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REVIEW PAPER

Nano-fluorophores as enhanced diagnostic tools to improve cellular imaging

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ABSTRACT

Biological events can be mapped in real-time using fluorescent images at high spatial resolution through the use of a powerful tool called fluorescence, and it is necessary to have ultra-bright fluorescent probes. The detrimental effects associated with the existing fluorescence imaging probes and contrast agents are the primary reason behind the greater involvement of nanotechnology. Developing advanced particles at the molecular and supramolecular levels is the only way to address the constraints underlying the current scenario. Nanosized structures dominate in multiple fields, especially in nanotheranostics, due to their higher quantum yield, negligible photobleaching, excellent biocompatibility, tunable optical properties, and improved circulation half-lives. Nanofluorophores, which are nanoparticles encapsulated or doped with fluorescent dyes, play a crucial role in fluorescence-based imaging modality by providing noninvasive real-time monitoring of the inner machinery of the anatomical and cellular structures. In addition to fluorescent inorganic and organic nanoparticles, there are labeled hydrophilic and hydrophobic nanostructures, semiconducting dots, carbon dots, as well as upconversion nanomaterials, etc., which are widely used in fluorescent imaging. A comprehensive literature survey has been provided in this review since intense studies are needed to clear the preclinic stage, thus opening up opportunities for future biomedical applications.

Keywords: *Biomedical imaging, Carbon dots, Fluorescence imaging, Nanoformulation, Nanotheranostics, Quantum dots*

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INTRODUCTION

Non-invasive imaging has traditionally been used to define gross anatomy and physiology at the level of the entire organ in order to establish a better understanding of the organ. There has been a gradual advancement in technology over the past few years, which has made it possible for non-invasive imaging techniques to be used to characterize molecular expression and physiology at the cellular level. The growing trend of an emerging field called "molecular imaging" has the potential to have a significant impact on the basic sciences and clinical setups [1]. It must have a high degree of specificity for the target that can be analyzed as an imaging tool or probe in order to be able to monitor interactions at the molecular level, the spatial resolution is high enough to image a diseased model, and the sensitivity high enough to study interactions. Therefore, to make a proper choice of technique, it is crucial to consider the benefits and drawbacks of each imaging modality. It is now possible to house and operate in diagnostic laboratories that are capable of performing MRI, CT, nuclear imaging, fluorescence imaging, and bioluminescence imaging [2]. It is also important to note that besides their diagnostic abilities, the costs of these systems also have an important impact on routine medical care. As a result, fluorescence imaging plays a significant role in diagnosis.

Fluorescence technology has been widely employed in biomedical imaging and diagnostic applications in research for decades due to its improved adaptability and accuracy. Fluorescence imaging provides outstanding spatial and temporal resolutions when Compared with other conventional imaging methods, enabling a

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thorough and detailed study in the visualization of the organism [3-5]. The rich and broad spectrum of technological advances engulfs the medical sector. Although radiological techniques such as X-ray imaging and computer tomography imaging are utilized for clinical evaluation, their capacity to assist the healthcare professional during medical therapy is restricted [6, 7]. Despite significant advances in imaging technology over the years, disease detection during treatments continues to rely on visible irradiation or the interpretation of white light images through a microscope on a computer screen. To improve the diagnostic and therapeutic research findings, nanomaterials provide a flexible framework for loading a range of payloads, such as imaging agents, nucleic acids, anticancer treatments, oxidizing agents, and antibodies [8, 9]. Furthermore, because of the unique properties of nanoparticles, such as their fluorescent and magnetic properties, nanoengineered drug carriers are gaining popularity as therapeutic agents. Fluorescence-based detection methods are frequently utilized in small-animal research because of their precise measurement, inherent biological safety, and relative ease of usage in terms of cost, length, flexibility, and compatibility among a diverse set of technologies [10, 11].

The source of fluorescence in any object mainly depends on a free electron in the orbital. Generally, the electrons will be in the lowest energy state, called a ground state. When photon sources of energy hit an electron in the range, the electron will absorb the energy and jumps to the excited state [12]. To revert to the lowest energy state from the highest excitation point, the electron will release excess energy in the form of photons. As a result of emission with lower energy, emission light possesses a larger wavelength than the excitation light. If fluorophores are sufficiently stimulated, emitted photons often lie within the visible spectrum and can be viewed using a microscope. Almost every fluorophore releases a photon at a specific wavelength, which makes fluorescence imaging relatively easy [13]. The photons that are emitted from the fluorescent probes help to identify the different parts and tissues in the body. They provide adaptability and flexibility for the detection system by using proteins, peptides, and other nanoparticles. In fluorescence imaging, specific wavelengths of light can be predicted because fluorophores release light at specific wavelengths.

The fluorescence of any particle can be either increased or quenched by several factors like the intrinsic properties of the molecule, the environment of a molecule, and other factors involved. Intrinsic properties of a molecule include structural rigidity, substituent groups, the lifetime of the fluorophores, quantum yield, etc. Any fluorescent molecule must absorb in the range of UV-Vis spectrum in order to emit fluorescence [14]. This sort of absorption is characterized by the index of hydrogen deficiency (IHD) or degree of unsaturation. IHD denotes the availability of π bonds and the presence of the number of rings [15]. A molecule needs to be unsaturated in order to absorb radiation and also to emit fluorescence. Elucidating the relationship between the quantum yield and the magnitude of the conjugation, the length of π bonds in its singlet-excited state could pave the way to design an organic compound of high fluorescence [16, 17]. The rigidity of the molecule is usually decided by the number of bonds. Single bonds can rotate, making the compound more flexible, whereas the double and triple bonds cannot rotate as free as a single bond making the compound remain more rigid. This rigidity plays an important role in fluorescence. A rigid compound like fluorene increases the fluorescence intensity, whereas a flexible compound like biphenyl quenches the fluorescence [12].

The free electrons on the substituent groups have a greater impact on the fluorescence intensity. The substituent groups with an electron-donating capability such as NH_2, NO, NO_2, NR_2, NHR, CN, OMe, OEt, OH, and NH_2OH increase the fluorescence [18, 19]. In contrast, the groups with electron-withdrawing properties such as COOR, COOH, COR, CHO, F, Cl, Br, and SH quenche the fluorescence. Certain groups, including NH_4^+, SO_3H, and alkyl groups, do not affect the fluorescence. The hydrocarbons with ring structure often decide the intensity of fluorescence. In the aromatic compounds, the fluorescence intensity is directly proportional to the number of rings present [20].

Several photophysical events like internal conversions, intersystem crossing, vibrational relaxations, fluorescence, and phosphorescence occur in an organic molecule after absorbing a photon with suitable energy [21]. The Jablonski diagram is the best possible way to represent the entire process (Figure 1). Each and every process happens in fluorescence with a certain probability,

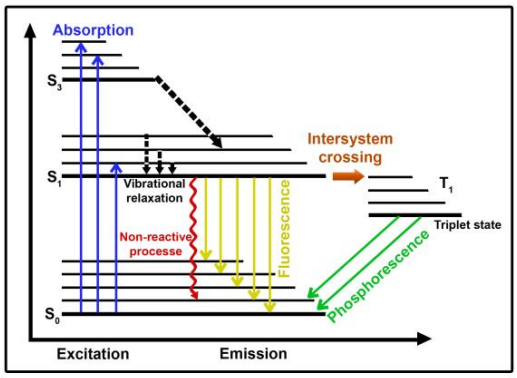

Fig. 1. Jablonski diagram to represent the photophysical processes during fluorescence

characterized by the decay rate constant. The lifetime of the fluorophore represents the average time the molecule takes to decay from one state to another, which is reciprocally proportional to the decay rate [22]. It can be simplified that the fluorescence lifetime is the time necessary for a population of excited molecules or fluorophores to decrease exponentially by the loss of energy through radiative or non-radiative pathways. The fluorescent lifetime is independent of the emission intensity and the concentration of the fluorophores [23]. The lifetime is mostly affected by the structure of fluorophores and external factors like polarity, quenchers, temperature, and pH [21, 24]. The other important parameter, quantum yield, is very important to represent the efficacy of a fluorophore. The quantum yield is defined as the efficiency of a fluorophore to convert the light absorbed to the emitted light [25, 26]. The emitted light is usually in the form of fluorescence. Thus, the quantum yield is directly proportional to the intensity of the fluorescence and independent of the concentration of the sample. Even at low concentrations, if the quantum yield of the fluorophore is higher, they emit strong fluorescence.

The environment of a molecule and other factors involved

Oxygen is a well-known fluorescence quencher molecule. The presence of oxygen leads to the oxidation of fluorescent substances and converts them to non-fluorescent ones. This quenching of fluorescence occurs due to the paramagnetic property of the molecular energy. The effect of temperature on fluorescence can be very prominently noticed [27]. High temperature may cause a high rate of collision between atoms, due to which the fluorescence intensity decreases.

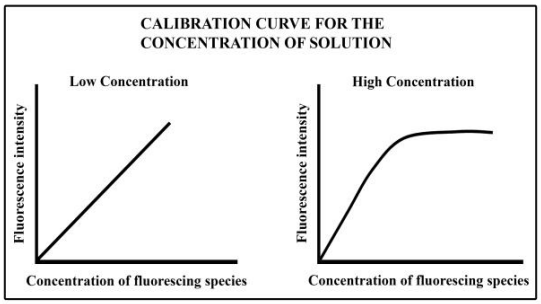

Fig. 2. Graphical representation shows the effect of concentration on fluorescence

This is vice versa with the thickness of the sample. A high viscous solution exhibits less collision, which leads to an increase in fluorescence [28]. In the case of concentrations, deviations at greater concentrations create self-quenching or self-absorption. Increasing the concentration of the sample eventually decreases the intensity (Figure 2). This can be explained by the inner filter effect, which occurs due to the high absorption rate of the sample. In order to avoid the inner filter effect, the sample can be diluted before the measurements.

Nano-based fluorophores for cellular imaging

In addition to high contrast, imaging in targeted areas, and reduced cytotoxicity, different nanomaterials are being developed for molecular and cellular imaging (Figure 3). A nanomaterial can provide images for a longer period than molecular imaging probes because of its high retention time. As a result of surface plasmon resonance, nanomaterials are able to display finer optical properties after interacting with specific wavelengths of light that cause electronic oscillation. Each fluorescent nanomaterial is discussed below.

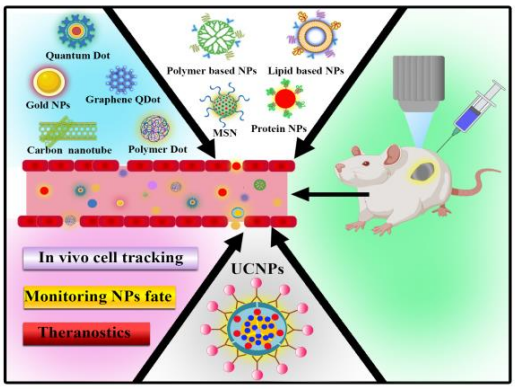

Fig. 3. Application of nano-based fluorophores

Quantum dots

A new class of nanoscale light-emitting particles called quantum dots (QA) is emerging as fluorescent probes for biomolecular and cellular imaging [29, 30]. The inorganic particles have optical, magnetic, or electronic properties that can be harnessed by these semiconducting nanoparticles with unique size and shape configurations. Most of these are derived from elements in the II and VI or III and V groups. When a photon of visible light hits a semiconductor, it leads to an electron jumping from a lower electronic state to a higher electronic state by causing excitation. When it comes to a lower energy level, it releases energy in the form of fluorescence. The diameter of an excitation spectrum, the width of the emission spectrum, photostability, as well as the decaying lifespan are all factors that influence adaptability to various conditions [31, 32]. Compared with typical fluorophores, these quantum dots have better bright imaging efficacy with improved molar excitation, which is higher than 100 times. Many biological applications require solubilization of QDs, but this is a challenging task. They are often employed as nanocarriers for signal enhancement, which includes electron transfer promoters, nanozymes, detectors, and electrocatalyst labeling components, resulting in innovative biosensing techniques [33, 34]. As fluorescent nanoparticles, QDs can be used for many applications, including detection of cancer indicators, labeling of specimens for classification, and identifying even the smallest lesions through medical imaging. The use of QDs for early cancer detection and classification has been investigated in clinical trials.

The quantum yield of QDs can be enhanced by attaching shell layers keeping the semiconductor at the core [35]. The optical properties of the QDs vary with the varying diameters of shells and cores to optimize the image quality. Most of the biomolecules absorb light either in the UV or visible range and therefore interfere with biomedical imaging. Therefore, there is a need to develop an optical probe that absorbs NIR, and tunable QDs are well known in this account to generate the diversity of the absorption spectrum. In addition to the tunable excitation wavelength, QDs possess a narrow emission spectrum and higher Stokes shift that benefits the fluorescence signal to generate multicolor bioimaging, where conventional fluorescent organic dyes fall short [36, 37].

Poor resolution, photobleaching, and autofluorescence are the two major concerns in visualizing cancer cells using a fluorescence microscope that affect the proper diagnosis. QDs are useful in this respect due to their high quantum yield to visualize tumors with higher spatial resolutions *in vivo*. Due to the enhanced

Table 1. Fluorescent nanodots used in theranostics

No.	Type	Conjugation	Approach	Application	Ref.
			Quantum Dots (QDs)		
1.	Black phosphorus quantum dots (BPQDs)	Paclitaxel	Theranostics	Imaging agent of photothermal optical coherence tomography and combined chemotherapy-photothermal cancer therapy	[44]
2.	Cu_2S QDs	Zn doping	Theranostics	Tumor cell imaging and chemodynamic therapy (CDT)	[45]
3.	MXene QDs	Chitosan	Immunomodulation	Treatment of degenerative diseases through the material-based tissue repair system	[46]
4.	Hydrophobic QDs-lipid nanocarriers	siRNAs and anti-EGFR aptamer-lipid conjugates	Theranostics	Carrier for RNA interference and fluorescence imaging of triple-negative breast cancer (TNBCs)	[47]
5.	Fluorescent carbon QDs	Rose Bengal (RB) photosensitizers	Theranostics	Drug delivery, tracking of mitochondria, and mitochondria-targeted Photodynamic	[48]
6.	ITK-CARBOXYL QDs 605, 655, and 705 nm	Cholera toxin B (CTB)	Cell Labeling and tracking	Multigeneration mammalian live cell tracking	[49]
7.	PEG-coated QDs 705 nm	Thiolated arginine-glycine-aspartic acid (RGD) peptide	Bioimaging	Tumor vascular targeted imaging through strong integrin avb3-binding affinity	[50]
8.	ZnxHg1-xSe QDs 630 nm	Bovine Serum Albumin (BSA)	Cell labeling and bioimaging	Specific fluorescence labeling and imaging of biomolecules.	[51]
9.	PEG-coated fluorescent Streptavidin QDs	Biotinylated ligand of dendritic cell-specific pathogen-uptake receptor (DC-SIGN)	Cellular tracking and monitoring	Investigation of cellular signaling processes.	[52]
10.	CdSe/CdS/ZnS QDs	Doxorubicin	Drug delivery	Treatment of pulmonary disease through targeted macrophage-selective therapy.	[53]

Continued Table 1.

11.	Streptavidin QDs 655 nm	Tat peptide	Theranostics	Delivery of nanoparticles to living cells, Intracellular imaging, and therapy	[54]
12.	Magnetic-QDs	ZnO	Theranostics	MR imaging and combinational photodynamic and radiation therapy for cancer	[55]
		Carbon Dots (CDs)			
1.	N-hydroxyphthalimide-derived CDs	Manganese-Doping	Theranostics	Strong MRI signal with better anti-tumoral properties against breast cancer	[56]
2.	Near-infrared fluorescence CDs	S, N doping	Theranostics	Fluorescence (FL) imaging, photoacoustic (PA) imaging, and photothermal therapy (PTT) for cancer	[57]
3.	Green synthesized CDs Source: watermelon juice	-	Theranostics	Effective probe for NIR-II imaging and Photo thermal therapy (PTT) for cancer	[58]
4.	Fluorescent CDs	PEGylated shell and hyperbranched poly (amido amine) (HPAP)	Theranostics	Gene delivery and monitoring of the microenvironment	[59]
5.	Nitrogen-enriched CDs (NCDs)	DNA nanostructures	Theranostics	Near-red fluorescence or photothermal therapy for non-small cell lung cancer	[60]
6.	Negatively charged carbon quantum dots	Tetraplatinated porphyrin complex (PtPor)	therapy	Photodynamic therapy for cancer	[61]
7.	L-CDs	Gadolinium doping	Theranostics	Multimodal magnetic-fluorescence imaging and gene delivery for cancer	[62]
8.	Janus-like poly (methyl methacrylate)-*b*-poly (ethylene glycol)-folic acid *block*-copolymer-grafted fluorescent CDs	Doxorubicin	Theranostics	Delivery of anti-cancer drugs and monitoring the real-time response	[63]
9.	Fluorescent CDs	Hafnium-doping	Bioimaging	Effective probes for multimodal fluorescence-computed tomography imaging of liver tumor microenvironment	[64]
10.	Green synthesized CDs Source: crab shell	Nitrogen doping and folic acid	Bioimaging	Imaging agent for cancer	[65]
11.	red emissive CDs (RCDs)	Chlorine e6	Therapy	Photodynamic and photothermal cancer therapy	[66]
12.	dual emissive carbon dots	Mn2+ complex-modified polydopamine (PDA)	Bioimaging	Multimodal photothermal-fluorescent-magnetic resonance imaging of tumor microenvironment	[67]
		Upconversion nanoparticles (UCNPs)			
1.	UCNPs	Bismuth nanoparticle, silicon dioxide nanoparticles	Theranostics	Multimodal luminescence-computed tomography and photothermal therapy for cancer	[68]
2.	UCNPs	Poly (lactic-co-glycolic Acid) (PLGA) and nanocurcumin	Theranostics	Anticancer therapy for lung cancer by selective accumulation and monitoring the live response	[69]
3.	UCNPs	Aptamer, Photosensitizer-*Pyropheophorbide a and doxorubicin*	Theranostics	Image-guided chemo and photodynamic theranostic approach for cancer	[70]
4.	Rattle structured UCNPs	hollow mesoporous silica shell, pillarene-based supramolecular valves, and doxorubicin	Theranostics	Drug delivery and multimodal luminescence-magnetic resonance imaging of tumor cells	[71]
5.	UCNPs	MnO_2	Theranostics	Image-guided photodynamic cancer therapy	[72]
6.	Sodium gadolinium fluoride UCNPs lanthanide	Lanthanide	Theranostics	Photodynamic cancer therapy and monitors the real-time response of the therapy	[73]
7.	UCNPs	Tin dioxide, BSA, and Doxorubicin	Theranostics	Image-guided dual hyperthermia and photodynamic cancer therapy	[42]
8.	Core–shell structured UCNPs	Nanographene oxide (NGO), PEG, Chlorin e6	Theranostics	Luminescent probe for image-guided combinational photodynamic-photothermal cancer therapy	[74]
9.	UCNPs	Gold nanoparticles	Theranostics	Temperature-controlled photothermal cancer therapy and live cell imaging	[75]
10.	UCNPs	Pyrolipid and rifampin	Theranostics	Image-guided photodynamic therapy against tuberculosis	[76]
11.	UCNPs	miRNA (miR122), gold nanocages, and glycyrrhetinic acid	Theranostics	Image-guided therapy for drug-induced liver injury	[77]
12.	UCNPs	Bismuth selenide	Theranostics	Dual luminescence - computed tomography and photothermal cancer therapy	[78]

permeability and retention effect of QDs, long-term tumor imaging is possible without photobleaching. They can be used as intravascular probes for theragnostic. Akerman et al. first reported in 2002 about the *in vivo* applications of QDs in cancer imaging [38]. The research showed that ZnS-CdSe functionalized with lung targeting peptides accumulates in the lungs of mice after injection. Yu et al. synthesized QDs bioconjugated with alpha-fetoprotein to monitor the *in vivo* hepatoma imaging by immunofluorescence. Alpha-fetoprotein antibody is an important biomarker for hepatocellular carcinoma and is used for active targeting [39]. Tiwari et al. reported anti-HER2ab conjugated CdSe-ZnS for *in vivo* imaging, and the study clearly showed the biocompatibility of conjugated particles in breast cancer imaging [40]. In 2015, Lin et al. reported size-dependent tunable photoluminescent colloidal MoS_2 quantum dots with well dispersed and low toxicity for real-time optical cellular imaging [41]. Brunetti et al. reported in 2018 that NT4 peptide-functionalized NIR QDs with highly selective accumulation and retention properties at the colon cancer site were very promising in theranostics [42]. There is an immense demand to develop potential fluorescent probes in biomedical imaging having bioavailability and biocompatibility. FeSe QDs with multiple photoexcitation nature have similar properties that were reported by Kwon et al. for second window imaging. The biocompatibility was judged by functionalizing the particles with human epidermal growth factor receptor 2 (HEGFR2) after PEGylation (polyethylene glycol conjugation) [43]. (Table 1).

Besides several useful applications, researchers have shown that QDs are cytotoxic in vitro in various cell lines and *in vivo* in a range of animal studies. Though QDs demonstrated substantial promise in clinical tests, notably for diagnostic and therapeutic applications of cancer, extensive work has to be done before QDs employment in clinical practice. QDs have been reported to cause health problems in laboratory animals when administered *in vivo*. There are many ways proposed to put forward explaining the cytotoxicity of QDs. The release of free radicals, particularly hydroxyl radicals, has been linked to cytotoxicity. The composition of the core as well as the representation of core size, appear to affect toxicity. Nevertheless, interpreting cytotoxicity data is challenging because of differences in QD cellular functioning and the potential involvement of unanticipated factors in toxicity.

Carbon dots

Carbon dots (C-dots), known for their characteristic size of less than 10 nm, have emerged as a rising star in the carbon nanomaterial field. C-dots contain sp^2-sp3 carbon skeleton and many other active functional groups making them a potential candidate for imaging, catalysis, biological, and energy-related applications [79, 80]. Its excellent water solubility and ease of combining with other materials without phase separation make it a perfect platform for many important environmental, biological, and energy-related applications. They possess higher quantum fluorescent yields and exhibit lower toxicity, lower costs, and improved biocompatibility. Carbon dots have been extensively used in the bioimaging of cells and tissues in both *in vivo* and in vitro applications [81]. Tumor suppressor gene therapy is a revolutionary approach to tumor growth control. The siRNAs are widely used in studying the silencing of genes. Fluorescent C-dots coated with polyetherimide and functionalized with SiRNA due to electrostatic interaction enhanced cellular uptake for visualization [82]. C-dots-driven PDT (photodynamic therapy), and PTT (photothermal therapy) agents help in targeting and inhibiting the tumors.

Conventional C-dots still have a long way to go in terms of improving their optical capabilities. NIR (Near-infrared) light is more penetrating, less cytotoxic, and can be easily used for *in vivo* imaging since it penetrates more deeply [83]. At the same time, C-dots did not produce near-infrared energy until sometime after their entry. In the future, it will only be possible to achieve the full potential of these materials through the development of NIR C-dots. It is also essential to address issues relating to C-dots heterogeneity since it has the potential to cause inconsistencies in size or quantum yield that will cause waveband ambling and fluorescence attenuation to occur in C-dots [84].

Fluorescent proteins

Fluorescent proteins (FP) are members of the structurally homologous class of proteins, which are gaining importance day by day in emerging biological and biomedical fields [85, 86]. FP is commonly used to monitor the dynamic cellular

process in a variety of biological systems. FP covers a wide range of fluorescent colors, from short violets to almost infrared. They are classified into a wide variety of colors so that they can be used to differentiate the phenotypic or genotypic marker for cancerous cells. For example, highly malignant tumor cells will be labeled with Green-shifted fluorescent proteins (GFPs), while the low malignant tumor cells will be labeled with Red-shifted fluorescent proteins (RFPs) [87]. This allows a better comparison of cancerous cells in contrast to non-cancerous cells *in vivo*. RFP has been found to have numerous advantages over other fluorescent protein types in terms of applications involving multiple colors. These methods have enabled the study of biomolecular pathways, biological functions of cells, and molecular biology, in a way that has generated a massive change in the biological world. During the year 2008, three researchers including Roger Tsien, Martin Chalfie, and Osamu Shimomura were awarded the Nobel Prize in Chemistry for their breakthrough research that led to "the discovery and development of the GFP" [88]. The emission range between 442–650 nm represents the family of fluorescent proteins, including GFP. It was discovered that Aequorea Victoria had GFP of a molar mass of 27–30 kDa in the year 1992 [89]. There are eleven strands comprising the barrel structure of GFP, characterized by certain strand topologies, which we all know as β-strands. The C-terminal strand carries a majority of the non-covalently attached amino acid residues that come into contact with the fluorophore which affects its color and excited-state properties. It is superimposed with α -helices along its central axis. During internal helix synthesis, amino acids 65-67 are responsible for chromophore synthesis. GFP is characterized by the unique property to act as a pH sensor [90]. The pKa value of GFP is determined by many factors, like presence of amino acids that make up the chromophore, as well as amino acids that may be closely associated, like H148 and T203. At the beginning of the GFP revolution, it was discovered that the internal tripeptide Ser65-Tyr66-Gly67 on the F-helix synthesized the chromophores by inducing intramolecular cyclization. This resulted in one of the most widely used types of GFP, the S65T mutant, in which the chromophore is present in its deprotonated form 4-(phydroxybenzylidene)-5-imidazolinone, p-HBI) at basic pH [91, 92].

During a study, researchers noticed that the RNA aptamers were able to bind to DFHBDI (a GFP chromophore-3,5-difluoro-4-hydroxyb enzylidenedimethylimidazolinone) [93]. The fluorescent proteins are non-genetic molecules that can be used for labeling cells, organelles, and enzymes, aside from being genetically encoded. Because of these properties, FPs are superior for *in vivo* imaging in comparison with chemically synthesized dyes. Chromophores do not require any additional enzymes or cofactors other than oxygen molecules to produce a chromophore. A target protein that is genetically labeled with FP has been shown to have no adverse effects when delivered to living cells. The fluorescent proteins can emit light between the wavelengths of 650–900 nm, which places them within the range of the near-infrared spectrum. Fluorescent proteins have many obstacles to working *in vivo* and one of the greatest hurdles is reacting with water, hemoglobin, and fats [94].

Aggregation-induced emission NPs

The use of organic light-emitting materials, specifically NIR materials in organic light-emitting diodes, has usually remained constrained by aggregation-caused quenching (ACQ) [95]. Organic luminous materials are typically investigated in dilute solutions. The molecules in the dilute solution interact through intermolecular interactions. The photophysical properties of many organic compounds in a dilute solution are very different from those in a concentrated solution. Hydrophobic aromatic compounds are the most commonly used organic luminescence probes since they cannot dissolve in water. Consequently, they tend to precipitate and aggregate in water, which results in the ACQ effect [96]. A dilute organic solution with five phenyl rotors undergoes intramolecular rotations that could allow the excited states to be non-radioactively annihilated, which leads to the absence of luminescence. These aggregates do not emit IR because the molecules are densely packed. In the formation of an aggregate state, these IRs and π−π interactions can be limited, turning off the non-radiative transitions and activating the radiative pathways. Hence, the process is named aggregation-induced emission (AIE). This AIE material can be actively used in various fields including luminescence probes bioimaging, and the ACQ effect is a major hurdle in fluorescence imaging [97-99].

Multiplexed in vivo imaging

The use of nanomaterials in living organisms has increased drastically in recent times for better understanding and visualizing of the diseases and diagnostic therapy and drug administration. These findings have a goal to reach the level of therapeutic standards. Moreover, no single material has been satisfied or capable of meeting all the requirements in the imaging models. Spatial resolution and penetration depth were seen as the major drawbacks of *in vivo* imaging but these are having many advantages in molecular study. In MRI the nanoparticles help to visualize the more spatial resolution compared with the others, as well as having no limit for the tissue penetration. Moreover, the nuclear nanoparticles offer excellent sensitivity and help for a complete scan. As a result, nanoparticles have shown novel prospective applications that are corroborated by the size of the particles and their surface charge.

Nuclear-fluorescence imaging

Nuclear medicine often referred to as radionuclide imaging, is a type of noninvasive, painless medical diagnostic technique that helps in diagnosing medical disorders. Nanomaterials adorned with radioactive isotopes are used as diagnostic agents that depend on radioactive decay [100]. In addition, these devices can generate images of in-depth living organisms as well as detectable capabilities, which range from bone to soft tissue. Compared with conventional imaging, nuclear imaging with PET/SPECT offers several benefits, including noninvasive properties that can track cancer cells. When imaging agents target cancer-specific changes, the gamma camera is utilized to record the energy emissions produced by the radiotracers and convert the data into images [101, 102]. Combining radioactive tracers with PET imaging has enhanced *in vivo* cancer functional assessment. FDG or fluoro-2-deoxy-D-glucose [18F], plays a key role in PET's success story and is frequently used for cancer diagnosis, staging, and therapeutic management [103, 104]. According to clinical and pathological studies, IRDye 800CW had no toxicological effects on the experimental group of rats [105]. A radio chemically synthesized 64Cu-E4-Fl was found to be a good bimodal agent for detecting and confirming the individual specifying GLP-1R-binding and had higher sensitivity than radioactive isotopes labels. These were used in PET imaging to enhance pancreatic autoradiography and also in preoperative fluorescence imaging. Based on hematological, clinical chemistry, and histopathological analyses of tissues harvested from rats, it was found that NIRF dye IRDye 800CW does not cause any harm to organs. The breakthrough of diagnostic imaging offers an additional path for *in vivo* imaging advancements. In animal models, nuclear imaging has high penetration levels but superior temporal signals can be found by fluorescence imaging [106, 107].

Magnetic resonance-fluorescence imaging

Magnetic Resonance Imaging is a non-ionizing imaging method, which offers cellular and morphological analysis of live organisms with a spatial resolution of 1 mm and the capacity to scan the whole body. The major downside of imaging modality is inability to acquire cellular imaging level information [108]. Fluorescence imaging in living animals is hampered by a lack of light penetration. High image resolution and sensitivity imaging of both cells and tissues could be obtained by combing cellular-sensitive fluorescence imaging with MR imaging. Iron oxide nanoparticles have both multifunctional magnetic targeted platforms for high effectiveness of traceable amounts of drug delivery in vitro and have long been employed as contrast agents in MR imaging and are able to produce a preliminary picture of reticuloendothelial and tumor-targeting probes. Quantum dots (QDs) with high luminescence paired with magnetic nanoparticles or ions offer a unique special generation of bio-imaging nanomaterials. By combining two capabilities in one single particle, a sensitive imaging agent can be created that can be used for two very strong and complementary imaging modalities. It has been demonstrated that combining two functions of nanoparticles can result in a highly sensitive contrast agent that can be used for two complementary imaging techniques that are extraordinarily efficient and powerful [109, 110].

NIR imaging

NIR (Near-infrared) spectroscopy is a non-destructive, in-situ analytical technique that employs near-infrared light to interact with materials. The electromagnetic spectrum represents the wavelengths of light based on the absorption of electromagnetic radiation in the range of 780 to 2,500 nm [111, 112]. Near-infrared spectroscopy tests for electromagnetic

absorption at wavelengths in the detector to detect the sample's transmittance and absorbance after the light interacts with it. The quantity of light is transmitted entirely through the sample and reaches the detectors which are referred to as transmittance. Absorbance is an estimate of how much light a sample absorbs. The detector detects the light that passes through the samples and turns the signal into a digital output and absorbance. Light in the infrared spectrum absorbs and causes molecules to vibrate and spin. Light absorption in materials is generally not uniform and is influenced by molecular organization. More strong absorbance may be seen at particular intervals, as shown by the broad absorption bands. Overtones and combination bands caused by CH, OH, and NH bonds dominate the NIR area. Overtones, combination modes, anharmonicity, and vibrational potentials can be studied using NIR spectroscopy. NIR spectroscopy is indeed another method for studying hydroxyl groups, intermolecular and intramolecular interaction, and hydration [113]. Non-contact investigation and analysis employing an optical light fiber probe are possible with NIR spectroscopy. In a risky setting, NIR spectroscopy may be used to remotely adjust the probe. One of the reasons why NIR spectroscopy is suitable for online analysis is because of this attribute. This analysis was much more useful for in-situ analysis of non-destructive materials and suitable for aqueous solution analysis.

A wider variety of samples can be analyzed using NIR spectroscopy including particles that are particularly absorbent, and dense materials. For separating samples, these systems use long fiber optic cables to separate measurement positions. They can also be used to monitor industrial processes online. By measuring multiple physicochemical properties from one spectrum, it is possible to determine several properties of chemical compounds. Using the blood flow and oxygen consumption responses to cerebral activation, it is possible to measure changes in oxygenated and deoxygenated hemoglobin that can help resolve ambiguities.

NIR fluorophores in the emission range of 700–900 nm such as ICG (Indocyanine green) and MB (Methylene blue) were approved by FDA for clinical use [58, 114]. The advancements of fluorophores made drastic progress in biomedical fluorescence imaging techniques. ICG emits around 800 nm and MB emits around 700 nm. It is possible to use ICG and MB simultaneously during intraoperative imaging to visualize the vasculatures, including the digestive tract, ureters, bile duct, and blood and lymphatic vessels. Image-guided surgeries can also be performed with the assistance of NIR fluorophore. Despite its high serum protein binding and greater quantum yield, ICG exhibits minimal extravasation and provides clear imagery of the anatomical structures. A major concern is that ICG cannot be used in a clinical setting to visualize a wide range of anatomical features because they lack biochemical information. IR 800 dye was the first to get approval from the FDA in the first phase of human clinical trials. Cetuximab is an anti–EGFR therapeutic antibody to treat head and neck cancers labeled with IR 800 dye that can target the tumor-specific receptors across the various types of cancers [115, 116]. The application of IR-783 in cervical cancer detection and diagnosis has yet to be fully explored (Table 2).

Upconversion nanoparticles

The upconversion nanoparticles (UCNPs) are rare earth metals, which contain a unique class of lanthanide series of elements featuring a wealth of electronic transitions with 4f inner shell configurations with unique and abundant energy levels [117-120]. The intra-4f electron shifts of lanthanide ions generate UC emissions without breaking chemical bonds. Upconversion nanomaterials are the latest imaging contrast agents. These upconversion nanomaterials utilize multiple photons as a source of energy, which converts low to high-energy light under a shorter wavelength. As the result of the upconversion process, the nanoparticles display impeccable luminescence properties, allowing for high photovoltaic stability, a long lifetime, and tunable emission. Biological samples can be successfully visualized even at high penetration depths by using upconversion nanoparticles. Their unique feature is a new family of fluorophores, which use a nonlinear optic mechanism to transform near-infrared energy into visible radiation. UCNPs can be strongly doped due to extensive energy flow contact by slightly adjusting the spectrum tunability of photon upconversion. Doping large quantities of lanthanide ions into UCNP has proven to be a feasible approach to increasing photon upconversion emission intensity. Desirable targets can be easily linked and their visible light emitting

Table 2. Various NIR fluorophores and their properties.

Dye	Emission	Excitation	Application	Advantages
IR- 125	783	833	Ability to serve as an ICG equivalent fluorophore	Demonstrate not only apoptotic changes in cells but also peripheral blood cell groups
IR- 140	700-800	840-915	High stability in aqueous solution	Widely used as photothermal agent and photosensitizer
IR- 780	777	823	Tumor imaging, Vessel imaging	Potential in dual imaging and tumor-killing in cancer cells
IR-783	745	820	Cervical cancer detection and diagnosis	Possesses cancer targeting and anticancer effects
IR- 800	774	789	Tumor imaging and targeting ligands	Excellent localization of the tumor and promising agent for fluorescence-guided surgery
IR- 813	815	863	IR-813, which is utilized for near-infrared real-time trafficking in sub-capsular areas of the draining lymph nodes, represents the most common location for initial nodal micrometastases	Capable of concentrating chemotherapeutic delivery directly to the targeted region
IR- 820	818	867	High dual-therapeutic agent loading for immunotherapy	Similar to ICG but with improved stability and a longer plasma half-life *in vitro* and *in vivo*
Cy5	650	670	Combination of proteins and nucleic acids for imaging, flow cytometry, and genomic study	It has the potential fundamental ability for intraoperative detection and resection of malignancies
Cy7	743	767	Imaging of tumors, tissues, and organs from the structural and functional perspective	Brighter and much more photostable

under the NIR irradiation makes them suitable for bioimaging [121]. There are several applications for UCNPs in theranostics, and they are uniquely suited for this field since they combine imaging, drug delivery, and therapy.

Over the years of advancement and development of the UCNP-based carriers, UCNPs are developed by surface modifications allowing them to deliver the DNA and RNA [122]. The luminance of the UCNPs helps to visualize the tracking of gene delivery to the target site. Indocyanine fluorescent probes are used to visualize tumor progression in choroidal melanoma and help the treatment development [123]. By using polycations, which are extensively used for transport materials across membranes, surface modifications of UCNPs can be accomplished by direct incorporation. Photodynamic therapy (PDT) is a noninvasive treatment executed by using photosensitizer molecules and oxygen for killing cancer cells [124, 125]. Most of the photosensitizer molecules can be excited in the visible and UV regions which limits their penetration levels into the tissues. Using these UCNPs which are normally activated by the NIR region has great penetration depths and also acts as an energy donor. UCNPs have been promising in the diagnosis and treatment of tumors inside the body by providing luminescent bio-labels with high stability.

Fluorescence image-guided therapy

Nanoparticles are of huge interest as their surface can be functionalized with multiple moieties such as drugs, contrast agents, peptides, receptors, and fluorophores. Oncological surgery lacks success rate, as it cannot clear out complete cancerous spots even though the surgery was planned with advanced imaging reports [126]. Once the body is cut open, surgeons struggle to find the cyst that is spread along. In order to help surgeons with a successful surgery and to minimize the mortality rate, the surgery can be assisted with an imaging technique. Nanoparticles tagged with fluorophores can well assist the surgery to find even a minute cyst and provide real-time tracking. Several studies concluded that gold nanoparticles could serve as the best candidate for carrying fluorophores to track cancer cells during surgery. Besides open surgery, endoscopy, laparoscopy, and robotic surgery can also be assisted by detecting the fluorescently labeled structures. Fluorescence image-guided surgery (FGS) is superior in its detection and resolution [127, 128]. Despite their advantages, several reasons limit the use of FGS. The number of clinically recognized fluorescent dyes is very less. Sometimes in order to avoid the interference of the surgical light with the fluorescent emission channel, the surgical light head needs to be turned off while detecting the glowing cancer cells. This makes the surgery personnel involved feel uncomfortable and might also increase uncertainty. Thus, FGS needs immense validation studies and clinical trials for

its implementation in routine patient care [129].

CONCLUSION

Fluorophores used in fluorescence imaging have seen enormous progress in recent years. Due to the limitations possessed by the conventional dyes, nanobased fluorophores have made a great entry into the imaging sector space required. Despite their excellent features, nanoparticles show adverse effects that need to be addressed for their vital use *in vivo*. Because the nanoparticles have a large diameter compared with the conventional fluorophores or an incompatible surface charge, reaching the desired site can be difficult, which can reduce imaging effectiveness dramatically. Therefore, the development of fluorescent nanoparticles is essential that retain their outstanding optical properties and must be accompanied by smaller sizes and appropriate charges. Additionally, some nanoparticles, especially quantum dots, can be toxic to living organisms. Nanoparticles can cause cytotoxicity and genotoxicity in the cells when dispersed throughout the living body. A quantum dot, for instance, contains heavy metals that may irreversibly affect the structure of proteins. The activity of several proteins depends on the metal ion conjugation, and these heavy metal replacement can cause severe effects, even death. To avoid such limitations, a scientist should concentrate on the solubility and homogeneity of the nanomaterial, which is important for the stability of nanomaterials for fluorescent imaging. Low toxicity of nanomaterials by making them biocompatible and biodegradable are the other essential parameters. Additionally, to meet the demands of a variety of experiments, the nanomaterial should be able to absorb and emit a wide spectrum of light. In conclusion, more research has to be carried out to better understand and tune the properties of fluorescent nanomaterials, such as biocompatibility, biodistribution, cellular uptake, clearance, and toxic response. Further continuous efforts can bring out multi-modality imaging into the picture without producing adverse effects. A strong motive to develop stable and biocompatible material can fulfill the concerns regarding imaging techniques to monitor the cellular process much better, which will help in better diagnosis followed by treatment. The intrinsic therapeutic ability of nanomaterials is an added advantage along with the diagnosis. Sometimes it can be the natural properties or gained through a surface modification that can help in therapy in combination with fluorescence-based diagnosis. As a whole, one can conclude that the fluorescence-based theranostic particles can be fabricated that would help in targeted diagnosis and therapy.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

REFERENCES

1. Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Genes Dev. 2003;17(5):545-580.
2. Pichler BJ, Wehrl HF, Judenhofer MS. Latest advances in molecular imaging instrumentation. J Nucl Med. 2008;49:5S-23S.
3. Hsiao WW-W, Hui YY, Tsai P-C, Chang H-C. Fluorescent nanodiamond: a versatile tool for long-term cell tracking, super-resolution imaging, and nanoscale temperature sensing. Acc Chem Res. 2016;49(3):400-407.
4. Gowtham P, Haribabu V, Prabhu AD, Pallavi P, Girigoswami K, Girigoswami A. Impact of nanovectors in multimodal medical imaging. Nanomed J. 2022;9(2):107-130.
5. Sharmiladevi P, Girigoswami K, Haribabu V, Girigoswami A. Nano-enabled theranostics for cancer. Mater Adv. 2021;2:2876-2891.
6. Haribabu V, Girigoswami K, Sharmiladevi P, Girigoswami A. Water–Nanomaterial Interaction to Escalate Twin-Mode Magnetic Resonance Imaging. ACS Biomater Sci Eng. 2020;6(8):4377-4389.
7. Haribabu V, Sharmiladevi P, Akhtar N, Farook AS, Girigoswami K, Girigoswami A. Label free ultrasmall fluoromagnetic ferrite-clusters for targeted cancer imaging and drug delivery. Curr Drug Del. 2019;16(3):233-241.
8. Sharmiladevi P, Haribabu V, Girigoswami K, Farook AS, Girigoswami A. Effect of mesoporous nano water reservoir on MR relaxivity. Sci Rep. 2017;7(1):1-7.
9. Girigoswami A, Yassine W, Sharmiladevi P, Haribabu V, Girigoswami K. Camouflaged nanosilver with excitation wavelength dependent high quantum yield for targeted theranostic. Sci Rep. 2018;8(1):1-7.
10. Yao J, Yang M, Duan Y. Chemistry, biology, and medicine of fluorescent nanomaterials and related systems: new insights into biosensing, bioimaging, genomics, diagnostics, and therapy. Chem Rev. 2014;114(12):6130-6178.
11. Farka Z, Jurik T, Kovář D, Trnkova L, Skládal P. Nanoparticle-

based immunochemical biosensors and assays: recent advances and challenges. Chem Rev. 2017;117(15):9973-10042.
12. Lakowicz JR. Principles of fluorescence spectroscopy: Springer; 2006.
13. Lichtman JW, Conchello J-A. Fluorescence microscopy. Nat Methods. 2005;2(12):910-919.
14. Feng S, Zhu L, Wang D, Li C, Chen Y, Chen X, et al. Rigidity-Tuned Full-Color Emission: Uncommon Luminescence Change from Polymer Free-Volume Variations. Adv Mater. 2022:2201337.
15. Xu Z, Zhang Q, Li X, Huang X. A critical review on chemical analysis of heavy metal complexes in water/wastewater and the mechanism of treatment methods. Chem Eng J. 2022;429:131688.
16. Godumala M, Kumar AV, Chandrasekar R. Room-temperature phosphorescent organic materials for optical waveguides. J Mater Chem C. 2021;9(40):14115-14132.
17. Sasaki S, Drummen GP, Konishi G-i. Recent advances in twisted intramolecular charge transfer (TICT) fluorescence and related phenomena in materials chemistry. J Mater Chem C. 2016;4(14):2731-2743.
18. Qi S, Kwon N, Yim Y, Nguyen V-N, Yoon J. Fine-tuning the electronic structure of heavy-atom-free BODIPY photosensitizers for fluorescence imaging and mitochondria-targeted photodynamic therapy. Chem Sci. 2020;11(25):6479-6484.
19. Wu Q-Y, Zhou T-H, Du Y, Ye B, Wang W-L, Hu H-Y. Characterizing the molecular weight distribution of dissolved organic matter by measuring the contents of electron-donating moieties, UV absorbance, and fluorescence intensity. Environ Int. 2020;137:105570.
20. Geng T, Zhu Z, Zhang W, Wang Y. A nitrogen-rich fluorescent conjugated microporous polymer with triazine and triphenylamine units for high iodine capture and nitro aromatic compound detection. J Mater Chem A. 2017;5(16):7612-7617.
21. Berezin MY, Achilefu S. Fluorescence lifetime measurements and biological imaging. Chem Rev. 2010;110(5):2641-2684.
22. Chang CW, Sud D, Mycek MA. Fluorescence lifetime imaging microscopy. Methods Cell Biol. 2007;81:495-524.
23. Hutchinson CL, Lakowicz J, Sevick-Muraca EM. Fluorescence lifetime-based sensing in tissues: a computational study. Biophys J. 1995;68(4):1574-1582.
24. Munishkina LA, Fink AL. Fluorescence as a method to reveal structures and membrane-interactions of amyloidogenic proteins. Biochim Biophys Acta Biomembr. 2007;1768(8):1862-1885.
25. Würth C, González MG, Niessner R, Panne U, Haisch C, Genger UR. Determination of the absolute fluorescence quantum yield of rhodamine 6G with optical and photoacoustic methods–Providing the basis for fluorescence quantum yield standards. Talanta. 2012;90:30-37.
26. Rurack K. Fluorescence quantum yields: methods of determination and standards. Standardization and quality assurance in fluorescence measurements I: Springer; 2008. p. 101-45.
27. Mohanty J, Jaffe JS, Schulman ES, Raible DG. A highly sensitive fluorescent micro-assay of H2O2 release from activated human leukocytes using a dihydroxyphenoxazine derivative. J Immunol Methods. 1997;202(2):133-141.
28. Hasegawa M, Sugimura T, Suzaki Y, Shindo Y, Kitahara A. Microviscosity in water pool of Aerosol-OT reversed micelle determined with viscosity-sensitive fluorescence probe, Auramine O, and fluorescence depolarization of xanthene dyes. J Phys Chem. 1994;98(8):2120-2124.
29. Jaiganesh T, Rani JDV, Girigoswami A. Spectroscopically characterized cadmium sulfide quantum dots lengthening the lag phase of Escherichia coli growth. Spectrochim Acta A Mol Biomol Spectrosc. 2012;92:29-32.
30. Jamieson T, Bakhshi R, Petrova D, Pocock R, Imani M, Seifalian AM. Biological applications of quantum dots. Biomaterials. 2007;28(31):4717-4732.
31. Yariv E, Schultheiss S, Saraidarov T, Reisfeld R. Efficiency and photostability of dye-doped solid-state lasers in different hosts. Opt Mater. 2001;16(1-2):29-38.
32. Demchenko AP. Photobleaching of organic fluorophores: quantitative characterization, mechanisms, protection. Methods Appl Fluoresc. 2020;8(2):022001.
33. Gupta N, Todi K, Narayan T, Malhotra B. Graphitic carbon nitride-based nanoplatforms for biosensors: design strategies and applications. Mater Today Chem. 2022;24:100770.
34. Zheng Y, Zhou Y, Cui X, Yin H, Ai S. Enhanced photoactivity of CdS nanorods by MXene and ZnSnO3: Application in photoelectrochemical biosensor for the effect of environmental pollutants on DNA hydroxymethylation in wheat tissues. Mater Today Chem. 2022;24:100878.
35. Eagle FW, Park N, Cash M, Cossairt BM. Surface Chemistry and Quantum Dot Luminescence: Shell Growth, Atomistic Modification, and Beyond. ACS Energy Lett. 2021;6(3):977-984.
36. Ding F, Fan Y, Sun Y, Zhang F. Beyond 1000 Nm Emission Wavelength: Recent Advances in Organic and Inorganic Emitters for Deep-Tissue Molecular Imaging. Adv. Healthc Mater. 2019;8(14):1900260.
37. McHugh KJ, Jing L, Behrens AM, Jayawardena S, Tang W, Gao M, et al. Biocompatible semiconductor quantum dots as cancer imaging agents. Adv Mater. 2018;30(18):1706356.
38. Åkerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E. Nanocrystal targeting in vivo. Proc Natl Acad Sci USA. 2002;99(20):12617-12621.
39. Yu X, Chen L, Deng Y, Li K, Wang Q, Li Y, et al. Fluorescence analysis with quantum dot probes for hepatoma under one- and two-photon excitation. J Fluoresc. 2007;17(2):243-247.
40. Tiwari DK, Jin T, Behari J. Bio-distribution and toxicity assessment of intravenously injected anti-HER2 antibody conjugated CdSe/ZnS quantum dots in Wistar rats. Int J Nanomed. 2011;6:463.
41. Lin H, Wang C, Wu J, Xu Z, Huang Y, Zhang C. Colloidal synthesis of MoS 2 quantum dots: size-dependent tunable photoluminescence and bioimaging. New J Chem. 2015;39(11):8492-8497.
42. Brunetti J, Riolo G, Gentile M, Bernini A, Paccagnini E, Falciani C, et al. Near-infrared quantum dots labelled with a tumor selective tetrabranched peptide for in vivo imaging. J Nanobiotechnology. 2018;16(1):1-10.
43. Kwon J, Jun S, Choi S, Mao X, Kim J, Koh E, et al. FeSe

quantum dots for in vivo multiphoton biomedical imaging. Sci Adv. 2019;5(12):eaay0044.
44. Chen H, Liu Z, Wei B, Huang J, You X, Zhang J, et al. Redox responsive nanoparticle encapsulating black phosphorus quantum dots for cancer theranostics. Bioact Mater. 2021;6(3):655-665.
45. Li S-L, Jiang P, Hua S, Jiang F-L, Liu Y. Near-infrared Zn-doped Cu 2 S quantum dots: an ultrasmall theranostic agent for tumor cell imaging and chemodynamic therapy. Nanoscale. 2021;13(6):3673-3685.
46. Rafieerad A, Yan W, Sequiera GL, Sareen N, Abu-El-Rub E, Moudgil M, et al. Application of Ti3C2 MXene quantum dots for immunomodulation and regenerative medicine. Adv Healthc Mater. 2019;8(16):1900569.
47. Kim MW, Jeong HY, Kang SJ, Jeong IH, Choi MJ, You YM, et al. Anti-EGF receptor aptamer-guided co-delivery of anti-cancer siRNAs and quantum dots for theranostics of triple-negative breast cancer. Theranostics. 2019;9(3):837.
48. Hua X-W, Bao Y-W, Chen Z, Wu F-G. Carbon quantum dots with intrinsic mitochondrial targeting ability for mitochondria-based theranostics. Nanoscale. 2017;9(30):10948-10960.
49. Chakraborty SK, Fitzpatrick JA, Phillippi JA, Andreko S, Waggoner AS, Bruchez MP, et al. Cholera toxin B conjugated quantum dots for live cell labeling. Nano Lett. 2007;7(9):2618-2626.
50. Cai W, Chen X. Preparation of peptide-conjugated quantum dots for tumor vasculature-targeted imaging. Nat Protoc. 2008;3(1):89-96.
51. He X, Gao L, Ma N. One-step instant synthesis of protein-conjugated quantum dots at room temperature. Sci Rep. 2013;3(1):1-11.
52. Cambi A, Lidke DS, Arndt-Jovin DJ, Figdor CG, Jovin TM. Ligand-conjugated quantum dots monitor antigen uptake and processing by dendritic cells. Nano Lett. 2007;7(4):970-977.
53. Chakravarthy KV, Davidson BA, Helinski JD, Ding H, Law W-C, Yong K-T, et al. Doxorubicin conjugated quantum dots to target alveolar macrophages/inflammation. Nanomedicine. 2011;7(1):88.
54. Ruan G, Agrawal A, Marcus AI, Nie S. Imaging and tracking of tat peptide-conjugated quantum dots in living cells: new insights into nanoparticle uptake, intracellular transport, and vesicle shedding. J Am Chem Soc. 2007;129(47):14759-14766.
55. Singh SP. Multifunctional magnetic quantum dots for cancer theranostics. J Biomed Nanotechnol. 2011;7(1):95-97.
56. Tiron A, Stan CS, Luta G, Uritu CM, Vacarean-Trandafir I-C, Stanciu GD, et al. Manganese-Doped N-Hydroxyphthalimide-Derived Carbon Dots—Theranostics Applications in Experimental Breast Cancer Models. Pharmaceutics. 2021;13(11):1982.
57. Bao X, Yuan Y, Chen J, Zhang B, Li D, Zhou D, et al. In vivo theranostics with near-infrared-emitting carbon dots—highly efficient photothermal therapy based on passive targeting after intravenous administration. Light Sci Appl. 2018;7(1):1-11.
58. Li Y, Bai G, Zeng S, Hao J. Theranostic carbon dots with innovative NIR-II emission for in vivo renal-excreted optical imaging and photothermal therapy. ACS Appl Mater Interfaces. 2019;11(5):4737-4744.
59. Zhao H, Duan J, Xiao Y, Tang G, Wu C, Zhang Y, et al. Microenvironment-driven cascaded responsive hybrid carbon dots as a multifunctional theranostic nanoplatform for imaging-traceable gene precise delivery. Chem Mater. 2018;30(10):3438-3453.
60. Wu D, Li BL, Zhao Q, Liu Q, Wang D, He B, et al. Assembling Defined DNA Nanostructure with Nitrogen-Enriched Carbon Dots for Theranostic Cancer Applications. Small. 2020;16(19):1906975.
61. Wu F, Yue L, Su H, Wang K, Yang L, Zhu X. Carbon dots@ platinum porphyrin composite as theranostic nanoagent for efficient photodynamic cancer therapy. Nanoscale Res Lett. 2018;13(1):1-10.
62. He X, Luo Q, Zhang J, Chen P, Wang H-J, Luo K, et al. Gadolinium-doped carbon dots as nano-theranostic agents for MR/FL diagnosis and gene delivery. Nanoscale. 2019;11(27):12973-12982.
63. Pei M, Jia X, Liu P. Design of Janus-like PMMA-PEG-FA grafted fluorescent carbon dots and their nanoassemblies for leakage-free tumor theranostic application. Mater Des. 2018;155:288-296.
64. Su Y, Liu S, Guan Y, Xie Z, Zheng M, Jing X. Renal clearable Hafnium-doped carbon dots for CT/Fluorescence imaging of orthotopic liver cancer. Biomaterials. 2020;255:120110.
65. Dehvari K, Liu KY, Tseng P-J, Gedda G, Girma WM, Chang J-Y. Sonochemical-assisted green synthesis of nitrogen-doped carbon dots from crab shell as targeted nanoprobes for cell imaging. J Taiwan Inst Chem Eng. 2019;95:495-503.
66. Chen Q, Sun S, Lin H, Li Z, Wu A, Liu X, et al. Supra-Carbon Dots Formed by Fe3+-Driven Assembly for Enhanced Tumor-Specific Photo-Mediated and Chemodynamic Synergistic Therapy. ACS Appl Bio Mater. 2021;4(3):2759-2768.
67. Zhang M, Zheng T, Sheng B, Wu F, Zhang Q, Wang W, et al. Mn2+ complex-modified polydopamine-and dual emissive carbon dots based nanoparticles for in vitro and in vivo trimodality fluorescent, photothermal, and magnetic resonance imaging. Chem Eng J. 2019;373:1054-1063.
68. Zhao S, Tian R, Shao B, Feng Y, Yuan S, Dong L, et al. Designing of UCNPs@ Bi@ SiO2 hybrid theranostic nanoplatforms for simultaneous multimodal imaging and photothermal therapy. ACS Appl Mater Interfaces. 2018;11(1):394-402.
69. Lakshmanan A, Akasov RA, Sholina NV, Demina PA, Generalova AN, Gangadharan A, et al. Nanocurcumin-Loaded UCNPs for Cancer Theranostics: Physicochemical Properties, In Vitro Toxicity, and In Vivo Imaging Studies. Nanomaterials. 2021;11(9):2234.
70. Jin X, Zeng Q, Zheng J, Xing D, Zhang T. Aptamer-functionalized upconverting nanoformulations for light-switching cancer-specific recognition and in situ photodynamic–chemo sequential theranostics. ACS Appl Mater Interfaces. 2020;13(8):9316-9328.
71. Zhou S, Ding C, Wang Y, Jiang W, Fu J. Supramolecular valves functionalized rattle-structured UCNPs@ hm-SiO2 nanoparticles with controlled drug release triggered by quintuple stimuli and dual-modality imaging functions: a potential theranostic nanomedicine. ACS Biomater Sci Eng. 2019;5(11):6022-6035.

72. Wang Y, Li Y, Zhang Z, Wang L, Wang D, Tang BZ. Triple-Jump Photodynamic Theranostics: MnO2 Combined Upconversion Nanoplatforms Involving a Type-I Photosensitizer with Aggregation-Induced Emission Characteristics for Potent Cancer Treatment. Adv Mater. 2021;33(41):2103748.
73. Wang M, Zhang Y, Ng M, Skripka A, Cheng T, Li X, et al. One-pot synthesis of theranostic nanocapsules with lanthanide doped nanoparticles. Chem Sci. 2020;11(26):6653-6661.
74. Gulzar A, Xu J, Yang D, Xu L, He F, Gai S, et al. Nano-graphene oxide-UCNP-Ce6 covalently constructed nanocomposites for NIR-mediated bioimaging and PTT/PDT combinatorial therapy. Dalton Trans. 2018;47(11):3931-3939.
75. Ramírez-García G, Honorato-Colin MÁ, De la Rosa E, López-Luke T, Panikar SS, de Jesús Ibarra-Sánchez J, et al. Theranostic nanocomplex of gold-decorated upconversion nanoparticles for optical imaging and temperature-controlled photothermal therapy. J Photochem Photobiol A Chem. 2019;384:112053.
76. Shiah JV, Grandis JR, Johnson DE. Targeting STAT3 with Proteolysis Targeting Chimeras and Next-Generation Antisense Oligonucleotides. Mol Cancer Ther. 2021;20(2):219-228.
77. Meng L, Wang Q, Wang L, Zhao Z, Xin G-Z, Zheng Z, et al. miR122-controlled all-in-one nanoplatform for in situ theranostic of drug-induced liver injury by visualization imaging guided on-demand drug release. Mater Today Bio. 2021;12:100157.
78. Zhao S, Tian R, Shao B, Feng Y, Yuan S, Dong L, et al. UCNP–Bi2Se3 upconverting nanohybrid for upconversion luminescence and CT imaging and photothermal therapy. Chem Eur J. 2020;26(5):1127-1135.
79. Liu C, Zhang F, Hu J, Gao W, Zhang M. A mini review on pH-sensitive photoluminescence in carbon nanodots. Front Chem. 2021:1242.
80. Sharmiladevi P, Akhtar N, Haribabu V, Girigoswami K, Chattopadhyay S, Girigoswami A. Excitation wavelength independent carbon-decorated ferrite nanodots for multimodal diagnosis and stimuli responsive therapy. ACS Appl Bio Mater. 2019;2(4):1634-1642.
81. Islam M, Lantada AD, Mager D, Korvink JG. Carbon-Based Materials for Articular Tissue Engineering: From Innovative Scaffolding Materials toward Engineered Living Carbon. Adv Healthc Mater. 2022;11(1):2101834.
82. Tarvirdipour S, Huang X, Mihali V, Schoenenberger C-A, Palivan CG. Peptide-based nanoassemblies in gene therapy and diagnosis: paving the way for clinical application. Molecules. 2020;25(15):3482.
83. Wang J, Liu G, Cham-Fai Leung K, Loffroy R, Lu P-X, Wang XJ. Opportunities and challenges of fluorescent carbon dots in translational optical imaging. Curr Pharm Des. 2015;21(37):5401-5416.
84. Zhou B, Guo Z, Lin Z, Zhang L, Jiang B-P, Shen X-C. Recent insights into near-infrared light-responsive carbon dots for bioimaging and cancer phototherapy. Inorg Chem Front. 2019;6(5):1116-1128.
85. Shaner NC, Patterson GH, Davidson MW. Advances in fluorescent protein technology. J Cell Sci. 2007;120(24):4247-4260.
86. Mansouri M, Strittmatter T, Fussenegger M. Light-controlled mammalian cells and their therapeutic applications in synthetic biology. Adv Sci. 2019;6(1):1800952.
87. Hoffman RM. Application of GFP imaging in cancer. Lab Invest. 2015;95(4):432-452.
88. Deng H, Yan S, Huang Y, Lei C, Nie Z. Design strategies for fluorescent proteins/mimics and their applications in biosensing and bioimaging. TrAC, Trends Anal Chem. 2020;122:115757.
89. Gilad AA, Winnard Jr PT, van Zijl PC, Bulte JW. Developing MR reporter genes: promises and pitfalls. NMR Biomed. 2007;20(3):275-290.
90. Tsien RY. The green fluorescent protein. Annu Rev Biochem. 1998;67(1):509-544.
91. Pakhomov AA, Martynov VI. GFP family: structural insights into spectral tuning. Chem Biol. 2008;15(8):755-764.
92. Christou NE, Giandoreggio-Barranco K, Ayala I, Glushonkov O, Adam V, Bourgeois D, et al. Disentangling Chromophore States in a Reversibly Switchable Green Fluorescent Protein: Mechanistic Insights from NMR Spectroscopy. J Am Chem Soc. 2021;143(19):7521-7530.
93. Romei MG, Boxer SG. Split green fluorescent proteins: scope, limitations, and outlook. Annu Rev Biophys. 2019;48:19.
94. Razansky D, Klohs J, Ni R. Multi-scale optoacoustic molecular imaging of brain diseases. Eur J Nucl Med Mol Imag. 2021;48(13):4152-4170.
95. Wu W, Li Z. Nanoprobes with aggregation-induced emission for theranostics. Mater Chem Front. 2021;5(2):603-626.
96. Zhang L, Che W, Yang Z, Liu X, Liu S, Xie Z, et al. Bright red aggregation-induced emission nanoparticles for multifunctional applications in cancer therapy. Chem Sci. 2020;11(9):2369-2374.
97. Zalmi GA, Jadhav RW, Mirgane HA, Bhosale SV. Recent Advances in Aggregation-Induced Emission Active Materials for Sensing of Biologically Important Molecules and Drug Delivery System. Molecules. 2021;27(1):150.
98. Zhao E, Gu X. Aggregation-Induced Emission (AIE) Probes for Cell Imaging. Fluorescent Materials for Cell Imaging: Springer; 2020. p. 181-215.
99. Wang Y, Zhang Y, Wang J, Liang X-J. Aggregation-induced emission (AIE) fluorophores as imaging tools to trace the biological fate of nano-based drug delivery systems. Adv Drug Del Rev. 2019;143:161-176.
100. Srivatsan A, Pera P, Joshi P, Marko AJ, Durrani F, Missert JR, et al. Highlights on the imaging (nuclear/fluorescence) and phototherapeutic potential of a tri-functional chlorophyll-a analog with no significant toxicity in mice and rats. J Photochem Photobiol B Biol. 2020;211:111998.
101. Loudos G, Rouchota MT. In vivo Imaging as a Tool to Noninvasively Study Nanosystems. Drug Delivery Nanosystems. 2019:339-364.
102. Chaudhari AJ, Badawi RD. Application-specific nuclear medical in vivo imaging devices. Phys Med Biol. 2021;66(10):10TR01.
103. Iravani A, Hicks RJ. Imaging the cancer immune environment and its response to pharmacologic intervention, part 1: the role of 18F-FDG PET/CT. J Nucl Med. 2020;61(7):943-950.
104. Rahman WT, Wale DJ, Viglianti BL, Townsend DM, Manganaro MS, Gross MD, et al. The impact of infection

and inflammation in oncologic 18F-FDG PET/CT imaging. Biomed Pharmacother. 2019;117:109168.
105. Bernhard W, Barreto K, El-Sayed A, Gonzalez C, Viswas RS, Toledo D, et al. Pre-clinical study of IRDye800CW-nimotuzumab formulation, stability, pharmacokinetics, and safety. BMC Cancer. 2021;21(1):1-13.
106. Wellens LM, Deken MM, Sier CF, Johnson HR, de la Jara Ortiz F, Bhairosingh SS, et al. Anti-GD2-IRDye800CW as a targeted probe for fluorescence-guided surgery in neuroblastoma. Sci Rep. 2020;10(1):1-12.
107. Kurbegovic S, Juhl K, Sørensen KK, Leth J, Willemoe GL, Christensen A, et al. IRDye800CW labeled uPAR-targeting peptide for fluorescence-guided glioblastoma surgery: Preclinical studies in orthotopic xenografts. Theranostics. 2021;11(15):7159.
108. Xie R, Wu Z, Zeng F, Cai H, Wang D, Gu L, et al. Retro-enantio isomer of angiopep-2 assists nanoprobes across the blood-brain barrier for targeted magnetic resonance/fluorescence imaging of glioblastoma. Signal Transduct Target Ther. 2021;6(1):1-13.
109. Xu S, Shi X, Chu C, Liu G. A TME-activated in situ nanogenerator for magnetic resonance/fluorescence/photoacoustic imaging. Methods Enzymol. 657: Elsevier; 2021. p. 145-156.
110. Zhu Y-L, Shen Y-C, Liu F, Chen S, Yan G-P, Liang S-C, et al. Dual-modal fullerenol probe containing glypican-3 monoclonal antibody for electron paramagnetic resonance/fluorescence imaging. Fuller Nanotub Carbon Nanostructures. 2021;29(4):280-287.
111. Yusnaini R, Ikhsan I, Idroes R, Munawar A, Arabia T, Saidi N, et al., editors. Near-infrared spectroscopy (NIRS) as an integrated approach for rapid classification and bioactive quality evaluation of intact Feronia limoni. IOP Conf Ser: Earth Environ Sci; 2021;667:012028.
112. Chandrasekaran I, Panigrahi SS, Ravikanth L, Singh CB. Potential of near-infrared (NIR) spectroscopy and hyperspectral imaging for quality and safety assessment of fruits: An overview. Food Anal Methods. 2019;12(11):2438-2458.
113. Fausto R, Ildiz GO, Nunes CM. IR-induced and tunneling reactions in cryogenic matrices: the (incomplete) story of a successful endeavor. Chem Soc Rev. 2022; 51:2853-2872.
114. Cwalinski T, Polom W, Marano L, Roviello G, D'Angelo A, Cwalina N, et al. Methylene Blue—Current knowledge, fluorescent properties, and its future use. J Clin Med. 2020;9(11):3538.
115. Wang L, Liang M, Xiao Y, Chen J, Mei C, Lin Y, et al. NIR-II Navigation with an EGFR-Targeted Probe Improves Imaging Resolution and Sensitivity of Detecting Micrometastases in Esophageal Squamous Cell Carcinoma Xenograft Models. Mol Pharm. 2022.
116. Polikarpov DM, Campbell DH, McRobb LS, Wu J, Lund ME, Lu Y, et al. Near-infrared molecular imaging of glioblastoma by Miltuximab®-IRDye800CW as a potential tool for fluorescence-guided surgery. Cancers (Basel). 2020;12(4):984.
117. Wu Y, Ang MJY, Sun M, Huang B, Liu X. Expanding the toolbox for lanthanide-doped upconversion nanocrystals. J Phys D Appl Phys. 2019;52(38):383002.
118. Akhtar N, Wu P-W, Chen CL, Chang W-Y, Liu R-S, Wu CT, et al. Radiolabeled Human Protein-Functionalized Upconversion Nanoparticles for Multimodal Cancer Imaging. ACS Appl Nano Mater. 2022;5:7051-7062.
119. Yamini S, Gunaseelan M, Kumar G, Singh S, Dannangoda GC, Martirosyan KS, et al. NaGdF 4: Yb, Er-Ag nanowire hybrid nanocomposite for multifunctional upconversion emission, optical imaging, MRI and CT imaging applications. Microchim Acta. 2020;187:1-10.
120. Yamini S, Gunaseelan M, Gangadharan A, Lopez SA, Martirosyan KS, Girigoswami A, et al. Upconversion, MRI imaging and optical trapping studies of silver nanoparticle decorated multifunctional NaGdF4: Yb, Er nanocomposite. Nanotechnology. 2021;33(8):085202.
121. Wen S, Zhou J, Zheng K, Bednarkiewicz A, Liu X, Jin D. Advances in highly doped upconversion nanoparticles. Nat Commun. 2018;9(1):1-12.
122. Gee A, Xu X. Surface functionalisation of upconversion nanoparticles with different moieties for biomedical applications. Surf. 2018;1(1):96-121.
123. Perumal V, Sivakumar PM, Zarrabi A, Muthupandian S, Vijayaraghavalu S, Sahoo K, et al. Near infra-red polymeric nanoparticle based optical imaging in Cancer diagnosis. J Photochem Photobiol B Biol. 2019;199:111630.
124. Vimaladevi M, Divya KC, Girigoswami A. Liposomal nanoformulations of rhodamine for targeted photodynamic inactivation of multidrug resistant gram negative bacteria in sewage treatment plant. J Photochem Photobiol B Biol. 2016;162:146-152.
125. Pallavi P, Girigoswami A, Girigoswami K, Hansda S, Ghosh R. Photodynamic Therapy in Cancer. Handbook of Oxidative Stress in Cancer: Therapeutic Aspects. 2022:1-24.
126. Sun W, Luo L, Feng Y, Qiu Y, Shi C, Meng S, et al. Gadolinium–Rose Bengal Coordination Polymer Nanodots for MR-/Fluorescence-Image-Guided Radiation and Photodynamic Therapy. Adv Mater. 2020;32(23):2000377.
127. Ito R, Kamiya M, Urano Y. Molecular probes for fluorescence image-guided cancer surgery. Curr Opin Chem Biol. 2022;67:102112.
128. Mondal SB, Tsen SWD, Achilefu S. Head-Mounted Devices for Noninvasive Cancer Imaging and Intraoperative Image-Guided Surgery. Adv Funct Mater. 2020;30(37):2000185.
129. Li D, Zhang J, Chi C, Xiao X, Wang J, Lang L, et al. First-in-human study of PET and optical dual-modality image-guided surgery in glioblastoma using 68Ga-IRDye800CW-BBN. Theranostics. 2018;8(9):2508.