

Optical Properties of Fluorescent Tags Based on Gold Nanoparticles at Physiological Salt Content: *In Vitro* and *In Vivo* Study

Andrei Demenshin¹, Maria Istomina^{2,3}, and Elena Solovyeva^{1*}

¹ Saint-Petersburg State University, 7/9 Universitetskaya nab., Saint Petersburg 199034, Russian Federation

² Institute of Experimental Medicine, Almazov National Medical Research Centre, 2 Akkuratova str., Saint-Petersburg 197341, Russian Federation

³ Saint-Petersburg Electrotechnical University, 5 Professora Popova str., Saint-Petersburg 197376, Russian Federation

*e-mail: e.solovieva@spbu.ru

Abstract. The study describes the optical properties of fluorescent tags based on gold nanoparticles coated with a polymer shell with incorporated cyanine 5.5 in water and medium with a physiological salt background. The radiant efficiency was assessed by fluorescence tomography for the tag suspensions placed in a black plate and after their local subcutaneous injection into ICR mice. Partial aggregation of tags is noted, which occurs at changing water on isotonic solution, which leads to the increase of dye fluorescent intensity. *In vivo* imaging was performed in a bimodal fluorescence and computed tomography regime with varying the dose of the tags. The fluorescence enhancement 30 min after the tags injection was revealed. Computed tomography images allow reliable identification of the area of gold tags localization when their content in the injected suspension is at least 23.7 mg/mL. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: bioimaging; fluorescence tomography; computed tomography; gold nanoparticles; aggregation; cyanines.

Paper #9161 received 31 Aug 2024; revised manuscript received 22 Oct 2024; accepted for publication 6 Nov 2024; published online 3 Dec 2024. [doi: 10.18287/JBPE24.10.040312](https://doi.org/10.18287/JBPE24.10.040312).

1 Introduction

The implementation of nanomaterials in biomedicine is currently occurring at an increasing rate. Nanoparticles (NPs) of various compositions are considered to be ones of the most promising objects for a wide range of biomedical applications. In particular, many potentially biocompatible structures based on nanodiamonds [1], iron oxide [2, 3], silicon [4], silicon oxide [5, 6] and upconversion [7] nanoparticles have already been proposed for targeted drug delivery [8], instrumental diagnostics [9] and therapy [10, 11]. Some of the developments have already reached clinical trials [12–14]. Plasmonic metal NPs, primarily gold, are among the most promising candidates for solving some problems of photomedicine. On their basis, agents for contrast computed tomography (CT) [15] and photothermal therapy [16, 17] have been actively developed. In combination with molecular dyes, gold NPs can be used as fluorescent tags [18–20] and surface-

enhanced Raman scattering tags [21–23]. Many relevant systems have been proposed to date, in which gold NPs are combined with cyanines [24, 25], BODIPYs [26, 27] and other dyes. The advantage of hybrid structures over molecular dyes is that they allow not only imaging of target cells, but also their treatment, i.e. theranostics.

At design of theranostic agents based on noble metal NPs, it is necessary to take into account all the features of their physical and chemical properties. In particular, chloride-induced aggregation is characteristic for almost all metal nanoparticles, which consists of the formation of NPs aggregates under a high salt background [28, 29]. Often, even stabilization of NPs by coating with various shells does not allow avoiding this effect completely. For practical purposes, nanostructures are of interest that retain colloidal stability in an aggregated state and are thus subject to injection. The aggregation of NPs affects their optical properties. As a rule, the absorption of light by gold NPs aggregates occurs at longer wavelengths compared to individual particles [30]. This can be

considered as a positive side for bioimaging, since it allows one to perform the measurements in the first transparency window of biological tissues.

It has already been noted that when developing photoactive agents based on molecules and NPs prone to aggregation, it is necessary to study their optical properties change that occurs during the aggregation [31, 32]. It is worth considering that aggregation can lead not only to an absorption wavelength shift, but also to a significant change in the intensity of the optical response. Quenching of the optical response may occur, for example, in the case of H-aggregates formation for polyaromatic dyes [33], or amplification of the optical response also may be observed, for example, the effect of aggregation-induced emission enhancement for molecular dyes [34] or the hot spots effect for plasmonic nanoparticles [35]. It is rationally to carry out a spectral evaluation of fluorophores and hybrid structures with them in conditions close to potential *in vivo* use, i.e. at physiological salt background and in the concentration range supposed for administration. This study is addressed to the evaluation of optical properties of tags based on gold NPs and cyanine 5.5 in saline solution used for injections and to testing of the tags as contrast for *in vivo* bimodal imaging by fluorescence tomography and computer tomography.

2 Materials and Methods

Gold nanoparticles of rod shape with an average aspect ratio of 2.76 were obtained by the two-stage seed-mediated method [36]. Further modification of the NPs included the coating with a polymer shell using a layer-by-layer technique accompanied with a dye incorporation. To coat the gold NPs, sodium polystyrene sulfonate (PSS) and polydiallyldimethylammonium (PDDA) chloride were used. These polymers are widely used in the fabrication of microcapsules for targeted drug delivery [37] that is the reason for their choice. Cyanine 5.5 was used as a fluorophore, the emission range of which falls within the first transparency window of biological tissues. The NPs coating with polymers was carried out sequentially in 4 stages, positively charged PDDA was used as layers 1 and 3, negatively charged PSS was used as layers 2 and 4. At each stage, NPs suspension was added dropwise to the polyelectrolyte solution under vigorous stirring, the mixture was kept for 2 h, and then the excess polymer was removed by centrifugation at 8000 rpm for 20 min. The previous study of nanorods coated via applied technique by transmission electron microscopy showed the polymer shell thickness of 2.5–3 nm [38]. The overall size of the obtained core-shell structures is about 50 nm in length and 18 nm in diameter. Cyanine 5.5 was introduced into the polymer shell by dropwise addition its solution with a concentration of 1×10^{-3} M into the NPs suspension in a ratio of 1:9 before applying the final fourth layer of polymer. Immobilization of the dye on NPs surface occurred due to non-specific adsorption proceeding during 2 h. The excess of not attached dye was removed

by centrifugation at 8000 rpm for 20 min. The absorption spectra recorded for the stock solution of cyanine 5.5 and supernatant after centrifugation showed that about 40% of the added dye is retained in the polymer shell, which corresponds to $\sim 10^5$ molecules per particle since the NPs concentration in the suspension is about 3.8×10^{11} particles/mL.

UV-Vis absorption spectra were recorded on a spectrophotometer UV-1800 (Shimadzu). Spectra were acquired in the range of 200–900 nm with a step of 1 nm. Quartz cuvettes with an optical path length of 1 cm were used. The baseline was recorded with deionized water under identical conditions.

Iv vivo imaging was performed with 25–30-day-old male ICR (CD-1) mice weighing 18–20 g obtained from the vivarium of Almazov National Medical Research Centre. Imaging was performed with test and control animals. The background radiation was taken into account based on the signal from the control mouse. Fluorescence emission was measured using an IVIS spectrum CT tomograph (PerkinElmer), which also allows working in computed tomography regime. Taking into account the optical properties of the fluorophore used, imaging was performed with the filters of 675 nm for excitation and 720 nm for emission. CT images were obtained at 50 kV and 1 mA, exposure time 20 ms, 4-4 binning and increment angle of 0.5. At imaging, mice were anesthetized and injected subcutaneously with a saline suspension of NPs in a volume of 30 μ L. In a dose-dependent experiment, the fluorescent tags were studied at gold concentrations of 15–45 mg/mL. The control mouse was injected subcutaneously with 30 μ L of physiological solution (0.9% NaCl). The fluorescence efficiency was recorded 5 and 30 min after the tags administration, and computed tomography was performed 30 min after the administration. During the experiment, the animals were kept on a thermostatic operating table, which maintained body temperature at a level of 37.0 ± 0.5 °C (TCAT-2LV, Physitemp Instruments Inc.).

The laboratory animals were kept on a standard diet with sufficient water in accordance with veterinary legislation and in accordance with the requirements for the humane maintenance and use of animals in experimental studies (National research Council, 2011).

3 Results and Discussion

Optical properties of nanoobjects depend on their size and morphology. Gold nanoparticles are a clear example: with a change in size and shape, not only their light scattering changes, but also the absorption wavelengths and extinction coefficient. The size of gold NPs increases with their partial or complete aggregation, which can occur when the salt content in the surrounding solution changes. Fig. 1 shows the UV-Vis absorption spectra of the obtained fluorescent tags based on gold NPs in water and isotonic solution. The spectrum 2 was measured 5 min after salt addition. This time was chosen based on the time that usually passes between the dilution of nanoparticles suspension

with saline and their injection in the experiments with laboratory mice. As can be seen, the main absorption band shifts from 710 to 750 nm. This shift indicates a partial aggregation of NPs, which progresses over time, but it is not dramatic during yet in the selected time period. Nevertheless, the change in the optical properties of NPs that occurs during the environment exchange from water to salt should be taken into account when choosing the conditions for fluorescence tomography performed using them.

The preparation of core-shell nanostructures based on gold NPs modified with fluorescent dyes can only be carried out in water, since with a high salt background the stability of the NPs is significantly reduced, and they do not withstand multiple centrifugation-resuspension cycles. The transfer of NPs into a medium with physiological salt background, suitable for injection, is carried out immediately before their use by diluting the concentrated suspension with a saline solution. The degree of aggregation and the shift of the absorption band maximum, which affects the optical efficiency of the hybrid nanostructure, depend on the concentration of the gold NPs themselves in the suspension.

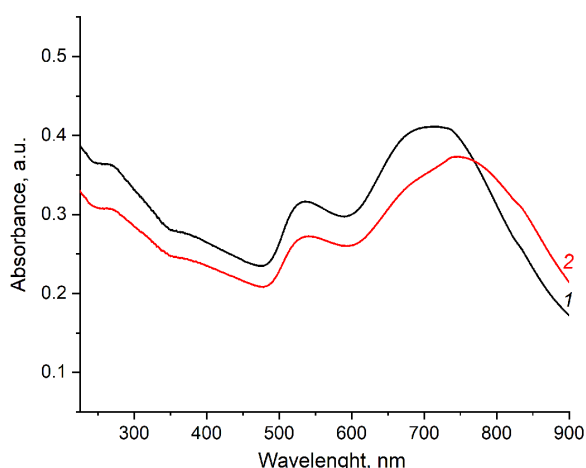


Fig. 1 UV-Vis absorption spectra of modified gold nanoparticles (1) in water and (2) isotonic solution ($C_{Au} = 35 \text{ mg/L}$).

Gold NP-based contrast agents are used in *in vivo* experiments for injection in sufficiently high concentrations, which allow obtaining a detectable signal after natural dilution in physiological fluids. According to the literature, the concentration of suspensions based on gold NPs introduced into a laboratory animal can reach up to 33 g/L on gold [39]. The study of such concentrated and, accordingly, optically dense suspensions of gold NPs by traditional methods of absorption and fluorescence spectroscopy is extremely difficult due to the dominance of self-absorption and scattering processes. In turn, the optical scheme of signal registration in fluorescence tomography differs greatly from that implemented in conventional measurements in the cuvette holder of a spectrometer, carried out with solutions, films and solid samples. Therefore, it is advisable to evaluate the change in the optical properties of concentrated NPs suspensions when replacing water with a salt medium directly on a fluorescence tomograph. Fig. 2 shows the images of the fluorescent tags suspensions obtained in a black plate on a fluorescence tomograph before and after a two-fold dilution with a 1.8% sodium chloride solution. Since the luminosity of gold core-shell nanostructures can also vary depending on their concentration, a similar volume of distilled water was added to the well on the right as a control sample.

As can be seen, there is an increase in the signal from the fluorescent contrast by almost 40% in the isotonic solution. Comparison of radiant efficiency values from the well, to which the saline solution was added with the control well, to which water was added, shows that this observation is due to the influence of chloride background. Apparently, the plasmon resonance band of the tags shifts to the long-wavelength region as a result of aggregation and the emission of cyanine 5.5 becomes more pronounced due to the less spectral overlap with the absorption region of the gold core. It is worth noting that after aggregation, the particles do not precipitate and are suitable for injection, which was carried out on laboratory mice. Fig. 3 represents the images of mice obtained 5 and 30 min after subcutaneous injection of a suspension of gold-cyanine tags into the thigh.

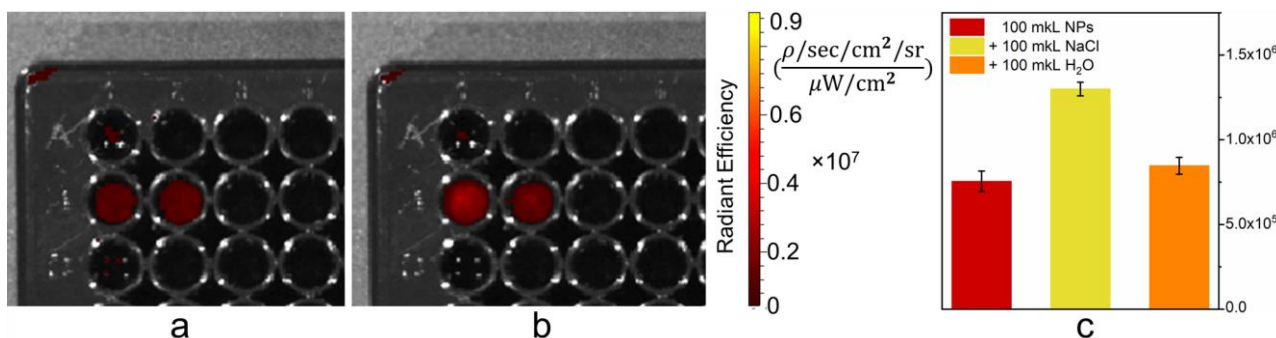


Fig. 2 Fluorescent images of the tags suspension (a) before and (b) after dilution with saline and water. (c) Radiant efficiency recorded from the test wells before and after addition of solvent.

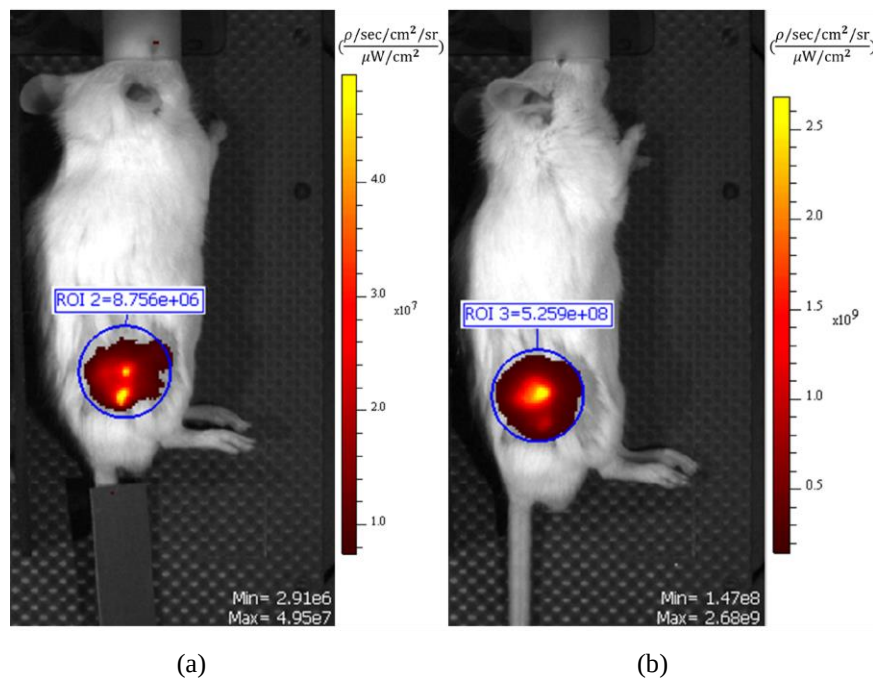


Fig. 3 Fluorescence tomography images after (a) 5 and (b) 30 min of tags subcutaneous administration.

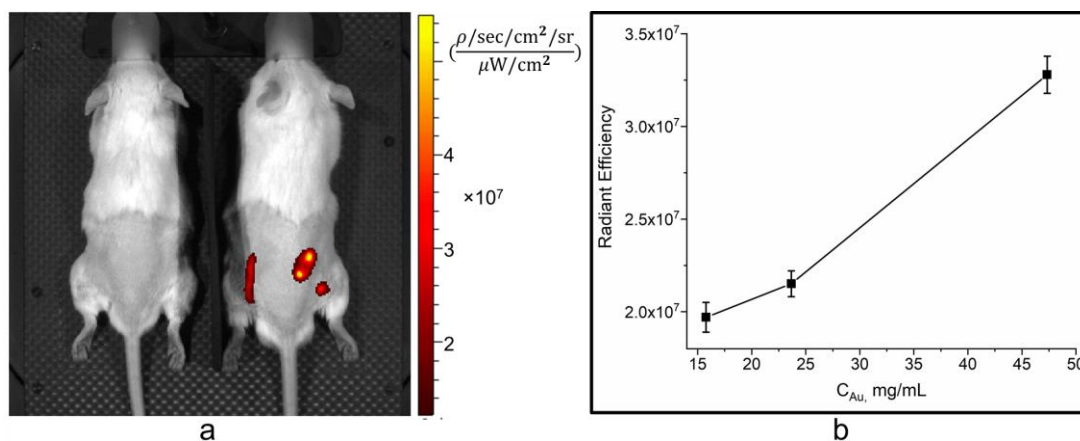


Fig. 4 (a) Fluorescence tomography images of control and injected mice after administration of tags in doses of 15.8, 23.7, and 47.3 mg/mL. (b) Dependence of the recorded radiant efficiency on the dose of gold tags administered. Error bars in graph indicate standard deviation of radiant.

Analysis of the radiant efficiency reveals that 30 min after tags injection, the signal level increases by almost two orders of magnitude. This flare-up of the introduced fluorescent tags can be associated with their interaction with the components of the subcutaneous fat tissue. The observed result is certainly extremely interesting, but requires further research.

When developing diagnostic contrasts, it is necessary to conduct dose-dependent experiments. Since hybrid nanostructures based on gold NPs and molecular fluorophores are capable of providing imaging in a bimodal mode, it is advisable to perform such experiments, scanning in both, the computer tomography and fluorescence tomography, regimes. The possibility of working with contrasts based on gold NPs in a bimodal mode has already been demonstrated previously and is one of their main advantages [40]. Fluorescence and CT

images obtained with varying doses of injected contrast from 15 to 47 mg/mL on gold are shown in Figs. 4 and 5, respectively.

As can be seen, the signal intensity in both regimes increases as expected proportionally to the dose of tags administered. At the same time, a dose of 23.7 mg/mL on gold is sufficient to obtain a contrast image.

4 Conclusions

The study highlights the physicochemical properties of hybrid tags based on gold nanoparticles with a polymer shell labeled with a fluorescent dye, which are important for their potential use for bioimaging purposes. In particular, partial aggregation of the tags is shown, which occurs when a medium in their suspension is exchanged from water to saline with a high salt content.

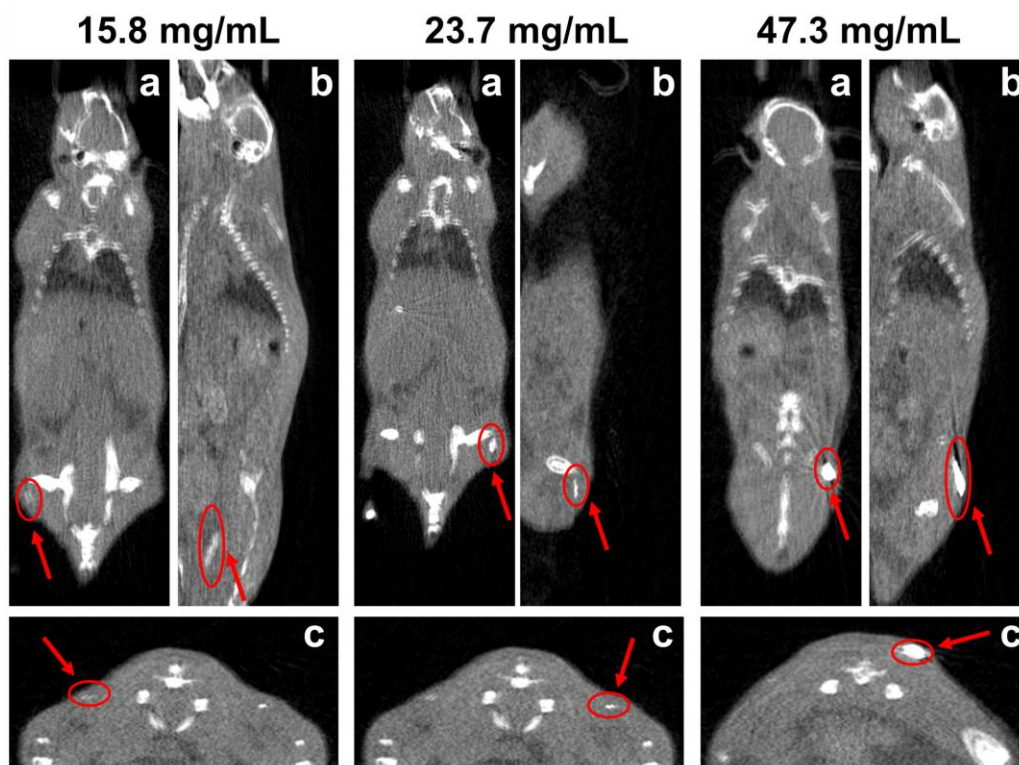


Fig. 5 *In vivo* CT images of ICR mouse after subcutaneous local injection of gold tags in three doses: (a) sagittal plane, (b) coronal plane, (c) transaxial plane. In the highlighted areas, the arrow indicates the accumulation of gold tags.

The aggregates formation causes a shift in the absorption maximum to the long-wavelength region, which is accompanied by an increase in the recorded emission intensity of cyanine 5.5.

In vivo experiments carried out on ICR mice using the gold-based tags clearly demonstrated the possibility of bimodal imaging in combined fluorescence tomography and computed tomography regime. Tags fluorescence increasing was revealed 30 min after their subcutaneous injection. The observed increase in radiative efficiency by almost two orders of magnitude is an extremely interesting effect that requires further research. CT images allow identification of the area of tags localization, the minimum tags content in the injected suspension required for acceptable contrast is of 23.7 mg/mL.

Acknowledgements

This work was funded by the Russian Science Foundation, grant № 22-73-10052. The authors acknowledge Saint-Petersburg State University for infrastructural support (Laboratory of Plasmon-Enhanced Spectroscopy and Bioimaging). The authors are grateful to the Resource Centers of SPBU: “Optical and Laser Materials Research”, “Chemical Analysis and Material Research” and “Center for Molecular and Cell Technologies” for access to equipment and for performing some measurements.

Disclosures

The authors declare no conflicts of interest.

References

1. Mumtaz, N. Hussain, S. Salam, and M. Bilal, “[Multifunctional nanodiamonds as emerging platforms for cancer treatment, and targeted delivery of genetic factors and protein medications—a review](#),” *Journal of Materials Science* 57(17), 8064–8099 (2022).
2. J. Dulińska-Litewka, A. Łazarczyk, P. Hałubiec, O. Szafranski, K. Karnas, and A. Karewicz, “[Superparamagnetic iron oxide nanoparticles-current and prospective medical applications](#),” *Materials* 12(4), 617 (2019).
3. R. Mahbuba, “[Magnetic Resonance Imaging and Iron-oxide Nanoparticles in the era of Personalized Medicine](#),” *Nanotheranostics* 7(4), 424–449 (2023).
4. A. F. Alykova, V. G. Yakunin, V. Yu. Timoshenko, and I. N. Zavestovskaya, “[Optical methods of silicon nanoparticle diagnostics for applications in biomedicine](#),” *Journal of Biomedical Photonics & Engineering* 5(2), 020304 (2019).
5. M. C. Goncalves, “[Sol-gel silica nanoparticles in medicine: A natural choice. Design, Synthesis and Products](#),” *Molecules* 23(8), 2021 (2018).

6. D. V. Korolev, G. A. Shulmeyster, N. V. Evreinova, M. S. Syrovatkina, M. S. Istomina, V. N. Postnov, I. V. Aleksandrov, A. S. Krasichkov, and M. M. Galagudza, “[Theranostic Platforms Based on Silica and Magnetic Nanoparticles Containing Quinacrine, Chitosan, Fluorophores, and Quantum Dots](#),” *International Journal of Molecular Sciences* 23(2), 932 (2022).
7. I. Yu. Yanina, N. A. Navolokin, I. V. Vidyasheva, I. Y. Goryacheva, V. I. Kochubey, and V. V. Tuchin, “[Functionalized upconversion luminescent nanoparticles for theranostics](#),” in *Diffuse Optical Spectroscopy and Imaging VII*, H. Dehghani and H. Wabnitz (Eds.), 11074, 11074_43 (2019).
8. Y. Toropova, D. Korolev, M. Istomina, G. Shulmeyster, A. Petukhov, V. Mishanin, A. Gorshkov, E. Podyacheva, K. Gareev, A. Bagrov, and O. Demidov, “[Controlling the Movement of Magnetic Iron Oxide Nanoparticles Intended for Targeted Delivery of Cytostatics](#),” *International Journal of Nanomedicine* 16, 5651–5664 (2021).
9. N. Mhlana, N. Mphuthi, H. Van der Walt, S. Nyembe, T. Mokhena, and L. Sikhwihlu, “[Nanostructures and nanoparticles as medical diagnostic imaging contrast agents: A review](#),” *Materials Today Chemistry* 40, 102233 (2024).
10. M. Bravo, B. Fortuni, P. Mulvaney, J. Hofkens, H. Uji-I, S. Rocha, and J. A. Hutchison, “[Nanoparticle-mediated thermal Cancer therapies: Strategies to improve clinical translatability](#),” *Journal of Controlled Release* 372, 751–777 (2024).
11. A. B. Bucharskaya, N. G. Khlebtsov, B. N. Khlebtsov, G. N. Maslyakova, N. A. Navolokin, V. D. Genin, E. A. Genina, V. V. Tuchin, “[Photothermal and Photodynamic Therapy of Tumors with Plasmonic Nanoparticles: Challenges and Prospects](#),” *Materials* 15(4), 1606 (2022).
12. L. Tang, R. Zhang, Y. Wang, M. Liu, D. Hu, Y. Wang, and L. Yang, “[Nanoparticle delivery for central nervous system diseases and its clinical application](#),” *Nano Research* 17(7), 6305–6322 (2024).
13. Y. Liu, W. Cheng, H. Xin, R. Liu, Q. Wang, W. Cai, X. Peng, F. Yang, and H. Xin, “[Nanoparticles advanced from preclinical studies to clinical trials for lung cancer therapy](#),” *Cancer Nanotechnology* 14(1), 28 (2023).
14. L. Yao, D. Bojic, and M. Liu, “[Applications and safety of gold nanoparticles as therapeutic devices in clinical trials](#),” *Journal of Pharmaceutical Analysis* 13(9), 960–967 (2023).
15. N. Chanda, A. Upendran, E. J. Boote, A. Zambre, S. Axiak, K. Selting, K. V. Katti, W. M. Leevy, Z. Afrasiabi, J. Vimal, J. Singh, J. C. Lattimer, and R. Kannan, “[Gold nanoparticle based X-ray contrast agent for tumor imaging in mice and dog: A potential nano-platform for computer tomography theranostics](#),” *Journal of Biomedical Nanotechnology* 10(3), 383–392 (2014).
16. A. Bucharskaya, G. Maslyakova, G. Terentyuk, A. Yakunin, Y. Avetisyan, O. Bibikova, E. Tuchina, B. Khlebtsov, N. Khlebtsov, and V. Tuchin, “[Towards effective photothermal/photodynamic treatment using plasmonic gold nanoparticles](#),” *International Journal of Molecular Sciences* 17(8), 1295 (2016).
17. W. Zhang, X. Ding, H. Cheng, C. Yin, J. Yan, Z. Mou, W. Wang, D. Cui, C. Fan, and D. Sun, “[Dual-Targeted Gold Nanoprism for Recognition of Early Apoptosis, Dual Model Imaging and Precise Cancer Photothermal Therapy](#),” *Theranostics* 9(19), 5610–5625 (2019).
18. D. K. Tuchina, I. G. Meerovich, O. A. Sindeeva, V. V. Zherdeva, N. I. Kazachkina, I. D. Solov’ev, A. P. Savitsky, A. A. Bogdanov, and V. V. Tuchin, “[Prospects for multimodal visualisation of biological tissues using fluorescence imaging](#),” *Quantum Electronics* 51(2), 104–117 (2021).
19. O. Mostafa, H. M. Saleh, T. A. Salaheldin, A. K. Ibrahim, and S. A. Elfeky, “[Fluorescein/gold nanoparticles conjugated EGFR antibody for imaging and P53 upregulation in hamster mucosal cells carcinoma](#),” *Journal of Drug Delivery Science and Technology* 76, 103293 (2022).
20. S. K. Pirutin, M. V. Efremova, A. I. Yusipovich, V. B. Turovetskii, G. V. Maksimov, A. B. Druzhko, and A. G. Mazhuga, “[Visualization and Cytotoxicity of Fluorescence-Labeled Dimeric Magnetite-Gold Nanoparticles Conjugated with Prostate-Specific Membrane Antigen in Mouse Macrophages](#),” *Bulletin of Experimental Biology and Medicine* 166(3), 386–389 (2019).
21. V. O. Svinko, A. I. Shevchuk, A. N. Smirnov, D. V. Makeeva, and E. V. Solovyeva, “[Gold nanostars-based labels for surface-enhanced Raman scattering imaging with red medical lasers](#),” *Opt. Spectrosc.* 130(10), 1590–1595 (2022).
22. A. I. Shevchuk, V. O. Svinko, A. N. Smirnov, and E. V. Solovyeva, “[SERS study of cyanine dyes: optimization of metal core and molecular label choice for plasmonic nanotags](#),” *Dyes and Pigments* 216, 111329 (2023).
23. B. Andreiuk, F. Nicolson, L. M. Clark, S. R. Panikkanvalappil, Kenry, M. Rashidian, S. Harmsen, and M. F. Kircher, “[Design and synthesis of gold nanostars-based SERS nanotags for bioimaging applications](#),” *Nanotheranostics* 6(1), 10–30 (2022).
24. A. N. Smirnov, S. F. Aslanov, D. V. Danilov, O. Y. Kurapova, and E. V. Solovyeva, “[One-Pot Synthesis of Silica-Coated Gold Nanostructures Loaded with Cyanine 5.5 for Cell Imaging by SERS Spectroscopy](#),” *Nanomaterials* 13(7), 1267 (2023).
25. A. N. Smirnov, A. I. Shevchuk, A. V. Volkova, V. D. Kalganov, and E. V. Solovyeva, “[Gold-silica plasmonic nanobones with tunable size and optical bimodality for bioimaging](#),” *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 684, 133115 (2024).

26. A. Rahman, K. Praveen, P. Panangattukara, P. Yadav, T. Goswami, A. Shanavas, H. N. Ghosh, and P. P. Neelakandan, “[Gold-BODIPY Nanoparticles with Luminescence and Photosensitization Properties for Photodynamic Therapy and Cell Imaging](#),” *ACS Applied Nano Materials* 5(5), 6532–6542 (2022).
27. H. Y. Lee, H. Son, J. M. Lim, J. Oh, D. Kang, W. S. Han, and J. H. Jung, “[BODIPY-functionalized gold nanoparticles as a selective fluoro-chromogenic chemosensor for imaging Cu²⁺ in living cells](#),” *Analyst* 135(8), 2022–2027 (2010).
28. S. Alizadeh, Z. Nazari, “[A Review on Gold Nanoparticles Aggregation and Its Applications](#),” *Journal of Chemical Reviews* 2(4), 228–242 (2020).
29. M. Chan, W. Leng, and P. Vikesland, “[Surface-Enhanced Raman Spectroscopy Characterization of Salt-Induced Aggregation of Gold Nanoparticles](#),” *ChemPhysChem* 19(1), 24–28 (2018).
30. L. Catanzaro, V. Scardaci, M. Scuderi, M. Condorelli, L. D’Urso, and G. Compagnini, “[Surface plasmon resonance of gold nanoparticle aggregates induced by halide ions](#),” *Materials Chemistry and Physics* 308, 128245 (2023).
31. D. S. Farrakhova, I. D. Romanishkin, D. V. Yakovlev, Yu. S. Maklygina, V. A. Oleinikov, P. V. Fedotov, M. V. Kravchik, L. Bezdetnaya, and V. B. Loschenov, “[Correlation of spectroscopic and structural properties of indocyanine green J-aggregates](#),” *Biomedical Photonics* 11(3), 4–16 (2022).
32. I. V. Klimenko, T. Yu. Astakhova, E. N. Timokhina, and A. V. Lobanov, “[Molecular Aggregation of Aluminum Phthalocyanine Chloride in Organic and Water-Organic Media](#),” *Journal of Biomedical Photonics & Engineering* 9(3), 030301 (2023).
33. J. Kang, O. Kaczmarek, J. Liebscher, and L. Dahne, “[Prevention of H-Aggregates Formation in Cy5 Labeled Macromolecules](#),” *International Journal of Polymer Science* 2010(1), 264781 (2010).
34. H. He, T. Li, L. Yao, M. Liu, H. Xia, and L. Shen, “[Aggregation-induced emission enhancement \(AIEE\)-active donor-acceptor type fluorophores based on the 1,3-diaryl pyrazole unit: Conspicuous mechanochromic fluorescence and biosensing of iodide ions](#),” *Dyes and Pigments* 203, 110309 (2022).
35. T. Haug, P. Klemm, S. Bange, and J. M. Lupton, “[Hot-Electron Intraband Luminescence from Single Hot Spots in Noble-Metal Nanoparticle Films](#),” *Physical Review Letters* 115(6), 067403 (2015).
36. A. Yoshida, N. Uchida, and N. Kometani, “[Synthesis and spectroscopic studies of composite gold nanorods with a double-shell structure composed of spacer and cyanine dye J-aggregate layers](#),” *Langmuir* 25(19), 11802–11807 (2009).
37. N. Pargaonkar, Y. M. Lvov, N. Li, J. H. Steenekamp, and M. M. De Villiers, “[Controlled release of dexamethasone from microcapsules produced by polyelectrolyte layer-by-layer nanoassembly](#),” *Pharmaceutical Research* 22(5), 826–835 (2005).
38. V. O. Svinko, A. N. Smirnov, A. I. Shevchuk, A. I. Demenshin, A. A. Smirnov, and E. V. Solovyeva, “[Comparative study of fluorescence core-shell nanotags with different morphology of gold core](#),” *Colloids and Surfaces B: Biointerfaces* 226, 113306 (2023).
39. G. Sonavane, K. Tomoda, and K. Makino, “[Biodistribution of colloidal gold nanoparticles after intravenous administration: Effect of particle size](#),” *Colloids and Surfaces B: Biointerfaces* 66(2), 274–280 (2008).
40. S. Kozomara, N. L. Ford, “[Detectability of fluorescent gold nanoparticles under micro-CT and optical projection tomography imaging](#),” *Journal of Medical Imaging* 7(2), 026002 (2020).