Chapter

Fluorescent Nanomaterials for Cellular Imaging

Kalyan Sundar Ghosh and Anchal Sharma

Abstract

This chapter will provide an overview of different nanomaterials, which are being used for nondestructive imaging of living entities such as cells and tissues. The chapter begins with the basics of fluorescence imaging followed by a discussion on the advantages of fluorescent nanomaterials as compared to commonly used molecular fluorophores and imaging probes. Specific features and applications of nanomaterials frequently exploited in bioimaging are summarized. These include fluorescent silicon-based nanomaterials, hydrogels, polymer dots, magnetic nanoparticles, fluorescent quantum dots, carbon dots and other carbon-based nanomaterials, noble metal nanoparticles, micelles, dendrimers, lipid nanoparticles, and so on. Specific examples on their applications in bioimaging including multimodal imaging and targeted imaging are illustrated.

Keywords: nanomaterials, fluorescence imaging, multimodal imaging

1. Introduction

In today's modern era, nanotechnology has emerged as one of the major discoveries, which become an inexorable part of our daily life. It has a wide range of applications in areas such as nanoelectronics, consumer products, biomaterials, and nanomedicines. This implies its interdisciplinary nature by combining different subject domains such as chemistry, biology, physics, material science, chemical, electrical, and mechanical engineering. Nanomaterials find remarkable biomedical applications due to: (i) their small size; (ii) fascinating chemical, physical, and optical properties; (iii) a highly loadable surface; and (iv) good biocompatibility [1]. On account of the resemblance in size of nanomaterials with biomolecules such as DNA, antibodies, oligonucleotides, glucose, proteins, and virus (a size range of 1–100 nm), various *in vitro* and *in vivo* studies involving nano and biomaterials are being reported [2]. Among different biomedical applications, the potential use of nanomaterials in bioimaging of cells and tissues has grabbed a significant interest of several researchers across the world. This technique has become an essential tool to investigate various biological processes occurring at the cellular and subcellular levels and gaining importance in both fundamental research and clinical diagnostics. Though myriad organic and inorganic imaging probes are already available, applications of nanomaterials as imaging probes are proliferating. The applications of nanomaterials in bioimaging cover from in-depth imaging of tissues to point of care (POC) testing [3]. There are numerous imaging modalities available in the medical field to access images of various cells and tissues. These include radio imaging, magnetic resonance imaging (MRI), CT imaging, electrochemical imaging,

and even more futuristic imaging techniques such as laser ablation-inductively coupled plasma-mass spectroscopy (LA-ICP-MS) along with others. Some of the above methods are destructive in nature, require substantial preparation of samples, and possess limited resolution. These factors limit applications of these methods for imaging of cellular and subcellular structures in the body. This has paved the way for the development of imaging probes, which will have high resolution, can provide images in nondestructive way, and offer multimodal imaging to gain in-depth information. With the development of new functional materials, bioimaging using nanomaterials attracts substantial attention in biomedical research due to its versatility, selectivity, sensitivity, rich in contrast, and high-resolution properties obtainable in this technique. This also opens up a window to incorporate conventional imaging techniques with newly developed probes to record cellular events with high contrast and clarity. Earlier, optical imaging could be resoluted only to a limited range (~200 nm). But afterward with the advancement of fluorescence imaging methods such as photo-activated localization microscopy (PALM) and stimulated emission depletion (STED) microscopy, there was a substantial ramping up in the resolution [4, 5]. But still there are sort of limitations and those appear during the studies of animal models, which require high resolution and in-depth imaging. This clampdown can be curbed by using recently developed fluorescent nanomaterials imaging probes. Bioimaging using nanomaterials mostly adopts the following three techniques—(i) fluorescent nanomaterials are injected into the cells and the tissues to make them fluorescent; (ii) targeted bioimaging; and (iii) using nanomaterials as sensors to sense the cellular biochemical species, which are not fluorescent intrinsically. In targeted bioimaging, specific target sites are imaged using fluorescent nanomaterials. This is performed through the fabrication of suitably functionalized nanomaterials. The functional groups in those cases may be oligomers, receptors, ligands, and so on to detect a particular target site.

In this chapter, different types of fluorescent nanomaterials, which are being used as nanoprobes for fluorescence imaging to study cellular events, are discussed.

2. Fluorescence imaging

Cells, tissues, and organelles in the living beings are of such a tiny size that it is quite difficult to get clear and discernible images of them. This facilitates the need in microscopy to have certain kind of contrast in color of the entire cells and tissues and the localized areas of these tiny structures to get more information about the biological processes occurring in them. Fluorescence enables to solve both the issues of contrasting the overall structure and the localized area of that structure. Fluorescence is a type of luminescence that involves the absorption of radiations followed by emission of light of certain wavelength by the matter. The molecules, which have the ability to emit light on absorption, are called fluorophores. Fluorescence imaging is an invasive technique that enables to envision various biological processes occurring in living systems. It covers a wide range of observations, which includes protein expression, gene expression, and interactions occurring in cells and tissues. This technique basically involves the picturization of fluorescent proteins and dyes to understand various molecular mechanisms occurring in living cells, thus serving the purpose of being a potential tool for biochemical applications. In fluorescence microscopy, the samples are labeled with a fluorescent probe, and then, the sample is illuminated with a bright light. The labeled fluorophore absorbs and then emits at a certain wavelength. Therefore, on a black background, an image of high contrast can be obtained. Fluorescence imaging is the most widely used tool to study biological processes. There are two types of fluorescence imaging namely intrinsic and extrinsic

fluorescence imaging. The first kind of imaging usually refers to the fluorescence emission by intrinsic fluorophoric species such as tryptophan residues in proteins, NADH in tissues, and so on. The second type of imaging is based on extrinsic fluorescence, which is exhibited by synthetic dyes and probes, nanomaterials, sensors, and so on. They basically help in the detection of species, which are not visible through direct fluorescence imaging. Various fluorescence imaging techniques are presently available to study the structures of cells and tissues in the body. These include fluorescent wide field microscopy, point scanning confocal microscopy, parallel confocal microscopy, two-photon microscopy, light sheet microscopy, total internal reflection fluorescence (TIRF) microscopy, and super resolution microscopy.

2.1 Fluorescence imaging over bright field

A bright field microscopy involves illumination of the sample with transmission of white light, which resulted in an image of high contrast. The image thus obtained is a dark image on white background. But this technique cannot differentiate transparent, translucent, and stained cells as well as the structure of the cells. On the contrary, fluorescence microscopy enables to study a specific area of cellular structure as the fluorophores have the ability to illuminate only the targeted area of activity. As a result, unwanted areas will have no or negligible fluorescence, which will be helpful in getting proper images and information of the targeted area.

2.1.1 Fluorescence microscopy imaging range

The imaging range for fluorescence microscopy is quite higher than the usual range that a human eye is able to see. This extended range can be obtained with the help of charge-coupled device (CCD) cameras, which accumulate light emitted by the sample [6].

2.1.2 Advantages of fluorescence imaging

The advantages of fluorescence imaging are as follows:

i. labeling only the desired area of interest in cellular physiology and investigating the biological processes;

ii. having the high resolving power as compared to conventional microscope, thus known as super resolution microscopy;

iii. enabling the identification of microelements of interest with the help of Raman spectroscopy;

iv. giving an insight into the structural dynamics using FRET, fluorescence anisotropy, and other fluorescence techniques; and

v. producing the high contrast images as compared to conventional microscope.

3. Fluorescent nanomaterials versus molecular fluorophores as imaging probes

Different nanomaterials are developed for imaging of cellular structures because of the advantages such as high contrast, imaging in targeted area, reduced

cytotoxicity, and better selectivity [7]. For example, polyethylene glycol (PEG) coating on nanoparticles prevents undesirable interactions with other biomolecules [8]. The optical properties of intrinsically fluorescent nanoparticles are also not altered much on interactions with proteins [7]. In contrast to molecular imaging probes, nanomaterials have high retention time, which enables them to provide images for a prolong period [9]. Photostability of nanomaterials is usually superior to the conventional imaging probes. In fact, nanodiamonds do not show any photobleaching and can resist bleaching up to 100 MW cm^{-2} [10]. Another important advantage is the ease in internalization of nanomaterials into the cells. The internalization depends on the surface charges, and positive charge usually facilitates the internalization of nanomaterials. Thus, it can be easily targeted to the desired area of investigation, which is not feasible with common molecular imaging probes [11]. Also, to label a large number of cells at the same time to study any biological processes, nanomaterials are considered to be the simplest tool in high-throughput screening [12]. Most of the organic imaging fluorophores have size less than 1 nm as compared to the size of nanomaterials (1–100 nm). This provides a highly loadable surface for nanomaterials. Another important feature of nanomaterials is their use as multimodal imaging probes. Attachment of multiple components with nanomaterials enables them for application in more than one imaging techniques [13]. The nanomaterials also have the ability to show finer optical properties on account of surface plasmon resonance (SPR) that arises from collective electronic oscillations after interacting with the light of certain wavelength [14]. Nanoparticles can scatter absorbed light very efficiently and thus can be used as a major fluorescence imaging tool. Due to low quantum yield and low labeling ratio of dyes to targeted molecules in case of organic molecular fluorophores, low detection limit is difficult to be achieved. This limitation can be overcome by using fluorescent nanomaterials [15]. Another advantage of nanomaterials over traditional imaging probes is in theronaustics, which basically involves the combination of both therapeutic drugs and diagnostics [16]. Along with the binding of ligands and fluorophores on the surface of nanoparticles, drugs can also be incorporated in nanomaterials for simultaneous discernment and therapy [17].

3.1 Different types of nanomaterials frequently used in bioimaging and their features

Considering numerous exciting properties, different nanomaterials are developed for fluorescence imaging of cellular and subcellular structures. These include quantum dots, metal nanoparticles, magnetic nanoparticles, silica nanoparticles, nanodiamonds, hydrogels, and so on.

3.1.1 Fluorescent metallic nanoparticles

Use of metallic nanoparticles in molecular imaging in recent years has been keeping pace as it helps in attenuating the photophysical constraints of organic molecular fluorophores. These nanoparticles possess advantageous properties such as better photostability, improvised low detection limits, localized detection, high quantum yield, and clinical applicability [18, 19]. Suitably functionalized silver NPs are considered as efficient nano probe in bioimaging [20]. Metallic nanoparticles can also be used as imaging agents with high X-ray contrast because of their potency to absorb X-ray radiations. As a result, diseased tissue appeared with high contrast than the normal tissue in the image [21, 22]. Gold nanoparticles (AuNPs) have acquired notable attention owing to their low toxicity, good biocompatibility, and high absorption coefficient [23]. Gold nanorods and silver NPs are used in two-photon luminescence

imaging of cancer cells [24, 25]. Molecularly targeted gold nanorods were established as a bright contrast agent for two-photon luminescence (TPL) imaging of cancer cells up to at a depth of 75 μm [24]. The TPL intensity of nanorod labeled cells was found to be much higher than the two-photon autofluorescence (TPAF) emission intensity from unlabeled cancer cells under deep imaging (**Figure 1**). The nanoclusters made from silver, gold, and copper show intrinsic fluorescence quite effectively, and their surface can be protected by coating them with alkanethiolate monolayers [26]. If their structures are modified effectively, then they can be used as agents in targeted imaging. Metallic nanoparticles enhance the fluorescence properties of fluorophores in their proximity on account of the interaction between the surface plasmon of the metal nanoparticles and the dipole moment of fluorophores [19]. Silver islands or silver particles deposited on a surface act as a scaffold in bioassays using metal-enhanced fluorescence (MEF) technique. Glass surface coated with silver island film can enhance the signal up to forty fold [27, 28]. Silver island films increase the sensitivity of the fluorescence signals of cell membrane as the fluorescence of the fluorophores enhances due to silver island films [29]. Coating of gold nanoclusters with ruthenium(II) polypyridyl provides the ability for DNA binding and thus leads to cellular uptake and imaging of cellular physiology [30].

3.1.2 Magnetic nanoparticles

Fluorescent magnetic nanoparticles play a major role in the biomedical applications and are widely used in bioimaging. Magnetic nanoparticles show various properties such as biocompatibility, superparamagnetic character, possibility of desired surface functionalization, and so on. They have been functionalized with various enzymes, proteins, and antibodies for improved targeting [31, 32]. Aminopropyl silica coated γ-Fe_2O_3 nanoparticles labeled with fluorescein can be used in both magnetic resonance and fluorescence imaging of cells and tissues [33].

3.1.3 Quantum dots

Quantum dots (QDs) are semiconducting nanocrystals with distinctive optical properties, which are quite unique from large sized particles because of quantum mechanical phenomenon. QDs are currently used extensively in fluorescence microscopy based on their high photostability, brightness, and tunable emission of light as compared to traditional organic and inorganic fluorophores [34–37]. Functionalization of quantum dots with protective ligands can alleviate toxicity and also reduces nonspecific binding with biomolecules. This also increases aqueous solubility and localized

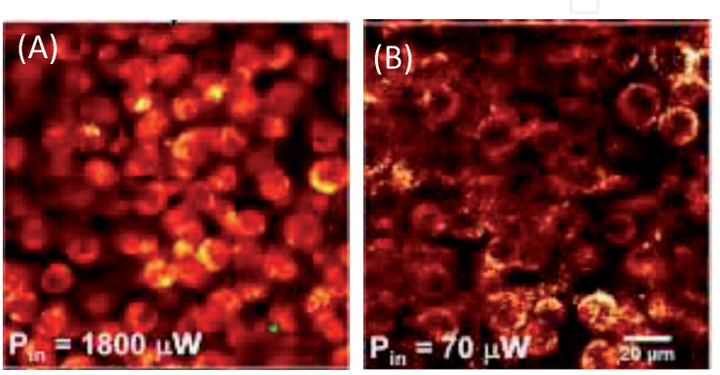

Figure 1.
Two-photon luminescence imaging of cancer cells embedded in a collagen matrix at 75 μm depth. (A) TPAF imaging of unlabeled cells and (B) TPL imaging of nanorods labeled cells. This figure is reprinted (adapted) with permission from Durr et al. [24]. Copyright © 2007, American Chemical Society.

targeting ability [36, 38–40]. On account of their small size, QDs have the tendency to get accumulated in liver or spleen, but this can be avoided by coating them with polyethylene glycol (PEG) [41]. A 3-mercaptopropanoic acid capped CdS QDs were used for the imaging of *Salmonella typhimurium* cells (**Figure 2A**) [42], and bioconjugated CdSe/ZnS QDs were used in multicolor imaging of HeLa cells [44]. ZnO QDs doped with Gd intensify the fluorescence, thus providing the QDs with the ability to act as nanoprobes for the rapid detection of cells [45]. Other QDs coated with polyethylene glycol and conjugated with peptides can target the nucleus and cytosol for imaging purpose [45–48]. Hybrid fluorescent nanocomposites such as silica-coated Fe_3O_4/CdTe quantum dots have been proven a promising agent for immune-labeling and fluorescence imaging of HeLa cells [49]. Bioimaging (**Figure 2B**) was also performed with the help of a TAT peptide conjugated fluorescent, radio-opaque, and paramagnetic CdS:Mn/ZnS QDs [43], and these conjugated QDs had efficiently crossed the blood-brain barrier.

Polymer-coated QDs bound with cancer-specific antibody offer the ability to image tumor targeted areas [50]. Fabrication of CdSe/ZnS QDs encapsulated in phospholipid micelles enables *in vivo* imaging of embryo [51]. The injected QDs had also exhibited greater stability without any toxicity. QDs conjugated with career peptide can be transfected into the cells for bioimaging in living organisms. An insect allatostatin has been identified that transfected into NIH 3T3 and A431 human squamous cells. QDs can be conjugated with allatostatin to make cellular entry easy for the purpose of imaging [52].

3.1.4 Hydrogels or hydrophilic polymers

Hydrogels are three-dimensional (3D) network of hydrophilic polymeric chains, which undergo swelling in water. The polymer chains are hitched in cross-link formation, and the structure contains a lot of water molecules inside [53]. The hydrophilicity is due to the presence of hydrophilic groups on its surface such as –COOH, $-SO_3H$, and $-NH_2$ [54]. Hydrogels are considered to be a potential candidate to act as an imaging agent for bioimaging. On account of their biodegradability, 3D crosslinking network, biocompatibility, sensitivity, nontoxicity, and quick gel to sol transformation in response to biological stimuli, they are being considered as promising fluorescent bioimaging probes [55, 56]. Hydrogels are fabricated using different procedures that involve metal-ligand complex formation, hydrogen bonding, host-guest complexation, and so on [57]. Fluorescent supramolecular metallohydrogel based on ruthenium(II) tris(bipyridine) complex had been used for cell imaging (**Figure 3**) [58].

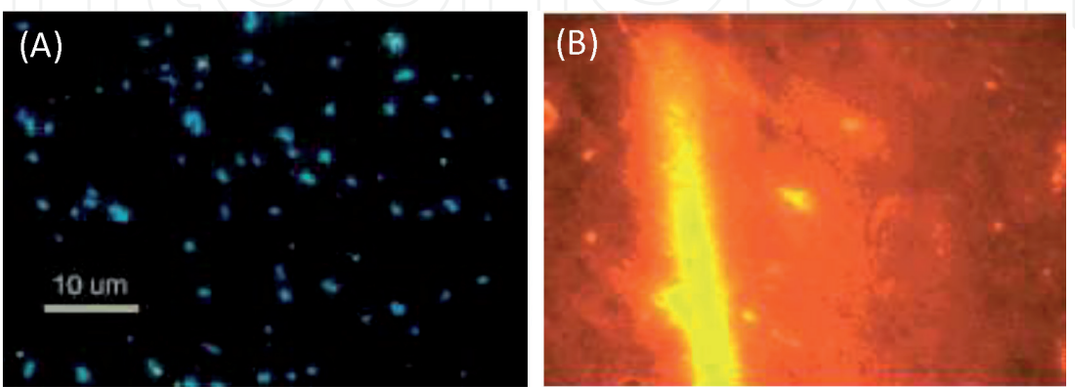

Figure 2.
(A) Fluorescence imaging of Salmonella typhimurium *cells by the aqueous CdS QDs. This figure is reprinted (adapted) with permission from Li et al. [42]. Copyright © 2007, American Chemical Society. (B) Fluorescence image of the branches of right middle cerebral artery of a cross section of rat brain after intra-arterial administration of TAT-conjugated QDs. This figure is reprinted (adapted) with permission from Santra et al. [43]. Copyright © 2005, American Chemical Society.*

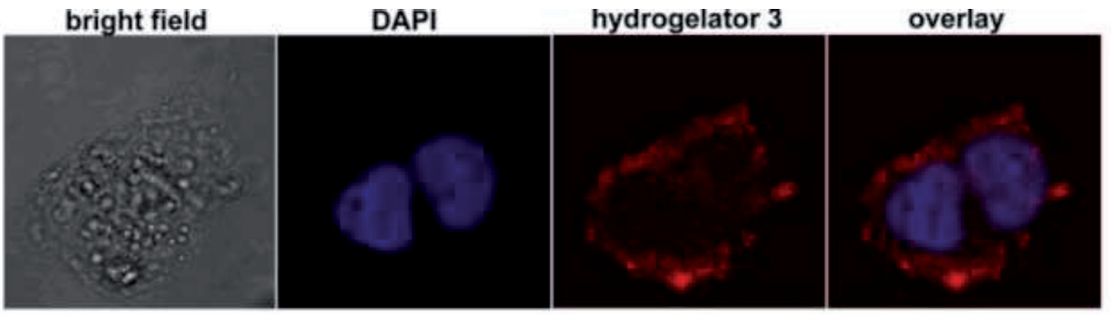

Figure 3.
Fluorescent image of HeLa cells incubated with metallohydrogel. From left to right: phase-contrast image, live cell stain DAPI (blue), luminescence emission of metallohydrogel (red), and the overlay image. This figure is reprinted (adapted) with permission from Zhang et al. [58]. Copyright © 2013, American Chemical Society.

Interactions between the hydrogels (based on gelatin cross linked with lysine diisocyanate ester) and the tissue were studied using multimodal imaging techniques [59]. Poly(ethylene glycol)-co-poly(ethylene oxide), which is also called pluronic hydrogel, was synthesized and proved to be a promising fluorescent nanoprobe for bioimaging [60].

3.1.5 Polymer dots

Polymer dot nanoparticles are fluorescent π-conjugated polymers having size of only few nanometers. They can be functionalized and encapsulated easily. They are highly fluorescent in both single-photon scanning microscopy and two-photon scanning microscopy [61, 62]. Conjugated polymers (CPs) acquire the maximum fluorescence brightness as compared to any other nanoparticles till date on account of high fluorescent decay rates, high quantum yield, high cross-sectional area ($\sim 10^{-14}$ cm^2) for absorption, and immoderate chromophore density [63]. Owing to small particle size, high cellular uptake through endocytosis, nontoxicity, and photostability, extraordinary emission rates endow the particle to act as efficient imaging probes. They have excellent fluorescence brightness that often widens to NIR region [64]. These semiconducting CPs have the tendency to get attached with peptides like chlorotoxin to image brain tumors [65]. The pH value inside the HeLa cells was demonstrated by imaging these cells using CPs with fluorescein, which is a pH indicator [66]. The fluorescence of anionic CPs is quenched by ions such as Cu(II) [67]. NIR-emissive CPs poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] doped with silicon 2,3-naphthalocyanine bis(trihexylsilyloxide) offers efficient FRET. A combination of bioluminescence resonance energy transfer (BRET) and FRET techniques was adopted with these CPs instead of their optical excitation, which does not provide enough in-depth information for imaging of cancer and lymph nodes [68]. In this case, the use of probes with longer life times helped in the separation of fluorescence signal from the background fluorescence and improved the signal-to-noise ratio. Considering this advantage, CPs were fabricated by encapsulating Ln complexes into the host polymer. Polyvinylcarbazole (PVK) (donor) was used as semiconducting host polymer incorporated with Eu complex (acceptor). These PVK/Eu complex CPs were found to show a luminescence lifetime of 509 μs. Cells labeled with these synthesized polymer dots were imaged and that showed very less background fluorescence as compared to the unlabeled ones [69]. When it comes to label receptors on the cells, monovalent or single chain CPs are considered to be an excellent labeling agent, for instance, alkyne terminated linear poly(p-phenylenevinylene) [70]. Furthermore, CPs have been establishing themselves as an efficient platform for the development of probes

for multimodal imaging. Various polymer dots have been fabricated to combine two imaging techniques for molecular imaging. Conjugated polymers modified with BSA and functionalized with Au clusters act as a promising imaging probe that integrates two imaging techniques, that is, photoacoustic imaging and fluorescence imaging [71]. After injecting this fabricated polymer dots into an animal model, signals for both the imaging techniques were obtained. Thus, polymer dots incorporated with characteristics as per clinical point of view seem to be a promising nanoprobe agent as compared to the conventional imaging probes.

3.1.6 Silicon-based nanomaterials

Silicon nanoparticles (SiNPs) are being used in the manufacture of finest sensors and nanoprobes for various bioimaging applications. These nanoparticles are highly porous with high surface area to volume ratio and possess unique chemical, biodegradable, electrical, and optical properties [72, 73]. The product of biodegradation of silicon nanomaterials, for instance, orthosilicic acid is congenial with tissues in the body. Besides, silicon is present in human body as a trace element [74]. Owing to good biocompatibility, these nanoparticles are not cytotoxic, which are prerequisite for a good bioimaging agent. Fluorescent SiNPs are considered to be a consummate nanoprobe agent over traditional imaging probes due to high photostability, brightness, biocompatibility, and biodegradability [75]. The major limitation is the poor dispersibility of SiNPs in aqueous media, which constrained their use as a biological probe [76]. By taking into account the above limitation, luminescent SiNPs with hydrophilic ligands were synthesized that enhance the aqueous dispersibility and hence their utility in molecular imaging. Modified SiNPs were fabricated by encapsulating SiNPs in phospholipid micelle and used for *in vivo* imaging [77, 78]. Various other modified SiNPs were also synthesized with hydrophilic surface and were conjugated with proteins to use them for immunofluorescent cell imaging [79]. But due to large hydrodynamic radius (>50 nm), these modified SiNPs become less useful for *in vivo* imaging. Considering this, silicon nanowires (SiNWs) incorporated with glutaric acid were prepared with a size of <5 nm. They have excellent photostability and good aqueous dispersibility also [80, 81]. SiNPs capped with allylamine were also fabricated and used for labeling the HeLa cells. The image (**Figure 4**) showed brilliant bright SiNPs in the cytoplasm [82].

Commonly, two-step process is used to synthesize functional SiNPs. In the first step, SiNPs are synthesized, and then their surfaces are modified. To achieve this in one step, bottom-up approach was also used. In this method, hydrophilic molecules

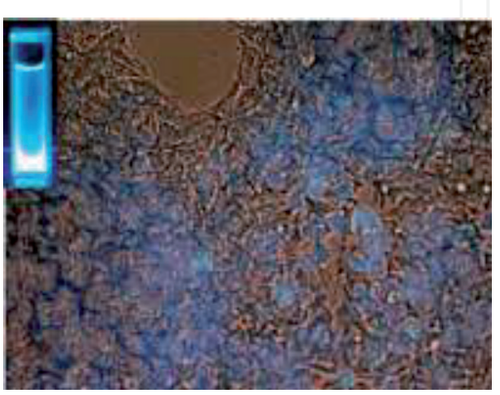

Figure 4.
Cell imaging using silicon quantum dots capped with allylamine. This figure is reprinted (adapted) with permission from Shiohara et al. [82]. Copyright © 2010, American Chemical Society.

containing silicon (e.g., 3-aminopropyl trimethoxysilane) were used as a source of silicon. The fluorescent SiNPs fabricated in this way were found to possess excellent aqueous dispersibility and high photostability over common organic fluorophores as well as II-VI QDs [83]. SiNWs coated with AuNPs are multicolored and are highly fluorescent nanomolecular beacons. They are highly sensitive for DNA detection and have the ability to detect up to ~50 pM [84]. Silica-based nanoparticles with fluorescence and magnetic properties can act as a nanoimage probe agent. Silica-coated iron oxide nanoparticles attached to the fluorescein dye have the ability to act as a multimodal probe agent to label bone marrow cells [85]. Organic fluorophores functionalized silica having gadolinium oxide (Gd_2O_3) NPs embedded in its shell were fabricated and then administered into mice and investigated further for its ability to act as a multimodal probe agent for both fluorescence microscopy and MRI (**Figure 5**). Gd_2O_3 core enhances the contrast of MRI, and the shell imparted excellent fluorescence thus suitable for multimodal imaging [86].

Another example for multimodal imaging is the nanoparticles having silica doped with dye-encapsulated in gadolinium silylated shell, which were used to label monocyte cells *in vitro* responsible for immune response in the body [87].

3.1.7 Carbon dots

Carbon dots or carbon-based quantum dots are small-sized semiconductor quasispherical luminescent nanoparticles with size below 10 nm and can also be as less than as 1 nm [88]. They were initially discovered from carbon nanotubes whose surface modification had resulted highly fluorescent emissions at different excitation wavelengths ranging between UV and near-IR [89]. C-dots are attributed with various characteristics that have grabbed the attention of several research groups.

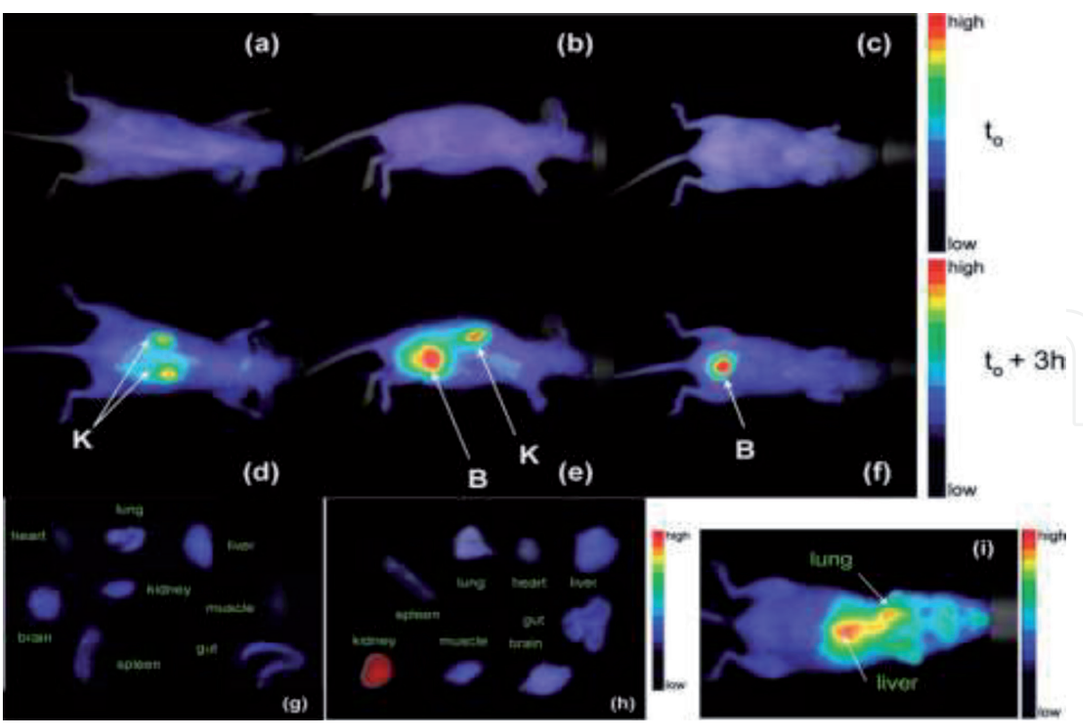

Figure 5.
Fluorescence reflectance imaging of a nude mouse (a–c) before and (d–f) 3 hours after the injection of Gd_2O_3-embedded silica NPs (K, kidneys; B, bladder). Fluorescence reflectance imaging of some organs after dissection (g) of a control mouse (no particles injected) and (h) of the nude mouse visualized on pictures (a–f). (i) Fluorescence reflectance imaging of a nude mouse after the injection of $GadoSi_2C$ (particles without PEG). This figure is reprinted (adapted) with permission from Bridot et al. [86]. Copyright © 2007, American Chemical Society.

They are noncytotoxic and have sufficient photostability and chemical inertness with ease in surface modification [90, 91]. Their surface is full of defects, and after suitable modifications, the surface can be made highly fluorescent [92]. C-dots can be easily taken up by cells through a process called endocytosis and can image cells using both single and two-photon excitations [93]. The intracellular uptake can be enhanced by coupling them with peptides, which can translocate the active membrane. C-dots also undergo rapid renal excretion [80]. Aqueous dispersible C-dots processed from nanodiamonds using one-pot hydrothermal treatment were administered to NIH-3T3 cells, which exhibited different colored fluorescence (yellow and green) at different excitation wavelengths [94]. Highly luminescent C-dots fabricated from citric acid were used to image MC3T3 cells [95]. C-dots were also prepared through green synthesis from natural products, proteins, carbohydrates, biowastes, and food products [91]. The C-dots obtained from barbeque meat and surface modified with PEG resulted in high quantum yield and excellent fluorescence emission [96]. C-dot surface passivated with polyethyleneimine was used for labeling the HeLa cells, which were further viewed with green fluorescence. These quantum dots have also been proved to show astounding applications in theronaustics to attain both drug delivery and fluorescence imaging [97]. C-dot SiO_2 nanoparticles further functionalized with PEG molecules were fabricated that resulted in the augmentation of the brightness, biocompatibility, and stability. These synthesized nanoparticles were able to deliver anticancer drug doxorubicin inside the HeLa cells efficiently [98]. Doxorubicin was also loaded on C-dots synthesized from BSA and then administered into A549 cells. C-dots and the drug both were found localized in the cells and thus served the purpose for simultaneous bioimaging and drug delivery [99]. Carbogenic nanodots doped with iron oxide were fabricated for multimodal imaging (MRI and fluorescence microscopy). These nanoparticles were injected into rats and were uptaken by RAW264.7 cells and visualized in the cytoplasm. Fluorescence and MRI enhanced signals were observed because of T1 and T2 relaxation [100].

3.1.8 Various other carbon-based nanomaterials

Apart from C-dots, various other carbon containing nanomaterials such as graphene oxide, graphene, nanodiamonds, graphite oxide, and carbon nanotubes are available. The graphite oxide is sensitive to pH and is photostable like C-dots [101]. They can be synthesized in one-pot hydrothermal process. They give different colored fluorescence emission at different excitation wavelengths, and the photoluminescence is size-dependent. Nanographite oxide was taken up by A549 and was imaged in cell cytoplasm [102]. Graphene quantum dots (GQDs) are fabricated using hydrothermal treatment of graphene oxide. Both bottom-up and top-down methods were used for the synthesis of GQDs [103]. To enhance the fluorescence of GQDs, they are either reduced or surface passivated. They were further employed for the bioimaging of cell physiology, for instance, GQDs labeled MG-63 cells exhibited fluorescence at different excitation wavelengths (405 and 488 nm) [104]. Graphene is not often used for the purpose of imaging owing to its negligible fluorescence. Similarly, fullerenes too do not show any fluorescence and cannot be used for fluorescence imaging except fullerene C_{70}, which exhibits fluorescence and thus can be used as an efficient imaging probe [105]. Carbon nanotubes (CNTs) are known to be fluorescent in the near infrared region (NIR) with low quantum yield [106]. CNTs embedded in single-stranded DNA remain in active form up to 3 months [107]. On account of its fluorescence emission in NIR range, CNTs can be used for NIR fluorescence imaging of tumors in a targeted way [108]. Nanodiamonds contain nitrogen vacant spots and are being utilized extensively for

bioimaging purposes based on their photoluminescence properties, photostability, and biocompatibility. They show red and green fluorescence at different excitation wavelengths [109].

3.1.9 Micelles, dendrimers, and lipid nanoparticles

NIR region is being extensively used for the imaging of cells and tissues due to its tendency to penetrate deeply and has an access to in-depth information. NIR dyes like indocyanine green (ICG) are being used for these days as image contrast agents. To enhance the specific targeting of ICG, it has been incorporated in several nanoparticles, but due to certain limitations, applications become restricted. Recently, ICG was formulated in micelles that include ICG and polycaprolactone (PCL). The ICG-PCL micelle has highly loadable surface and uniform size. They were found to have better fluorescence brightness, retention time, biocompatibility, no toxicity, and enhanced accumulation of ICG when compared with only ICG fluorescence imaging [110]. These micelles have also been proved to be useful for the surgery of tumors using intraoperative images. Another example is the fabrication of supramolecular micelles that involve perylenediimide and poly (D, L-lactide)-*b*-poly(ethyl ethylene phosphate), which is a block copolymer as precursors. Camptothecin drug was then loaded on the surface of synthesized supramolecular micelle and injected into mice having tumor cells. The fluorescence images suggest alleviation in the growth of tumor as compared to only drug [111]. Dendrimers are branched molecules having three-dimensional network and spherical morphology. Owing to their small size than normal nanoparticles, they can easily undergo endocytosis. They are highly fluorescent with very high molar absorbance. Thus, they can be considered as alternative of quantum dots for imaging [112]. Dendritic nanoprobes conjugated with cyanine dyes resulted in higher photostability, brightness, localization, and resolution for bioimaging [113]. Polyamide amine (PAMAM) dendrimer has been used widely for the fluorescence imaging of cancer cells. The receptor (sialoglycoprotein) present on cancer cells helped in the identification of this dendrimer by exhibiting blue emission. Dendrimers embedded with pH and NIR sensitive probes can be used to localize and target cancer cells [114]. Lipid NPs are being extensively used as drug delivery system and in bioimaging. They have been identified as excellent nanocarriers for the treatment of cancer. They can overcome various barriers in the physiological environment, thus enhancing the delivery of drug [115]. On account of their bioavailability, low-toxicity, and production in large scale, they are being widely used in bioimaging [116, 117]. The nonpolar lipid NPs are labeled with various probes and dyes (emitting in NIR region) for the purpose of bioimaging [118].

4. Conclusion and future directions

Advantages of fluorescence imaging of cellular events in the body have captured the attention of several research groups. This is on account of the advancement made in the bioimaging techniques. Various nanoparticles have been developed as remarkable imaging probes compared to the conventional imaging fluorophores, and many others are in the process. Cell is a biological entity through which the entire biological mechanism of an organism can be observed. Thus, such an image probe, which has the ability to go deep into the cells and helps in extracting the information out of it, was the necessity. Thus, various nanomaterials were identified that have been discussed in this chapter to be superior probes than the traditionally used fluorophores. Nanoparticles are often preferred to the conventional

imaging probes for the intracellular biological activities owing to their ability to have an access to in-depth information. These nanoprobes have also been proved finer than other fluorophores owing to their high contrast, which can help to get brighter image. It makes easier to discern information with these highly fluorescent materials. Their high photostability aids in visualization of the image for a longer period of time. Better localizing ability of these materials assists to target only the affected cells and tissues and reduces nonspecific binding with other biomolecules. In addition to that, in-depth penetration and better endocytosis help in the easy uptake of these probes by the cells. Good biocompatibility and highly loadable surface add beneficial features in these probes. As a result, they can be used for both drug delivery and diagnosis by imaging. All these features make the nanomaterials more efficient than any other probes in the field of bioimaging. Still there are some limitations associated with them like toxicity that is a major issue that need to be addressed before its use in future. Its ability to combine various imaging modalities to act as a multimodal imaging probe is a remarkable feat that can bring revolution in cellular imaging.

Abbreviations

C-dots	carbon dots
CNTs	carbon nanotubes
CPs	conjugated polymers
GQDs	grapheme quantum dots
ICG	indocyanine green
NPs	nanoparticles
QDs	quantum dots
SiNPs	silicon nanoparticles
SiNWs	silicon nanowires

Author details

Kalyan Sundar Ghosh* and Anchal Sharma
Department of Chemistry, National Institute of Technology Hamirpur, Himachal Pradesh, India

*Address all correspondence to: kalyan@nith.ac.in

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