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# 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 nanospheres 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 nanospheres are especially promising because of their simple and fast preparation

and bioconjugation. Gold nanospheres 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<sup>+</sup> 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 nanospheres and nanorods that result from their unique optical properties, especially in the area of cancer photodiagnostics and phototherapy. We discuss important recent advances in

**Keywords:** cancer diagnostics, gold nanoparticles, gold nanorods, plasmonic photothermal therapy, Mie scattering, surface-enhanced Raman scattering, two-photon luminescence

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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

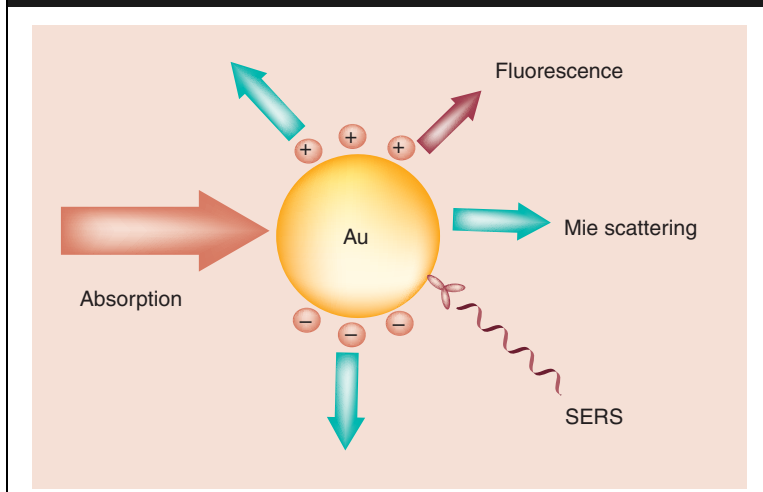
The Rayleigh (Mie) scattering by gold nanoparticles is enhanced greatly owing to the excitation

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.

**Figure 1. Important optical processes resulting from the interaction of light with a gold nanoparticle, viz. light absorption, Mie scattering, surface-enhanced luminescence and surface-enhanced Raman scattering from adsorbed molecules.**



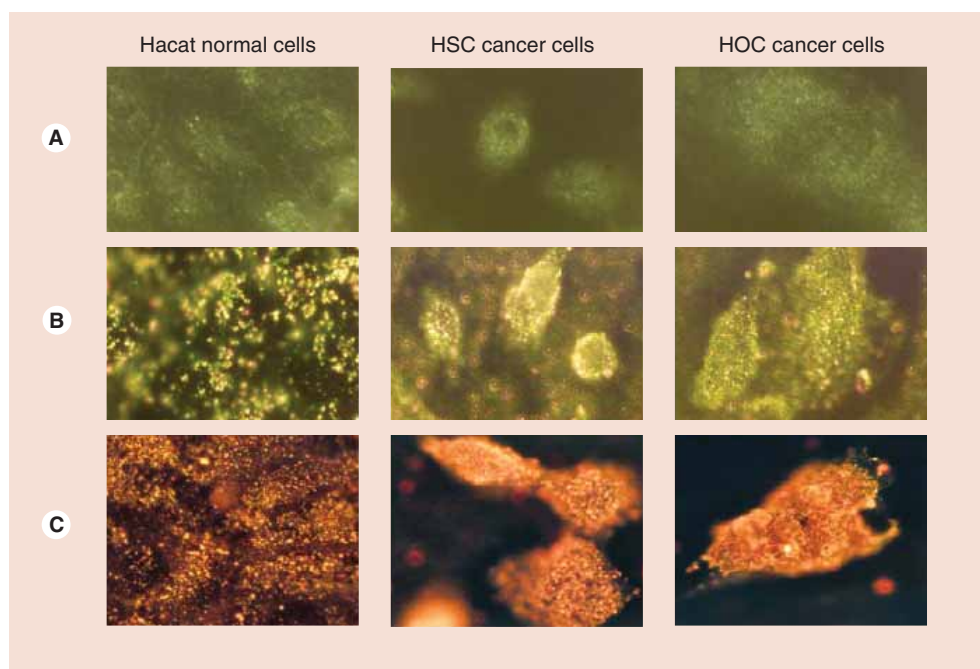
Compared with single-wavelength illumination, white-light illumination is more advantageous owing to its simplicity and availability. White-light excitation also allows the possibility of the simultaneous differentiation of the scattering from nanoparticles of different size and/or shape (and hence different 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 [116]. 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

**Figure 2. Cancer-cell diagnostics using dark-field light-scattering imaging of gold nanoparticles.**



**(A)** Light-scattering images of normal and cancer cells without nanoparticles. (Reproduced with permission from El-Sayed IH, Huang X, El-Sayed MA. *Nano Lett.* 5(5), 819–825 (2005). © ACS 2005) **(B)** Light-scattering images of normal and cancer cells after incubation with anti-EGFR antibody-conjugated gold nanospheres. (Reproduced with permission from El-Sayed IH, Huang X, El-Sayed MA. *Nano Lett.* 5(5), 819–825 (2005) © ACS 2005) **(C)** Light-scattering images of normal and cancer cells after incubation with anti-EGFR antibody-conjugated gold nanorods. The anti-EGFR-conjugated gold nanoparticles are bound to the cancer cells assembled in an organized fashion, whereas they are distributed randomly around normal cells, thus enabling the optical differentiation and detection of the cancer cells.

HOC: Human osteocalcin; HSC: Hematopoietic stem cells.

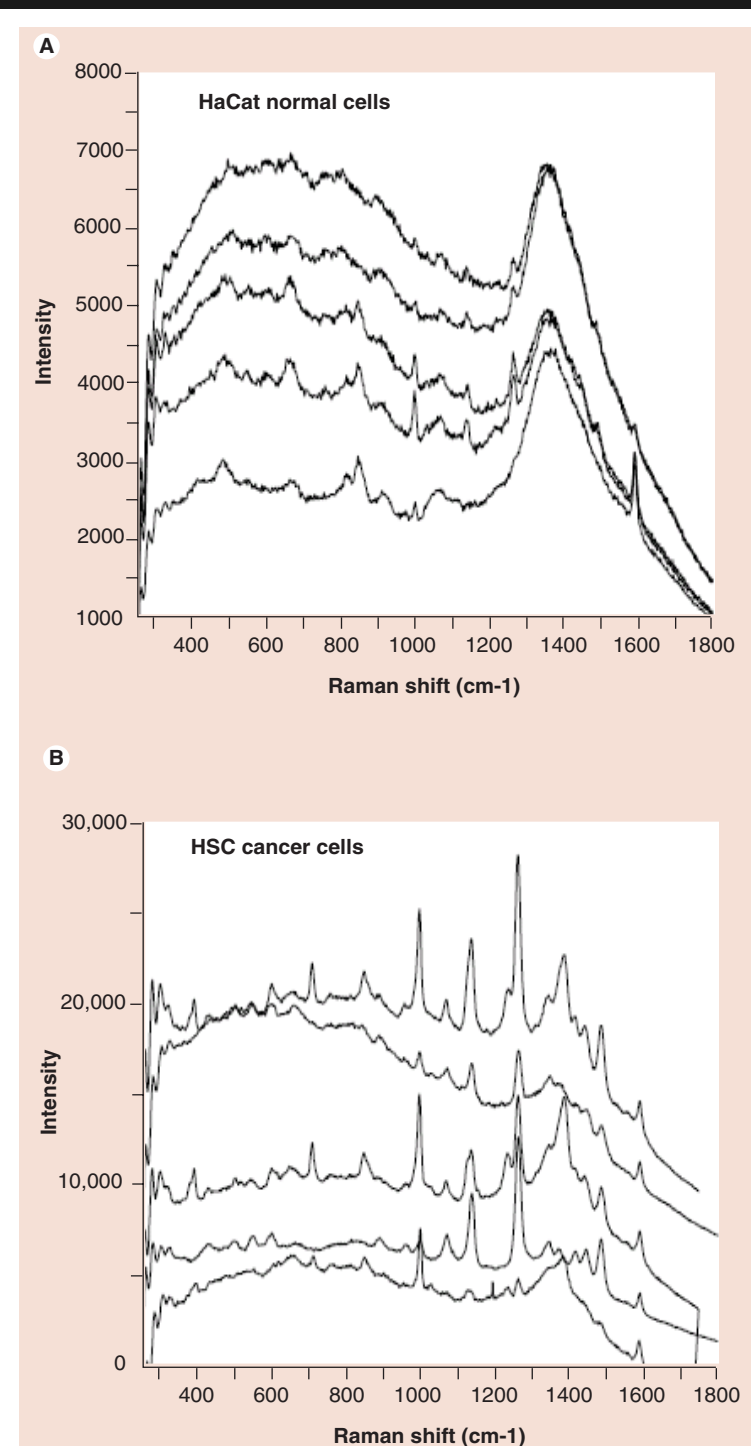
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minimal absorption. Thus, for highly scattering tissue, the use of the TPL of the gold nanorods is highly appropriate.

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  $\mu\text{m}$  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 silane linkers, following which biomolecules are conjugated

**Figure 3. SERS of anti-EGFR antibody-conjugated gold nanorods.**



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. Reproduced with permission from Huang X, El-Sayed IH, Qian W, El-Sayed MA: *Nano Lett.* 7(6), 1591–1597 (2007). ©ACS 2007.

covalently to the linkers [11]. In addition, the toxicity of quantum dots is in question [121], whereas gold nanoparticles are thought to be biosafe [75].

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

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 non-spherical 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

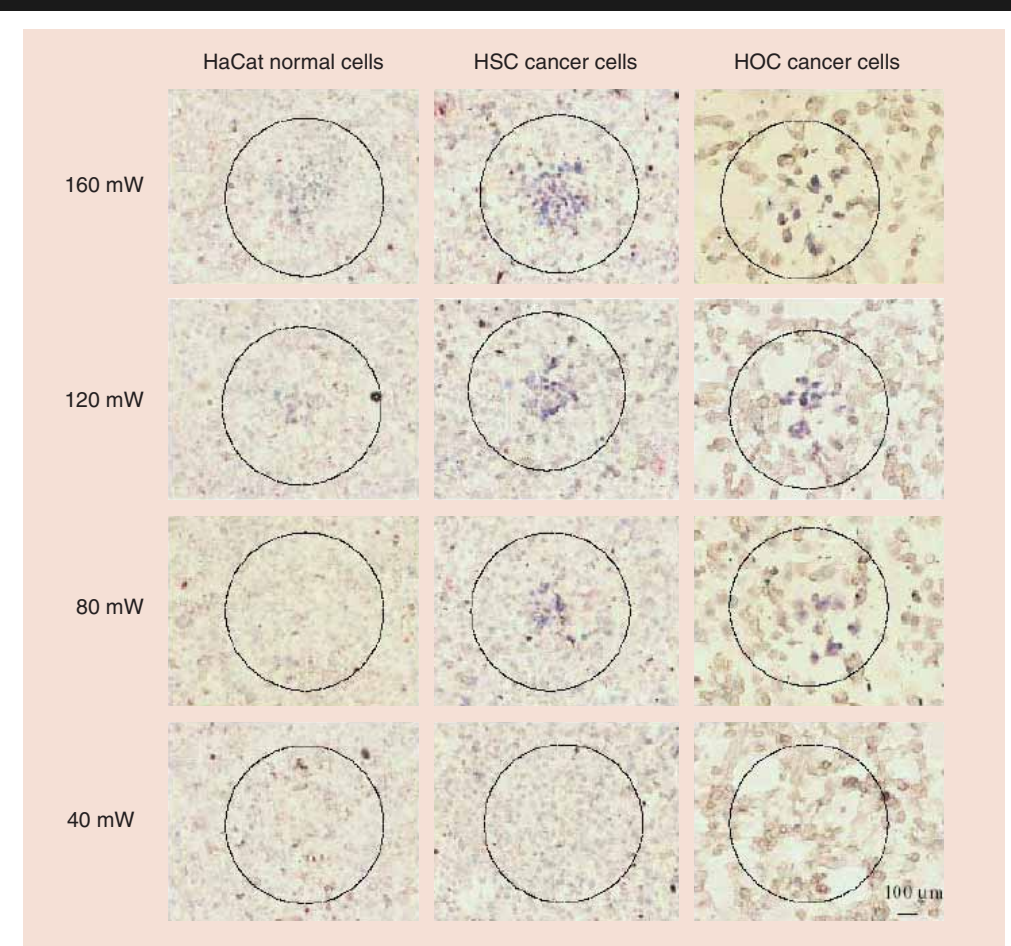
heating of the local environment by using light radiation with a frequency strongly overlapping with the nanoparticle SPR absorption band. The highly efficient and localized light-to-heat conversion by gold nanoparticles makes them very useful for the photothermal therapy of cancers and other diseases.

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<sup>+</sup> 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

**Figure 4. Plasmonic photothermal therapy of cancer cells using anti-EGFR antibody-conjugated gold nanorods and NIR cw light at 800 nm.**



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.

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cells [101]. 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 ( $10 \text{ W/cm}^2$ ) to be photothermally damaged as compared with the normal cells ( $20 \text{ W/cm}^2$ ) (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].

## 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.

#### Financial & competing interests disclosure

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#### Executive summary

- Gold nanoparticles exhibit unique and tunable optical properties owing to the phenomenon of surface plasmon resonance (SPR). SPR-enhanced properties include Mie scattering, surface plasmon absorption, surface-enhanced luminescence and surface-enhanced Raman scattering (SERS) from adsorbed molecules.
- Colloidal gold nanoparticles also provide a surface for easy bioconjugation of a variety of ligands, including antibodies, which can be used for the immunotargeting of the nanoparticles to particular biomarkers on cancer cells, because of which molecular-level specificity can be achieved.
- Gold nanospheres and gold nanorods conjugated to anti-EGFR antibodies have been targeted selectively to cancer cells that overexpress EGFR on their surface.
- Conventional light-scattering microscopy under dark-field illumination enables facile detection and distinction of cancer cells from normal cells based on the strongly enhanced SPR scattering of the nanoparticles bound specifically to the cancer cells. Additionally, anti-EGFR-conjugated gold nanorods organized on the surface of cancer cells give very strong SERS, thus providing an additional spectroscopic diagnostic tool.
- The two-photon luminescence imaging of gold nanorods has also been demonstrated *in vivo*.
- The intense surface plasmon absorption of the gold nanoparticles, followed by rapid photothermal conversion, has been used for the selective photothermal therapy of cancer, by using a suitable immunotargeting strategy.
- The change in the shape of the gold nanoparticles from spherical to rod-shaped enables the optical tuning of the SPR to the near-infrared biological window region, in which biological tissue has high transmissivity. This enables the use of gold nanorods for *in vivo* imaging and therapy, making them highly promising for clinical applications.

#### Bibliography

1. Bruchez Jr M, Moronne M, Gin P, Weiss S, Alivisatos AP: Semiconductor nanocrystals as fluorescent biological labels. *Science* 281(5385), 2013–2016 (1998).
2. Chan WCW, Nie S: Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 281(5385), 2016–2018 (1998).
3. Mitchell P: Turning the spotlight on cellular imaging. *Nat. Biotechnol.* 19, 1013–1017 (2001).
4. Michalet X, Pinaud F, Lacoste TD *et al.*: Properties of fluorescent semiconductor nanocrystals and their application to biological labeling. *Single Mol* 2(4), 261–276 (2001).
5. Chan WCW, Maxwell DJ, Gao X, Bailey RE, Han M, Nie S: Luminescent quantum dots for multiplexed biological detection and imaging. *Curr. Opin. Biotechnol* 13, 40–46 (2002).
6. Wu X, Liu H, Liu J *et al.*: Immunofluorescent labeling of cancer marker Her2 and other cellular target with

- semiconductor quantum dots. *Nat. Biotechnol.* 21, 41–46 (2003).
7. Jaiswal JK, Mattoussi H, Mauro JM, Simon SM: Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat. Biotechnol.* 21, 47–51 (2003).
  8. Gao X, Cui Y, Levenson RM, Chung LWK, Nie S: *In vivo* cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol.* 22(8), 969–976 (2004).
  9. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS: Noninvasive imaging of quantum dots in mice. *Bioconjugate Chem.* 15(1), 79–86 (2004).
  10. Michalet X, Pinaud FF, Bentolila LA *et al.*: Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science* 307(5709), 538–544 (2005).
  11. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H: Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater.* 4(6), 435–446 (2005).
  12. Vashist SK, Tewari R, Bajpai RP, Bharadwaj LM, Raiteri R: Review of quantum dot technologies for cancer detection and treatment. *J. Nanotechnol.* 2, 1–14 (2006).
  13. Rhyner MN, Smith AM, Gao X, Mao H, Yang L, Nie S: Quantum dots and multifunctional nanoparticles: new contrast agents for tumor imaging. *Nanomedicine* 1(2), 1–9 (2006).
  14. Stechell CH: Magnetic separations in biotechnology – a review. *J. Chem. Technol. Biotechnol.* 35(B), 175–182 (1985).
  15. Olsvik O, Popovic T, Skjerve E *et al.*: Magnetic separation techniques in diagnostic microbiology. *Clin. Microbiol. Rev.* 7 (1), 43–54 (1994).
  16. Pankhurst QA, Connolly J, Jones SK, Dobson J: Applications of magnetic nanoparticles in biomedicine. *J. Phys. D Appl. Phys.* 36(13), R167–R181 (2003).
  17. Ito A, Shinkai M, Honda H, Kobayashi T: Medical application of functionalized magnetic nanoparticles. *J. Biosci. Bioeng.* 100(1), 1–11 (2005).
  18. Bonnemain B: Superparamagnetic agents in magnetic resonance imaging: physicochemical characteristics and clinical applications. A review. *J. Drug Target* 6(3), 167–174 (1998).
  19. Josephson L: Magnetic nanoparticles for MR imaging. *BioMEMS Biomed. Nanotechnol.* 1, 227–237 (2006).
  20. Alexiou C, Jurgons R, Seliger C, Iro H: Medical applications of magnetic nanoparticles. *J. Nanosci. Nanotechnol.* 6(9/10), 2762–2768 (2006).
  21. Duguet E, Vasseur S, Mornet S, Devoisselle JM: Magnetic nanoparticles and their applications in medicine. *Nanomedicine* 1(2), 157–168 (2006).
  22. Mornet S, Vasseur S, Grasset F *et al.*: Magnetic nanoparticle design for medical applications. *Prog. Solid State Chem.* 34(2–4), 237–247 (2006).
  23. Bery CC, Curtis ASG: Functionalisation of magnetic nanoparticles for applications in biomedicine. *J. Phys. D Appl. Phys.* 36, R198–R206 (2003).
  24. Josephson L: Magnetic nanoparticles for MR imaging. In: *BioMEMS and Biomedical Nanotechnology Volume I Biological and Biomedical Nanotechnology*. Ferrari M, Lee AP, Lee LJ. (Eds) Springer, NY, USA 227–237 (2006).
  25. Häfeli UO, Chastellain M: Magnetic nanoparticles as drug carriers. In: *Nanoparticulates as Drug Carriers*. Torchilin VP (Ed.), Imperial College Press, London, UK, 397–418 (2006).
  26. Duguet E, Vasseur S, Mornet S *et al.*: Towards a versatile platform based on magnetic nanoparticles for *in vivo* applications. *Bull. Mater. Sci.* 29(6), 581–586 (2006).
  27. Dobson J: Magnetic nanoparticles for drug delivery. *Drug Del. Res.* 67(1), 55–60 (2006).
  28. Jurgons R, Seliger C, Hilpert A, Trahms L, Odenbach S, Alexiou C: Drug loaded magnetic nanoparticles for cancer therapy. *J. Phys. Condens. Matter* 18, S2893–S2902 (2006).
  29. Hilger I, Hiergeist R, Hergt R, Winnefeld K, Schubert H, Kaiser WA: Thermal ablation of tumors using magnetic nanoparticles: an *in vivo* feasibility study. *Invest. Radiol.* 37(10), 580–586 (2002).
  30. Shinkai M: Functional magnetic particles for medical application. *J. Biosci. Bioeng.* 94(6), 606–613 (2002).
  31. Mornet S, Vasseur S, Grasset F, Duguet E: Magnetic nanoparticle design for medical diagnosis and therapy. *J. Mater. Chem.* 14, 2161–2175 (2004).
  32. Häfeli UO: Magnetically modulated therapeutic systems. *Inter. J. Pharm.* 277, 19–24 (2004).
  33. Pradhan P, Giri J, Samanta G *et al.*: Comparative evaluation of heating ability and biocompatibility of different ferrite-based magnetic fluids for hyperthermia application. *J. Biomed. Mater. Res. Part B Appl. Biomater.* 81(1), 12–22 (2006).
  34. Kreuter J: Drug targeting with nanoparticles. *Eur. J. Drug Metabol. Pharm.* 19(3), 253–256 (1994).
  35. Kwon GS, Kataoka K: Block copolymer micelles as long-circulating drug vehicles. *Adv. Drug Del. Rev.* 16(2), 295–309 (1995).
  36. Langer R: Drug delivery and targeting. *Nature* 392(Suppl. 6679), 5–10 (1998).
  37. Müller RH, Maeder K, Gohla S: Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. *Eur. J. Pharm. Biopharm.* 50, 161–177 (2000).
  38. Pillai O, Panchagnula R: Polymers in drug delivery. *Curr. Opin. Chem. Biol.* 5(4), 447–451 (2001).
  39. Sershen S, West J: Implantable, polymeric systems for modulated drug delivery. *Adv. Drug Del. Rev.* 54(9), 1225–1235 (2002).
  40. Hillaireau H, Couvreur P: Polymeric nanoparticles as drug carriers. In: *Polymers in drug delivery*. Uchegbu IF (Ed.), CRC Press LLC, Boca Raton, FL, USA, 101–110 (2006).
  41. Uchegbu IF: Pharmaceutical nanotechnology: polymeric vesicles for drug and gene delivery. *Expert Opin. Drug Del.* 3(5), 629–640 (2006).
  42. Moghimi SMVE, Garcia ML, Al-Hanbali OAR, Rutt KJ: Polymeric nanoparticles as drug carriers and controlled release implant devices. In: *Nanoparticulates as Drug Carriers*. Torchilin VP (Ed.), Imperial College Press, London, UK, 29–42 (2006).
  43. Shi Kam NW, O’Connell M, Wisdom JA, Dai H: Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc. Natl Acad. Sci. USA* 102(33), 11600–11605 (2005).
  44. Bianco A, Kostarelos K, Partidos CD, Prato M: Biomedical applications of functionalized carbon nanotubes. *Chem. Commun.* 5, 571–577 (2005).
  45. Bekyarova E, Ni Y, Malarkey EB *et al.*: Applications of carbon nanotubes in biotechnology and biomedicine. *J. Biomed. Nanotechnol.* 1(1), 3–17 (2005).
  46. Lin Y, Taylor S, Li H *et al.*: Advances toward bioapplications of carbon nanotubes. *J. Mater. Chem.* 14(4), 527–541 (2004).
  47. Link S, El-Sayed MA: Spectral properties and relaxation dynamics of surface plasmon electronic oscillations in gold and silver nanodots and nanorods. *J. Phys. Chem. B* 103(40), 8410–8426 (1999).
  48. Link S, El-Sayed MA: Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals. *Int. Rev. Phys. Chem.* 19(3), 409–453 (2000).

49. El-Sayed MA: Some interesting properties of metals confined in time and nanometer space of different shapes. *Acc. Chem. Res.* 34(4), 257–264 (2001).
50. Link S, El-Sayed MA: Optical properties and ultrafast dynamics of metallic nanocrystals. *Ann. Rev. Phys. Chem.* 54, 331–366 (2003).
51. Masala O, Seshadri R: Synthesis routes for large volumes of nanoparticles. *Annu. Rev. Mater. Res.* 34, 41–81 (2004).
52. Hao E, Schatz G, Hupp J: Synthesis and optical properties of anisotropic metal nanoparticles. *J. Fluor.* 14(4), 331–341 (2004).
53. Daniel MC, Astruc, D: Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem. Rev.* 104, 293–346 (2004).
54. Hutter E, Fendler JH: Exploitation of localized surface plasmon resonance. *Adv. mater.* 16(19), 1685–1706 (2004).
55. Xia Y, Halas NJ: Shape-controlled synthesis and surface plasmonic properties of metallic nanostructures. *MRS Bull* 30, 338–348 (2005).
56. Murphy CJ, Sau TK, Gole AM *et al.*: Anisotropic metal nanoparticles: synthesis, assembly, and optical applications. *J. Phys. Chem. B* 109(29), 13857–13870 (2005).
57. P´erez-Juste J, Pastoriza-Santosa I, Luis M: Liz-Marz´an A, Mulvaney P: Gold nanorods: Synthesis, characterization and applications. *Coord. Chem. Rev.* 249, 1870–1901 (2005).
58. Kelly KL, Coronado E, Zhao LL, Schatz GC: The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. *J. Phys. Chem. B* 107(3), 668–677 (2003).
59. Hirsch LR, Stafford RJ, Bankson JA *et al.*: Nanoshell-mediated near infrared thermal therapy of tumors under MR guidance. *Proc. Natl Acad. Sci. USA* 100(23), 13549–13554 (2003).
60. Loo CH, Lin A, Hirsch LR *et al.*: Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol. Cancer Res. Treat.* 3, 33–40 (2004).
61. O’Neal DP, Hirsch LR, Halas NJ, Payne JD, West JL: Photo-thermal Tumor Ablation in mice using near infrared absorbing nanoshells. *Cancer Lett.* 209(2), 171–176 (2004).
62. Loo C, Lowery A, Halas NJ, West JL, Drezek R: Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Lett.* 5(4), 709–711 (2005).
63. Chen J, Saeki F, Wiley BJ *et al.*: Gold nanocages: bioconjugation and their potential use as optical imaging contrast agents. *Nano Lett.* 5(3), 473–477 (2005).
64. Chen J, Wang D, Xi J *et al.*: Immuno gold nanocages with tailored optical properties for targeted photothermal destruction of cancer cells. *Nano Lett.* 7(5), 1318–1322 (2007).
65. Turkevich J, Stevenson PC, Hillier J: A study of the nucleation and growth processes in the synthesis of colloidal gold. *Disc. Farad. Soc.* 11, 55–75 (1951).
66. Frens G: Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nat. Phys. Sci.* 241, 20–22 (1973).
67. Thaxton CS, Rosi NL, Mirkin CA: Optically and chemically encoded nanoparticle materials for DNA and pprotein detection. *MRS Bull* 30(5), 376–380 (2005).
68. Glomm WR: Functionalized gold nanoparticles for applications in bionanotechnology. *J. Disp. Sci. Technol.* 26(3), 389–414 (2005).
69. Paciotti GF, Kingston DG, Tamarkin L: Colloidal gold nanoparticles: a novel nanoparticle platform for developing multifunctional tumor-targeted drug delivery vectors. *Drug Dev. Res.* 67(1), 47–54 (2006).
70. Niidome Y, Niidome T: Surface modification of gold nanorods for bio-application. *Jasco Report* 48(2), 37–41 (2006).
71. Han G, Ghosh P, Rotello VM: Functionalized gold nanoparticles for drug delivery. *Nanomedicine* 2(1), 113–123 (2007).
72. Yu YY, Chang SS, Lee CL, Wang CRC: Gold nanorods: electrochemical synthesis and optical properties. *J. Phys. Chem. B* 101(34), 6661–6664 (1997).
73. Jana NR, Gearheart L, Murphy CJ: Seed-mediated growth approach for shape-controlled synthesis of spheroidal and rod-like gold nanoparticles using a surfactant template. *Adv. Mater.* 13(18), 1389–1393 (2001).
74. Nikoobakht B, El-Sayed MA: Preparation and growth mechanism of gold nanorods (NRs) using seed-mediated growth method. *Chem. Mater.* 15(10), 1957–1962 (2003).
75. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD: Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 1(3), 325–327 (2005).
76. Mie G: Contribution to the optics of turbid media, especially colloidal metal suspensions. *Ann. Phys.* 25, 377–445 (1908).
77. Papavassiliou GC: Optical properties of small inorganic and organic metal particles. *Prog. Solid State Chem.* 12, 185–271 (1979).
78. Bohren CF, Huffman DR: *Absorption and scattering of light by small particles.* Wiley, NY, USA (1983).
79. Kreibig U, Vollmer M: *Optical Properties of Metal Clusters.* Springer, Berlin, Germany. (1995).
80. Gans R: Form of ultramicroscopic particles of silver. *Ann. Phys.* 47, 270–284 (1915).
81. Link S, Mohamed MB, El-Sayed MA: Simulation of the optical absorption spectra of gold nanorods as a function of their aspect ratio and the effect of the medium dielectric constant. *J. Phys. Chem. B* 103(16), 3073–3077 (1999).
82. Link S, El-Sayed MA: Additions and corrections to simulation of the optical absorption spectra of gold nanorods as a function of their aspect ratio and the effect of the medium dielectric constant. *J. Phys. Chem. B* 109(20), 10531–10532 (2005).
83. Kerker M: *The scattering of light and other electromagnetic radiation.* Academic Press, NY, USA (1969).
84. Van De Hulst HC: *Light Scattering by Small Particles.* Dover, NY, USA (1981).
85. Yguerabide J, Yguerabide EE: Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications. I. Theory. *Anal. Biochem.* 262, 137–156 (1998).
86. Yguerabide J, Yguerabide EE: Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications. II. Experimental characterization. *Anal. Biochem.* 262, 157–186 (1998).
87. Yguerabide J, Yguerabide EE: Resonance light scattering particles as ultrasensitive labels for detection of analytes in a wide range of applications. *J. Cell. Biochem. Suppl.* 37, 71–81 (2001).
88. Jin R, Cao Y, Mirkin CA, Kelly KL, Schatz GC, Zheng JG: Photoinduced conversion of silver nanospheres to nanoprisms. *Science* 294(5548), 1901–1903 (2001).
89. Sonnichsen C, Franzl T, Wilk T, Plessen GV, Feldmann J: Drastic reduction of plasmon damping in gold nanorods. *Phys. Rev. Lett.* 88(7), 077402–077406 (2002).

90. Raschke G, Kowarik S, Franzl T, Sonnichsen C, Klar TA, Feldmann J: Biomolecular recognition based on single gold nanoparticle light scattering. *Nano Lett.* 3(7), 935–938 (2003).
91. Orendorff CJ, Baxter SC, Goldsmith EC, Murphy CJ: Light scattering from gold nanorods: tackling material deformation. *Nanotechnology* 16(11), 2601–2605 (2005).
92. Aslan K, Lakowicz JR, Geddes CD: Nanogold plasmon resonance-based glucose sensing. 2. Wavelength-ratiometric resonance light scattering. *Anal. Chem.* 77(7), 2007–2014 (2005).
93. Orendorff CJ, Sau Tapan K, Murphy CJ: Shape-dependent plasmon-resonant gold nanoparticles. *Small* 2(5), 636–639 (2006).
94. Zhu J, Huang L, Zhao J *et al.*: Shape dependent resonance light scattering properties of gold nanorods. *Mater. Sci. Eng. B* 121, 199–203 (2005).
95. Jain PK, Lee KS, El-Sayed IH, El-Sayed MA: Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *J. Phys. Chem. B* 110(14), 7238–7248 (2006).
96. Lee KS, El-Sayed MA: Dependence of the enhanced optical scattering efficiency relative to that of absorption for gold metal nanorods on aspect ratio, size, end-cap shape, and medium refractive index. *J. Phys. Chem. B* 109(43), 20331–20338 (2005).
97. Sokolov K, Aaron J, Hsu B *et al.*: Optical systems for *in vivo* molecular imaging of cancer. *Technol. Cancer Res. Treat.* 2(6), 491–504 (2003).
98. Sokolov K, Follen M, Aaron J, Pavlova I, Malpica A, Lotan R, Richartz-Kortum R: Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Res.* 63, 1999–2004 (2003).
99. Raub CB, Orwin EJ, Haskell R: Immunogold labeling to enhance contrast in optical coherence microscopy of tissue engineered corneal constructs. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2, 1210–1213 (2004).
100. El-Sayed IH, Huang X, El-Sayed MA: Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. *Nano Lett.* 5(5), 829–834 (2005).
101. Huang X, El-Sayed IH, El-Sayed MA: Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *J. Am. Chem. Soc.* 128(6), 2115–2120 (2006).
102. Aaron JS, Oh J, Larson TA, Kumar S, Milner TE, Sokoly KV: Increased optical contrast in imaging of epidermal growth factor receptor using magnetically actuated hybrid gold/iron oxide nanoparticles. *Optics Express* 14(26), 12930–12943 (2006).
103. Aslan K, Lakowicz JR, Geddes CD: Plasmon light scattering in biology and medicine: new sensing approaches, visions and perspectives. *Curr. Opin. Chem. Biol.* 9, 538–544 (2005).
104. Taton TA, Lu G, Mirkin CA: Two-color labeling of oligonucleotide arrays via size-selective scattering of nanoparticle probes. *J. Am. Chem. Soc.* 123, 5164–5165 (2001).
105. Zsigmondy RA: *Colloids and the ultramicroscope—A manual of colloid chemistry and ultramicroscopy.* John Wiley and Sons, Inc., NY, USA (1914).
106. Schultz S, Smith DR, Mock JJ, Schultz DA: Single-target molecule detection with nonbleaching multicolor optical immunolabels. *Proc. Natl Acad. Sci. USA* 97(3), 996–1001 (2000).
107. Bao P, Frutos AG, Greef C *et al.*: High-sensitivity detection of DNA hybridization on microarrays using resonance light scattering. *Anal. Chem.* 74(8), 1792–1797 (2002).
108. Schatz DA: Plasmon resonant particles for biological detection. *Curr. Opin.* 44, 13–22 (2003).
109. Mooradian A: Photoluminescence of metals. *Phys. Rev. Lett.* 22, 185–187 (1969).
110. Boyd GT, Yu ZH, Shen YR: Photoinduced induced luminescence from the noble metals and its enhancement on roughened surfaces. *Phys. Rev. B: Condens. Matter* 33(12), 7923–7936 (1986).
111. Wilcoxon JP, Martin JE, Parsapour F, Wiedenman B, Kelley DF: Photoluminescence from nanosized gold clusters. *J. Chem. Phys.* 108(21), 9137–9143 (1998).
112. Link S, Beeby A, FitzGerald S, El-Sayed MA, Schaaff TG, Whetten RL: Visible to infrared luminescence from a 28-atom gold cluster. *J. Phys. Chem. B* 106(13), 3410–3415 (2002).
113. Zheng J, Zhang C, Dickson RM: Highly fluorescent, water-soluble, size-tunable gold quantum dots. *Phys. Rev. Lett.* 93(7), 077402.1–077402.4 (2004).
114. Mohamed MB, Volkov V, Link S, El-Sayed MA: The 'lightning' gold nanorods: fluorescence enhancement of over a million compared to the gold metal. *Chem. Phys. Lett.* 317(6), 517–523 (2000).
115. Eustis S, El-Sayed MA: Aspect ratio dependence of the enhanced fluorescence intensity of gold nanorods: experimental and simulation study. *J. Phys. Chem. B* 109(34), 16350–16356 (2005).
116. Li CZ, Male KB, Hrapovic S, Luong JHT: Fluorescence properties of gold nanorods and their application for DNA biosensing. *Chem. Commun.* 31, 3924–3926 (2005).
117. Bouhelier A, Beversluis MR, Novotny L: Characterization of nanoplasmonic structures by locally excited photoluminescence. *Appl. Phys. Lett.* 83(24), 5041–5043 (2003).
118. Imura K, Nagahara T, Okamoto H: Plasmon mode imaging of single gold nanorods. *J. Am. Chem. Soc.* 126(40), 12730–12731 (2004).
119. Wang H, Huff TB, Zweifel DA *et al.*: *In vitro* and *in vivo* two-photon luminescence imaging of single gold nanorods. *Proc. Natl Acad. Sci. USA* 102(44), 15752–15756 (2005).
120. Durr NJ, Larson T, Smith DK, Korgel BA, Sokolov K, Ben-Yakar A: Two-photon luminescence imaging of cancer cells using molecularly targeted gold nanorods. *Nano Lett.* 7(4), 941–945 (2007).
121. Shiohara A, Hoshino A, Hanaki K, Suzuki K, Yamamoto K: On the cyto-toxicity caused by quantum dots. *Microbiol. Immunol.* 48(9), 669–675 (2004).
122. Fleischman M, Hendra PJ, McQuillan AJ: Raman spectra of pyridine adsorbed at a silver electrode. *Chem. Phys. Lett.* 26(2), 163–166 (1974).
123. Jeanmaire DL, Van Duyne RP: Surface Raman spectroscopy. 1. Heterocyclic, aromatic, and aliphatic-amines adsorbed on anodized silver electrode. *J. Electroanal. Chem.* 84, 1–20 (1977).
124. Albercht M, Creighton JA: Anomalously intense Raman spectra of pyridine at a silver electrode. *J. Am. Chem. Soc.* 99, 5215–5217 (1977).
125. Persson BNJ: On the theory of surface-enhanced Raman scattering. *Chem. Phys. Lett.* 82(3), 561–565 (1981).
126. Chang RK, Furtak TE: *Surface enhanced Raman scattering.* Plenum Press, NY, USA (1982).
127. Schatz GC: Theoretical studies of surface enhanced Raman scattering. *Acc. Chem. Res.* 17, 370–376 (1984).
128. Moskovits M: Surface enhanced spectroscopy. *Rev. Mod. Phys.* 57(3), 783–826 (1985).
129. Garrell RL: Surface-Enhanced Raman Spectroscopy. *Anal. Chem.* 61(6), 401A–411A (1989).

130. Chumanov GD, Efremov RG, Nabiev IR: Surface-enhanced Raman spectroscopy of biomolecules. I: Water-soluble proteins, dipeptides and amino acids *J. Raman Spectrosc.* 21(1), 43–48 (1989).
131. Cotton TM, Kim JH, Chumanov GD: Application of surface-enhanced Raman spectroscopy to biological systems. *J. Raman Spectrosc.* 22(12), 729–742 (1991).
132. Otto A, Mrozek I, Grabhorn H, Akemann W: Surface-enhanced Raman scattering. *J. Phys. Condens. Matter* 4(5), 1143–1212 (1992).
133. Garrell RL, PJ, Cotton TM. *Fundamentals and Applications of Surface Raman Spectroscopy*. VCH Publishers Inc, FL, USA (1993).
134. Nabiev I, Chourpa I, Manfait M: Applications of Raman and surface enhanced Raman scattering spectroscopy in medicine. *J. Raman Spectrosc.* 25, 13–23 (1994).
135. Campion A, Kambhampati, P: Surface-enhanced Raman scattering. *Chem. Soc. Rev.* 27, 241–250 (1998).
136. Kneipp K, Kneipp H, Itzkan I, Dasari RR, Feld MS: Surface-enhanced non-linear Raman scattering at the single-molecule level. *Chem. Phys.* 247, 155–162 (1999).
137. Tian ZQ, Ren R, Wu DY: Surface-enhanced Raman scattering: from noble to transition metals and from rough surfaces to ordered nanostructures. *J. Phys. Chem. B* 106(37), 9463–9483 (2002).
138. Kneipp K, Kneipp H, Itzkan I, Dasari RR, Feld MS: Surface-enhanced Raman scattering and biophysics. *J. Phys. Condens. Matter* 14(18), R597–R624 (2002).
139. Kneipp K, Moskovits M, Kneipp H: Surface-enhanced raman scattering: physics and applications. NY, USA Springer (2006).
140. Stuart DA, Haes AJ, Yonzon CR, Hicks EM, Van Duyne RP: Biological applications of localised surface plasmonic phenomena. *IEE Proc-Nanobiotechnol.* 152(1), 13–32 (2005).
141. Haynes CL, Yonzon CR, Zhang X, Van Duyne RP: Surface-enhanced Raman sensors: early history and the development of sensors for quantitative biowarfare agent and glucose detection. *J. Raman Spectrosc.* 36, 471–484 (2005).
142. Manfait M, Morjani H, Millot JM, Debal V, Angiboust JF, Nabiev I: Drug target interactions on a single living cell. An approach by optical microspectroscopy. *Proc. Soc. Photo Opt. Instrum. Eng.-Int Soc Opt Eng* 1403, 695–707 (1991).
143. Nabiev IR, Morjani H, Manfait M: Selective analysis of antitumor drug interaction with living cancer cells as probed by surface-enhanced Raman spectroscopy. *Eur. Biophys. J.* 19(6), 311–316 (1991).
144. Manfait M, Nabiev I, Morjani H: Molecular events on single living cancer cells as studied by microspectrofluorometry and micro-SERS Raman spectroscopy. *J. Cell Pharm.* 3(1), 120–125 (1992).
145. Morjani H, Riou JF, Nabiev I, Lavelle F, Manfait M: Molecular and cellular interactions between intoplicine, DNA, and topoisomerase II studied by surface-enhanced Raman scattering spectroscopy. *Cancer Res.* 53, 4784–4790 (1993).
146. Kneipp K, Haka AS, Kneipp H *et al.*: Surface-enhanced Raman spectroscopy in single living cells using gold nanoparticles. *Appl. Spectrosc.* 56(2), 150–154 (2002).
147. Kneipp J, Kneipp H, Rice WL, Kneipp K: Optical probes for biological applications based on Ssurface-enhanced Raman scattering from indocyanine green on gold nanoparticles. *Anal. Chem.* 77(8), 2381–2385 (2005).
148. Tang HW, Yang XB, Kirkham J, Smith DA: Probing Intrinsic and Extrinsic Components in Single Osteosarcoma Cells by Near-Infrared Surface-Enhanced Raman Scattering. *Anal. Chem.* 79(10), 3646–3653 (2007).
149. Nabiev I, Chourpa I, Manfait M: comparative studies of antitumor DNA intercalating agents, aclacinomycin and saintopin, by means of surface-enhanced raman scattering spectroscopy. *J. Phys. Chem.* 98(4), 1344–1350 (1994).
150. Chourpa I, Morjani H, Riou JF, Manfait M: Intracellular molecular interactions of antitumor drug amsacrine (m-AMSA) as revealed by surface-enhanced Raman spectroscopy. *FEBS Lett.* 397(1), 61–64 (1996).
151. Beljebbar A, Morjani H, Angiboust JF, Sockalingum GD, Polissiou M, Manfait M: Molecular and cellular interaction of the differentiating antitumour agent dimethylcroctin with nuclear retinoic acid receptor as studied by near-infrared and visible SERS spectroscopy. *J. Raman Spectrosc.* 28(2–3), 159–163 (1997).
152. Allain LR, Vo-Dinh T: Surface-enhanced Raman scattering detection of the breast cancer susceptibility gene BRCA1 using a silver-coated microarray platform. *Anal. Chim. Acta* 469(1), 149–154 (2002).
153. Vo-Dinh T, Allain LR, Stokes DL: Cancer gene detection using surface-enhanced Raman scattering (SERS). *J. Raman Spectrosc.* 33, 511–516 (2002).
154. Culha M, Stokes D, Allain LR, Vo-Dinh T: Surface-enhanced Raman scattering substrate based on a self-assembled monolayer for use in gene diagnostics. *Anal. Chem.* 75(22), 6196–6201 (2003).
155. Hawi SR, Rochanakij S, Adar F, Campbell WB, Nithipatikom K: Detection of membrane-bound enzymes in cells using immunoassay and Raman microspectroscopy. *Anal. Biochem.* 259(2), 212–217 (1998).
156. Seballos L, Zhang JZ, Sutphen R: Surface-enhanced Raman scattering detection of lysophosphatidic acid. *Anal. Bioanal. Chem.* 383(5), 763–767 (2005).
157. Ansari DO, Stuart DA, Nie S: Surface-enhanced Raman spectroscopic detection of cancer biomarkers in intact cellular specimens. *Proc. Soc. Photo Opt. Instrum. Eng. – Inter. Soc. Opt. Eng.* 5699 82–90 (2005).
158. Kim JH, Kim JS, Choi H *et al.*: Nanoparticle probes with surface enhanced Raman spectroscopic tags for cellular cancer targeting. *Anal. Chem.* 78(19), 6967–6973 (2006).
159. Lee S, Kim S, Choo J *et al.*: Biological imaging of HEK293 cells expressing PLC $\zeta$ 1 using surface-enhanced Raman microscopy. *Anal. Chem.* 79(3), 916–922 (2007).
160. Schlücker S, Küstner B, Punge A, Bonfig R, Marx A, Ströbel P: Immuno-Raman microspectroscopy: In situ detection of antigens in tissue specimens by surface-enhanced Raman scattering. *J. Raman Spectrosc.* 37, 719–721 (2006).
161. Huang X, El-Sayed IH, Qian W, El-Sayed MA: Cancer cells assemble and align gold nanorods conjugated to antibodies to produce highly enhanced, sharp and polarized surface raman spectra: a potential cancer diagnostic marker. *Nano Lett.* 7(6), 1591–1597 (2007).
162. Kooij ES, Poelsema B: Shape and size effects in the optical properties of metallic nanorods. *Phys. Chem. Chem. Phys.* 8, 3349–3357 (2006).
163. Du H, Fuh RA, Li J, Corkan A, Lindsey JS: PhotochemCAD $\dagger\dagger$ : A computer-aided design and research tool in photochemistry. *Photochem. Photobiol.* 68(2), 141–142 (1998).
164. Pitsillides CM, Joe EK, Wei X, Anderson RR, Lin CP: Selective cell targeting with light-absorbing microparticles and nanoparticles. *Biophys. J.* 84(6), 4023–4032 (2003).

165. Zharov VP, Galitovsky V, Viegas M: Photothermal detection of local thermal effects during selective nanophotothermolysis. *Appl. Phys. Lett.* 83(24), 4897–4899 (2003).
166. Zharov VP, Galitovskaya E, Viegas M: Photothermal guidance for selective photothermolysis with nanoparticles. *Proc. Soc. Photo Opt. Instrum. Eng.* 5319, 291–300 (2004).
167. Zharov VP, Galitovskaya EN, Johnson C, Kelly T: Synergistic enhancement of selective nanophotothermolysis with gold nanoclusters: potential for cancer therapy. *Lasers Surg. Med.* 37, 219–226 (2005).
168. El-Sayed IH, Huang X, El-Sayed MA: Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. *Cancer Lett.* 239(1), 129–135 (2006).
169. Huang X, Jain PK, El-Sayed IH, El-Sayed MA: Determination of the minimum temperature required for selective photothermal destruction of cancer cells using immunotargeted gold nanoparticles. *Photochem. Photobiol.* 82(2), 412–417 (2006).
170. Takahashi H, Niidome T, Nariai A, Niidome Y, Yamada S: Gold nanorod-sensitized cell death: microscopic observation of single living cells irradiated by pulsed near-infrared laser light in the presence of gold nanorods. *Chem. Lett.* 35(5), 500–501 (2006).
171. Huff TB, Tong L, Zhao Y, Hansen MN, Cheng JX, Wei A: Hyperthermic effects of gold nanorods on tumor cells. *Nanomedicine* 2(1), 125–132 (2007).