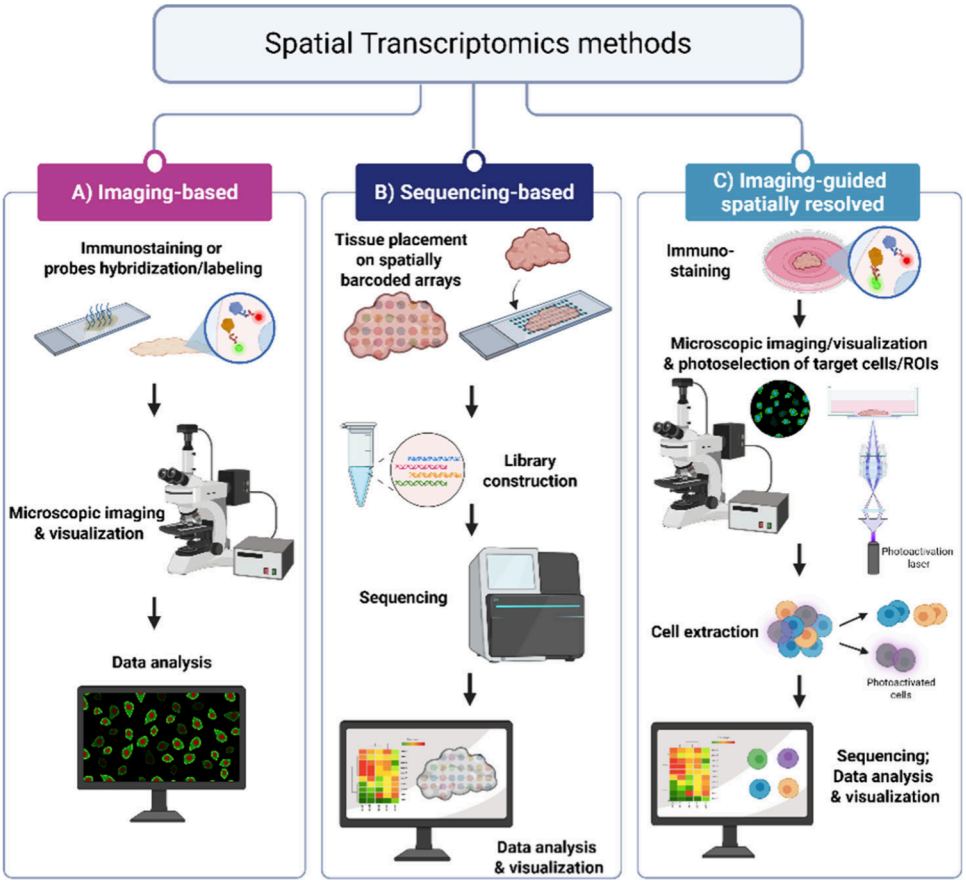

Figure 2. Three distinct categories of spatial transcriptomics methods. (A) Imaging-based methods, which visualize targeted genes under the microscope, such as SEQFISH+. (B) Sequencing-based methods, which capture RNA transcripts on a spatially barcoded array, such as spatial transcriptomics (ST). (C) Imaging-guided spatially resolved methods, which capture RNA transcripts with user-defined patterned illumination, such as spatial FUNseq. Adapted from ref 46. CC BY 4.0. Created with BioRender.

analyzed, as exemplified by MAGIC,[16] which profiled fewer than 150 micronucleated cells, a rare chromosomal instability event. Recent advances, however, have significantly increased the throughput. CIN-seq,[12] recently introduced by our group, achieved the isolation of more than 500 chromosomally unstable cancer cells per condition by using an ultrawide field of view (UFO) microscope that allows for screening of large cell populations (>150 K cells per sample[13−15]). This significantly enhances the applicability and statistical robustness of these methods for studying (intratumor) heterogeneity at single-cell resolution. Nonetheless, these next-generation photoactivatable approaches still face challenges, for example, in multiplexing different phenotypes, mainly due to the limited availability of photoactivatable dyes. In contrast, the microwell-based SCOPE-seq platform[17] overcomes this limitation by enabling multiplexed analysis using optically barcoded beads in combination with fluorescence microcopy. A limitation specific to microwell-based platforms, however, is their inability to capture information on cell−cell interactions, which forms a significant bottleneck for comprehensive cellular analysis. This challenge can be addressed by spatially resolved, photoactivatable approaches, which can detect interactions between individual cells, allowing microenvironmental crosstalk to be captured in the analysis.[20,21] All in all, each approach confers its own advantages and can be valuable in different biological contexts (Table 1A).

Even though these methods have uncovered valuable new insights into the linkage of phenotypic and molecular profiles, distinguishing driving from resulting mechanisms of specific biological events remains challenging, as cells are typically analyzed only after the phenotype has emerged. While thorough experimental validation remains essential, machine- and deep-learning approaches could address this problem, as previous research has shown that phenotypes could be predicted[22−24] and therefore potentially isolated for sequencing prior to the emergence of the event of interest, followed by (single-cell) sequencing. Such approaches potentially allow for better decoupling of the driving and resulting mechanisms, enabling more precise identification of the molecular events that initiate specific phenotypes. These attempts highlight the potential of integrating machine- and deep-learning strategies with imaging-guided single-cell omics techniques, as such approaches may reveal previously undetectable phenotypes and molecular mechanisms.

Photoactivation-based imaging-guided single-cell omics methods (e.g., FUNseq[13]) enable detailed characterization of essential cellular processes and in vitro cell−cell interactions in cancer, forming an interesting platform for many next-generation biological analyses. However, these approaches remain insufficient for questions that rely on the broader context of the TME. In such cases, tissue-level analyses are required to capture native spatial relationships. To address this need, methods have been developed to isolate individual cells

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directly from tissue (Figure 1), relying on either (1) micropipettes (e.g., Image-seq[25]) or (2) laser microdissection (e.g., LCM-seq,[26] TSCS,[27] Geo-seq[28]). These methods have proven their potential in isolating subpopulations of interest from their native tissue environment with high resolution, revealing interesting patterns that could not be captured using in vitro culture systems. However, these methods lack multiplexing ability, as they do not allow simultaneous comparison of multiple distinct regions within the same tissue section. This limitation is addressed by modern spatial transcriptomics, which enables region-resolved gene expression profiling and thus supports more comprehensive analyses.

SPATIALLY RESOLVED FUNCTIONAL TRANSCRIPTOMICS IN THE TUMOR MICROENVIRONMENT

Spatial biology has shown for decades the importance of spatial information in a biological context. The location of cells within a tissue provides crucial information about tissue architecture, intercellular interactions (e.g., tumor-immune crosstalk), tumor infiltration, and disease progression. For a long time, immunohistochemistry and immunofluorescence have been gold-standard methods for elucidating protein expression patterns in tissue samples, enabling identification of different cell types and visualizing the spatial organization of cells within the tissue.[29] However, these methods cannot comprehensively capture molecular mechanisms such as the drivers underlying certain phenotypes within the tissue. This limitation motivated the shift toward spatial transcriptomics (Figure 2), providing an additional layer of information within the spatial context of tissue.

Fluorescence in situ hybridization (FISH) was one of the first methods capable of visualizing DNA and RNA within tissue samples, thereby revealing their spatial distribution under the microscope.[30,31] FISH uses dye-labeled complementary probes to target specific DNA or RNA sequences. Over the past decades, this imaging-based technique (Figure 2A) has progressed from visualizing only a few genes/transcripts at a time in the 1980s and 1990s to multiplexing thousands of genes/transcripts in an automated manner (e.g., MERFISH, seqFISH+).[30,32,33] Although FISH is a powerful tool to use, it needs a prior panel design, meaning that selecting which genes to probe potentially leads to biased interpretation. To tackle this limitation, spatial transcriptomics or spatially resolved whole transcriptome methods were developed to capture RNA transcripts while retaining their spatial context.

In these spatially resolved whole transcriptome approaches, also known as sequencing-based spatial transcriptomics (Figure 2B), polyadenylated mRNA is primarily captured using a barcoded poly(T) oligonucleotide that hybridizes to the poly(A) tail, thereby priming it for reverse transcription and downstream processing. The readout of these barcodes in the sequencing data encodes the spatial information on the captured mRNA transcripts. Methods like spatial transcriptomics (ST)[34] and Slide-seq[35] use grids of barcoded poly(T) oligos fixed onto a slide, either directly linked or via bead deposition, allowing mRNA molecules to diffuse toward the capture oligos.[34,35] DBiT-seq[36] and spatial-Mux-seq[37] utilize microfluidics to deliver barcoded oligos horizontally and vertically to the mRNA transcripts, generating unique barcode combinations that encode spatial coordinates.[36,37] Stereo-seq uses a grid of DNA nanoballs carrying unique barcodes, which are first sequenced in situ and subsequently ligated to poly(T) oligos, allowing for mRNA capture.[38]

Lastly, a separate class of spatial transcriptomics methods rely on photoactivation to define spatial location based on imaging data, also termed imaging-guided spatial transcriptomics (Figure 2C). Instead of using a prepatterned grid of oligo barcodes, user-defined regions are assigned specific oligo barcodes through patterned illumination, enabling the isolation of rare cell types and the interrogation of specific cell−cell interactions with higher spatial resolution and sequencing depth. Multiple photoactivation-based imaging-guided spatial omics methods have been developed, tackling different modalities and photoactivation mechanisms: transcriptome in vivo analysis (TIVA), photo-isolation chemistry (PIC), and photoselective sequencing (PSS), which rely on photosensitive, caged oligos to capture mRNA (TIVA and PIC) or open chromatin regions (PSS);[39−41] ZipSeq, which uses photoactivatable antibody-labeled barcodes for single-cell sequencing;[42] Light-seq, which uses photoinduced covalent linking of barcodes to in situ cDNA;[43] and spatial FUNseq, which uses photoactivatable dyes paired with single-cell sequencing.[20,21]

At this moment, spatial transcriptomics has become a powerful tool applied in many applications, such as in investigations of TME interactions, tumor vulnerabilities, and immunotherapy efficiency, with different techniques being suited to address different biological questions (Table 1B).[44] Most current methods focus on a single modality, e.g., transcriptomics, proteomics, or epigenomics. Recent developments, however, aim to combine multiple modalities within the same sample while retaining spatial information. Spatial-Mux-seq and spatial-CITE-seq exemplify this trend, enabling spatially resolved measurements of both transcriptome and proteome.[37,45] Notably, spatial-Mux-seq can also capture epigenomic features like histone modifications and chromatin accessibility.[37] As the field advances toward spatial multiomics, it will provide a more comprehensive and integrated view of biological systems. In parallel, the maturation of spatial omics technologies, supported by nanotechnology, will facilitate deeper collaboration with other fields, with nanomedicine being a particularly promising partner.

INTEGRATION OF IMAGING-GUIDED SINGLE-CELL AND SPATIAL OMICS WITH NANOMEDICINE

These previously described imaging-guided single-cell and spatial omics methods have already demonstrated their impact on drug target discovery. For example, CIN-seq revealed a key role for *BCL2L1* in the survival of tripolar cell divisions in the MCF10A cell line, providing a potential starting point for the development of novel therapeutic strategies.[12] Therefore, they are expected to play an important role in the future of drug development, not only in the field of immunotherapies but also in nanomedicine. Nanomedicine has gained increasing attention over recent years, as it allows for tumor-targeted drug delivery, in contrast to conventional methods (e.g., chemotherapy), which frequently affect healthy cells alongside tumor cells.[47] This capability has the potential to greatly improve the safety, effectiveness, and personalization of anticancer therapies.

Various nanoparticles have been developed for the targeted delivery of chemotherapeutic molecules, immunotherapeutic agents, or therapeutic nucleic acids to tumor cells, including lipid-based polymeric and inorganic nanoparticles.[47] First-

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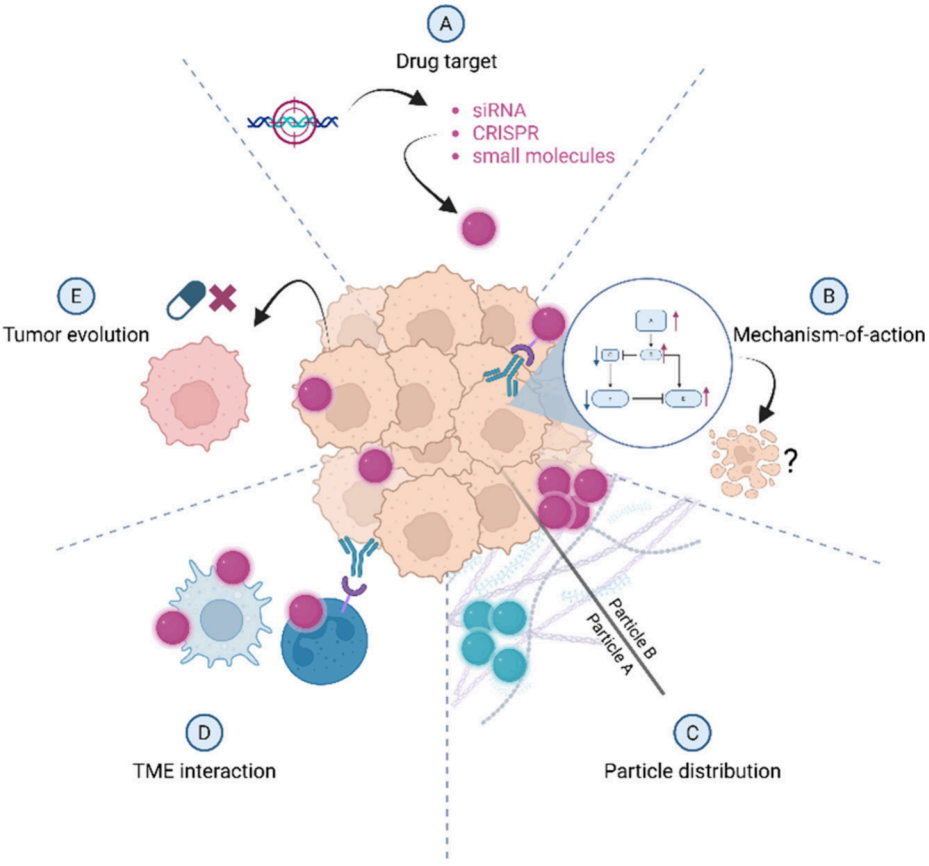

Figure 3. Applications of imaging-guided omics in nanomedicine research. Imaging-guided omics methods could be valuable in nanomedicine research, as they allow identification and elucidation of (A) drug targets, (B) mechanisms of action, (C) particle distributions, (D) tumor microenvironment (TME) interactions, and (E) tumor evolution. Created with BioRender.

generation nanoparticles exploited the enhanced permeability and retention effect to passively accumulate in tumors.[47] In contrast, second-generation nanomedicines employ cell-specific targeting strategies that rely on either (1) targeting moieties, such as antibodies, peptides, or carbohydrates, which selectively bind to specific antigens or receptors on the target cell membrane, or (2) biomimetic approaches in which nanoparticles are coated with membranes derived from the target cell.[47] Recently, self-propelling systems[48] (also called nanomotors), organelle-targeting nanoparticles,[49] and stimuli-responsive multistage delivery strategies[50] have been developed to further increase therapeutic efficiency.

These studies emphasize the revolutionary potential of nanotechnology in cancer treatment; however, translating fundamental research into clinically effective outcomes remains difficult.[47] Inefficient cellular uptake, ligand masking (i.e., formation of a biomolecular corona), and the often-conflicting requirements for nanoparticle properties at different stages of drug delivery collectively hinder therapeutic efficacy.[47] Imaging-guided omics methods are expected to provide valuable insights into the mechanisms underlying these bottlenecks, thereby facilitating the design and optimization of next-generation nanomedicines.

Single-cell and spatial omics approaches have been increasingly used for nanomedicine research to guide the design of cargo and delivery strategies and to evaluate existing treatments, providing insights that facilitate future iterations. Currently, the identification of drug targets is the most common application of omics methods in nanomedicine research. For example, omics analyses revealed a critical role for the Erbin gene in colorectal cancer progression and metastasis.[51] Building on these findings, nanoparticles were designed to deliver Erbin-targeting siRNA, which successfully knocked out the Erbin gene in megakaryocytes/platelets and reduced lung metastases in mice. Similar studies have repeatedly guided cargo selection, in which the cargos are not limited to siRNA but also include, among others, Cas9/sgRNA combinations, metabolites, and inhibitors.[52−54]

Additionally, omics methods have proven valuable for evaluating existing designs, for example by studying pathway alterations, metabolic differentiation, or TME regulation following nanoparticle exposure.[55−57] Although conventional single-cell omics techniques reveal valuable information about cellular states, they often lack information about actual nanoparticle uptake by individual cells. Single-cell nanoparticle targeting sequencing (SENT-seq) and ST address this limitation by mapping nanoparticle uptake using LNPs containing sequencing barcodes and by capturing the spatial distribution of nanoparticles using fluorescence microscopy, respectively. As a result, SENT-seq and ST studies have enabled the investigation of both the causes and consequences of nanoparticle distribution and uptake within tumors. These analyses reveal that heterogeneity in the cellular states of cancer cells and in their interactions with the TME are closely associated with patterns of nanoparticle localization and internalization.[58−60]

These studies emphasize the potential of using omics technologies to advance nanomedicine research and under-

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score the critical role of cellular heterogeneity within tumors. In future research, imaging-guided omics approaches are expected to be particularly valuable for capturing the full complexity of the molecular mechanisms in nanomedicine, as they consider complex dynamic (intercellular) phenotypic heterogeneity and characterize corresponding genetic profiles. By profiling nanomedicine-relevant phenotypes, such as those associated with nanoparticle uptake stages, response levels, or TME interactions, these methods could provide a more comprehensive view of the nanoparticle interaction with tumor cells and their surrounding microenvironment.

Imaging-guided omics methods can shed light on multiple aspects of nanomedicine−tumor interactions. First, these methods have shown potential for revealing drug targets,[12] and can therefore contribute to guiding cargo selection in nanomedicine (e.g., siRNA, CRISPR, small molecules) as well as the design of drug delivery systems (DDS) (Figure 3A). Second, these methods can help elucidate the mechanisms of action of nanomedicines (e.g., metabolic or proteomic effects and pathway alterations) by enabling single-cell-resolved profiling of cellular responses to nanomedicine treatments (Figure 3B). Such profiling allows for uncovering of heterogeneous responses of cells—such as differences in nanoparticle uptake or therapeutic sensitivity—which may provide insights into strategies for enhancing the proportion of effectively targeted cells, e.g., by combinational treatments. Besides nanoparticle−cell interactions, imaging-guided single-cell and spatial omics methods can also shine light on the heterogeneous distribution of nanoparticles (Figure 3C). Furthermore, interactions with the TME could be elucidated (Figure 3D), which is an essential consideration in nanomedicine, given that hypersensitivity reactions remain a major obstacle to effective clinical use.[47] Moreover, as imaging-guided omics methods can dynamically capture these mechanisms, they also offer the possibility of real-time monitoring of tumor evolution during nanoparticle treatment, which, for example, allows us to study drug resistance mechanisms (Figure 3E). As these methods are suitable for patient-derived samples, patient-specific variation in these processes can also be considered, which is expected to be valuable for the development of personalized treatments. Overall, imaging-guided omics methods are capable of capturing important, previously difficult to measure aspects of nanomedicine, ultimately advancing the field toward improved patient outcomes.

FUTURE DIRECTIONS AND CHALLENGES

In the past decade, nanotechnology, high-resolution imaging, and single-cell and spatial omics have advanced significantly, both individually and collectively. Progress within each field has led to new opportunities for collaboration, especially as improvements in spatial resolution have been aligned across modalities. State-of-the-art interdisciplinary tools are now enabling deeper insights into biologically relevant mechanisms such as those underlying cancer cell behavior and nanomedicine design and efficacy. Future developments will likely include the transition from 2D to 3D imaging in imaging-guided single-cell omics, allowing the characterization of more realistic biological structures and cellular interactions; going from fixed to live cells, organoids, or thicker tissues in spatial omics, opening new avenues to study dynamic phenotypes at a larger spatial scale; and the evolution of nanomedicine targeting, loading, and cargo-release strategies, ultimately reducing toxicity and hypersensitivity.

Furthermore, machine- and deep-learning strategies will not only assist in predicting specific phenotypes, thereby decoupling the driving from the resulting molecular mechanisms, but also accelerate biomarker identification, multiomics data integration, and in silico prediction of nanomedicine efficacy. In parallel, the increasing availability of multiomics datasets will provide a more comprehensive view of tumor-driving mechanisms and nanomedicine effects, filling the knowledge gap across different omics modalities.

These combinatorial advances in high-resolution imaging, single-cell and spatial omics, and nanotechnology will enable the establishment of next-generation multidisciplinary pipelines that support the development of scalable, affordable, and more effective nanomedicine treatments. Due to the novelty of imaging-guided omics technologies, multiple challenges may arise as the field matures. At this point, standardized experimental protocols and data-processing pipelines are often lacking, which limits the accessibility of these technologies. Furthermore, successful implementation of these technologies to specific biological applications, such as nanomedicine, may require substantial optimization. Moreover, to use these technologies and perform downstream analyses, nonstandard devices and/or materials are often necessary, such as specific imaging modalities, chemical compounds, or extensive computational resources. As noted above, these methods could offer valuable insights into patient variability. However, due to their limited throughput, these approaches currently facilitate functional studies of intratumoral heterogeneity in only a restricted number of patient samples rather than enable large-scale cohort screenings. To achieve meaningful clinical translation from such patient-derived data, these advances must also be supported by predictive translational models, ranging from animal models to organ-on-a-chip systems, which facilitate the transition from controlled in vitro environments to the complexity of human physiology. At the same time, patient privacy and clinical regulations must be carefully considered.

Altogether, the application of imaging-guided omics in nanomedicine research will generate new insights into cancer biology and improve therapeutic strategies. By resolving nanomedicine mechanisms of action, mapping particle distributions, and characterizing interactions within the native TME, these approaches will play a key role in accelerating the development of future cancer treatments.

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Notes

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