Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy

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Recent years have seen tremendous progress in the design and study of nanomaterials geared towards biological and biomedical applications, most notable among these being the noble metal nanoparticles. In this review, we outline the surface-plasmon resonance-enhanced optical properties of colloidal gold nanoparticles directed towards recent biomedical applications with an emphasis on cancer diagnostics and therapeutics. Methods of molecular-specific diagnostics/detection of cancer, including strongly enhanced surface plasmon resonance light-scattering, surface-enhanced emission of gold nanorods and surface-enhanced Raman scattering, are described. We also discuss the plasmonic photothermal therapy of cancer achieved by using the strongly enhanced surface-plasmon resonance absorption of gold nanoshperes and nanorods.

With the tremendous developments in nanotechnology over recent decades, a variety of nanoscale structures have emerged that possess novel properties suitable for a range of biological and biomedical applications. Major classes of biologically relevant nanostructures include semiconductor quantum dots, magnetic nanoparticles, polymeric particles, carbon-based nanostructures and metallic nanoparticles. Quantum dots are useful in biological labeling and detection due to their size-dependent fluorescence properties [1-13]. Magnetic nanoparticles have been used for cell sorting [14-17], MRI [18-24], drug delivery [25-28] and magnetic hyperthermia therapy [29-34]. Lipid and polymeric nanoparticles have been used to encapsulate therapeutic molecules to increase drug solubility, safety and delivery efficiency based on the enhanced permeability and retention (EPR) effect of the tumor tissue [34-42]. Carbon-based nanoparticles, especially carbon nanotubes, have found increasing interest from the point-of-view of biomedical applications, such as photothermal therapy [43] and drug delivery [44-46].

Compared with other nanostructures, metallic nanoparticles have proven to be the most flexible nanostructures owing to the synthetic control of their size, shape, composition, structure, assembly and encapsulation, as well as the resulting tunability of their optical properties. The synthesis and optical properties of various metallic nanoparticles can be found in recent reviews [47-58]. Compared with other metallic nanostructures that are useful in biomedical applications [59-64], colloidal gold nanoparticles are especially promising because of their simple and fast preparation and bioconjugation. Gold nanoparticles can be prepared easily by the reduction of auric acid with sodium citrate [65]. The size of the nanoparticles can be varied by changing the sodium citrate concentration [66]. Citrate-capped nanoparticles are very stable. In addition, the citrate-capping can be replaced easily and the gold surface can be functionalized with various ligands, such as DNA, peptides and antibodies, by means of covalent and noncovalent interactions [67-71]. Gold nanorods can be synthesized by the well developed electrochemical method through gold ionization and reduction [72] or the seed-mediated growth method involving the growth of spherical gold seed particles in the presence of Au+ ions and the rod-like cetyl trimethyl ammonium bromide (CTAB) surfactant [73,74]. The aspect ratio (length/width) of the rods can be tuned readily by changing the concentration of the silver ions. The nanorod surface also enables multifunctionalization. In addition to good synthetic control, gold is potentially biosafe. Recent in vitro studies show that gold nanoparticles do not cause cytotoxicity in human cells [75]. The recent promise of colloidal gold nanoparticles for modern medicinal applications, especially cancer diagnostics and photothermal therapy, has originated mainly from their strongly enhanced optical properties, on which we focus in this review.

In this review, we introduce the most recent biomedical applications of colloidal gold nanoparticles and nanorods that result from their unique optical properties, especially in the area of cancer photodiagnostics and phototherapy. We discuss important recent advances in
molecular-specific cancer detection and therapy using gold nanoparticles, including techniques of light-scattering imaging, two-photon luminescence imaging, surface-enhanced Raman scattering and plasmonic photothermal therapy.

Enhanced photophysical properties of gold nanoparticles

When matter is exposed to light, a number of processes can occur:

• The light can be absorbed
• The light can be scattered at the same frequency as the incoming light (Mie or Rayleigh scattering)
• The absorbed light can be re-emitted (i.e., fluorescence)
• The local electromagnetic field of the incoming light can be enhanced, thus enhancing any spectroscopic signals from the molecules at the material surface, that is, surface-enhanced spectroscopy, such as surface-enhanced Raman scattering.

In the case of gold nanoparticles, all these processes are enhanced strongly owing to the unique interaction of light with the free electrons in the metal particles. When gold nanoparticles are exposed to light radiation, the electric field of the light causes the collective oscillation of the conduction-band electrons at the surface of the particle, with respect to the ionic core of the nanoparticle [76]. The coherent oscillation of the metal free electrons in resonance with the electromagnetic field is called the surface plasmon resonance (SPR). A theoretical and experimental discussion of the SPR can be found in earlier and recent literature [47–50,76–79]. For gold nanospheres, this resonance occurs in the visible spectral region at approximately 520 nm, which is the origin of the brilliant red color of the nanoparticles in solution. For gold nanorods, the free electrons oscillate along both the nanorod long and short axis [80], resulting in a stronger resonance band in the near-infrared (NIR) region and a weaker band in the visible region (similar to the nanospheres), respectively [47–50,80–82]. The excitation of the SPR results in the enhancement of the photophysical properties of gold nanoparticles. Figure 1 summarizes the major optical processes that occur on the interaction of light with gold nanoparticles, which we discuss in detail in the following sections.

Light-scattering imaging

The Rayleigh (Mie) scattering by gold nanoparticles is enhanced greatly owing to the excitation of the SPR [76–79,83–85]. The SPR scattering frequency and intensity are sensitive to the size, shape, composition and environment of the nanoparticles [83–93] and can be quantified using the Mie theory for spherical gold nanoparticles [76]. Typically, nanoparticles of 30–100 nm diameter scatter intensely and can be detected easily by a commercial microscope under dark-field illumination conditions [93]. In fact, 40 nm gold nanoparticles can be detected easily by eye, down to a particle concentration of $10^{-14}$ M [85,86]. Likewise, the scattering from a 60 nm gold nanoparticle is $10^5$ stronger than the emission of a fluorescein molecule [86].

The light scattering of gold nanorods is dependent strongly on the aspect ratio of the nanorods [94]. With an increase in the aspect ratio, the ratio of the intensity of the longitudinal band to that of the transverse band increases and the SPR maximum of the longitudinal mode red shifts, whereas that of the transverse mode blue shifts only slightly. The wavelength shift of the longitudinal band depends linearly on the nanorod aspect ratio [94]. Recently El-Sayed and colleagues calculated the size and shape dependence of the contribution of the SPR scattering to the total extinction (the sum of the absorption and scattering) by using the discrete dipole approximation method [95,96]. The scattering-to-extinction ratio increases with the increase in the size of the nanospheres and the nanorods, which gives a handle for the choice of gold nanoparticles for either optimized imaging or photothermal therapy.

The high-scattering cross-sections of gold nanoparticles, together with their superior photostability (as compared with organic dyes), make them powerful for imaging-based medical applications. The use of the light-scattering property of gold nanoparticles for cellular imaging, especially cancer imaging, has advanced in recent years [97–102] (see ref. [103] for a review of other biological applications). The studies by Sokolov et al. showed that gold nanoparticles can be targeted molecularly to cancer cells and tissue by conjugation with anti-epidermal growth factor receptor (anti-EGFR) antibodies [98]. The cells or tissue labeled with the antibody-conjugated gold nanoparticles can be visualized clearly by the SPR scattering of the nanoparticles under monochromatic light illumination using a scanning laser-confocal reflectance microscope or even the light from a simple laser pen. The strong scattering from the gold nanoparticles thus provides effective optical labeling of the cancer biomarkers.
Compared with single-wavelength illumination, white-light illumination is more advantageous owing to its simplicity and availability. White-light excitation also allows the possibility of the simultaneous differentiation of the scattering from nanoparticles of different size and/or shape (and hence different optical resonances). White light can be delivered to the sample by a flexible optic-fiber light guide and the scattered light can be collected by the objective using a simple optical microscope [86,104]. Dark-field imaging using a conventional microscope is the simplest way to image the individual nanoparticles. Typically, a narrow beam of white light is delivered and focused with a dark-field condenser with high numerical aperture onto the sample. An iris objective is used to collect only the scattered light by the nanoparticles, either in transmission mode or reflection mode, thus giving a colored image of the nanoparticles on a dark background. Although the light scattering of individual gold nanoparticles using the dark-field mode was observed back in 1914 by Zsigmondy using an ultramicroscope [105], studies using a conventional microscope in the dark-field mode have been reported only in recent years [89–93,106–108]. In 2005, El-Sayed et al. demonstrated that the dark-field imaging of 40 nm gold nanospheres can be used for cancer-cell detection in vitro (Figure 2A and B) [100]. The 40 nm gold nanospheres scatter greenish light strongly owing to the SPR scattering in the visible region at approximately 530 nm. In these studies, the nanoparticles used for the optical imaging were conjugated to anti-EGFR antibodies, enabling their specific binding to the cancer cells owing to the overexpressed EGFR on the cancer-cell surface. As a result, the well-organized scattering pattern of the nanoparticles bound to the cancer cells could be distinguished clearly from the random distribution of the nanoparticles around the healthy cells. In the following year, Huang et al. conjugated gold nanorods to anti-EGFR antibodies and demonstrated that gold nanorod–antibody conjugates could also be used as a novel class of contrast agents for cancer-cell imaging by conventional dark-field microscopy owing to their strongly enhanced scattering in the NIR region (Figure 2C) [101]. Similar to the case of gold nanospheres, the antibody-conjugated nanorods are bound to the cancer cells specifically, whereas they are distributed randomly in the case of normal cells. Gold nanospheres and nanorods thus offer very effective analogs to fluorescent labels for cancer imaging.

Two-photon luminescence imaging

The disadvantage of light-scattering imaging is the interference of the scattered light from tissue. Thus, for highly scattering tissue, fluorescence-based imaging techniques are advantageous. Some nanostructures exhibit fluorescence relatively enhanced with respect to that from bulk materials [109,110]. These include gold nanoclusters with few to tens of atoms, which show size-dependent emission in the visible and NIR regions [111–113] with quantum yields up to 0.001, millions of times stronger than that of the bulk metal. Gold nanorods are found to have emission 6–7 orders of magnitude higher than that of bulk gold [114–116]. The fluorescence enhancement is attributed to the excitation of the longitudinal surface plasmon, which enhances the radiative rate of the interband electronic transitions relative to that in bulk metals. The quantum efficiency of gold nanorods increases with nanorod elongation [114] and the strong luminescence can be observed easily by eye for nanorods of over 200 nm in length [115]. Metal nanoparticles, especially gold nanorods, also exhibit enhanced two-photon and multiphoton luminescence [110,117]. Strongly enhanced two-photon luminescence (TPL) has been observed from individual particles [118,119] and particle solutions [119,120] under femtosecond NIR laser excitation, which provides a great potential for nonlinear optical imaging in the NIR region, where water and biomolecules have
minimal absorption. Thus, for highly scattering tissue, the use of the TPL of the gold nanorods is highly appropriate.

Although surface-enhanced single-photon and multiphoton luminescence of gold nanorods was observed years ago [110], its application in cancer diagnostics has emerged only recently. In 2005, Wang et al. demonstrated that single nanorods can be imaged in vivo in the mouse ear blood vessel on account of their strongly induced luminescence by two-photon excitation using a femtosecond NIR laser [119]. Durr et al. applied TPL for the molecular imaging of cancer cells and tissue using nanorods conjugated to antibodies [120]. The TPL signals of gold nanorods are three-orders of magnitude stronger than those from the two-photon autofluorescence of tissue, which has enabled the TPL imaging of cancer cells in a 3D tissue phantom down to 75 µm deep.

In the context of fluorescence-based imaging techniques, the semiconductor quantum dots, which show stronger, narrower and more tunable emission, offer much more sensitive and efficient imaging [1–13]. However, quantum dots do not show photothermal properties that the metal nanoparticles exhibit. The dual scattering/fluorescence and absorption properties of gold nanoparticles enable simultaneous cancer detection and therapy [62,101]. Further, the surface functionalization of gold nanoparticles is facile. Various biomolecules can be conjugated to gold nanoparticles directly through binding of the gold with sulfur-, phosphor-, nitrogen- or oxygen-based ligands or through noncovalent interactions between the water-soluble capping agents and the biomolecules [67–71]. Quantum dots require water solubilization through mercaptoacetic acid or slane linkers, following which biomolecules are conjugated.
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Another common class of nanostructured contrast agents includes the magnetic nanoparticles, which are used to improve the imaging contrast in MRI [18–24]. Clinical tests of magnetic nanoparticles have shown great potential [24], whereas the use of gold nanoparticles using dark-field light-scattering imaging or two-photon luminescence imaging is still in the lab stage. However, the light-scattering imaging using gold nanoparticles is simple and inexpensive, compared with the requirements of MRI. A conventional light microscope equipped with a dark-field condenser is the only instrument required for the imaging. An additional femtosecond laser enables two-photon luminescence imaging.

Surface-enhanced Raman scattering
Since its first discovery on pyridines adsorbed on a silver electrode roughened by oxidation-reduction cycles [122], surface-enhanced Raman scattering (SERS) has been studied extensively on various substrates with its impact ranging from fundamental research to medical applications [123–141]. The SERS mechanism is a result of two major enhancements that result in an increase in the Raman scattering cross-section of the adsorbed molecules. First, there is the long-range electromagnetic (EM) enhancement, which is owing to the resonance of the applied light field with the collective electron oscillations of the nanostructures resulting in strongly enhanced local electric fields at the particle surface. Second, there also exists a short-range chemical enhancement, which is owing to a change in the molecular polarizability by the charge-transfer interaction of the molecules with the metal surface and interaction with adatoms near the metal surface.

Colloidal gold nanoparticles have been used widely as SERS substrates to probe components in living cells [142–148], especially to study the interaction of various antitumor drugs with their pharmacological targets, such as DNA, within living cancer cells [143,145,149–151]. Other studies include cancer gene detection [152–154] and cancer protein-biomarker detection [155–160]. Recently, Huang et al. demonstrated the difference in the SERS of anti-EGFR conjugated gold nanorods between cancer and normal cells (Figure 3) [161]. Molecules near the nanorods on the majority of cancer cells give highly enhanced, sharp and

![Figure 3. SERS of anti-EGFR antibody-conjugated gold nanorods.](image)

SERS of anti-EGFR antibody-conjugated gold nanorods incubated with the (A) HaCat normal cells and (B) HSC cancer cells. The spectra from the cancer cell samples are stronger, sharper and better resolved, suggesting the potential of using surface-enhanced Raman spectroscopy for the molecular-specific diagnosis of cancer.

HSC: Hematopoietic stem cells; SERS: surface-enhanced Raman scattering.

polarized SERS, whereas no SERS is observed from the majority of the normal cells. This difference is attributable to the assembly of gold nanorods on cancer cells resulting from the binding of the anti-EGFR-conjugated rods to the overexpressed EGFR on the cancer cell surface and their resulting assembly. This study has thus added SERS to the existing nanoparticle toolkit for cancer diagnostics.

Plasmonic photothermal therapy
In addition to the strong Mie scattering, gold nanoparticles absorb light strongly [76] as a result of the SPR. This SPR absorption depends on the particle size and shape, the dielectric constant of the metal and that of the surrounding medium [47–50]. For particles smaller than 25 nm, the absorption cross-section is linearly dependent on the volume of the particle size and can be quantified by Mie theory [76]. As described earlier, when the shape of the nanoparticles is changed from nanospheres to nanorods, the SPR absorption splits into two bands [80]: a stronger long-wavelength band in the near-infrared region owing to the longitudinal oscillation of electrons and a weak short-wavelength band in the visible region at approximately 520 nm owing to the transverse electronic oscillation [47,48,77,78,80–82]. The position of the longitudinal absorption band of the gold nanorods is very sensitive to the aspect ratio (length/width), whereas that of the short wavelength is not. Gans [80] first studied the SPR absorption of gold nanorods by extending the Mie theory to nonspherical particle shapes. Link et al. [81,82] modeled the SPR absorption of gold nanorods according to Gans’ theory and found a linear relationship between the longitudinal SPR absorption maximum and the mean-aspect ratio, in agreement with their experimental observations. Additional calculations by Kooij et al. [162] showed that the linear relationship can be extended to an aspect ratio of nine. In addition to the resonance-wavelength tunability, the absorption intensity increases with increasing aspect ratio.

The absorption cross-section of gold nanoparticles [95] is typically 4–5 orders of magnitude stronger than the strongest absorbing Rhodamine 6G dye molecules [163]. In addition, the absorbed light is converted to heat efficiently on a picosecond time domain by rapid electron–phonon and phonon–phonon processes [48]. This strong SPR absorption followed by fast energy conversion and dissipation can be used readily for the heating of the local environment by using light radiation with a frequency strongly overlapping with the nanoparticle SPR absorption band. The highly efficient and localized light-to-heat conversion by gold nanoparticles makes them very useful for the photothermal therapy of cancers and other diseases.

Pitsillides et al. first reported, in 2003, the photothermal therapy of lymphocytes in vitro using gold nanoparticle immunoconjugates coupled with a nanosecond Nd:YAG-pulsed laser at 532 nm, which induced solvent bubbles around the particles that imposed enough mechanical stress to cause cell destruction [164]. Around the same time, Zharov et al. studied the factors that affect the killing energy, such as the number of pulses and particle size as well as the dynamics of the thermal events around the particles, which is important to understand the killing efficiency and mechanisms involved [165–167].

Recently, studies by El-Sayed and colleagues demonstrated the selective photothermal therapy of cancer cells in vitro by using 40 nm gold nanoparticles conjugated to anti-EGFR antibodies [168,169]. The cancer cells, following labeling by the antibody-conjugated nanospheres, were exposed to a visible cw Ar+ laser. The selectivity of this method is demonstrated by the fact that the malignant cells required less than half the laser energy to be killed as compared with the benign cells. In addition, no photothermal destruction was observed for any of the cell types without nanoparticle labeling, even at four times the energy required to kill the malignant cells labeled with anti-EGFR/Au conjugates. The selective photodamage of the cancer cells is clearly a result of the higher gold nanoparticle loading on the cancer cells owing to the overexpressed EGFR on the cancer cell surface. Thus, the method can be used for a variety of cancers by integrating the nanoparticles with an immunotargeting strategy specific to the particular cancer.

A step further from the gold nanosphere-based visible laser therapy discussed earlier, for the in vivo treatment of cancers under the skin and deep within tissue, gold nanorods become ideal. This is because of their tunable absorption in the NIR region of the biological window (650–900 nm). Biological tissue has high transmissivity in this spectral region. In vitro studies by Huang et al. show that gold nanorods conjugated to anti-EGFR antibodies enable selective photothermal therapy because of their preferential binding onto the cancer
A cw Ti:Sapphire NIR laser with a wavelength at 800 nm, overlapping with the longitudinal SPR absorption maximum of the gold nanorods was used for the photo-irradiation of the cells immunolabeled with the nanorods. The cancer cells required half the laser energy (10 W/cm²) to be photothermally damaged as compared with the normal cells (20 W/cm²) (Figure 4). Phosphatidylcholine-passivated gold nanorods [170] and gold nanorods conjugated to folate ligands [171] have been similarly demonstrated in vitro for the NIR therapy of cancer. Other NIR-resonant nanostructures that have shown potential for cancer therapy include gold nanoshells [59,61] and gold nanocages [64].

Cancer cells are damaged at half the energy of that required for normal cells, thus realizing the selective photothermal therapy of cancer. The strongly enhanced absorption of gold nanorods in the NIR region enables photothermal therapy in vivo owing to the minimal light absorption of tissue in the NIR region. HOC: Human osteocalcin; HSC: Hematopoietic stem cells; NIR: Near-infrared.


Conclusion
Noble metal nanoparticles have thus shown good experimental success in the field of nanomedicine, especially cancer, which has always been an area of high concern. The SPR-enhanced properties of gold nanospheres and nanorods, including Mie scattering, enhanced two-photon luminescence and SERS, have been used in a novel way for the optical diagnostics and detection of cancer. At the same time, the intense surface-plasmon absorption and efficient photothermal conversion has been used for the selective laser therapy of cancer. Molecular specificity and selectivity of both diagnostics and therapy is achieved owing to the ability to bioconjugate the gold nanoparticles.
with various immunotargeting functionalities. At the same time, the tunability of the SPR of the nanoparticles is also a great asset. As discussed, by changing the shape of the gold nanoparticles from spheres to rods, the SPR can be shifted to the NIR region of the 'biological window', enabling the imaging/therapy modalities to be used in vivo.

Future perspective
Recent research on the successful use of gold nanoparticles in cancer diagnostics and therapy has already set the stage for the development of clinical applications in the near future. Currently, there is increasing interest in the research on the optimization of the nanoparticle-based imaging and therapy techniques to physiological environments, which will determine the clinical-stage success of gold nanoparticle-based nanomedicine. The diagnostic and therapeutic strategies based on the unique optical properties of the gold nanoparticles discussed in this review are general and can be extended easily to other diseases and disorders besides cancer. This would require the identification of biomolecular signatures associated with the particular disorder being targeted. The synthesis and bioconjugation of the nanoparticles can be tuned easily for the desired application. The collaboration of biomedical researchers and materials scientists in the identification and characterization (in vitro and in vivo) of biomedical strategies using the interesting noble metal nanostructures will impact the future of nanomedicine greatly.

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