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Radiance Spectroscopy Tool Box for Characterizing Au Nanoparticles in Tissue Mimicking Phantoms as Applied to Prostate

Serge Grabtchak^{1,2,3*}, Tyler J. Palmer¹ and William M. Whelan^{1,4}

¹Department of Physics, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI C1A4P3, Canada ²Department of Electrical and Computer Engineering, Dalhousie University, 1360 Barrington Street, Halifax, NS B3J1Z1, Canada ³Department of Physics, Dalhousie University, 6310 Coburg Road, Halifax, NS B3H3J5, Canada ⁴Atlantic Veterinary College, 550 University Avenue, Charlottetown, PEI C1A4P3, Canada

Abstract

Background: We have developed a new approach to map localized inclusions of gold nanoparticles in the Intralipid-1% liquid phantom. Our goal was to show that combined spectroscopic and angular snapshots of liquid phantoms and phantoms with inclusions allow obtaining information relevant for prostate cancer diagnostics and treatment.

Methods: A combination of the point radiance spectroscopy and white light spectroscopy was used to measure angular resolved light distribution in 450-900 nm spectral range in Intralipid-1% liquid phantoms with and without localized inclusions of gold nanoparticles.

Results: Characteristic spectro-angular snapshots of the liquid phantom alone and with the localized inclusion of gold nanoparticles were obtained. For liquid phantoms without inclusions, the snapshots demonstrate wavelength dependent light distribution inside the turbid medium, visualize the transparency window and provide a quantification of angular spread of different wavelengths of light. For liquid phantoms with gold inclusions, the approach allows to isolate the spectroscopic signatures of the inclusions from the background, identify locations of the inclusions in the angular domain and quantify the detection limits in terms of the contrast value attainable for the selected quantity of gold nanoparticles located at the specific depth in tissue. A detection of 3x10¹³ particles up to 25 mm deep in Intralipid-1% was demonstrated.

Conclusions: The encouraging results indicate a promising potential of radiance spectroscopy in prostate treatment and diagnostics with gold nanoparticles.

Keywords: Intralipid; Gold nanoparticles (Au NPs); Prostate; Radiance; Light scattering; Spectro-angular mapping

Introduction

Nanotechnology utilizes nanoscale-size materials that offer new attractive properties and is positioned to revolutionize cancer medicine [1]. For prostate applications, the use of gold nanoparticles (Au NPs) in particular, has advanced along four major fronts: 1) interstitial nanoparticle-mediated laser thermal therapy [2], 2) cancer diagnostics via delineation of cancer affected areas with NPs conjugated to monoclonal antibodies [3], 3) enhanced radiation sensitivity and toxicity in prostate cancer cells containing Au NPs [4], 4) brachytherapy implants of radioactive ¹⁹⁸Au NPs placed into a prostate gland to deliver the radiopharmaceutical dose directly to the tumor site [5].

Unless NPs are injected directly to the tumor and followed by an immediate application, delivery methods and the time elapsed between the incubation and the application possess a serious problem for establishing NPs presence. Whether one uses functionalized NPs with specific binding to tumor sites or non-functionalized NPs with preferential accumulation at tumor sites, the main challenge is "...the inability to visualize or quantify the global concentration or spatial distribution of these particles within the tumors" [6]. Registering Au NPs in the particular region of the tumor prior application would provide the first encounter with the affected area thus facilitating the following treatment procedure (including optimization of irradiation conditions for thermal therapy). Therefore, for all applications an ability to identify, locate and quantify fixed loads of Au NPs in the prostate gland via non-invasive or minimally invasive methods is highly desirable. Optical-based techniques use a non-ionizing radiation, and light penetration depth can be optimized based on wavelength dependent tissue optical properties. However, in turbid media (i.e., tissues) photons undergo multiple scattering, and exhibit a chaotic random walk, which results in a significant spread in spatial distribution. Hence, information about localized optical properties is difficult to decode from light-tissue data. The ultimate goal of all optical techniques is in developing approaches for extracting the information about local optical properties of turbid media.

Among the techniques that have been successfully employed to address this challenge is Diffused Optical Spectroscopy (DOS) [7]. The technique is based on collecting reflected light and extracting relevant optical properties by coupling measured optical data to a model for diffuse reflectance based on a diffusion approximation. This technique was able to extract the concentration of gold nanoshells in tumors,

*Corresponding author: Serge Grabtchak, Department of Physics, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI C1A 4P3 Canada, Tel: +1-902-566-6078; Fax: +1-902-566-0483; E-mail: sgrabtchak@upei.ca

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but it cannot provide any spatial information about their location [7]. Another technique, narrow band infrared imaging can visualize Au nanoshell distribution but only in superficial tissue layers (~ 1mm depth) [8].

Point radiance spectroscopy is an optical technique whereby light is collected within a small, well-defined angular cone thus providing information on angular light distribution at a selected point in turbid medium. Interestingly, the radiance-based light dosimetry was first applied to prostate interrogations [9]. A later work from the same group was devoted to optimization of modeling algorithms for extracting global optical properties of phantoms from radiance measurements [10]. Building on this we have demonstrated a potential for radiancebased tissue coagulation detection [11] and optical property determination [12]; and more recently, radiance-based detection of localized Au NPs inclusions inside Intralipid phantoms [13,14].

The scattering properties of the Intralipid-1% are similar to those of human prostate. The absorption coefficient of Intralipid-1% in the VIS-NIR range is from 0.01 to 0.1 cm⁻¹. This is somewhat lower than the absorption coefficient of human prostate which exhibits significant inter-organ and intra-organ variability that translates to a broad range of 0.1-1.6 cm⁻¹ as measured at 732 nm in prostate carcinoma [14]. It is well known that it is the highly scattering nature of biological tissues that is responsible for loosing optical resolution and increasing blurring of optical images with depth. Hence, it was our intention to isolate scattering from absorption of the prostate and focus mostly on scattering for the phantom studies. We performed measurements within a 5 cm area to mimic scattering over a prostate of average dimension. Once the developed methodology is applied to prostate studies, new detectability limits in terms of the minimal concentration and detection distance can be established because of the different absorption coefficient of prostate. However, the spectro-angular approach would remain valid.

In this work, a new approach to detecting a specific payload of Au NPs in turbid media based on a combined spectral and optical data set is presented. The experimental data are acquired in a tissue equivalent liquid phantom with scattering properties similar to human prostate. We obtained a complete spectro-angular characterization of the phantom material (Intralipid-1%), visualized experimentally the biomedical transparency window and its evolution with distance and mapped localized inclusions of Au NPs in Intralipid-1% simultaneously in the spectroscopic and angular domains. The ease of manipulation with the source, the detector and the Au NPs sample inside the liquid phantom allowed targeting sample's placement geometries relevant for the actual prostate.

Materials and Methods

Intralipid phantom and gold nanoparticles

Intralipid-1% served as a background optical medium. It was prepared by a volume dilution of Intralipid-20% (Sigma-Aldrich, Oakville, ON, Canada) stock solution. Water-based 5-nm diameter Au colloid with a stock concentration of $5x10^{13}$ particles/mL (Ted Pella, Redding, CA, USA) filled a 3.5-mm diameter quartz capillary with a volume ~0.7 mL that was completely immersed into the Intralipid solution. The immersion length was ~6 cm. Therefore, the estimated maximum number of Au nanoparticles that were detected was ~3x10¹³. The extinction coefficient of 5-nm Au nanoparticles is dominated by the absorption due the localized plasmon resonance at a wavelength of ~ 520 nm as shown on Figure 1.

Instruments

A detailed description of the experimental setup for radiance measurements has been published elsewhere [13,14]. Therefore, only essential details are given here. Figure 2 shows the experimental geometry (cross-section) that consists of a source fiber, a detection fiber and an Au NPs filled tube (i.e., the target). A blackened, 18 cm sided Lucite box was filled with Intralipid-1%. A tungsten halogen white light source (20 W) was connected to a fiber terminated with an isotropic spherical diffuser. The total power output from the spherical diffuser was estimated to be ~18 mW. A side firing fiber (Polymicro Technologies, Phoenix, AZ, USA) with a well-defined angular acceptance window (~10 degrees in water) served as a radiance detector. The side firing fiber was mounted on a computer-controlled rotation stage ensuring a full 360-degree rotation with a 2-degree step. The output of the radiance detector was connected to spectrometer (USB 4000, Ocean Optics, Dunedin, FL, USA) for spectral acquisition. Signal averaging was based on four measurements at each angular step requiring approximately 10





Figure 2: 2D cross-section of the measurement box showing relative arrangement of light source, radiance detector and gold sample. Dotted line outlines a contour of a virtual prostate. minutes to acquire a single complete angular profile. Positions of both fibers and the tube were independently controlled in the x,y-plane by three translation stages with a sub-mm positioning accuracy.

The positioning of the fibers/tube in the Intralipid-1% phantom was chosen to mimic a prostate application in which the radiance detector is placed in the urethra, the diagnostic white light source is placed in the rectum and the Au NPs target is located at a peripheral zone of the prostate (Figure 2). This represents an idealized, minimally invasive approach to radiance-based detection in prostate, but at the same time the separations between the radiance detector and the Au NPs target (~2.5 cm) and between the white light source and the detector (~ 3 cm) correspond to worst case scenario in terms of target detectability due to limited optical penetration in tissues.

Results and Discussion

Spectral and angular information is presented as a spectro-angular map, hence obtaining a snapshot of the medium under selected experimental conditions. Spectral profiles measured at every angular step in Intralipid-1% were assembled into the Intralipid matrix where columns corresponded to angles from 0 to 360 degree and rows were wavelengths from 450 nm to 950 nm. To exclude spectral variations due to the white light source and the detector, all radiance data obtained in the Intralipid-1% were divided by corresponding values in air. This ratio of the Intralipid matrix to the air matrix represents a relative radiance that reflects properties of Intralipid-1% only. This normalization approach is similar to standard spectroscopic measurements using a blank target. An example of the contour plot of the relative radiance in Intralipid-1% obtained at 16.5 mm sourcedetector separation is shown on Figure 3. Same color and contour lines correspond to regions of equal intensity. Areas of highest transmission are more red while areas of lowest transmission are blue. The plot is essentially a snapshot of the wavelength-selective angular distribution of light in Intralipid-1% at the chosen distance. It is easy to show that viewing the plot along the vertical direction at the fixed angle gives the transmission spectrum of Intralipid-1% at this angle (see the inset corresponding to a-a line in Figure 3). Similar, dissecting the plot horizontally provides the angular distribution of light for the selected wavelength in Intralipid (see the inset corresponding to b-b line in



Figure 3: Spectro-angular map of Intralipid-1% obtained at 16.5 mm sourcedetector separation distance (no gold sample). Inset a-a: cross-section along the vertical line as indicated. Inset b-b: cross-section along the horizontal line as indicated.







Figure 3). Notice a substantial (relative to the maximum) value of the backscattered light (at 180 degrees). The amount of the backscattered light increases with increasing the source-detector separation because it increases a probability of reversing a path for photons after successive multiple scattering events [9]. A 2D analysis of the contour plot provides the wavelength and the angle of the maximum transmission, i.e. ~550 nm and 0 degree, correspondingly. This is not surprising because Intralipid-1% is known to be a homogeneous isotropic medium with preferential forward scattering properties [14] and therefore, the maximum of the transmitted signal will be observed when the detector is facing the light source (i.e. at 0 degrees). Notice the water absorption feature that is delineated as a valley at ~750 nm on the contour plot shown in Figure 3.

A similar snapshot can be obtained in the prostate by placing the detector into the urethra and illuminating the prostate through the rectal wall. Also, using a standard brachytherapy grid can provide multiple interstitial locations for placement of the illuminating fiber adding



Figure 6: Spectro-angular maps of a localized inclusion of gold nanoparticles in Intralipid-1% for selected sample-detector separations: a) 5 mm, b) 10 mm, c) 15 mm, d) 20 mm, e) 25 mm.

more flexibility to illumination conditions. The prostate snapshot would carry information about spectral properties of the prostate gland prior to incubating it with Au NPs. The ability to achieve a complete 360-degree rotation is essential for the technique. While it is relatively easy to obtain it in phantom studies, clinical testing in prostate would require the development of a special catheter/endoscope coupled to a miniaturized rotation stage similar to what has been developed for optical coherence tomography (OCT) applications [15].

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Obtaining radiance measurements at various source-detector separations provides an opportunity to analyze the combined effects of absorption and scattering on light transmission in phantoms and tissues. When the diffusion approximation is valid, it can be expressed in terms of the well-defined effective attenuation coefficient [16]. The effective attenuation coefficient defines the biomedical transparency window in tissues [17]. We performed distance dependent radiance measurements by varying the source-detector separation from 6.5 mm (the closest distance achievable with the experimental apparatus) to 30.5 mm and keeping the detector facing the light source (corresponding to 0 degree). Then all measured values were normalized by the value of radiance measured at the shortest distance, i.e. 6.5 mm. The spectral variation of the radiance with distance is presented on Figure 4 in the form of a spectro-spatial contour plot (note a logarithmic scale for radiance values). One can see that transmission peaks become more sharp and well-defined with distance. With inherent scattering by lipid particles and absorption by two chromophores (Intralipid and water), the wavelength range from ~550 nm to ~700 nm appears to be the optimal transmission window for Intralipid-1%.

Figure 5 demonstrates the effect of wavelength-dependent light scattering in Intralipid-1% on the full-width half-maximum (FWHM) of angular profiles of light measured at the extremes of source-detector separations, 6.5 mm and 30.5 mm. The plot was obtained from analyzing sets of angular profiles similar to the one shown at the inset b-b on Figure 3. It is known that multiple scattering in turbid media tends to homogenize light distribution. Knowledge of wavelength-dependent spectral broadening is important when considering intended localized light-tissue interactions like drug illumination in PDT, selective tissue ablation in thermal therapy, optimized light delivery to gold nanoparticles in interstitial thermal therapy or detection of localized optical inhomogeneities in bulk tissues. Results from Figure 5 indicate that even after passing 6.5 mm distance in Intralipid-1%, light spreads over ${\sim}145$ degree angle for 450 nm and over ${\sim}100$ degree angle for 950 nm. Increasing the illumination distance up to 30.5 mm changes the values to ~170 degrees (450 nm) and ~135 degrees (950 nm). This information may be used when optimizing light delivery to Au nanoparticles in tissues. A current practice is to use fibers with isotropic cylindrical diffusers for illuminating tissues containing embedded Au nanoparticles. During a uniform 360-degree illumination, a significant portion of light does not interact with the target (i.e. Au nanoparticles) unless the illuminating fiber is inserted directly in the nanoparticles containing area. Using a side firing fiber as a light source, one can explore target illumination from a distant location that doesn't have to coincide with the target spot. It may result in a better light delivery and optimized power budget depending on the illumination wavelength and distance to the target. A similar optimization can be performed for drug illumination in PDT.

Radiance measurements with the Au NPs tube for target-detector separations of 5mm, 10 mm, 15 mm, 20 mm and 25 mm are shown on Figure 6. The source-detector separation was chosen to be 3 cm even though in the actual prostate the distance between the prostate and the rectal wall is ~2 cm. The extra distance accounts for the fact that Intralipid-1% is a weaker absorber than the prostate. Similar to the previous approach, two matrices were constructed. The first was the Intralipid matrix corresponding to the homogenous phantom. The second was the Intralipid+Au matrix corresponding to the phantom with the localized Au inhomogeneity. Since we were interested in absorption rather than transmission properties of the system, we constructed the ratio of Intralipid/(Intralipid+Au). Our blank in this

case was pure Intralipid and our sample of interest was Intralipid with the inclusion. The obtained ratio excluded spectral contributions from the white light source, detection system and more important, Intralipid. The only visible features were due to the sample, i.e. colloidal Au. The series of snapshots corresponding to different sampledetector separations are shown on Figure 6. These are raw data that haven't been subject to any post-processing. As one can see, the red area corresponds to the maximum absorption, and it is located in the vicinity of (-90)-degree value (the actual position of the sample). The most distinct changes occur between 5- and 10-mm separations. The first contour line that is somewhat close to a circle resides firmly at the (-90)-degree mark for 5-mm separation. Shifting sample to the 10-mm location produces two effects: first, the contour lines tend to acquire a more elliptical shape and second, the center of the wouldbe ellipse shifts away from the source towards (-110)-degree mark. All other sample locations demonstrate about the same degree of concentricity of contour lines and an additional 10-degree shift towards (-120)-degree location. It appears that the sample develops some sort of a shadowing effect when seen from a larger distance. We'd like to note that different sample-detector separations correspond to probing the same photon distribution around the sample but at some distance away from the sample. (Changing sample-detector distance produces also some variation in the sample illumination condition by changing the source-sample separation as one can easily envision from Figure 1. However, our additional measurements indicated that the variation in sample illumination doesn't play a major role in the observed effect.) The shadowing effect is a signature of the strong absorber in the highly scattering medium. Because of the ratio approach introduced in the article, we always compare light distribution in the vicinity of the sample with the one in Intralipid with sample absent. Placing the sample inside Intralipid extends the perturbation in light distribution beyond the physical dimensions of the sample. Due to multiple scattering events, photons that cross the sample's area can be found in a large cloud around the sample. Introducing the absorbing sample effectively removes the photons from the cloud they would be normally present and forms the area of the lower photon density that is perceived as the shadow. A remarkable fact is that even with the 30-degree angular uncertainty at larger distances, the angular location of the sample and its spectroscopic signature can be reliably indentified.

By comparing the maximum ("on-sample") and the minimum ("off-sample") radiance values along the wavelength of the maximum light-sample interaction (520nm, corresponding to the plasmon resonance), we obtained the contrast value for the specified concentration of Au colloid in Intralipid-1%. The contrast value decreased with increasing the sample-detector separation at the fixed angle of 90-degree as follows: 5 mm – 47%, 10 mm – 11%, 15 mm – 4.8%, 20 mm – 2.1%, 25 mm – 1.1%. The increase in separation spreads photons over a larger angular range and increases a portion of backscattered light thus leading to lower contrast values.

We were able to obtain the snapshots similar to those shown on Figure 6 for the sample placed at other angular locations. The contrast value changed with the angle for the sample-detector separation fixed at 15 mm as follows: 0-degree – 3.6%, 45-degree – 8.2%, 90-degree – 4.8%, 135-degree – 4.6%, 180-degree – 2.6%. Notice, that the variation is not as dramatic as for the distance dependent measurements. The numbers reflect a combined effect of a variation in the sample illumination condition and the preferential forward scattering. It bears a practical significance when translated to measurements in the prostate. Illumination via rectum would provide a similar non-

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uniform illumination of the prostate implying that localized inclusions of Au nanoparticles can be detected at any angular location when placing the radiance detector into the urethra. As was discussed earlier [14], the experimental set-up has a 2D symmetry. For the in-plane measurements, the infinite cylinder represents a localized inhomogeneity. Therefore, in the phantom experiments, Au NPs always occupy a well-defined cylindrical volume. This ideal geometry is needed to evaluate the effect of multiple scattering on target blurring in the plane of detector rotation and to identify signatures of Au NPs in turbid media. However, due to the inherent 3D nature of scattering, the interaction between light and a capillary with Au NPs can't be confined to in-plane. The actual concentration and distribution of Au NPs in prostate cancer would likely be quite variable and would depend on a number of factors including the tumour vasculature, nanoparticle size and method of delivery. Hence it would be expected that the overall shape of the Au NP distribution would extend nonuniformly in various directions. While the proposed technique can't visualize individual nanoparticles, it can detect localized AuNP concentrations. An implementation of the technique (via a vertical translation of the detecting fiber) for adding a third dimension in locating Au NPs distribution was proposed earlier [14].

Au NPs detection limits depend strongly on the values of the contrast ratio attainable at the specified depth for the specified density of Au NPs. For the used in this work stock solution of 5 nm Au NPs, all reported above values correspond to ~3x1013 particles. In the previous work [14] where different Au NPs were used, the detected volume ~0.7 mL of 3.8x108 particles/ml of 250 nm Au produced ~3.8x108 particles detected at the distance of 6.5 mm with the contrast value of 3-4%. In a good approximation one can safely ignore the difference in values of the absorption cross-section between 5 nm and 250 nm Au NPs and attribute the detection limits to the concentration only. Our detectability range for Au NPs includes for the example the amount of nanoparticles used in hyperthermia treatment of cancer in mice when 100 μ L of 2.4x10¹¹ nanoparticles/mL were injected intravenously [6]. Given a size difference, the payload to the canine and human prostate would exceed this amount. We'd like to stress, that the minimally detectable number of nanoparticles should be always considered in conjunction with a value of contrast ratio achieved at a particular depth in a specific tissue. We are currently working on developing algorithms for extracting the sample-detector distance information and a concentration of Au NPs from the measured data. Once achieved, it would provide a more complete characterization of localized Au inclusions in the prostate therefore addressing the remaining problems mentioned in the introduction.

Finally, we'd like to comment on the effect of the increased optical absorption in prostate tissues on detectability of Au NPs. It is known from Monte-Carlo modeling and radiance measurements in phantoms with different values of the absorption coefficient, that increasing the background absorption leads to less backscattered signal [9]. The result is a reduction of the overall spread due to scattering thus improving light confinement. We expect that spectro-angular maps of the tissue and of the inhomogeneity in the tissue (Figure 3 and Figure 6) will exhibit less angular spread in the actual prostate facilitating angular detection of the target. At the same time, a total detected signal will be reduced due to additional absorption of prostate that would require revisiting the power budget and establishing new conditions for detectability. This most likely would lower the contrast

ratio values, decrease the detection depth and increase the minimal detectable concentration. However, to some degree the changes can be compensated by appropriate adjustments in acquisition parameters (e.g. integration time and number of averages). Also, the biomedical transparency window of the prostate differs from that of Intralipid because of the presence of additional chromophores, hemoglobin in particular. In the current article, the choice of 5-nm Au NPs that absorb at 525 nm was dictated by a need to match the wavelength of plasmon absorption to the transparency window in the turbid medium under study, Intralipid-1% from ~550 nm to ~700 nm. The same matching requirement would call for using different Au NPs with plasmon resonance in the biomedical transparency window from ~750 nm to ~1000 nm of the prostate.

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In conclusion, we presented very encouraging results on detecting spectral and angular signatures of localized inclusions of Au NPs within 6.5 mm- 30.5 mm distance from the radiance detector in Intralipid-1%. The spectro-angular mapping approach described here can be extended to prostate applications.

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