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Chapter 1

Introductory Chapter: Nano-bioimaging—Past, Present, and Future

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Additional information is available at the end of the chapter

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1. Introduction

From the 1960s to the present, the image processing technology was dramatically improved, and an enormous growth rate in the use of image processing technology can be seen today in almost every field. It is a computer vision technology with the ability to replace the human vision by an artificial vision for observation of objects and processes. Historically, the potential of image processing for biological applications has been understood by researchers in later years of the 1980s and in the early 1990s, and many attempts have been made to focus on conducting research for the employment of this technique in biological imaging [1]. For instance, the milestones of optical imaging in autophagy research are shown in **Figure 1**. As it has been reported by Wang et al. [2], these events involve specific fluorescence probing methods that localize the selected autophagic components and optical imaging platforms. They confirmed that most of the new investigative methods and advanced imaging equipment were developed during the past decade, during which some super-resolution optical imaging technologies have emerged.

Biological imaging or bioimaging is a precision technique for the recording of information relevant to biological materials using a variety of imaging equipments and processings. Usually, it is defined as a visualization method in which a biological process can be recognized noninvasively or to record the information from the biological specimen. Bioimaging aims to interfere as little as possible with life processes. Moreover, it is often used to gain information on the 3D structure of the observed specimen from the outside, i.e., without physical interference. In addition, the subcellular structures and the entire cells over tissues up to the entire multicellular organisms can be observed using bioimaging. Also, in cell biology, the cellular process or the ion or metabolite levels can be evaluated by bioimaging. Over three decades various biological imaging techniques such as X-ray, thermal imaging, X-ray computed

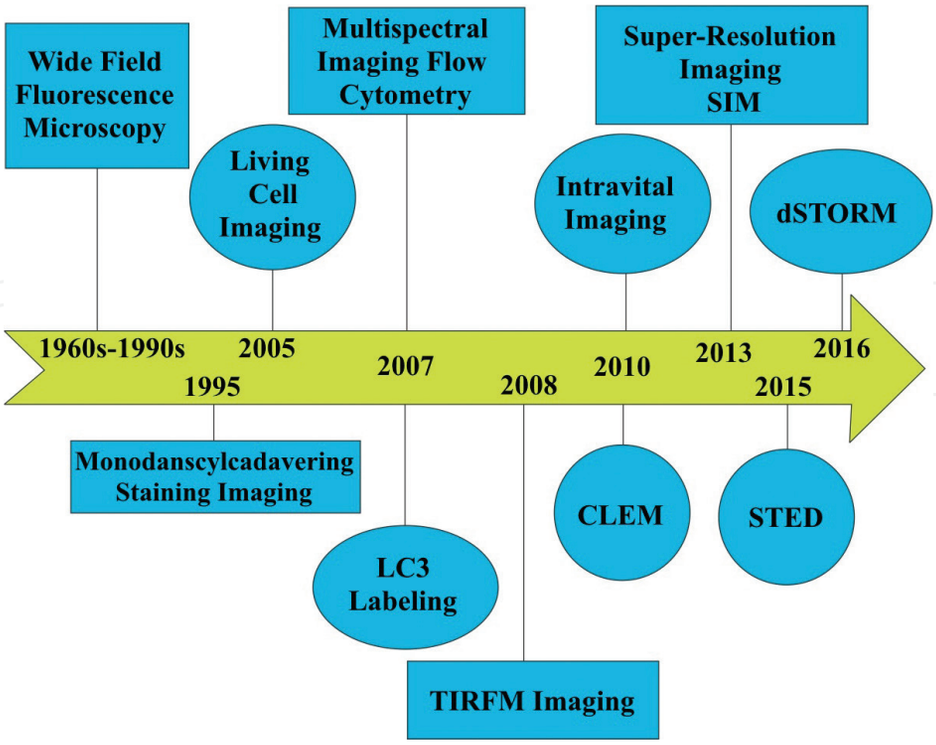

Figure 1. Milestones of optical imaging in autophagy research. All abbreviations are as follows: CLEM, correlated light and electron microscopy; dSTORM, direct stochastic optical reconstruction microscopy; LC3, light chain 3; LSCM, laser scanning confocal microscopy; SIM, structured illumination microscope; STED, stimulated emission depletion microscopy; TIRFM, total internal reflection fluorescence microscopy [2].

tomography (CT), hyperspectral imaging, optical, and magnetic resonance imaging (MRI) have been developed that are different in principles and equipment [3]. Due to their wide application potential in various fields, these techniques have continuously seen the rapid growths and the tremendous improvements. To carry out bioimaging, a setup of equipment is needed. Each kind of imaging requires different equipments, but the basic components in any imaging system are essentially the same. Usually, the following basic components such as a camera, illumination, frame grabber, and image processing hardware and software are used in a biological imaging system [1]. In bioimaging process, the image of the biological object is primary captured by a camera, and its external characteristics, such as color, shape, size, and surface texture, are evaluated using a sensor including a charge-coupled device (CCD) camera, X-ray, X-ray CT, ultrasound, and MRI. CCD camera is the most commonly used sensor for this purpose. After that, the conversion process named as digitization is used to convert the analog video signal from the camera into a digital signal. The conversion of pictorial images into digital form is performed by the frame grabber (or digitizer). Following that, digital image must be processed by the image processing hardware and software to analyze the captured images and obtain the necessary output. There are several steps including image acquisition, image preprocessing, enhancement, segmentation, representation, and description that need to be performed for image processing [1]. In each step, a process is performed. For example, the process of acquiring the raw binary data from the image of the area of interest is achieved by image acquisition, and the reduction of the unwanted features

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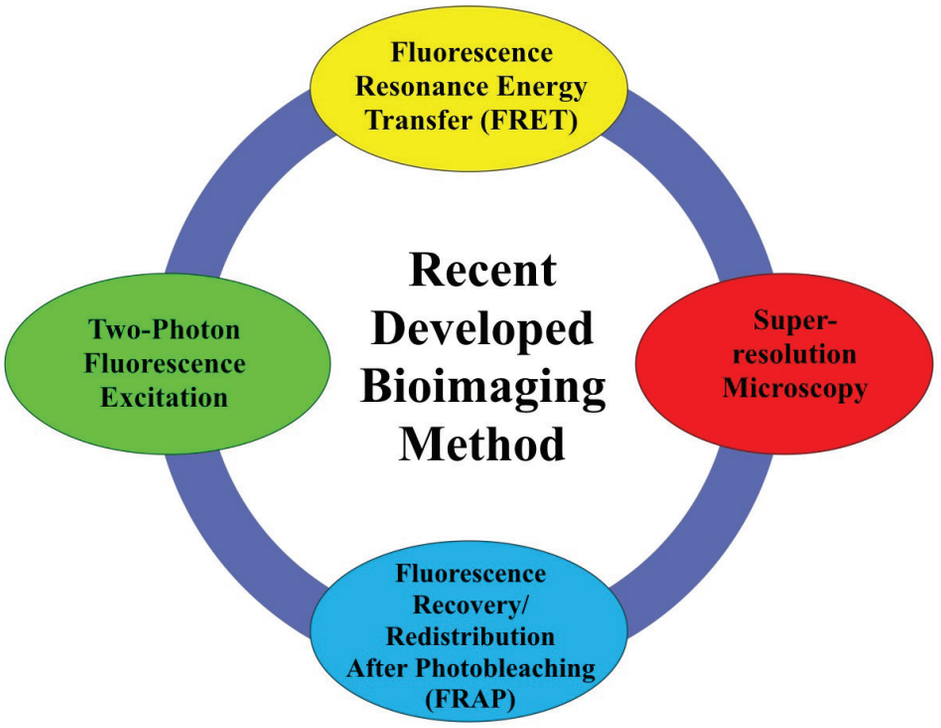

Figure 2. Recently developed techniques for bioimaging.

in the images is followed by the image processing. Over three decades, many developments have been achieved in equipments and image processing steps. As can be seen in **Figure 2**, recent progresses in bioimaging include super-resolution, two-photon fluorescence excitation microscopy, fluorescence recovery/redistribution after photobleaching (FRAP), and fluorescence resonance energy transfer (FRET).

2. Nano-bioimaging

As it is mentioned above, considerable advancements have been achieved in bioimaging. Nevertheless, the limitation of image resolution is a barrier that needs to circumvent for the next generation of bioimaging technology. Recently, the super-resolution microscopy was purposed for image resolution betterment [4]. It is found that the super-resolution microscopy can address some of the difficulties inherent in earlier microscopy techniques, provide new advances in biomedicine, and in those fields deal with the study of biological systems [4, 5]. However, it is suffering from the special equipment requirement which makes it as complex as it needs to be and hard to operate [6]. On the other hand, biologists believe that only a single type of technique cannot be used to obtain all information about a biological system [7]. Hauser et al. [8] found that the integration of two or more microscopy techniques performed on the same sample has been highly successful in overcoming the aforementioned limits of each method. They demonstrate how a correlative approach adds new dimensions of information and provides new opportunities in the fast-growing field of super-resolution microscopy. Additionally, other super-resolution microscopy methods such as structured

illumination microscopy (SIM) were developed for resolution enhancement. Despite their great success, all of these techniques are intrinsically involving with their limitations and challenges. For example, an expensive hardware and an expensive software are needed to overcome the spatial resolution improvement of SIM providing excellent super-resolution in 2D or 3D [9]. Ponsetto et al. explained that all of these methods have trade-offs between several factors such as resolution, speed, field of view, biocompatibility, sensitivity, and experimental complexity [10]. So et al. [11] compared the advancements of the widely used fluorescence microscopic techniques, stochastic optical reconstruction microscopy (STORM) or photoactivated localization microscopy (PALM), stimulated emission depletion (STED), and SIM. They explained that the label-free optical microscopic techniques whose functions are originated from nanoscale structures such as micro-curvilinear lens, super-oscillatory, and metamaterials can provide the conventional microscopy procedure with the ability for the new generation of nanoscopy. These approaches can break the barrier of diffraction limit, which is literally not a limit any more [11].

It is expected that the barrier of high-resolution image construction can be solved by nanotechnology. It seems that nanotechnology is able to advance medical imaging to the next level by increasing the resolution of current techniques [12]. This approach enhances the specificity of targeted imaging using the unique designed nanoconstructs. The increasing role of nanomaterials in biological and medical imaging research can reflect the impact of nanotechnology in bioimaging. As it has been mentioned, the contrast improvement of image is the most important reason for nanoparticle combination with imaging [13]. Usually, to utilize the enhancement of image contrast, contrast agents are needed. Traditionally, small-molecule contrast agents are used and biocompatible nanocrystals with the ability to provide a strong imaging signal. Also, plasmonic nanostructures can exhibit the field confinement effect which is locally amplified within subdiffraction-limited volume and is useful in many biomedical sensing and imaging applications [14]. Willets et al. reviewed the vital role of plasmonic nanoparticles in improvement of super-resolution imaging and described the growing partnership between super-resolution imaging and plasmonics, by describing the various ways in which the two topics mutually benefit one another to enhance our understanding of the nanoscale world [15]. They demonstrated that the plasmonic nanoparticles are explored as image contrast agents for super-localization and super-resolution imaging, offering benefits such as high photostability, large signal-to-noise ratio, and distance-dependent spectral features but presenting challenges for localizing individual nanoparticles within a diffraction-limited spot. In addition, they found that the subdiffraction-limited spatial resolution can be achieved by using the plasmon-tailored excitation fields [15]. Their study confirms that the localized surface plasmons and the surface plasmon polaritons can be used to create confined excitation volumes or image magnification to enhance spatial resolution. As powerful analytical tools, luminescent bioprobes are another example of the nanomaterial employment for optical imaging [16]. Shewring et al. described an Ir(III)-based small-molecule, multimodal probe for use in both light and electron microscopies [17]. Their finding confirmed the use of Ir(III) complexes as probes that provide excellent image contrast and quality for both luminescence and electron microscopy imagings. All of these correlative techniques are categorized in

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nano-bioimaging. Nanotechnology can also be used in other imaging techniques. For instance, Goel et al. reported that the hybridization of the dynamic synergism of positron emission tomography (PET) and nanotechnology can enhance the sensitivity and quantitative nature of PET which can help overcome certain key challenges in the field [18]. Fluorescence bioimaging in the second near-infrared spectral region (NIR-II, 1000–1700 nm) can be considered as attractive nanoparticles in nano-bioimaging. It can provide advantages of high spatial resolution and large penetration depth, due to low light scattering [18]. Their finding illustrates that NIR-II quantum dots (QDs) can be used in biomedical and clinical applications due to their excellent biocompatibility, photostability and brightness which require deep tissue imaging [19].

Lanthanide-doped upconversion nanoparticles (UCNPs) are the interesting nanomaterials with a great potential for nano-bioimaging applications [20, 21]. Their exceptional optical and physicochemical properties made them to be considered for design of the novel UCNP-based nanoplatform for luminescent and whole-body imaging [22]. Generalova et al. [22] demonstrated applications of UCNPs as a new nanoplatform for optical and multimodal cancer imaging in vitro and in vivo and extend discussions to delivery of UCNP-based therapeutic agents for photodynamic and photothermal cancer treatments. Similar to other semiconductor nanocrystals such as CdS, ZnO, TiO_2, and CdSe/ZnS core shells, lanthanide-doped upconversion nanoparticles can be used for magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET)/single photon emission computed tomography (SPECT) [23–25]. **Figure 3** shows the schematic applications of semiconductor nanocrystals in bioimaging. As it is mentioned by Chen et al. [26, 27], semiconductor polymer dots (SPDs) can also be utilized in biological imaging because of their outstanding photophysical properties, such as high brightness, extraordinary photostability, and favorable biocompatibility, in comparison with those of quantum dots (QDs), organic dyes, and reversibly switchable fluorescent proteins (RSFPs).

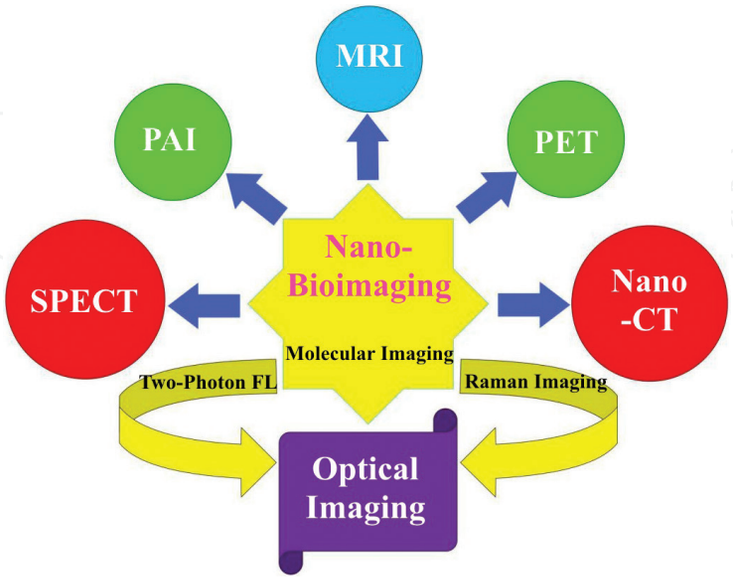

Figure 3. Schematic illustration of semiconductor nanocrystal applications in bioimaging.

3. Graphene-based materials for nano-bioimaging

Among all nanomaterials that are considered as potential candidates for nano-bioimaging applications, graphene has attracted great attentions. As it has been expressed by Lin et al. [28], the biomedical applications of graphene and its derivatives are extensively investigated for diagnostics, drug delivery, near-infrared (NIR) light-induced photothermal therapy, and bioimaging. Due to the versatile surface functionalization and ultrahigh surface area of graphene and its derivatives, they can be easily functionalized by small-molecule dyes, polymers, nanoparticles, drugs, or biomolecules to obtain graphene-based nanomaterials for different bioimaging applications [28]. Lin et al. [28], focused on applying graphene-based nanomaterials for different molecular imaging modalities, and their review confirmed that the graphene-based materials can be employed for optical imaging (FL, two-photon FL, and Raman imaging), PET/SPECT (positron emission tomography/single photon emission computed tomography), MRI (magnetic resonance imaging), PAI (photoacoustic imaging), CT (computed tomography), and multimodal imaging (**Figure 4**). Graphene quantum dots (GQDs) are one of the graphene-based materials that have a considerable potential for bioimaging applications. Due to the photoluminescence (PL) and high structural stability, low cytotoxicity, and good biocompatibility, fluorescent graphene quantum dots (GQDs) have

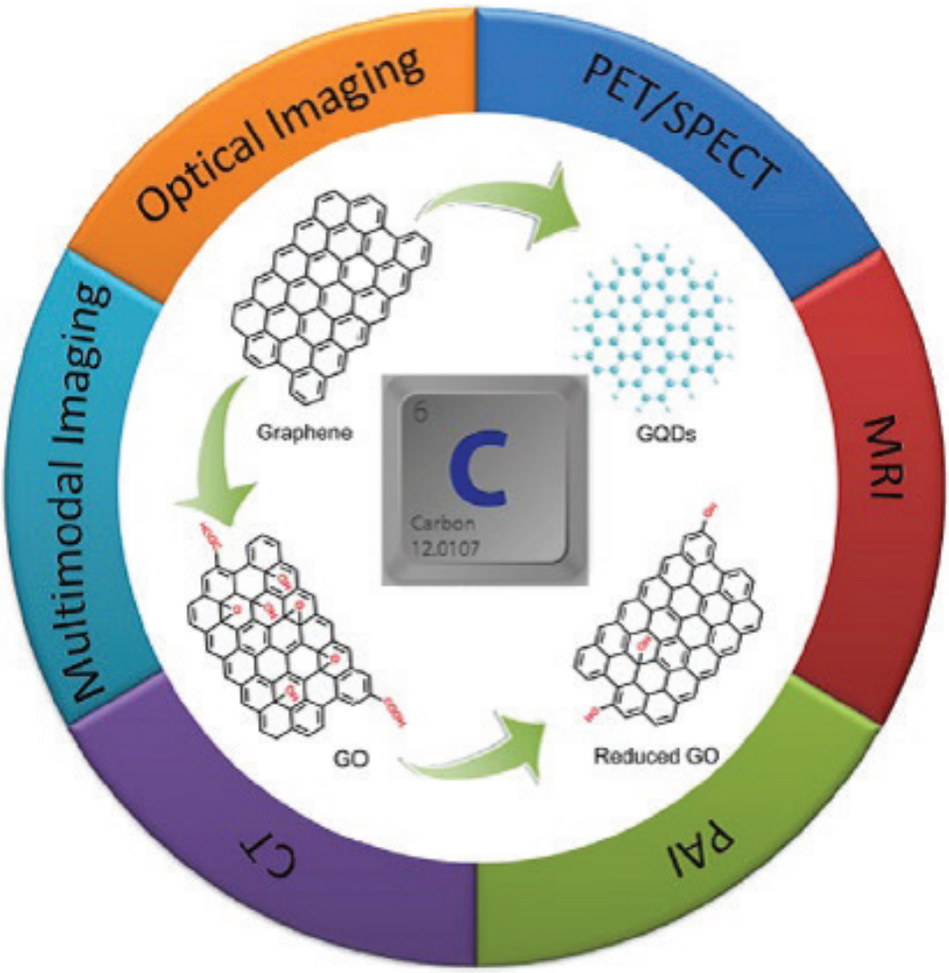

Figure 4. The biomedical applications of graphene. Adapted from Ref. [28].

recently attracted an increasing interest in cancer bioimaging [29, 30]. Chen et al. [31] evaluated the recent advances and future challenges of biomedical applications of graphene quantum dots. They expressed that the wide applications of graphene quantum dots in biological imaging can be attributed to the relatively high quantum yields with high molar extinction coefficients, broad absorption with narrow emission spectra and high photostability, the strong quantum confinement, and edge effects stir of GQDs. In addition, in comparison with a traditional imaging modality, the highly stable and bright fluorescence characters of GQD-derived nanomaterials lead to generate the optical signal with the small number of GQDs [31]. On the other hand, the ability of GQDs for near-infrared reflectance emission makes them as promising candidates for the imaging of deeper tissue samples. Based on the above reasons, applying GQDs as contrast agents for in vivo imaging has been the area of high expectations and recurring attention. The near-infrared emitting window shows great advantages for biomedical imaging because of the low tissue absorption and reduced light scattering in more than 650 nm wavelengths' region [31]. Functionalized graphene oxide (GO; 2D)-based nanocomposites are one of the graphene-based nanomaterials that have a great potential for biomedical applications. In recent years, because of their unique and highly enriched physical and chemical properties, such as excellent biocompatibility, ready cellular uptake, flexible chemical modifications, unique optical properties, and thermal and electrical conductivity, they have been used in biological fields, such as biomedicine, biosensors, drug delivery, and bioimaging [32]. Qiu et al. [33] developed the graphene oxide (GO)-wrapped gold nanorods (GO@GNRs) that can be used as a smart and robust nanoplatform for ultrafast NIR SERS bioimaging. Their finding demonstrated that the fabricated GO@GNRs could efficiently load various NIR probes, and the in vitro evaluation indicated that the nanoplatform could exhibit a higher NIR SERS activity in comparison with traditional gold nanostructures.

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References

[1] Vadivambal R, Jayas Digvir S, editors. Bio-Imaging Principles, Techniques and Applications. Boca Raton: CRC Press, Taylor & Francis Group; 2016

[2] Wang Y, Li Y, Wei F, Duan Y. Optical imaging paves the way for autophagy research. Trends in Biotechnology. 2017;**35**(12):1181-1193

[3] Bronzino JD, Peterson DR, editors. Biomedical Signals, Imaging, and Informatics. Boca Raton: CRC Press, Taylor & Francis Group; 2015

[4] Diaspro A, van Zandvoort MAMJ, editors. Super-resolution Imaging in Biomedicine. Boca Raton: CRC Press, Taylor & Francis Group; 2017

[5] Heintzmann R, Huser T. Super-resolution structured illumination microscopy. Chemical Reviews. 2017;**117**:13890-13908

[6] Peeters Y, Vandenberg W, Duwé S, Bouwens A, Lukeš T, Ruckebusch C, Lasser T, Dedecker P. Correcting for photodestruction in super-resolution optical fluctuation imaging. Scientific Reports. 2017;**7**(1):10470

[7] Hanssen E, editor. Cellular Imaging: Electron Tomography and Related Techniques. AG: Springer International Publishing; 2018

[8] Hauser M, Wojcik M, Kim D, Mahmoudi M, Li W, Xu K. Correlative super-resolution microscopy: New dimensions and new opportunities. Chemical Reviews. 2017;**117**:7428-7456

[9] Demmerle J, Innocent C, North AJ, Ball G, Müller M, Miron E, Matsuda A, Dobbie IM, Markaki Y, Schermelleh L. Strategic and practical guidelines for successful structured illumination microscopy. Nature Protocols. 2017;**12**:988-1010

[10] Ponsetto JL, Bezryadina A, Wei F, Onishi K, Shen H, Huang E, Ferrari L, Ma Q, Zou Y, Liu Z. Experimental demonstration of localized plasmonic structured illumination microscopy. ACS Nano. 2017;**11**:5344-5350

[11] So S, Kim M, Lee D, Nguyen DM, Rho J. Overcoming diffraction limit: From microscopy to nanoscopy. Applied Spectroscopy Reviews. 2017;**4**:1-23

[12] JWM B, MMJ M, editors. Design and Applications of Nanoparticles in Biomedical Imaging. Switzerland: Springer International Publishing; 2017

[13] Padmanabhan P, Kumar A, Kumar S, Kumar Chaudhary R, Gulyás B. Nanoparticles in practice for molecular-imaging applications: An overview. Acta Biomaterialia. 2016;**41**:1-16

[14] Kim K, Oh Y, Lee W, Kim D. Plasmonics-based spatially activated light microscopy for super-resolution imaging of molecular fluorescence. Optics Letters. 2010;**35**:3501-3503

[15] Willets KA, Wilson AJ, Sundaresan V, Joshi PB. Super-resolution imaging and plasmonics. Chemical Reviews. 2017;**117**(11):7538-7582

[16] Mei J, Huang Y, Tian H. Progress and trends in AIE-based bioprobes: A brief overview. ACS Applied Materials & Interfaces. 2018. DOI: 10.1021/acsami.7b14343

[17] Shewring JR, Cankut AJ, McKenzie LK, Crowston BJ, Botchway SW, Weinstein JA, Edwards E, Ward MD. Multimodal probes: Superresolution and transmission electron microscopy imaging of mitochondria, and oxygen mapping of cells, using small-molecule Ir(III) Luminescent complexes. Inorganic Chemistry. 2017;**56**(24):15259-15270

[18] Goel S, England CG, Chen F, Cai W. Positron emission tomography and nanotechnology: A dynamic duo for cancer theranostics. Advanced Drug Delivery Reviews. 2017;**113**:157-176

[19] Zebibula A, Alifu N, Xia L, Sun C, Yu X, Xue D, Liu L, Li G, Qian J. Ultrastable and biocompatible NIR-II quantum dots for functional bioimaging. Advanced Functional Materials. 2018;**1**:1703451

[20] Liu Y, Lu Y, Yang X, Zheng X, Wen S, Wang F, Vidal X, Zhao J, Liu D, Zhou Z, Ma C, Zhou J, Piper JA, Xi P, Jin D. Amplified stimulated emission in upconversion nanoparticles for super-resolution nanoscopy. Nature. 2017;**543**:229-233

[21] Reineck P, Gibson BC. Near-infrared fluorescent nanomaterials for bioimaging and sensing. Advanced Optical Materials. 2017;**5**:1600446

[22] Generalova AN, Chichkov BN, Khaydukov EV. Multicomponent nanocrystals with anti-Stokes luminescence as contrast agents for modern imaging techniques. Advances in Colloid and Interface Science. 2017;**245**:1-19

[23] Duan C, Liang L, Li L, Zhang R, Xu ZP. Recent progress in upconversion luminescence nanomaterials for biomedical applications. Journal of Materials Chemistry B. 2018;**6**:192-209

[24] Chandan HR, Schiffman JD, Balakrishna RG. Quantum dots as fluorescent probes: Synthesis, surface chemistry, energy transfer mechanisms, and applications. Sensors and Actuators B. 2018;**258**:1191-1214

[25] Martynenko IV, Litvin AP, Purcell-Milton F, Baranov AV, Fedorov AV, Gunko YK. Application of semiconductor quantum dots in bioimaging and biosensing. Journal of Materials Chemistry B. 2017;**5**:6701-6727

[26] Chen X, Li R, Liu Z, Sun K, Sun Z, Chen D, Xu G, Xi P, Wu C, Sun Y. Small photoblinking semiconductor polymer dots for fluorescence nanoscopy. Advanced Materials. 2017;**29**:1604850

[27] Chen X, Liu Z, Li R, Shan C, Zeng Z, Xue B, Yuan W, Mo C, Xi P, Wu C, Sun Y. Multicolor super-resolution fluorescence microscopy with blue and carmine small photoblinking polymer dots. ACS Nano. 2017;**11**:8084-8091

[28] Lin J, Chen X, Huang P. Graphene-based nanomaterials for bioimaging. Advanced Drug Delivery Reviews. 2016;**105**:242-254

[29] Li K, Zhao X, Wei G, Su Z. Recent advances in the cancer bioimaging with graphene quantum dots. Current Medicinal Chemistry. 2017;**24**:1-18

[30] Luo Z, Yang D, Yang C, Wu X, Hu Y, Zhang Y, Yuwen L, Yeow EKL, Weng L, Huang W, Wang L. Graphene quantum dots modified with adenine for efficient two-photon bioimaging and white light-activated antibacterial. Applied Surface Science. 2018;**434**:155-116

[31] Chen F, Gao W, Qiu X, Zhang H, Liu L, Liao P, Fu W, Luo Y. Graphene quantum dots in biomedical applications: Recent advances and future challenges. Frontiers in Laboratory Medicine. 2018. DOI: 0.1016/j.flm.2017.12.006

[32] Jang SC, Kang SM, Lee JY, Oh SY, Vilian AE, Lee I, Han YK, Park JH, Cho WS, Roh C, Huh YS. Nano-graphene oxide composite for in vivo imaging. International Journal of Nanomedicine. 2018;**13**:221-234

[33] Qiu X, You X, Chen X, Chen H, Dhinakar A, Liu S, Guo Z, Wu J, Liu Z. Development of graphene oxide-wrapped gold nanorods as robust nanoplatform for ultrafast near-infrared SERS bioimaging. International Journal of Nanomedicine. 2017;**12**:4349-4360

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