

Gold Nanorods as Molecular Probes for In-Vivo Imaging *

Optical imaging encompasses a multitude of techniques for the elucidation of morphology, molecular function, and metabolism of tissue with the general objective of detecting, diagnosing, staging, and treatment monitoring of disease. Progression of disease is usually accompanied by changes in physiology and pathology that are manifested as location specific changes in optical properties thereby providing contrast for optical imaging to study disease.

Optical imaging techniques span the range from surface to bulk imaging systems with applications ranging from “optical biopsies” to full human breast imaging with resolutions that cover the microscopic to macroscopic. Some important imaging techniques for superficial tissue imaging are confocal microscopy [1], two-photon microscopy [2], and optical coherence tomography (OCT) [3]. Techniques that permit subsurface to deep imaging are diffuse optical imaging (DOT) [4] and photoacoustic imaging [5].

The interaction of visible and near-infrared (NIR) light with tissue is dominated by

(a) absorption processes which are due to the presence of various chromophores such as hemoglobin, oxyhemoglobin, melanin, water, and lipids [6];

(b) scattering processes due to the cell membrane and cell structures such as the nucleus, mitochondria, lysosomes [6].

Penetration of light in tissue is dependent on the extent of the two processes above and is low in the high-energy visible region of the spectrum. This is due to high absorption by hemoglobin and severe light scattering. In the wavelength regime between 600nm and 1100 nm, absorption and scattering losses are minimal permitting high-light penetration. This is the so-called “optical imaging window” which is exploited for deep imaging in tissue [7].

The sensitivity and specificity of optical imaging techniques to visualize a pathological disorder are governed by contrast: the ability of the disease to differentially scatter or absorb light compared with non-pathological tissue and background noise. This native or endogenous

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contrast may not be sufficient and in any case, the interactions of light with tissue are not disease-specific. Therefore, there is a role for exogenously administered contrast enhancing agents which have affinity for the disease site through biochemical interactions, providing not only sensitive but also disease-specific signals.

Contrast agents for optical imaging thus far have near infrared dyes based on cyanine dyes [8] such as Indocyanine Green [9], but in the last few years, gold nanoparticles [10–12] have emerged as prime candidates due to their unusual optical properties and inherent biocompatibility.

Gold metal nanoparticles (NPs) exhibit narrow and intense absorption and scattering bands due to the phenomenon of plasmon resonance. This occurs at the resonance condition of the collective oscillation that the conduction electrons experience in an electromagnetic field of the appropriate wavelength [13]. The plasmon resonant condition of gold NPs depends upon their size, shape, structure (solid or hollow), and upon the refractive index of the embedding medium. Spherical gold nanoparticles have a single plasmon resonant extinction peak at around 520 nm, which does not shift extensively with changes in size and refractive index of the surrounding medium. This is a wavelength at which light penetration in tissue is poor due to strong scattering and absorption by hemoglobin, and gold nanospheres are not useful in contrast enhancement for deep tissue imaging.

Rod-shaped NPs exhibit two plasmon resonances due to oscillation of the conduction electrons along the short axis as well as along the long axis of the particles. The former plasmon band is called the transverse resonance and the latter the longitudinal resonance. While the transverse plasmon band occurs in the neighborhood of 520 nm, the longitudinal band is red-shifted. The extent of the red-shift depends on the aspect ratio of the nanorod; the higher the aspect ratio, the further the shift. Thus by tailoring the length and/or width of these particles, their extinction peaks may be made to cover the low-energy visible to infrared wavelength regions.

The intense scattering and absorption of light, that occurs under the plasmon resonant condition coupled with the ability to tune the resonance into the near-infrared (NIR) by manipulating the aspect ratio, make gold nanorods extremely attractive as contrast agents for optical imaging techniques. Further, gold-protein chemistry is well developed and several bioconjugation protocols are available in the literature, which allows the combination of the targeting functionality of antibodies with such gold NPs. The inertness and biocompatibility of gold in general hold promise the use of gold NPs for in vivo imaging applications.

Potential contrast enhancing applications The scattering and absorption bands of the synthesized nanorods span the wavelength regime between 675–850nm that is of interest to optical imaging. This occupies the most important part of the “optical imaging window” where light penetration in tissue is high due to reduced scattering and absorption coefficients. Optical imaging techniques (Table 1) that rely on scattering and/or absorption contrast to detect pathological tissue could benefit from the use of such nanoparticles with or without targeting capability.

Table 1. Important optical imaging techniques that utilize absorption and scattering contrasts in biology and medicine.

| Technique | Imaging Depth | Imaging Resolution | Mechanism | Typical Imaging Applications |
|----------------------------------|---------------|-------------------------------------|-----------------------|--|
| Confocal Microscopy [1] | 500 um | > 250 nm | Scattering/Absorption | Tissue surfaces |
| Two photon microscopy [2] | 800 um | > 250 nm | Absorption | Tissue surfaces |
| Optical Coherence Tomography [3] | 2 mm | 1 um | Scattering | Surfaces/subsurfaces of tissue |
| Diffuse Optical Tomography [4] | > 20 mm | ~ 10% depth | Scattering/Absorption | Small animal; human breast and brain |
| Photoacoustic Imaging [5] | > 20 mm | < 1 mm (detector bandwidth limited) | Absorption | Subsurface to deep imaging; small animal; human breast |

Oraevsky and coworkers [26, 27] have recently proposed gold Nanorods as photoacoustic imaging labels. Photoacoustic imaging relies on optical absorption for its signals. When photons are absorbed, nonradiative de-excitation of the absorbed optical energy takes place with the release of localized heat. The local thermal expansion that results produces pressure transients [5]. When illuminated with pulsed laser light, a tumor site by virtue of its higher absorption with respect to the healthy background tissue, due to angiogenesis [28], will act as a source of bipolar photoacoustic pulses. This ultrasound propagates with minimal distortion to the surface where it is detected using appropriate wideband detectors. The time-of-flight, amplitude, and peak-peak time of the bipolar PA pulse possess information regarding the location, absorption, and dimensions of the source, thereby permitting a reconstruction of the tumor site [29, 30].

It is known that the NIR optical absorption contrast of tumors versus healthy tissue, measured using optical mammographic methods, is between 1.5 and 3. Clinical trials of optical mammography are being conducted worldwide but at present, it seems implausible that intrinsic contrast alone will provide sufficient sensitivity and specificity, and targeted contrast

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enhancement is likely to be required [31]. Since the same contrast mechanism of optical absorption is operative in photoacoustic imaging as well, a similar conclusion may be anticipated. An impression of the feasibility of using the nanorods synthesized for contrast enhancement is now discussed. The absorption cross-section of a nanorod at a wavelength, say 800 nm, is estimated using discrete dipole approximation (DDSCAT) simulations [32, 33] as $C_{abs} = 2.8 \times 10^{-14} \text{ m}^2$. A typical average optical absorption coefficient for an invasive ductal carcinoma is $\mu_a = 0.008 \text{ mm}^{-1}$ at 800 nm. In order to achieve contrast enhancement, a certain number density of gold nanorods is required to exhibit higher absorption than the intrinsic value and may be calculated as

$$\rho_{NR} \geq \mu_a / C_{abs} \quad (1)$$

This gives $\rho_{NR} = 2.8 \times 10^8 \text{ NR/cm}^3$. Further photoacoustic signals can be enhanced by a thermal nonlinearity mechanism to 3 orders of magnitude higher [34], then the modified number density of nanorods required is only $\rho_{NR} = 2.8 \times 10^5 \text{ NR/cm}^3$.

Published studies report that most tumor cell types express from 2×10^4 to 20×10^4 ErbB2 receptors/cell [12]. Let us assume arbitrarily that 2×10^3 of these sites per cell are occupied by conjugated nanorods. Further, if we assume that 1% of cells at a tumor site overexpress HER2 results in a figure of 2×10^6 cancer cells/ cm^3 . This will then lead to an estimation of the density of binding sites of the order of 10^9 cm^{-3} . Comparison of ρ_{NR} and the estimated figure of density of binding sites shows that contrast enhancement will be possible. Accumulation of the contrast agent at the tumor site will depend on enhanced permeation and retention (EPR) but have been shown to be successful in Photothermal therapy experiments [35].

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