

The Role of Imaging in Evaluating Nanocarrier-Based Drug Delivery

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ABSTRACT

Imaging has become central to the evaluation of nanocarrier-based drug delivery because it can connect formulation behavior with pharmacokinetics, tissue distribution, microregional transport, cellular uptake, intracellular trafficking, drug release, and biological response. Yet an imaging signal is not intrinsically equivalent to intact carrier, encapsulated drug, bioavailable payload, or therapeutic action. This distinction is the main interpretive challenge in the field. This narrative review examines optical imaging, positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), magnetic particle imaging (MPI), computed tomography (CT), ultrasound, photoacoustic imaging, electron microscopy, autoradiography, and mass spectrometry imaging as complementary tools spanning whole-body to subcellular scales. Particular attention is given to label-carrier decoupling, the limitations of static organ-level accumulation, vascular and interstitial barriers, cell-type-specific sequestration, endosomal escape, and the need to measure carrier integrity and drug release directly. Representative studies demonstrate that size, vascular phenotype, endothelial transport, basement membrane structure, protein corona formation, and tumor-associated macrophages can each alter delivery in ways that are not captured by a single terminal biodistribution measurement. Clinically, radiolabeled liposomes and companion iron-oxide nanoparticles have revealed substantial interpatient and inpatient heterogeneity, supporting imaging-guided patient selection while also exposing the limitations of surrogate tracers. The most defensible studies therefore combine longitudinal whole-body imaging with tissue validation, orthogonal labels, pharmacokinetic mass balance, and pharmacodynamic endpoints. A practical decision framework and reporting checklist are provided to support rigorous study design and translation.

Keywords: nanocarrier, nanomedicine, drug delivery, molecular imaging, biodistribution, pharmacokinetics, intravital microscopy, payload release, theranostics

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1. INTRODUCTION

Nanocarriers are not just designed to carry an active agent, but also to change its pharmacology. Liposomes, polymeric nanoparticles, micelles, lipid nanoparticles, protein-based particles, inorganic constructs, nanodroplets and hybrid systems can be designed to extend circulation, alter organ exposure, protect labile cargo, provide controlled release, or target a drug to a specific biological compartment. However, their performance is controlled by a series of events starting from the formulation vial, through dilution, protein adsorption, vascular transport, tissue entry, interstitial transport, cell-type specific uptake, intracellular transport, release, metabolism and elimination. This cascade is only partially sampled using conventional bulk kinetic sampling.

The unique value of imaging is that it maintains the spatial and temporal context. A whole-body scan can be used to identify clearance routes and lesion-to-lesion heterogeneity; intravital microscopy can be used to reveal vascular dynamics and single-particle behavior; fluorescence lifetime or FRET can be used to report nanoscale proximity and carrier

disassembly; electron microscopy can be used to localize material at ultrastructural resolution; and functional imaging can be used to determine whether delivery changes perfusion, metabolism, receptor occupancy, cell death, or another biological process. Imaging is thus not only a visualization tool, but also a measurement tool that connects formulation properties to biological mechanism.

This is the same force, and it is always a source of error. The measured signal is from a fluorophore, radionuclide, magnetic core, acoustic droplet, elemental tag or reporter reaction. That signal can detach from the carrier, transfer to membranes or plasma proteins, metabolize, or become trapped following payload delivery, or change due to the microenvironment of the tissue. This can result in the bright accumulation while little or no nanocarrier, drug or therapeutic effect remains. The main question is what is being imaged and what inference can be legitimately drawn from the signal, both in terms of its physical and/or biological nature.

That is the measurement-centred approach that this review takes. It provides an overview of the roles of the major imaging modalities, imaging through the delivery cascade, pitfalls in quantitative and translational imaging, and a practical approach to selecting imaging endpoints and controls. While tumor delivery is highlighted as having the most comprehensive multiscale evidence base, the principles are applicable to infectious disease, inflammation, cardiovascular disease, neurological delivery, vaccines, nucleic acid therapeutics and organ-targeted gene editing.

2. SCOPE AND NARRATIVE LITERATURE APPROACH

A targeted narrative search was performed in PubMed, on publisher websites and in DOI records until 16 July 2026. The search concepts included nanocarrier, nanoparticle, liposome, micelle, lipid nanoparticle, drug delivery, biodistribution, intravital microscopy, fluorescence, FRET, PET, SPECT, MRI, MPI, CT, ultrasound, photoacoustic imaging, electron microscopy, mass spectrometry imaging, endosomal escape, radiolabeled liposome, and companion imaging. Primary studies that addressed mechanistic, quantitative, or translational questions using imaging were selected for priority, as were selected syntheses that influenced interpretation. References were selected to demonstrate principles and not to give a systematic or comprehensive evidence inventory. This review was not prospectively registered, searches and screening were not independently duplicated and no formal risk-of-bias or certainty-of-evidence assessment was conducted. Spatial-resolution and sensitivity are given as approximate ranges depending on the platform since instrumentation and reconstruction are still in a state of evolution.

3. IMAGING AS A MEASUREMENT AND INFERENCE SYSTEM

3.1 Define the imaged entity before selecting the modality

The first step in a rigorous imaging experiment is to have a clear definition of what is to be called the analyte. The analyte can be

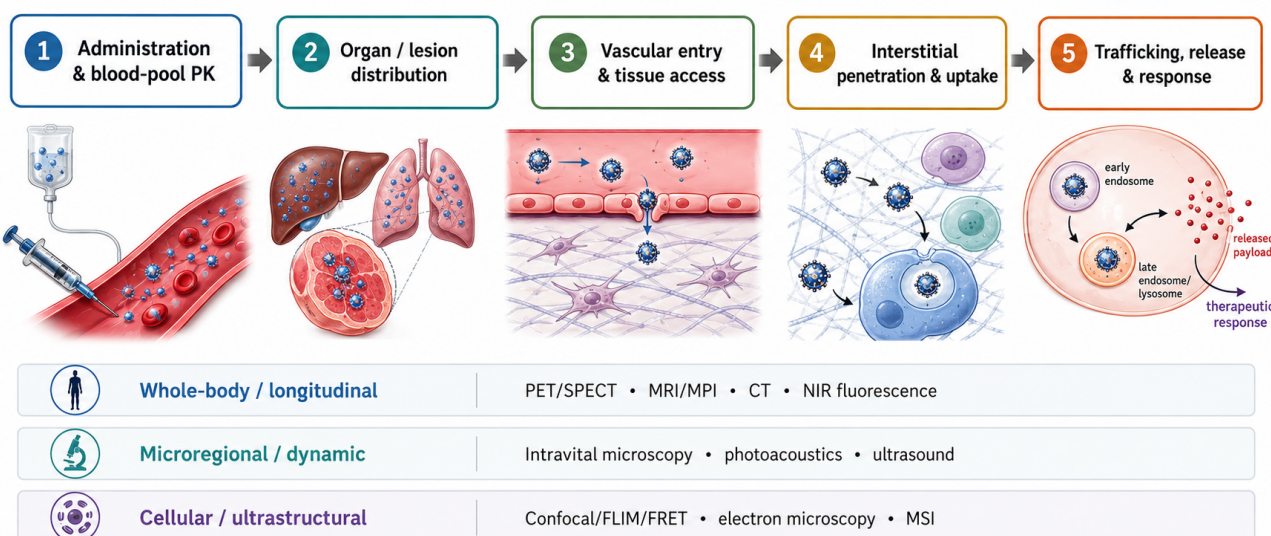
the carrier scaffold, surface ligand, encapsulated payload, co-encapsulated surrogate, released metabolite, elemental component, cell that has taken up material or downstream biological state. These do not interchange. A stable radiometal chelated to a lipid may be appropriate to assess particle biodistribution over a long period of time, while a small molecule drug directly radiolabeled may be appropriate to assess payload pharmacokinetics. A FRET pair can report on the maintenance of nanoscale proximity, however, if the FRET pair is lost, this could be due to dilution, lipid exchange, degradation or true release. Iron-oxide core can be tracked quantitatively by MPI, however, after the original organic coating has degraded it may remain in macrophages.

Therefore, the label should be considered a part of the formulation. Its location, molar fraction, charge, hydrophobicity, chelation chemistry, specific activity and stability may change the colloidal properties or produce a different tracer population in the biological system. Fluorescence labeling has been reported to alter biodistribution of nanoparticles and adsorbing a biomolecule corona can obscure targeting ligands and eliminate receptor mediated behavior [16,23]. Characterization is required to be repeated following labelling, following incubation in clinically relevant biofluids and following clinically realistic dilution.

3.2 The signal hierarchy

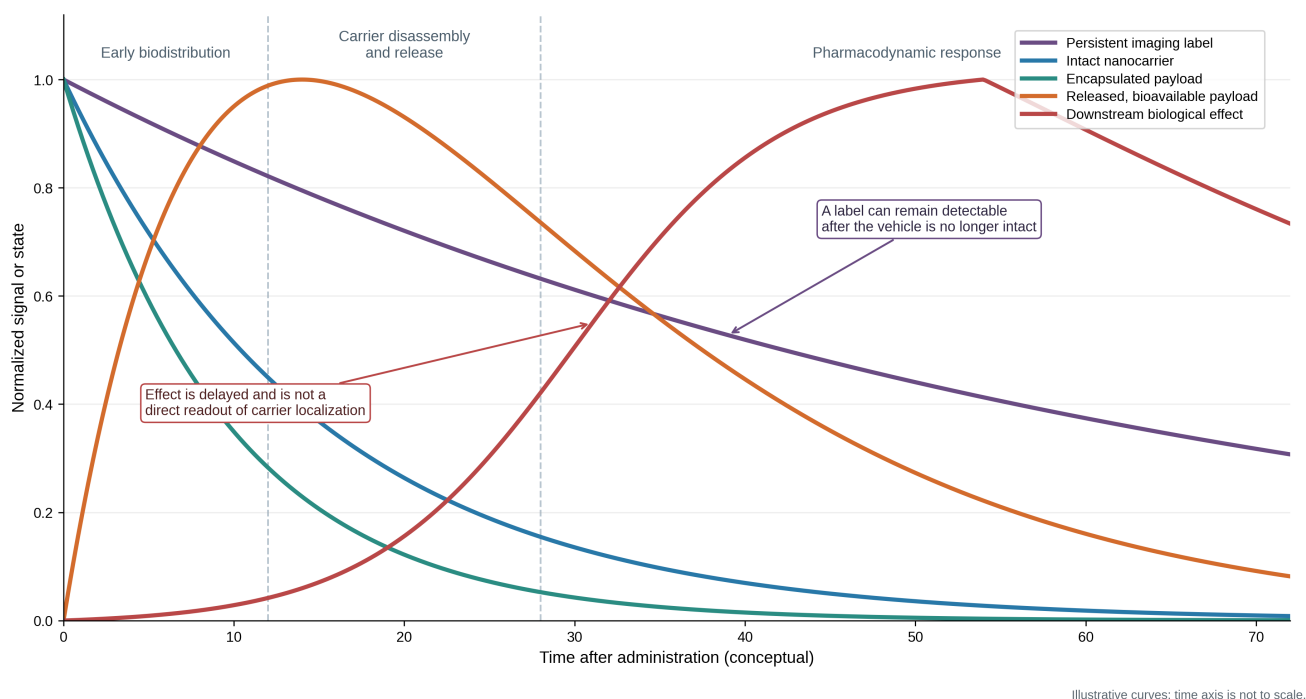
The hierarchy in Figure 2 distinguishes five variables that are frequently grouped together under the single term "claim": persistent label, intact nanocarrier, encapsulated payload, released bioavailable payload and biological effect. The time courses of these can differ significantly. A one-time-point colocalization image is not an integrity of carrier image, and persistent signal is not an image of payload retention. The amount of evidence required varies with claims. Calibrated whole body imaging and tissue recovery may be necessary for a distribution claim. A stability claim can only be made using a ratiometric, lifetime or dual label method. A release claim must be chemically confirmed, or have an activation mechanism that is specific to release. Mechanism claim must be associated with cells/organelles. Therapeutic claim must have target engagement or outcome data.

Imaging the nanocarrier delivery cascade across biological scales



The nanocarrier delivery cascade and the imaging scales used to interrogate it. Whole-body modalities support longitudinal pharmacokinetics and organ distribution; dynamic and microscopic methods resolve vascular entry, penetration, and cell-type-specific uptake; nanoscale or chemistry-sensitive methods address integrity, release, and intracellular fate. Original schematic.

The signal hierarchy: localization, integrity, release, and effect are distinct variables



Conceptual signal hierarchy. A persistent imaging label can outlast the intact nanocarrier; encapsulated and released payloads have different kinetics; and biological effects are delayed. Curves are illustrative, normalized, and not tied to a particular experiment or time scale. Original schematic.

Box 1. Evaluation criterion	Key question
Signal Source	What exact chemical or biological entity generates the imaging signal?
Label Stability	Can the label detach, exchange, degrade, or be retained independently of the carrier?
Quantitative Accuracy	Is the measurement quantitative under the relevant depth, concentration, and tissue conditions?
Validation Method	What orthogonal assay validates localization, integrity, or payload release?
Pharmacological Relevance	Does the imaging endpoint explain or predict pharmacological activity rather than simply accompany it?

4. IMAGING MODALITIES ACROSS SPATIAL AND TEMPORAL SCALES

There is no one modality that is always better. Selection is an optimization problem based on sensitivity, spatial and temporal resolution, penetration depth, quantitative accuracy, chemical specificity, label burden, radiation exposure, availability and compatibility with repeated measurements. The following

narrative focusses on what each modality can and cannot establish, while Table 1 offers a practical comparison. The reported resolution and sensitivity ranges are dependent on the field of view, specimen size, depth, tracer, hardware, acquisition and reconstruction and should not be viewed as fixed specifications.

Comparison of Imaging Modalities for Nanocarrier-Based Drug Delivery

Modality	Typical spatial resolution (approx.)	Sensitivity / quantification	Best-suited questions	Principal limitations
Planar fluorescence / NIR	≈0.1–1 mm superficially; degrades with depth	High; usually relative or semiquantitative	Rapid screening; superficial or intraoperative imaging; ex vivo mapping; dual-label studies	Depth-dependent attenuation and scattering; autofluorescence; spectral overlap; dye leakage; limited absolute quantification
Bioluminescence imaging	≈1–10 mm localization in small animals	Very high; reporter- and physiology-dependent	Reporter-cell tracking; gene expression; therapeutic response in small animals	Requires a viable reporter, substrate, oxygen, and ATP; weak anatomy; not a direct particle measure; limited clinical use
Confocal / multiphoton / intravital microscopy	≈0.2–2 μm lateral; depth typically ≈0.1–1 mm	High spatial and temporal detail	Single-particle and cell tracking; vascular dynamics; extravasation; intracellular trafficking	Limited field and depth; window and sampling bias; motion; photobleaching and phototoxicity
FRET / FLIM / ratiometric fluorescence	Proximity ≈1–10 nm; image resolution platform-dependent	High; ratio- or lifetime-based	Nanoscale proximity; carrier integrity; payload release; endosomal escape	Requires calibration; stoichiometry, pH, quenching, and bleaching matter; loss of proximity is not uniquely drug release

Modality	Typical spatial resolution (approx.)	Sensitivity / quantification	Best-suited questions	Principal limitations
PET	≈0.7–2 mm preclinical; ≈3–6 mm clinical	Very high; quantitative with calibration and correction	Whole-body PK; biodistribution; lesion heterogeneity; kinetic modeling; dosimetry	Ionizing radiation; radiochemistry and metabolites; partial-volume effects; label may outlast the carrier
SPECT	≈0.3–1 mm preclinical; ≈7–15 mm clinical	High; quantitative with calibration and correction	Longer-lived tracking; organ function; multi-isotope studies	Clinical resolution is generally coarser than PET; radiation; collimator sensitivity-resolution trade-off; label stability
MRI	≈25–500 μm preclinical; ≈0.5–2 mm clinical	Moderate to low molar sensitivity; excellent anatomy	Anatomy; perfusion; permeability; diffusion; oxygenation; macrophage uptake	Longer scans; relaxivity and compartment effects; susceptibility artifacts; endogenous confounders
MPI	≈0.5–5 mm preclinical, tracer- and system-dependent	High for iron tracers; approximately linear mass quantification	Blood-pool kinetics; iron-tracer biodistribution; cell tracking; clearance studies	Tracks magnetic material rather than the intact composite carrier; limited availability and clinical experience; no intrinsic anatomy
CT / spectral CT	≈10–100 μm micro-CT; ≈0.3–1 mm clinical	Low to moderate contrast-agent sensitivity	Dense or high-Z carriers; vascular imaging; material decomposition; image-guided delivery	Ionizing radiation; high local dose often required; low sensitivity for many organic carriers; beam-hardening and decomposition errors
Ultrasound	≈30 μm–1 mm, frequency- and depth-dependent	High for gas agents; real-time	Perfusion; microbubble or nanodroplet activation; cavitation-mediated delivery and release	Operator and acoustic-field dependence; nonlinear signal; gas agents may not represent nanocarrier biodistribution
Photoacoustic imaging	≈10 μm–1 mm; resolution decreases with depth	Moderate to high for strong absorbers	Absorber mapping; vascular oxygenation; photothermal and theranostic monitoring	Depth-dependent fluence and spectral coloring; difficult absolute quantification and unmixing
Electron microscopy / AFM	Sub-nm to ≈100 nm	Direct structural visualization	Particle morphology; membrane interactions; ultrastructural localization	Terminal or ex vivo; tiny sampled volume; preparation and identification artifacts; low throughput
Autoradiography	≈10–100 μm	High for radionuclides	High-resolution radionuclide mapping; lesion heterogeneity; validation of in vivo scans	Terminal; section-dependent; isotope-specific calibration and spatial-registration uncertainty
Mass spectrometry imaging	≈1–200 μm, platform-dependent	Moderate to high; chemically specific	Label-free or isotope-resolved mapping of drug, metabolites, lipids, and carrier components	Terminal; matrix effects and ion suppression; variable resolution; demanding calibration and identification

4.1 Optical imaging: speed, mechanism, and molecular responsiveness

Fluorescence is the most experimentally flexible platform as a carrier, payload, targeting ligand or responsive element can be labeled with different spectra. The use of near infrared excitation minimizes autofluorescence and allows for greater depth than visible wavelengths, while confocal and multiphoton microscopy allow for cellular resolution. The use of bright, photostable nanocrystal probes for in vivo tumor imaging was pioneered by quantum dots, which also demonstrated how imaging can be used to correlate particle size with elimination by renal clearance [30,31]. However, planar fluorescence intensity is highly sensitive to tissue absorption, scattering, illumination geometry, detector settings and fluorophore environment. It should generally be considered as relative distribution, unless it is backed up by tomographic reconstruction and tissue-specific calibration.

Intravital microscopy is particularly powerful for causal questions. Time-lapse imaging allows to differentiate free-flowing, marginating, adherent, extravasating and phagocytosed particles, quantify vascular permeability and burst-like leakage and map penetration in relation to perfused vessels [3,11]. The drawback of it is that it is not representative. A small optical field in an accessible window is not necessarily a representation of the entire tumor, organ or patient. Regional, animal and biological phenotypical sampling is crucial.

FRET, fluorescence lifetime imaging, and activatable probes provide answers to a more challenging question: Do the labeled components stay close together or do they experience a specific environment? The stability and premature drug release in vivo have been monitored using FRET-enabled micelles and

polymeric nanoparticles [20–22]. While these methods may be more informative than intensity, the transfer efficiency depends on the distance between the donor and the acceptor, the donor/acceptor ratio, the orientation, the local refractive index, the dilution, and the photophysics of the probe. Lifetime based readouts are less concentration dependent, but do require controls to account for pH, protein binding, quenching and degradation.

4.2 Nuclear imaging: quantitative whole-body pharmacology

PET and SPECT are highly sensitive, can detect regardless of depth, can image longitudinally and can quantify whole body. Using appropriate kinetic modeling, it is possible to separate vascular and tissue compartments, estimate uptake rates and compare lesion level exposures. Resolution depends on the system: multi-pinhole preclinical SPECT can be submillimetric, while clinical SPECT is typically less than clinical PET. In studies, system resolution, reconstructed voxel size, recovery coefficients and partial-volume methods should be reported in addition to modality labels. The radiolabel can be attached to the carrier, to the membrane, to a payload or as a matched companion nanoparticle – this dictates the biological inference.

Thus, the critical control point is radiochemistry. Residualizing radiometals can be retained in cells following degradation of the carrier, while radiohalogens can dehalogenate or form diffusible metabolites. Serum stability alone is not enough; sometimes in vivo metabolite analysis, radiochromatography in urine and plasma, and dual-label studies and terminal gamma counting are necessary. Partial-volume correction is significant for small lesions and organs; cross validation of image derived concentration with recovered activity is important.

Radiolabeled pegylated liposomes were used in clinical studies, which showed considerable heterogeneity of nanocarrier accumulation between tumours and patients [2]. More recently, ^{64}Cu -MM-302 PET was used to quantify the heterogeneous uptake in metastatic breast cancer, and correlate uptake with treatment response, creating a model for image-guided nanotherapy [28]. As can be seen in these examples, the utility of nuclear imaging is that it can measure the distribution of the labeled construct, but it cannot determine on its own whether it is intact, whether it has penetrated the tumor, or whether the released drug has reached the tumor.

4.3 MRI and MPI: anatomy, tissue physiology, and magnetic tracer fate

MRI offers the unique combination of nanocarrier-sensitive contrast and high-resolution anatomy and functional measures of perfusion, permeability, diffusion, oxygenation, and spectroscopy. Superparamagnetic iron-oxide particles can be used to probe macrophage uptake or vascular delivery, and create susceptibility effects. One of the greatest strengths is the capability of correlating the signal of nanoparticles with the phenotype of the tissue in the same examination. The major limitation is that the contrast is indirect, meaning that the changes in relaxivity due to aggregation, compartmentalization, field strength, pulse sequence, and endogenous iron affect the contrast. Hemorrhage, calcification, air and motion are also causes of signal loss on T2- or T2*-weighted images.

The nonlinear magnetization of superparamagnetic iron is directly detected by MPI and yields positive, background-free signal, which is approximately linear with tracer mass over a useful range. It is highly favorable for blood-pool kinetics, cell tracking and quantitative biodistribution in combination with CT or MRI for anatomical information. However, MPI is not able to track the original magnetic composite carrier, and the clinical infrastructure is still limited.

Translational potential of companion nanoparticle MRI. In preclinical studies and in a pilot study of patients with advanced solid tumors, uptake of Ferumoxytol was shown to be correlated with deposition of nanoliposomal irinotecan and response [26,27]. These findings corroborate the idea of imaging access to tissues either prior to or early in the course of therapy, and highlight the importance that a surrogate particle should be similar to the therapeutic formulation with respect to size, surface chemistry, circulation time, and clearance pathways.

4.4 CT, ultrasound, and photoacoustic imaging

The high anatomical precision, speed and reproducibility of CT comes at the cost of its relatively low sensitivity to contrast agents, which typically need to be present at high local concentrations or be a material with a high atomic number. Targeted molecular CT has been achieved in preclinical cancer models using gold nanoparticles [32]. Spectral and photon-counting methods can enhance material decomposition and separation of multiple contrast elements but dose, beam-hardening and long-term fate are factors to consider.

Real-time imaging that does not involve the use of ionizing radiation is possible with ultrasound. Conventional microbubbles are not nanocarriers in the true sense of the word, but are relevant as an intravascular companion agent and cavitation nuclei for triggered delivery, and are typically micron-sized. Depending upon the composition and biological context, nanoscale phase-change droplets can extravasate prior to acoustic vaporization. Acoustic dosimetry, temperature monitoring (when applicable), and sham-insonation controls are essential due to the fact that acoustic pressure, frequency, pulse sequence, vessel geometry, and bubble or droplet destruction all affect the signal and bioeffects.

The photoacoustic imaging is based on the detection of ultrasonic waves that are produced following the optical absorption. It can be used to achieve optical molecular contrast and greater spatial localization than microscopy, and it can also measure vascular oxygenation. Multimodal optical and photoacoustic carriers such as porphyrin-based nanovesicles are examples [33]. The quantitative interpretation is challenging due to the local light fluence being heterogeneous and wavelength dependent; depth effects may be interpreted as concentration changes when performing spectral unmixing without fluence correction.

4.5 Ex vivo microscopy, autoradiography, and mass spectrometry imaging

The direct evidence of morphology and subcellular localization is provided by electron microscopy, particularly in the presence of intrinsic electron density of the particles or when elemental mapping is used. Cryogenic TEM is also of key importance for formulation characterization. For instance, cholesterol analogues altered the morphology of LNPs and their ability to deliver mRNA into the cell, connecting nanoscale structure to function [24]. However, in tissue it may be challenging to differentiate a degraded nanoparticle from dense material that is naturally occurring. Specificity is improved by immunogold, elemental spectroscopy, correlative light-electron microscopy and blinded morphology criteria.

Autoradiography is useful for fine scale mapping of radionuclide distribution and is useful for validating lesion heterogeneity, which is beyond the resolution of clinical scanners. Mass spectrometry imaging provides chemical specificity in the form of mapping of parent drug, metabolites and lipids without a fluorescent surrogate. It can directly demonstrate that a carrier label and active drug are in different tissue areas. Both are terminal, section dependent and sampling sensitive, and tissue handling and calibration sensitive, but are among the best tools for anchoring in vivo signal to tissue chemistry.

5. IMAGING THE NANOCARRIER DELIVERY CASCADE

A delivery study is most informative when imaging endpoints are aligned with sequential biological barriers. The same particle can perform well at one stage and fail at the next. Table 2 maps common questions to preferred readouts and minimum controls.

Imaging Endpoints, Modalities, and Validation Across the Drug Delivery Cascade

Delivery Stage	Key Question	Imaging Modalities / Readouts	Validation & Controls
Formulation Integrity	Is the carrier stable before administration?	DLS, Cryo-EM, FRET/FLIM, leakage assay	Labeled vs. unlabeled formulations; serum stability
Blood Pharmacokinetics	How long does the carrier remain in circulation?	PET, SPECT, MPI, MRI, blood analysis	Free-label control, metabolite analysis, mass balance
Organ Biodistribution	Which organs accumulate the carrier?	PET/SPECT, MPI, CT/MRI, optical imaging	Dose correction, perfused vs. non-perfused tissue
Lesion Delivery	How heterogeneous is lesion uptake?	PET, SPECT, MRI, fluorescence imaging, MSI	Partial-volume correction, multi-lesion analysis
Vascular Transport	Does the carrier adhere or extravasate?	Intravital microscopy, EM, permeability MRI/CT	Perfusion markers, endothelial labeling, motion correction
Interstitial Penetration	How far does the carrier penetrate tissue?	Multiphoton microscopy, MSI, autoradiography	Vessel-distance analysis, ECM and necrosis mapping
Cellular Uptake	Which cell types internalize the carrier?	Multiplex microscopy, imaging mass cytometry, flow cytometry, EM	Cell segmentation, extracellular signal removal
Endosomal Escape	Does the payload reach the cytosol?	Confocal microscopy, FLIM/FRET, EM	Free-cargo and pH controls, functional assays
Payload Release	Where and when is the drug released?	Activatable probes, PET, MSI, microdialysis	Dual labeling, metabolite separation, calibration
Target Engagement	Does delivery induce the desired biological effect?	Receptor PET, diffusion/metabolic MRI, pathology	Baseline and longitudinal imaging, free-drug comparison
Safety Assessment	Is there off-target accumulation or toxicity?	Whole-body imaging, organ imaging, histology	Long-term follow-up, degradation product analysis

5.1 Circulation, protein corona, and clearance

Early blood-pool imaging sets the stage to determine if a formulation will achieve the desired exposure window. The distribution phase can be measured by dynamic PET, SPECT or MPI and rapid extraction by the liver, spleen, kidneys or lungs can be identified. A blood ROI is only useful after correction for spillover and partial volume and image derived input functions should be validated by blood samples. The concentration of interest may also vary from plasma to erythrocytes, leukocytes and platelets.

Nanocarriers are constantly changing in biological fluids. Size, charge, receptor accessibility and uptake can be altered by adsorbed proteins. A protein corona can be used to mask transferrin and remove the targeting behaviour of functionalized nanoparticles as demonstrated by Salvati et al. [16]. Therefore, imaging studies of targeted and untargeted constructs should measure the biological identity of the construct, rather than simply the synthetic identity. In vitro targeting not in vivo can be explained by corona composition, receptor binding after serum exposure and circulation kinetics.

Clearance imaging should differentiate between intact carrier and label products. Small fluorescent pieces or radio-metabolites may be excreted in the urine, but the scaffold will be left in the liver or spleen. In contrast, phagolysosomal biogenesis of radiometals and iron can be retained in phagolysosomes following degradation. To complete the mass balance, serial urine and feces collection, chromatographic analysis, elemental balance and terminal organ assays are required.

5.2 Organ and lesion biodistribution

Biodistribution to the organ is required but not sufficient. In a tumor, a large portion of the injected dose can be trapped in the blood, perivascular macrophages, necrotic tissue or a few vascular hotspots. Meta-analytic studies have shown that the average percentage of systemically administered nanoparticles which reach tumors is low [1] but this is less important than the mechanisms and the heterogeneity of a given formulation-disease combination.

The size, circulation time, surface chemistry, dose and tumor phenotype are interdependent. With gold nanoparticles, polymeric micelles, and other constructs, there have been studies that indicate that there are size dependent trade-offs between blood half-life, extravasation, penetration, cellular uptake, and renal and reticuloendothelial clearance [5,6,12,14]. Imaging designs should not be interpreted as intrinsic targeting efficiency,

but rather as area under the concentration-time curve, vascular exposure and tissue retention are all different quantities.

Lesion-level imaging is particularly useful in the clinical setting as there is inter- and intra-patient heterogeneity. The uptake of radiolabeled liposomes has been found to vary significantly in studies [2,28]. This heterogeneity can be used for biopsy guidance, identify nonresponse due to delivery, and inform adaptive dosing if the timing of the scan and the formulation of the tracer are aligned with the therapeutic process.

5.3 Vascular entry: permeability, active transport, and transient events

Although the EPRE is still valid in terms of prolonged circulation and interaction with leaky tumor vessels and reduced clearance, it is not a universal mechanism or a binary tumor property. Vascular pore size, vascular perfusion, endothelial cell phenotype, pericyte coverage, pressure and basement membrane structure is heterogeneous and changes with time. Carrier size was shown to affect permeability and penetration by Dreher et al. [3] and vascular normalization was shown to increase delivery in a size-dependent manner [6].

Transient vascular bursts have been observed by high-speed and intravital imaging, which leads to episodic entry of nanoparticles [11]. Multiscale imaging and electron microscopy studies have suggested that active trans-endothelial transport can be a significant pathway in the particular tumor models studied and that specialized endothelial subpopulations affect the site of transport [7,8]. These findings cannot be applied as a general rule for the replacement of paracellular leakage. Route assignment depends on particle size and surface chemistry, type of tumor model, perfusion and fixation, time of sampling and three dimensional verification of localization. The most defensible interpretation is that the three mechanisms, transcytosis, paracellular leakage and transient vascular events, can all occur at the same time and the proportions of each can be determined by experiments.

A good vascular-entry experiment should co-image perfusion, endothelial boundaries, basement membrane, and particles and should differentiate stationary intravascular signal from extravascular signal in three dimensions and quantify trajectories over time. Luminal and adherent particles are reduced by tissue perfusion prior to harvest but not completely removed. Transcellular localization may be supplemented by electron microscopy, taking into account the orientation of the sections and the sampling.

5.4 Interstitial penetration and exit

Once the vessels are entered, diffusion and convection are counteracted by the extracellular matrix, cell density, binding-site barriers, increased interstitial pressure, and distance from working vessels. Smaller carriers are likely to penetrate deeper while larger or more tightly binding systems may have higher local retention but lack perivascular distribution [5,12-14]. Multistage particles that are smaller after arrival or release of smaller particles in deep tissue, are examples of how imaging can be used to differentiate initial tumor deposition from deep tissue access [13].

Distance-to-vessel is more informative than average fluorescence. Three-dimensional segmentation can quantify radial penetration, accessible tissue fraction and co-localization with viable tumor, stroma, immune cells, hypoxia or necrosis. Optical clearing increases sampled volume while autoradiography and MSI give chemical maps section wide.

Retention is also a dynamic process. Nanoparticles are able to exit tumors via intratumoral or peritumoral lymphatics and in certain circumstances, via blood vessels, with the predominant pathway depending on the size and context of the nanoparticles [9]. Basement membrane itself may be a significant barrier and modification or permeation of the basement membrane was shown to enhance delivery in experimental tumors [10]. Therefore, longitudinal imaging should not just assess influx, but also should assess efflux, and should not assume that the accumulation in the tumor is irreversible.

5.5 Cell-type-specific uptake and macrophage sequestration

The bulk tumor signal is not able to identify the cell responsible for uptake. Depending on the properties of the particles and time, endothelial cells, perivascular macrophages, resident phagocytes, fibroblasts, tumour cells and infiltrating leukocytes can each dominate. Multiplex microscopy, extracellular-signal quenching flow cytometry, imaging mass cytometry and EM can be used to assign signal to cell types. The segmentation of cells should be reported along with the percentage of the total tissue signal that is present in each compartment.

Although the uptake by macrophages is referred to as a loss mechanism, imaging has shown that the uptake by macrophages has more complex functions. A nanoparticle prodrug can be stored in a slow-release depot in the form of TAMs and then be released gradually over time [15]. However, in other environments, macrophages can also isolate cargo from target cells or induce inflammatory toxicity. The functional effect will thus depend on the kinetics of drug release, the localization of the macrophages and the diffusion of the active species to the neighbouring cells.

Targeting ligands often lead to higher cellular internalization but do not necessarily result in higher whole tumor accumulation. Kirpotin et al. discovered that antibody targeting of long circulating lipid nanoparticles resulted in increased internalization but not tumor localization [4]. This is important: a negative whole body targeting result can mask a positive cellular delivery result and vice versa.

5.6 Intracellular trafficking, endosomal escape, and payload release

Cytosolic delivery is the key limiting step for many macromolecular drugs and for nucleic acids. Image-based

analysis of siRNA LNPs revealed that very little internalized LNP material is delivered to the cytosol and that endocytic recycling can deliver particles or cargo to the extracellular space [17,18]. Live-cell imaging also revealed episodic release from endosomes and correlated this with knockdown of target genes [19]. These studies shifted the target of the optimization from total uptake to productive uptake.

Endosomal escape is not easy to quantify as disappearance of the puncta could be due to photobleaching, vesicle movement, degradation or failure of deconvolution. More powerful methods involve the use of orthogonal reporters like galectin recruitment, split luciferase, activation of cytosolic enzymes, RNA interference, protein expression or genome editing, in addition to spatial redistribution. Rare, transient events must be sampled. It is important to report the denominator: escape can be reported per internalized particle, per cell, per delivered cargo molecule, or per dose—and these are not equivalent.

Special probes are necessary for the disassembly of the carrier and the release of the drug. FRET has been used to characterize micelle and polymeric-nanoparticle stability and, in some systems, in vivo premature release [20-22] and dual labeling can be used to compare scaffold and payload. Direct imaging of a pharmacologically active isotope-labeled drug or MSI of parent drug and metabolites leads to more chemical specificity. Recent structure-guided studies have used both cryo-EM/cryo-TEM and functional assays, in addition to live-cell imaging, to correlate LNP morphology or endosomolytic chemistry to mRNA escape and expression [24,34,35]. The core-shell study from 2026 employed engineered proof-of-concept gold cores coated with ionizable lipids; the results presented in this study should not be extrapolated to conventional LNPs without validation in clinically relevant materials, cargoes, doses, cell types, and species.

5.7 Target engagement, pharmacodynamics, and response

Only after delivery is the pharmacologically meaningful molecular or cellular effect achieved is delivery pharmacologically meaningful. The imaging endpoints can be receptor occupancy, reporter-gene modulation, apoptosis, proliferation, immune activation, perfusion, diffusion restriction, metabolism or tissue function. A delayed pharmacodynamic signal is not necessarily a carrier localization signal, but rather a downstream effect that is dependent on potency, resistance, and host biology.

The most robust analysis is spatial exposure to spatial effect. For instance, lesion level PET of nanoparticles could be correlated with volumetric response and diffusion MRI or metabolic PET could detect early biological change. MSI or autoradiography can be correlated to histology and molecular markers within tissues. Baseline lesion phenotype, free-drug comparators and uncertainty in imaging and outcome measurements should be included in exposure-response models.

6. REPRESENTATIVE STUDIES: WHAT IMAGING CHANGED

The value of imaging is most evident when it alters a mechanistic conclusion or a translational decision. Table 3 summarizes illustrative primary studies across scales.

Representative Studies on Imaging-Guided Nanocarrier Drug Delivery

Study	Imaging Approach	Key Findings
Harrington et al. (2001) [2]	Radiolabeled PEGylated liposomes with clinical gamma imaging	Demonstrated tumor accumulation and inter-patient variability, supporting clinical nanocarrier imaging.
Dreher et al. (2006) [3]	Intravital fluorescence imaging	Showed that carrier size independently influences permeability, accumulation, and tissue penetration.
Kirpotin et al. (2006) [4]	Fluorescence imaging and tissue analysis	Targeting improved cellular uptake without increasing overall tumor accumulation.
Cabral et al. (2011) [5]	Optical imaging of size-tunable polymeric micelles	Demonstrated superior accumulation of sub-100 nm carriers in poorly permeable tumors.

Study	Imaging Approach	Key Findings
Matsumoto et al. (2016) [11]	High-resolution intravital microscopy	Identified transient vascular bursts as a mechanism of nanoparticle entry.
Sindhwani et al. (2020) [7]	Multiscale imaging and electron microscopy	Reported active trans-endothelial transport as a major entry pathway in the tumor models studied.
Nguyen et al. (2023) [9]	Longitudinal multiscale imaging	Demonstrated size- and context-dependent lymphatic and vascular egress, challenging the assumption of irreversible retention.
Miller et al. (2015) [15]	Intravital microscopy and mass spectrometry	Identified tumor-associated macrophages as slow-release reservoirs for Pt(IV) nanoparticles.
Gilleron et al. (2013) [17]	Live-cell and tissue imaging of siRNA LNPs	Showed limited endosomal escape and proposed a framework for delivery analysis.
Wittrup et al. (2015) [19]	Live-cell fluorescence imaging	Visualized endosomal release events linked to effective gene silencing.
Pérez-Medina et al. (2016) [25]	Nanoreporter PET imaging	Predicted nanotherapy response using companion nanoparticles.
Ramanathan et al. (2017) [27]	Ferumoxylot MRI	Correlated companion-particle uptake with treatment response in patients.
Lee et al. (2017) [28]	⁶⁴ Cu-MM-302 PET	Quantified lesion-level EPR heterogeneity and its relationship with therapeutic response.
Patel et al. (2020) [24]	Cryo-TEM and 3D particle tracking	Linked LNP morphology and intracellular transport to mRNA delivery efficiency.
Li et al. (2026) [35]	Cryo-EM, live-cell imaging, functional assays	Engineered ionizable-lipid-coated gold-core LNPs with improved endosomal escape and mRNA expression.

7. MULTIMODAL AND CORRELATIVE IMAGING

Multimodal imaging is most useful when each component resolves a different ambiguity. PET/CT combines sensitive tracer quantification with anatomy; PET/MRI adds soft-tissue phenotype and functional parameters; fluorescence enables surgical or microscopic validation after nuclear imaging; and MPI can be co-registered with CT or MRI to provide quantitative magnetic-tracer maps. Orthogonality should mean independent analytes or failure modes, not merely different instruments detecting the same persistent label. Adding modalities that report the same entity at the same scale increases complexity without necessarily increasing validity.

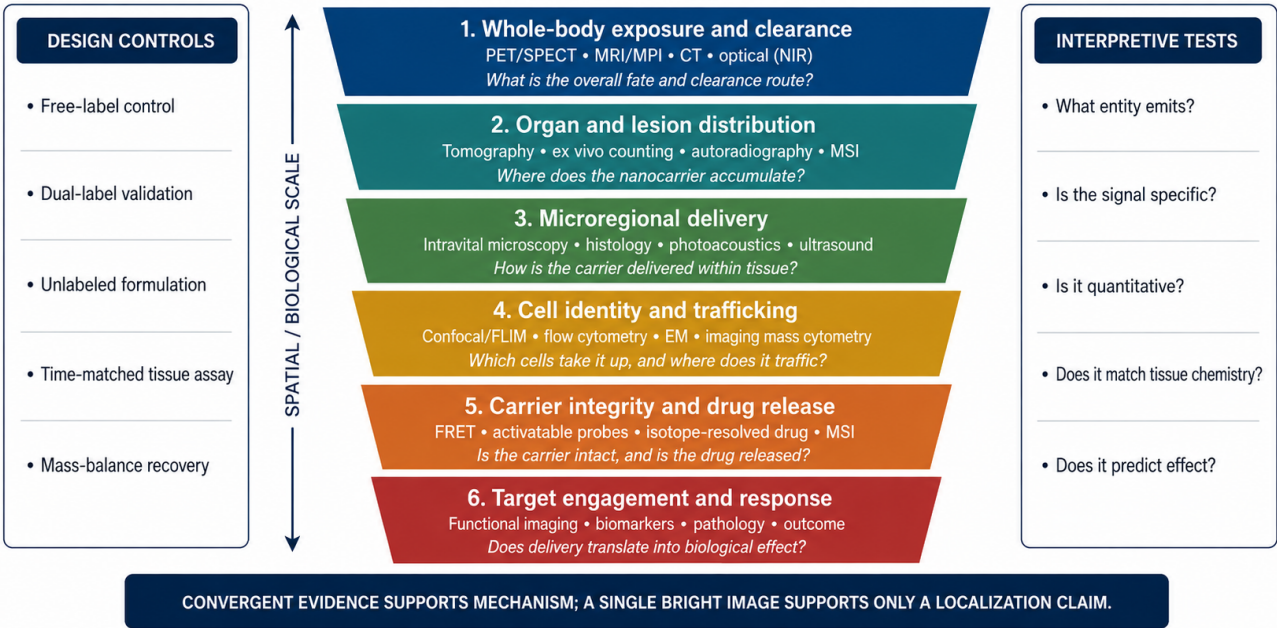
Correlative imaging links scales. A longitudinal PET scan can identify high- and low-uptake lesions, which are then sampled for

autoradiography, MSI, histology, and microscopy. Intravital microscopy can identify dynamic entry events, followed by EM of the same or matched region to resolve ultrastructure. Image registration must account for tissue deformation, section thickness, sampling time, and coordinate transformation. The uncertainty of registration should be reported rather than hidden.

Dual- or triple-label designs can separate scaffold, surface component, and payload, but they increase the risk of perturbation and spectral or kinetic mismatch. Labels should have comparable detection sensitivity and validated stability. A useful design is to pair a long-lived carrier label with a chemically distinct payload label and a pharmacodynamic reporter. Concordance supports co-delivery; divergence reveals disassembly, redistribution, or delayed response.

Evidence triangulation for nanocarrier imaging studies

Complementary scales answer different questions; concordance strengthens mechanism.



Evidence triangulation. Each layer answers a different question. Confidence increases when independent whole-body, tissue, cellular, molecular, and pharmacodynamic measurements agree. Controls and interpretive tests reduce false mechanistic conclusions. Original schematic.

8. QUANTITATIVE INTERPRETATION, ARTIFACTS, AND VALIDATION

8.1 Calibration and image formation

Quantification begins with a calibration model that reproduces the relevant matrix, geometry, concentration range, and acquisition settings. Optical calibration in a clear solution does not correct for depth-dependent tissue attenuation. MRI relaxivity measured in buffer may not apply after aggregation or cellular uptake. Ultrasound and photoacoustic signals depend on acoustic or optical fields. PET and SPECT require dose calibration, decay correction, attenuation and scatter correction, reconstruction harmonization, and recovery coefficients for small structures.

ROIs should be defined independently of the signal being tested when possible. Pre-specified anatomic segmentation, blinded

analysis, and sensitivity testing across thresholds reduce circularity. Dynamic data should be analyzed using time-activity curves or kinetic models rather than a single maximum-intensity image. Report both concentration and total organ burden when organ size differs across groups.

Normalization can create misleading results. Percentage of injected dose per gram emphasizes concentration, whereas percentage of injected dose per organ emphasizes total burden. Tumor-to-blood or tumor-to-muscle ratios can rise because background clears rather than because tumor uptake increases. All relevant denominators, including injected dose, recovered dose, body weight, tissue mass, and plasma exposure, should be stated.

8.2 Labeling artifacts and minimum controls

Common Labeling Strategies, Limitations, and Recommended Controls

Labeling Strategy	Advantages	Common Limitations	Recommended Controls
Covalent fluorophore	Stable, multiplex imaging, microscopy compatible	Charge alteration, fluorophore cleavage, environment-dependent intensity	Reassess particle size, zeta potential, pharmacokinetics, label density, and serum stability
Lipophilic membrane dye	Simple, bright, suitable for liposomes/LNPs	Dye transfer, micelle formation, persistent false signals	Free-dye controls, chromatography, plasma stability, secondary scaffold label
Encapsulated dye/FRET pair	Monitors carrier integrity and payload release	Self-quenching, dye leakage, pH effects	Intact vs. disrupted carrier calibration, pH/protein controls, lifetime or ratio imaging
Radiometal–chelator	Highly sensitive and quantitative (PET/SPECT)	Transchelation, residual radioactivity after degradation	Radiometabolite analysis, free-chelator control, dual labeling
Radiohalogen	Direct labeling of carrier or drug	Dehalogenation, diffusible metabolites	Metabolite analysis, blocking studies, free-label comparison
Iron oxide core	MRI/MPI compatible, long-term tracking	Persistent core, aggregation, endogenous iron interference	MPI/elemental assays, histology, EM, long-term degradation studies
High atomic-number tag	CT/X-ray/ICP-MS compatible	High dose requirement, ion exchange, tissue retention	Elemental mass balance, dissolution studies, payload quantification
Isotope-labeled drug	Direct assessment of payload pharmacokinetics	Metabolite formation, complex synthesis	Radiometabolite analysis, comparison with unlabeled drug, scaffold labeling
Activatable probe	Reports release or biological activation	Off-target activation, nonlinear response	Inhibitor or knockout controls, calibration, chemical validation
Reporter gene/Bioluminescence	Highly sensitive functional imaging	Depends on substrate, oxygen, ATP, and gene expression	Constitutive reporter controls, substrate timing, protein/RNA validation

8.3 Study design and statistics

Imaging studies often produce many voxels, cells, frames, and lesions, but few independent, biological units. Typically the experimental unit is not a single cell or a single pixel, but the animal or patient. When lesions are clustered within patients, or regions within tumors, hierarchical models are suitable. For repeated scans, models that incorporate within-subject correlation are needed. The automated segmentation should be tested on a held out set and presented with error metrics.

Randomization, blinded analysis, pre-specified primary endpoints, power calculations based on biological units, and inclusion of both sexes where applicable help to minimize bias. Imaging time points should be chosen based on a mechanistic hypothesis and not convenience, and should be based on pilot PK. Negative results are informative for the setting of the assay sensitivity and positive control.

Data presented should be individual values, distributions and uncertainty, not only representative images. Unless otherwise specified, image display ranges should be the same for comparative panels. The following parameters should be reported: saturated pixels, nonlinear look up tables, background subtraction parameters. Images and analysis code that are raw or minimally processed materially enhance reproducibility.

9. CLINICAL TRANSLATION AND IMAGING-GUIDED NANOTHERAPY

9.1 From biodistribution to patient selection

Clinical imaging can answer a question that animal models cannot: Does the therapeutic nanocarrier make it to the lesions in a particular patient? Wide interpatient and interlesion variability has been observed for radiolabeled liposomes and ⁶⁴Cu-labeled HER2-targeted liposomal doxorubicin constructs [2,28]. Ferumoxytol MRI has been tested as a co-marker for nanoliposomal irinotecan [27]. These studies validate a precision delivery paradigm, where imaging can be used to identify lesions that are favorable for delivery, differentiate between access failure and pharmacologic resistance, and enrich trials.

A companion tracer can only be believed if it is biologically matched with the therapeutic. The size distribution, morphology, surface chemistry, protein adsorption, clearance, circulation time, and degradation must be similar. If clearance systems become saturated or if the therapeutic dose alters vascular permeability, a trace imaging dose may not reflect the PK of a therapeutic dose. There are different questions to be answered by co-administration, microdosing, and separate-day imaging, and each of these approaches needs pharmacologic justification.

Prospective assessment of predictive performance should be performed at the patient and lesion level. Retrospectively determined thresholds are susceptible to overfitting. Clinical utility should be determined by evidence that uptake is associated with change in treatment selection and improved outcomes or trial efficiency, not just that uptake is associated with response in a small cohort.

9.2 Safety, radiation, and manufacturability

Constraints in translation are less apparent in laboratory imaging. The imaging label and conjugation process needs to be compatible with GMP and release testing, sterility, shelf life and transport. Radionuclide half-life should be similar to that of the carrier: short half-life may not be able to follow late accumulation, long half-life increases radiation dose. SPECT radionuclides may allow for longer tracking, but with lower clinical resolution.

The use of repeated MRI is free of ionizing radiation but can have safety concerns and susceptibility artifacts related to iron or gadolinium. Optical, ultrasound, and photoacoustic approaches are appealing for superficial or interventional applications, but suffer from poor whole-body penetration and standardization. The imaging component should be taken into account as part of the product or combination strategy for regulatory assessment, and determine if the labeling/ conjugation alters the therapeutic formulation. The FDA guidance for nanomaterial-containing drug products and liposome drug products highlights characterization of product attributes, free and associated drug fractions, release, degradation, and pharmacokinetics or bioavailability [36,37]. Although neither is an imaging-methods standard, both underscore the importance of orthogonal analytical validation of what an imaging signal is purported to be.

The clinically meaningful endpoints need to be easily implementable across multiple centers. The following are required: harmonized acquisition, phantom calibration, scanner qualification, centralized or validated local analysis, and pre-defined quality-control criteria. Quantitative biomarkers are not valid if they rely on one scanner, one analyst, or an unreported reconstruction pipeline.

9.3 Lessons from established nanoparticle contrast agents

Nanoparticle imaging agents have already shown clinical utility, apart from drug delivery. Occult lymph-node metastases in prostate cancer were better detected with ultrasmall superparamagnetic iron oxide [29]. This experience demonstrates that nanoparticles can be used to identify disease through biological trafficking, and that their adoption will require manufacturing continuity, workflow, regulatory approach and a clear clinical decision. The same biological validity and operational feasibility will be required for drug-delivery imaging.

10. EMERGING DIRECTIONS

10.1 Quantitative whole-organ microscopy and spatial omics

The volume and molecular depth of ex vivo analysis is growing, with tissue clearing, light-sheet microscopy, multiplex immunofluorescence, imaging mass cytometry, and spatial transcriptomics. These methods can be used to map carrier signal throughout the entire organ and correlate with vascular, stromal, immune and transcriptional states. The problem is no longer just image acquisition, but also the registration, segmentation, batch correction and statistical analysis of nested spatial data.

Spatial omics can be used to identify delivery-permissive and delivery-resistant niches, and MSI can be used to map active drug and metabolites. Combining these data with longitudinal PET or MRI might identify which noninvasive phenotypes are predictive of cellular access. Careful matching of time points and cohorts is necessary as many spatial assays are terminal.

10.2 Label-free and minimally perturbing measurements

Label-free approaches reduce the risk that the imaging tag changes biology. Raman and coherent Raman imaging, photothermal microscopy, X-ray fluorescence, elemental analysis, and MSI can identify some carrier or drug chemistries without bulky fluorophores. Stable-isotope labeling combined with MSI or secondary-ion mass spectrometry can distinguish formulations with minimal chemical perturbation. Sensitivity, throughput, and clinical access remain limiting.

A practical compromise is orthogonal minimal labeling: use the lowest label density compatible with detection, verify that pharmacology is unchanged, and pair imaging with chemical assays. The objective is not a label-free ideal at all costs, but an explicit uncertainty budget for how the label may alter the system.

10.3 AI-assisted image analysis and mechanistic transport modeling

Machine learning can be used for segmentation of vasculature and cells, tracking of particles, classification of uptake phenotypes, and integration of imaging with omics and clinical variables. Physics-informed and mechanistic transport models can be used to estimate parameters, including permeability, binding, interstitial mobility, release, and clearance, and can be used to test the sensitivity of tissue exposure to changes in formulation or vascular state. Patient-specific forecasting should be considered as a research goal and not as a fact unless validated in a prospective manner.

The major threats are domain shift, leakage between training and test sets, uncalibrated confidence, and biologically plausible but incorrect correlations. Models should be tested on instruments, locations, disease models and perturbations. Explainable features should be linked to measurable transport processes and predictions should be tested in a prospective manner.

10.4 Theranostic and responsive systems

Theranostic nanocarriers have both therapeutic and imaging capabilities or a companion construct. The most informative designs report a state that is mechanistically very close to efficacy, including carrier disassembly, enzyme triggered release, cytosolic access, or target engagement. The mere addition of a constitutive contrast label can help localize but does not make a predictive theranostic.

Rapidly evolving are responsive probes that vary their signal as a function of pH, redox potential, enzymes, mechanical stress, or release of nucleic acids. Their validation should include selectivity in the complete biological matrix, dynamic range at relevant concentrations and correlation with independent chemical or functional measurements. Several responsive channels multiplexed can help differentiate delivery failure from release failure or biological resistance.

11. A PRACTICAL DECISION FRAMEWORK

The following sequence converts a broad desire to “image delivery” into a testable experiment. It is intentionally claim-first: the biological claim determines the analyte, label, modality, controls, and validation.

1. STATE ONE PRIMARY CLAIM.

Examples: the intact carrier reaches the lesion; payload is released in acidic endosomes; mRNA reaches the cytosol; lesion uptake predicts response.

2. DEFINE THE ANALYTE AND ITS FAILURE MODES.

Specify whether the signal belongs to scaffold, lipid, ligand, payload, metabolite, cell, or biological response. List detachment, exchange, degradation, retention, and activation pathways.

3. CHOOSE THE COARSEST MODALITY THAT ANSWERS THE PRIMARY QUESTION, THEN ADD ONE COMPLEMENTARY SCALE.

Use whole-body imaging for PK and heterogeneity; microscopy for mechanism; chemistry-sensitive methods for payload and metabolites; functional imaging for effect.

4. BUILD THE CONTROLS BEFORE COLLECTING IMAGES.

Include free label, unlabeled carrier, free drug, formulation-matched comparator, disruption calibration, and time-matched tissue validation as appropriate.

5. PRE-SPECIFY QUANTIFICATION.

Define calibration, ROI or segmentation, denominators, time points, kinetic model, primary endpoint, experimental unit, and uncertainty analysis.

6. TRIANGULATE THE CONCLUSION.

Require concordance among imaging, recovered tissue chemistry, histology or cell analysis, and pharmacodynamic response. Explain discordance rather than averaging it away.

7. DESIGN FOR TRANSLATION EARLY.

Match tracer and therapeutic biology, assess manufacturability and dosimetry, use clinically feasible time points, and test whether imaging changes a treatment decision.

Minimum reporting checklist for nanocarrier imaging studies

Domain	Minimum Information to Report
Formulation	Composition, cargo, particle size, morphology, surface chemistry, charge, encapsulation efficiency, release profile, dose, and batch information.
Label	Label type/location, labeling density or specific activity, purity, stability, free-label fraction, and impact on particle properties or pharmacokinetics.
Biological System	Species, strain, sex, age, disease model, lesion characteristics, immune status, anesthesia, and physiological conditions.
Imaging Protocol	Imaging system, acquisition/reconstruction settings, probe dose, imaging schedule, anesthesia, temperature, and motion correction.
Calibration	Phantom/standard curve, sensitivity, attenuation or partial-volume correction, and validation with tissue assays.
Image Analysis	Preprocessing, ROI selection, segmentation/tracking method, blinding, background correction, software, and version.
Quantitative Endpoints	Measurement units, normalization method, kinetic model, uncertainty, blood correction, and primary analysis time point.
Controls	Free label, unloaded or unlabeled carrier, free drug, targeting-negative control, disrupted carrier, and positive controls where applicable.
Validation	Confirmation using gamma counting, LC-MS/MS, ICP-MS, MSI, histology, flow cytometry, EM, or pharmacodynamic assays.
Statistics	Experimental unit, sample size, randomization, blinding, statistical model, effect size, and confidence intervals.
Data Transparency	Individual datasets, representative images, raw/minimally processed data, analysis code, negative results, and metadata availability.
Translation	Tracer-therapy similarity, dose effects, dosimetry, GMP feasibility, multicenter reproducibility, and clinical decision thresholds.

12. CONCLUSIONS

Imaging has transformed nanocarrier evaluation from a terminal organ-counting exercise into a multiscale analysis of transport, cellular processing, and pharmacology. It can reveal why a formulation succeeds or fails: inadequate circulation, dominant off-target clearance, heterogeneous vascular access, poor interstitial penetration, sequestration by the wrong cell, endosomal trapping, premature release, or biological resistance.

The field's central methodological lesson is that signal identity must be separated from carrier identity and drug action. A bright image is evidence that a detectable label is present within the validated detection model; every stronger conclusion requires additional validation. Longitudinal whole-body quantification, microscopic mechanism, chemical tissue analysis, and pharmacodynamic endpoints provide complementary—not redundant—information.

The next advances are likely to come from biologically matched companion imaging, responsive probes that report productive delivery, chemistry-resolved tissue mapping, and integrated spatial models. Their impact will depend less on visual sophistication than on calibration, orthogonal controls, transparent analysis, and prospective demonstration that the imaging result improves a formulation decision, trial design, or patient outcome.

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Abbreviation	Meaning
AFM	Atomic force microscopy
BLI	Bioluminescence imaging
CT	Computed tomography
EM	Electron microscopy
EPR	Enhanced permeability and retention
FLIM	Fluorescence lifetime imaging microscopy
FRET	Förster resonance energy transfer
LNP	Lipid nanoparticle
MPI	Magnetic particle imaging
MRI	Magnetic resonance imaging
MSI	Mass spectrometry imaging
NIR	Near-infrared

Abbreviation	Meaning
PAI	Photoacoustic imaging
PET	Positron emission tomography
PK	Pharmacokinetics
ROI	Region of interest
SPECT	Single-photon emission computed tomography
TAM	Tumor-associated macrophage