Optical Imaging for In Vivo Assessment of Tissue Pathology

Rebekah Drezek
Rice University
Houston, Texas

Introduction

For hundreds of years, optical imaging at both macroscopic and microscopic levels has been used as a tool to aid clinicians in establishing a diagnosis. Pathologists routinely use a simple compound microscope to examine stained and sectioned tissue at the microscopic level to definitively determine a diagnosis of cancer. At a macroscopic level, clinicians often rely on observed colors as indicators of physiologic status, for instance, associating yellow skin with jaundice, blue or purple hues with cyanosis, or red areas with inflammation. In each of these examples, the human eye is used to gather *qualitative* information about the patient's status based on either the gross visual appearance of tissue or microscopic evaluation of stained tissue sections or cytologic samples. Despite the clear importance of such qualitative optical approaches in current medical practice, these strategies are sensitive only to a highly limited subset of the diverse array of optical events that occur when light interacts with biologic tissue. There is a compelling need for the development of more *quantitative* optical imaging strategies, which can probe tissue physiology in vivo in real time with high resolution and relatively low costs. This Frontiers in Engineering talk will describe emerging technologies for quantitative optical imaging and the use of these technologies to diagnose and monitor cancer. In particular, emphasis will be placed on how advances in nanobiotechnology are enabling new approaches to *in vivo* medical diagnostics. As another talk in the session will consider luminescence-based nanomaterials (i.e., quantum dots), here we will focus our discussion on nanomaterials which provide a scatter or absorption based optical signal. We will focus most strongly on gold-based materials as the general biocompatibility of gold coupled with extensive prior medical applications of gold colloid provides a somewhat more straightforward regulatory path towards ultimate clinical use than exists for many other nanomaterials currently under development.

A Role for Nanotechnology in Optical Imaging of Cancer

For over fifty years, cancer remained the second leading cause of death in the United States, accounting for over 25% of the deaths in the population. However, over the past two years, cancer mortality has exceeded deaths due to heart attacks, and cancer has become the primary cause of United States deaths. Early detection is well-recognized as a highly effective approach to reducing the morbidity and mortality associated with cancer today. When diagnosed at an early stage when the cancer is still localized and risk for metastasis is low, cancer is highly treatable with favorable prognosis. However, five year survival is quite poor across a wide variety of organ sites if cancer is diagnosed when metastasis to distant sites has already occurred (Table 1.) [1]. Thus, there is a significant clinical need for novel methods for early detection and treatment of cancer which offer improved sensitivity, specificity, and cost-effectiveness. In recent years, a number of groups have demonstrated that photonics-based technologies are valuable in addressing this need. Optical technologies promise high resolution, noninvasive functional imaging of tissue at competitive costs. However, in many cases, these technologies are limited by the inherently weak optical signals of endogenous chromophores and the subtle

spectral differences of normal and diseased tissue. Over the past several years, there has been increasing interest in combining emerging optical technologies with the development of novel exogenous contrast agents, designed to probe the molecular specific signatures of cancer, to improve the detection limits and clinical effectiveness of optical imaging. For instance, Sokolov *et al.* [2] recently demonstrated the use of gold colloid conjugated to antibodies to the epidermal growth factor receptor (EGFR) as scattering contrast agents for biomolecular optical imaging of cervical cancer cells and tissue specimens. In addition, optical imaging applications of nanocrystal bioconjugates have been described by multiple groups including Bruchez *et al.* [3], Chan and Nie [4], and Akerman *et al.* [5]. More recently, interest has developed in the creation of nanotechnology-based platform technologies which couple molecular specific early detection strategies with appropriate therapeutic intervention and monitoring capabilities.

Example Nanomaterial: Metal Nanoshells

Metal nanoshells are a new type of nanoparticle composed of a dielectric core such as silica coated with an ultrathin metallic layer, which is typically gold. Gold nanoshells possess physical properties similar to gold colloid, in particular, a strong optical absorption due to the collective electronic response of the metal to light. The optical absorption of gold colloid yields a brilliant red color which has been of considerable utility in consumer-related medical products, such as home pregnancy tests. In contrast, the optical response of gold nanoshells depends dramatically on the relative size of the nanoparticle core and the thickness of the gold shell. By varying the relative core and shell thicknesses, the color of gold nanoshells can be varied across a broad range of the optical spectrum that spans the visible and the near infrared spectral regions (Figure 1) [6, 7]. Gold nanoshells can be made to either preferentially absorb or scatter light by varying the size of the particle relative to the wavelength of the light at their optical resonance. In Figure 2, a Mie scattering plot of the nanoshell plasmon resonance wavelength shift as a function of nanoshell composition for the case of a 60 nm core gold/silica nanoshell is depicted. In this figure, the core and shell of the nanoparticles are shown to relative scale directly beneath their corresponding optical resonances. In Figure 3, a plot of the core/shell ratio versus resonance wavelength for a silica core/gold shell nanoparticle is displayed [7]. The extremely agile "tunability" of the optical resonance is a property unique to nanoshells: in no other molecular or nanoparticle structure can the resonance of the optical absorption properties be so systematically "designed."

Halas and colleagues have completed a comprehensive investigation of the optical properties of metal nanoshells [8]. Quantitative agreement between Mie scattering theory and the experimentally observed optical resonant properties has been achieved. Based on this success, it is now possible to predictively design gold nanoshells with the desired optical resonant properties, and then to fabricate the nanoshell with the dimensions and nanoscale tolerances necessary to achieve these properties [7]. The synthetic protocol developed for the fabrication of gold nanoshells is very simple in concept:

(1) grow or obtain silica nanoparticles dispersed in solution,
(2) attach very small (1-2 nm) metal "seed" colloid to the surface of the nanoparticles via molecular linkages; these seed colloids cover the dielectric nanoparticle surfaces with a discontinuous metal colloid layer,
(3) grow additional metal onto the "seed" metal colloid adsorbates via chemical reduction in solution.

This approach has been successfully used to grow both gold and silver metallic shells onto silica nanoparticles. Various stages in the growth of a gold metallic shell onto a functionalized silica nanoparticle are shown in Figure 4. Based on the core/shell ratios that can be achieved with this protocol, gold nanoshells with optical resonances extending from the visible region to approximately 3 □m in the infrared can currently be fabricated. This spectral region includes the 800-1300 nm "water window" of the near infrared, a region of high physiological transmissivity which has been demonstrated as the spectral region best suited for optical bio-imaging and biosensing applications. The optical properties of gold nanoshells, when coupled with their biocompatibility and their ease of bioconjugation, render these nanoparticles highly suitable for targeted bioimaging and therapeutics applications. By controlling the physical parameters of the nanoshells, it is possible to engineer nanoshells which primarily scatter light as would be desired for many imaging applications, or alternatively, to design nanoshells which are strong absorbers permitting photothermal-based therapy applications.

Because the metal layer of gold nanoshells is grown using the same chemical reaction as gold colloid synthesis, the surfaces of gold nanoshells are virtually chemically identical to the surfaces of the gold nanoparticles universally used in bioconjugate applications. The use of gold colloid in biological applications began in 1971 when Faulk and Taylor invented the immunogold staining procedure [9]. Since that time, the labeling of targeting molecules, especially proteins, with gold nanoparticles has revolutionized the visualization of cellular or tissue components by electron microscopy. The optical and electron beam contrast qualities of gold colloid have provided excellent detection qualities for such techniques as immunoblotting, flow cytometry, and hybridization assays. Conjugation protocols exist for the labeling of a broad range of biomolecules with gold colloid, such as protein A, avidin, streptavidin, glucose oxidase, horseradish peroxidase, and IgG. The vast prior history of gold colloid based materials has greatly facilitated development of biomedical applications of newer gold-based nanoparticles. As one example of the type of medical application enabled using this class of material**,** Figure 5 shows an ***in vitro*** proof-of-principle example of gold nanoshells designed to simultaneously scatter (for imaging) and absorb (for photothermal therapy) near infrared light. Here, scattering and absorbing NIR nanoshells are conjugated to an antibody for a common breast cancer surface marker. This enables both "lighting up" and if desired, destroying, cells which express this marker while other cells remain unharmed. In Figure 5, the top row shows scatter-based imaging of carcinoma cells. By increasing the laser power, it is then possible to selectively destroy the cells as shown in the middle row which depicts viability of cells (green = live cells) after laser irradiation. The left column shows a no nanoshell (cells only) control, and the middle column displays a non-specific antibody control. The right column indicates successful imaging of cells followed by photothermal destruction (black circle = laser irradiation spot) based on the presence of a chosen marker. Results using these same dual imaging/therapy nanoshells ***in vivo*** in animal tumor models will be presented in the talk. Although ***in vitro*** demonstrations can be completed using simple microscopes, ***in vivo*** use of this type of nanomaterials requires coupling development of appropriate materials with development of the optical devices which will enable imaging of these materials in tissue. By careful design of these optical systems, it is possible to generate multiple order of magnitude improvements in optical contrast which can be achieved using nanomaterial imaging agents potentially allowing detection of much smaller lesions. In addition to examples from our own group, work by other labs employing a variety of other gold-based nanomaterials will be discussed. In all cases, an emphasis will be placed on how the move

from *in vitro* cell-based demonstrations to *in vivo* clinical use is enabled by rapid developments in photonics-based strategies for real-time, low cost *in vivo* imaging

Summary

Numerous research groups throughout the country are leveraging emerging techniques in optical imaging and nanotechnology to develop powerful new approaches for detecting the molecular specific signatures of precancers and early cancers. These groups are developing several classes of ultrabright contrast agents which strongly scatter and/or absorb at tunable wavelengths throughout the visible and near IR spectral bands, as well as necessary methods to target these agents to molecular markers of neoplasia. They are demonstrating the efficacy of these agents in biological samples of progressively increasing complexity. These projects represent initial efforts which will be subsequently expanded in future studies. Ultimately, the use of these agents will extend the limits of detection of optical technologies, increasing the achievable sensitivity and specificity and promoting improved screening and detection of early lesions. We believe there is tremendous potential for synergy between the rapidly developing fields of biophotonics and nanotechnology. Combining the tools of both fields – together with the latest advances in understanding the molecular origins of cancer – provides a fundamentally new approach to detection of cancer, a disease responsible for over one quarter of all deaths in the United States today.

Note: Portions of the text and figures have previously been published. I will request reprint permission to use here.

References

1. Cancer Facts and Figures 2006. www.cancer.org. 2006.
2. Sokolov, K., Follen, M., Aaron, J., Pavlova, I., Malpica, A., Lotan, R., and Richards-Kortum, R. Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Research*. 63, 1999-2004 (2003).
3. Bruchez, M, Moronne, M, Gin, P, Weiss, S, and Alivisatos, AP. Semiconductor labels as fluorescent biological labels. *Science.* 281, 2013-2016 (1998).
4. Chan, WCW and Nie, S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science*. 281, 2016-2018 (1998).
5. Akerman, M.E., Chan, W., Laakkonen, P., Bhatia, S.N., and Ruoslahti, E. Nanocrystal targeting in vivo. *PNAS*. 99, 12617-12621 (2002).
6. Brongersma, M.L. Nanoshells: gifts in a gold wrapper. *Nature Materials*. 2, 296-297 (2003).
7. Oldenburg, S.J., Averitt, R.D., Westcott, S.L., and Halas, N.J. Nanoengineering of optical resonances. *Chemical Physics Letters***.** 288, 243-247 (1998).
8. Averitt R.D., Sarkar D., and Halas N.J. Plasmon resonance shifts of Au-coated Au2S nanoshells: insights into multicomponent nanoparticles growth. *Phys Rev Letters.* 78, 4217-4220 (1997).
9. Faulk, W.T., and Taylor G. An immunocolloid method for the electron microscope. *Immunochemistry*. 8, 1081-1083 (1971).

Table 1. Cancer Survival at Five Years as a Function of Stage at Diagnosis

Organ	Localized	Regional	Distant
Prostate	~100%	>85%	30%
Oral	>80%	50%	25%
Breast	>90%	80%	25%

Figure Captions

Figure 1: Visual demonstration of the tunability of metal nanoshells.

Figure 2: Optical resonances of gold shell-silica core nanoshells as a function of their core/shell ratio. Respective spectra correspond to the nanoparticles depicted beneath.

Figure 3: Core/shell ratio as a function of resonance wavelength for gold/silica nanoshells.

Figure 4: Transmission electron microscope images of gold/silica nanoshells during shell growth.

Figure 5: Dual imaging/therapy nanoshell bioconjugates.

Figure 1.

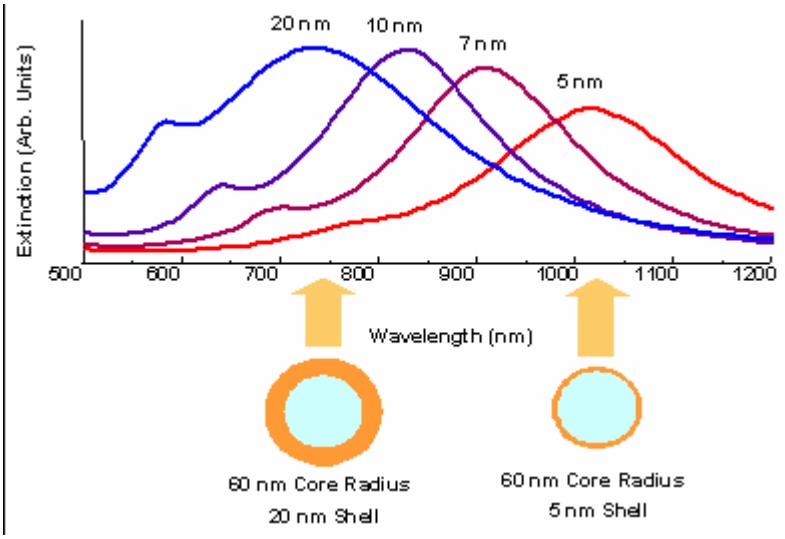
20 nm
10 nm
7 nm
5 nm
Extinction (Arb. Units)
500
600
700
800
900
1000
1100
1200
Wavelength (nm)
60 nm Core Radius
20 nm Shell
60 nm Core Radius
5 nm Shell

Figure 2.

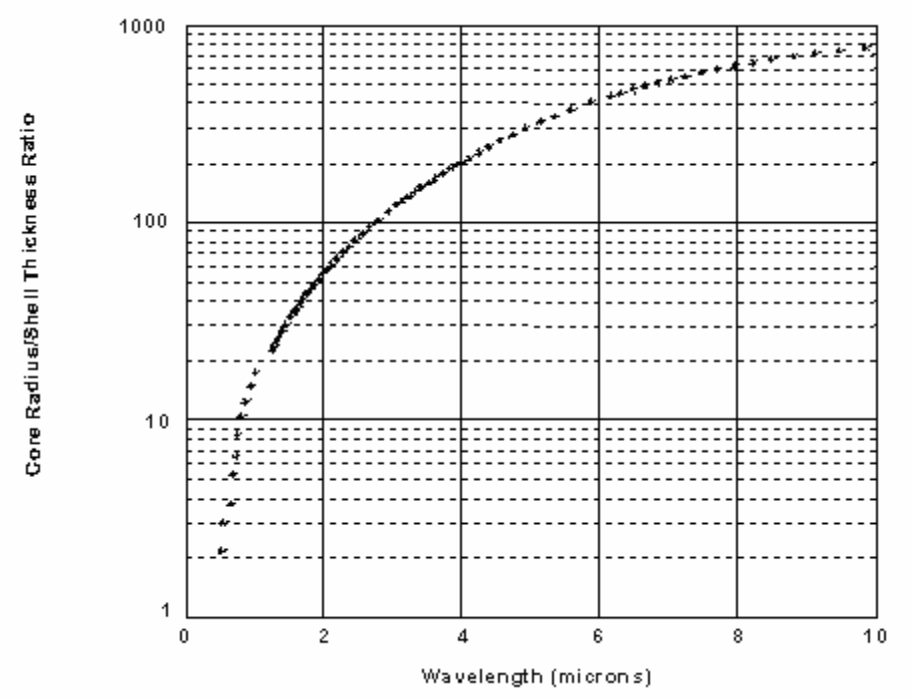
Core Radius/Shell Thickness Ratio
1000
100
10
1
0
2
4
6
8
10
Wavelength (microns)

Figure 3.

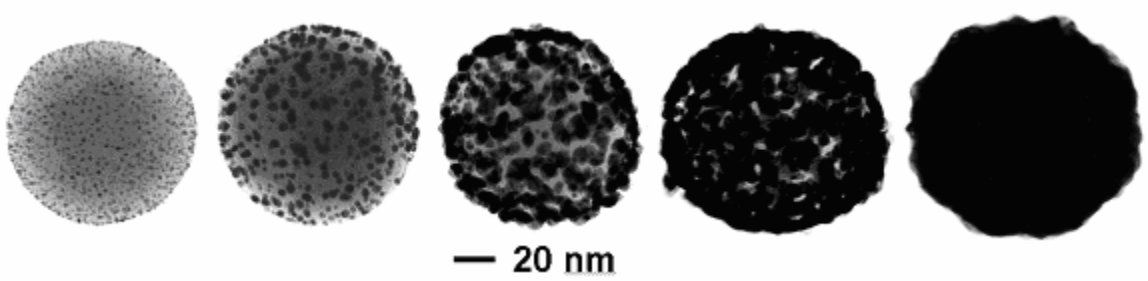

Figure 4.

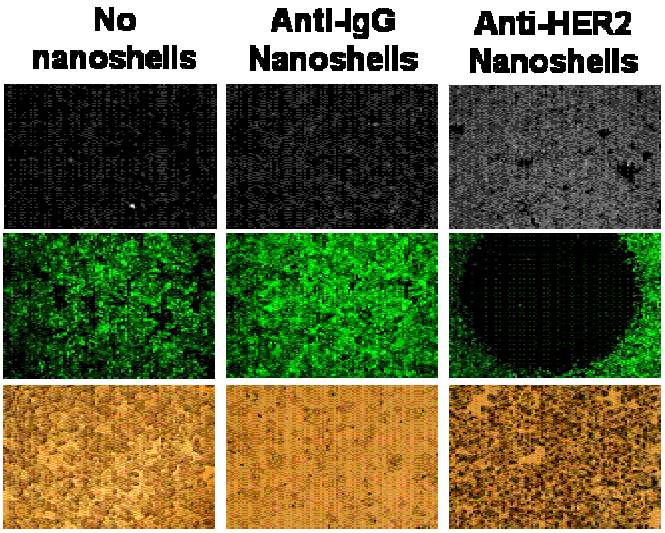

Figure 5.