Gold nanorod light scattering labels for biomedical imaging

Le Qiu,¹ Timothy A. Larson,² Edward Vitkin,¹ Lianyu Guo,¹ Eugene B. Hanlon,^{1,3}
Irving Itzkan,¹ Konstantin V. Sokolov,^{2,4} and Lev T. Perelman^{1,*}

¹Biomedical Imaging and Spectroscopy Laboratory, Beth Israel Deaconess Medical Center,
Harvard University, Boston, Massachusetts 02215, USA

²Departments of Biomedical Engineering and Chemical Engineering,
University of Texas at Austin, Austin, Texas 78712, USA

³Medical Research Service and Geriatric Research Education and Clinical Center,
Department of Veterans Affairs, Bedford, Massachusetts 01730, USA

⁴Departments of Biomedical Engineering and Imaging Physics,
The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA
[*lperel@bidmc.harvard.edu](mailto:lperel@bidmc.harvard.edu)

Abstract: Gold nanorods can be used as extremely bright labels for differential light scattering measurements using two closely spaced wavelengths, thereby detecting human disease through several centimeters of tissue *in vivo*. They have excellent biocompatibility, are non-toxic, and are not susceptible to photobleaching. They have narrow, easily tunable plasmon spectral lines and thus can image multiple molecular targets simultaneously. Because of their small size, gold nanorods can be transported to various tissues inside the human body via the vasculature and microvasculature, and since they are smaller than vascular pore sizes, they can easily cross vascular space and enter individual cells.

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References and links

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1. Introduction

There are two problems in visualizing tumors in deep tissue, lack of contrast and tissue turbidity. In this paper we discuss the potential of gold nanorods to overcome these problems. They can serve as extremely bright light scattering labels for the detection of disease through several centimeters of tissue in humans *in vivo*. There is a common misconception, based on the high background signal associated with light absorption and scattering measurements, that absorption and scattering probes should always perform poorly compared to fluorescence probes such as quantum dots and molecular fluorophores. This is because the absorption and scattering labels are detected at the same wavelength as the excitation wavelength and are seen against a high background of light coming directly from the source. However, in the case of gold nanorods, this problem is overcome by two unusual properties of the nanoparticles. The first is their narrow absorption and scattering plasmon resonant linewidths [1,2]. It allows one to perform differential absorption and scattering measurements at two closely spaced wavelengths. This effectively eliminates the background signal, although the noise associated with the subtracted background signal will still be present in the measurement. The second is their extremely high absorption and scattering plasmon resonant cross sections. These very large narrowband cross sections more than compensate for the effect of the noise associated with the subtracted background signal.

At the same time gold nanorods have excellent biocompatibility, are not toxic [3], and are not susceptible to photobleaching. Their narrow and easily tunable plasmon spectral lines can thus be used to image multiple molecular targets simultaneously [1]. Finally, because, nanorod labels are smaller than vascular pore sizes, they can easily cross the vascular space to be delivered to various tissues and individual cells inside the human body via the vasculature and microvasculature. (Nanorod labels are 10-12 nm diameter and 25-50 nm length or approximately 3 to 5 times smaller than available quantum dots).

Some of the material described in sections 2 and 3 has been previously published elsewhere. We include it here for the convenience of the readers of this journal who may not be familiar with the nanorod literature.

2. Theory of the optical properties of gold nanorods

Nanoparticles with sizes small compared to the wavelength of light, made from metals with an appropriate complex index of refraction, such as gold and silver, have absorption and scattering resonance lines in the visible part of the spectrum. These lines are due to the in-phase oscillation of free electrons and are called surface plasmon resonances.

Since nanorods have spectra very similar to nanospheroids with the same aspect ratio, the absorption and scattering cross sections of gold nanospheroids are used to describe the optical properties of gold nanorods [4,5]. A rigorous solution for nonspherical particles, which can be considered an extension of Mie theory, was developed by Waterman and is called the T-matrix approach [6]. For spherical particles the T-matrix approach becomes identical to Mie theory. Although this solution is exact it is not transparent. For particles much smaller than a wavelength, only the dipole term contributes significantly to the absorption cross-section [7], and thus a great deal of physical insight can be gained from conclusions drawn by examining the dipole approximation [2].

In the dipole approximation the angle averaged absorption cross-section for a nanospheroid is given by [8,9]

$$\langle \sigma_{abs} \rangle = \frac{2\pi}{3\lambda} \epsilon_m^{3/2} V \sum_i \frac{\epsilon_2 / (n^{(i)})^2}{\left(\epsilon_1 + \frac{1 - n^{(i)}}{n^{(i)}} \epsilon_m \right)^2 + \epsilon_2^2}. \quad (1)$$

and the angle averaged scattering cross-section is

$$\langle \sigma_{sca} \rangle \cong \frac{8\pi^3 \varepsilon_m^2}{9\lambda^4} V^2 \sum_i \frac{((\varepsilon_1 - \varepsilon_m)^2 + \varepsilon_2^2) / (n^{(i)})^2}{\left(\varepsilon_1 + \frac{1 - n^{(i)}}{n^{(i)}} \varepsilon_m \right)^2 + \varepsilon_2^2}. \quad (2)$$

Here $\varepsilon_1(\lambda) = n(\lambda)^2 - \kappa(\lambda)^2$, $\varepsilon_2(\lambda) = 2n(\lambda)\kappa(\lambda)$ and $\varepsilon_m = n_m^2$ where $n(\lambda)$ and $\kappa(\lambda)$ are the real and imaginary parts of the complex refractive index of the nanospheroid's material, n_m is the refractive index of the surrounding medium, and $n^{(i)}$ is the depolarization factor in the i -th direction [10].

The explicit dependence of the cross-sections on the aspect ratio, α , which is the ratio of the length to the diameter of the nanoparticles can be derived using two simple approximations for the depolarization factor [2], yielding a good small α approximation of $n^{(a)} \cong 1/3\alpha$. Using this approximation, for the range of aspect ratios from 1 to 4, Eq. (1) can be simplified

$$\langle \sigma_{abs} \rangle \cong \frac{6\pi}{\lambda} \varepsilon_m^{3/2} \varepsilon_2 V \alpha^2 \left[F_L(\alpha, \lambda) + \frac{8}{(3\alpha - 1)^2} F_T(\alpha, \lambda) \right]. \quad (3)$$

where the first term $F_L(\alpha, \lambda) = \left[(\varepsilon_1 + (3\alpha - 1)\varepsilon_m)^2 + \varepsilon_2^2 \right]^{-1}$ represents the longitudinal plasmon optical mode and the second term $F_T(\alpha, \lambda) = \left[\left(\varepsilon_1 + \left(1 + \frac{2}{3\alpha - 1} \right) \varepsilon_m \right)^2 + \varepsilon_2^2 \right]^{-1}$ represents the transverse plasmon optical mode. We see that the red shift of the longitudinal mode $\varepsilon_1(\lambda) = -(3\alpha - 1)\varepsilon_m$ is sensitive to the aspect ratio, varying almost linearly with it, and the position of the peak of the transverse mode $\varepsilon_1(\lambda) = -\left(1 + \frac{2}{3\alpha - 1} \right) \varepsilon_m$ is insensitive to the aspect ratio, shifting only slightly from the position of the well known nanosphere plasmon spectral peak $\varepsilon_1(\lambda) \cong -\varepsilon_m$. In addition the plasmon transverse mode is heavily damped by the coefficient $\frac{8}{(3\alpha - 1)^2}$. This coefficient is less than 7% of the main peak at $\alpha = 4$.

While the scattering and absorption cross-sections exhibits similar behavior for longitudinal and transverse plasmon modes [1,2], the scattering cross-section has a stronger wavelength dependence than the absorption cross-section. Indeed, the ratio of the scattering to

absorption cross-sections is approximately proportional to $\frac{(\kappa(\lambda)^2 + n_m^2)^2}{2n(\lambda)\kappa(\lambda)}$ [1]. This ratio is

10 times greater at 700 nm than at 520 nm and explains why the transverse mode is barely seen in the scattering cross-section. (See Fig. 1 where we compare T-matrix calculations of normalized absorption and scattering cross-sections for several aspect ratios and diameters of nanospheroids. Here, the transverse mode for scattering disappears.)

Fig. 1. Normalized absorption and scattering cross sections for three aspect ratios, α and diameters d of nanospheroids modeled using the T-matrix approach [11]. The solid curves are for scattering while the dotted lines are for absorption. From left to right: $\alpha = 2.18$ and $d = 10$ nm, $\alpha = 3.2$ and $d = 10$ nm, and $\alpha = 4.0$ and $d = 10$ nm. Inset magnifies the curves in the vicinity of the 520 nm transverse mode.

Our approximation agrees with the well known result of the dipole model that the spectral properties of nanospheroids depend only on the complex permittivity of the material and their aspect ratio but not size. Size enters only as a coefficient, changing the overall value of the cross-sections. Thus to tune the absorption and scattering lines of gold nanorods we should control for aspect ratio. Note however, that though the aspect ratio is the main factor deciding the position of the plasmon spectral peak, it is shifted slightly with other factors such as diameter and end geometry when non-dipole terms are taken into account [12].

3. Optical spectroscopy of single gold nanorods

Samples containing a large number of gold nanorods usually exhibit relatively broad spectral lines. This broadened linewidth limits the use of nanorods with uncontrolled aspect ratios as effective molecular labels, since it would be rather difficult to image several types of nanorod markers simultaneously.

Fig. 2. Schematic of the single gold nanorod experiment using the CLASS system.

Fig. 3. Normalized scattering spectrum for a single gold nanorod. Dots: CLASS measurements. Other lines are T-matrix calculations for a nanorod with an aspect ratio of 3.25 and a diameter of 16.2 nm and various A values. Solid line is for the natural linewidth, $A = 0$. Also included are lines for $A = 0.5$ and $A = 1$. The curve for $A = 0.13$ is the best fit for measurements made on eight different nanorods.

However, the calculated linewidth of a single gold nanorod is narrow. To confirm this we measured the linewidth of single gold nanorods using the CLASS microscope (see Fig. 2) we developed [13]. The experimental data from one of these measurements is shown in Fig. 3. The measured CLASS spectra were compared with numerical calculations which use the complex refractive index of gold and various values of the A-parameter, a phenomenological correction introduced in the literature to account for homogeneous broadening caused by finite size and interface effects. The curve for $A = 0.13$ is the best fit for measurements made on eight different nanorods. This agrees very well with an A-parameter calculation using a quantum mechanical jellium model [14].

Fig. 4. Optical properties of an ensemble of gold nanorods. (a) TEM image of a sample of gold nanorods with an average length and standard deviation of 48.9 ± 5.0 nm and an average diameter and standard deviation of 16.4 ± 2.1 nm. (b) Experimentally measured extinction of the same sample of gold nanorods as in the TEM image in aqueous solution (blue dots) vs. T-matrix calculation for a single nanorod with length and diameter of 48.9 and 16.4 nm respectively (red solid line). (c) Aspect ratio distribution of gold nanorods as in the TEM image. (d) Ensemble spectrum calculated using aspect ratio distribution demonstrates good agreement with experimentally measured extinction of the sample of gold nanorods in aqueous solution.

From these measurements one can draw the conclusion that single gold nanorods indeed exhibit a scattering line significantly narrower than the lines routinely observed in experiments that involve multiple nanorods. These experiments also suggest that the observed broad spectra of nanorod samples containing large number of nanoparticles could be explained by the inhomogeneous line broadening caused by the contribution of nanorods with various aspect ratios (see Fig. 4). Indeed, since the position of a nanorod spectral peak is very sensitive to its aspect ratio, we can demonstrate [1] that the ensemble spectrum calculated using aspect ratio distribution extracted from the TEM image of a sample of gold nanorods exhibits good agreement with the experimentally measured extinction of the same sample.

A possible technique for obtaining a narrow aspect ratio distribution might employ devices already developed for cell sorting. These would use the position of the narrow plasmon spectral line for particle discrimination. Narrow, easily tunable spectra might allow several biochemical species to be imaged simultaneously with molecular markers which employ gold nanorods of several different, controlled, aspect ratios as labels. These markers could be used for cellular microscopic imaging where even a single nanorod can be detected. Minimizing the number of nanoparticles should reduce possible damage to a living cell. For optical imaging of tumors, multiple gold nanorods with a narrow aspect ratio distribution might be used.

Thus we conclude that nanorod-based molecular markers selected for a narrow aspect ratio and, to a lesser degree, size distribution, could provide spectral lines sufficiently narrow for effective biomedical imaging.

4. Comparison of the gold nanorod labels vs. fluorescent labels

To evaluate the sensitivity of the gold nanorod label vs. various fluorescence dye and quantum dots labels, we performed numerical experiments modeling the propagation of light through slabs of turbid tissues of various thicknesses (from 0.25 cm to 7 cm). Light is delivered through a 1 mm fiber bundle and collected with a detector of the same size. The collection time was 1 sec. The power of the illumination light was 100 mW, which is well below the maximum permissible exposure (MPE) level of 440 mW set by American National Standard Institute [15]. However, since nanorods are strong absorbers of light, they can raise local tissue temperature. Thus, we estimated the temperature increase in tissue for proposed nanorod labels. The characteristic size of the region around an individual nanorod which will be heated in $t = 1$ second is $r \cong \sqrt{4kt/\rho c} \approx 700 \mu\text{m}$ where k is heat diffusion coefficient, ρ is tissue density, and c is tissue heat capacity. Since this size is significantly larger than the average distance between nanorods ($10 \mu\text{m}$), the temperature increase in tissue will be limited by $\Delta T \leq (\sigma \cdot N \cdot P \cdot t) / (\rho c V)$ where N is concentration of nanorods and P is power of the incoming light. For our nanorods, near the surface of the tissue where P is the highest, we get $\Delta T \approx 0.01^\circ\text{C}$, which poses no danger to the tissue.

Fig. 5. Geometry and typical wavelength pair of the numerical simulation used to evaluate the sensitivity of various optical labels.

The 1 mm spherical “tumor” was placed half way across the slab (Fig. 5). We assumed that in addition to the probes inside the tumor we will have probes distributed in the surrounding tissue and used various contrast ratios from 0 (no probes outside the tumor) to 0.1. Concentration of the probes in tissue varied from 102 to 1014 probes per cubic centimeter.

To evaluate the ability to detect the “tumor” we calculated the signal to noise ratio (S/N) which is the ratio of the measured signal to the overall measured noise:

$$\frac{S}{N} = \frac{qF_T t}{\sqrt{qF_0 t + N_d t + N_r^2}}. \quad (4)$$

where q is quantum efficiency of the detector, F_T is the tumor-related photon flux (photons/spot/second), F_0 is the total photon flux which is the sum of F_T and background flux, t is the integration time per spot, N_d is dark current of the detector (electrons/spot/sec), and N_r is the read noise (electrons RMS/spot). To minimize the effect of the detector we have chosen characteristics of a standard available high-end CCD camera: $q = 0.5$, $N_d = 0.01$ electrons/spot/sec $N_r = 10$ electrons RMS/spot.

In the case of fluorescent dyes and quantum dots $F_T \approx F_0$, and in the case of scattering probes $F_T \ll F_0$, which significantly reduces, as we already discussed above, the efficiency of the scattering probes. However, to evaluate the overall effect of the increased background vs. increased cross section for the gold nanorod probes we need to perform accurate modeling. The results of this modeling are presented in Fig. 6. To compare various labels (ICG, Rhodamine 6G, DDI and NIR1 fluorescence dyes, CdTe/CdS and CdTe/ZnS quantum dots, 11 nm diameter, 3.3 aspect ratio and 10 nm diameter, 4.0 aspect ratio gold nanorods) we calculated concentration of labels (per mm^3) needed to achieve $S/N = 10$ which we consider to be minimum detectable level (Fig. 6a). As we can see the concentration of gold nanorod label (red curves) is three orders of magnitude lower than that of NIR1 fluorescence dye (green curve) and CdTe/CdS quantum dot (blue curve). In the vicinity of 5 cm tissue slab, which can be considered a realistic thickness of a breast during mammographic imaging, the concentration of gold nanorod probes is approximately 109 per cubic centimeter or 1 $\mu\text{g/kg}$. Even though the concentration of nontoxic probes is low, the procedure will need to be experimentally validated in terms of safety.

Fig. 6. Sensitivity of gold nanorod labels vs. available fluorescence molecular labels and quantum dots. (a) Concentration of labels (per mm^3) needed to achieve $S/N = 10$. The concentration of gold nanorod label (red curves) is three orders of magnitude lower than that of NIR1 fluorescence dye (green curve) and CdTe/CdS quantum dot (blue curve). (b) Detectability of several common fluorescent molecular and quantum dot labels and gold nanorod labels (concentration of probes is 1 nM). Gold nanorod labels (red curves) provide more than two orders of magnitude increase in S/N over NIR1 and DDI fluorescence dyes (green curves) and CdTe/CdS quantum dot (blue curve).

Another way to evaluate the detectability of the probe is to calculate S/N as a function of tissue thickness. Here (Fig. 6b) gold nanorod labels (red curves) provide more than two orders of magnitude increase in S/N over NIR1 and DDI fluorescence dyes (green curves) and CdTe/CdS quantum dot (blue curve) for the same generic detector. More importantly gold nanorod labels can be employed at depths as great as 5 cm and still retain a S/N ratio of 10 which is approximately a factor of two deeper than fluorescent labels.

5. Discussion and conclusion

We see that nanorod scattering labels can achieve two to three orders of magnitude improvement in detectability compared to fluorescence labels, because of their extremely high plasmon resonant scattering. What is even more important, this improvement enables nanorod scattering probes to be detected in deep tissues where fluorescence probes perform poorly. Furthermore, nanorod labels can be tuned to any particular color simply by changing the aspect ratio, and thus could be used to image multiple molecular targets simultaneously. In the examples in the previous section the separation between two sets of nanorods is 80 nm which is several times the width of the plasmon resonance line.

It is important to emphasize that in scattering cross-section measurements, the contribution of the 520 nm transverse plasmon mode is significantly lower than in absorption cross-section measurements. Thus, biomedical markers based on scattering would have a better line shape by reducing the background signals and possible interference from the transverse peak.

Gold nanorod probes have several properties that make them especially suitable for *in vivo* application: because of their small size (approximately 100 times smaller volume than available quantum dots) nanorod labels can be delivered to various tissues in the human body via the vasculature and microvasculature; they are not toxic; they can be tuned for near-infrared detection wavelengths, which can propagate through several centimeters of tissue; finally, because of their extreme brightness, they potentially can achieve imaging sensitivity needed for single-cell detection in human organs.

Overall, gold nanorod light scattering labels with a single, well defined aspect ratio might provide important advantages over fluorescent probes for biomedical imaging.

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