Fabrication and characterization of biological tissue phantoms with embedded nanoparticles

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Abstract. Phantoms are imitations of biological tissue, which are used for modelling of the light propagation in biological tissues. Carrying out any biophysical experiments requires an indispensable constancy of the initial experiment conditions. The use of solid undegradable phantoms is the basis to obtain reliable reproducible experimental results. The fabrication of biological tissues phantoms containing high absorbance or fluorescence nanoparticles and corresponding to specific mechanical, optical properties is an actual task. This work describes development, fabrication and characterization of such solid tissue phantoms with embedded CdSe/ZnS quantum dots, gold and upconversion nanoparticles. Luminescence of samples with CdSe/ZnS quantum dots and upconversion nanoparticles were recorded. A sample of gold nanorods was analyzed using thermal gravimetric analysis. It can be concluded that the samples are well suited for experiments on laser thermolysis.

1. Introduction

Development of new kinds of cancer diagnosis and treatment methods requires extensive testing before the introduction into clinical practice. Using natural biological tissues instead natural human or animal tissue is practical for several reasons. Optical properties of the natural biological tissues depend on temperature [1, 2] and denaturation degree [3, 4] and may vary from source to source, change rapidly in various atmospheric conditions and quickly degrade over time [5]. Thus, the topical task of this work is the developing method to fabricate biological tissue phantoms with the specified optical properties that can be used as standard samples.

Despite there are a variety of liquid and solid phantoms, in carrying out experimental work preference is given to solid phantoms [6]. Solid phantoms consist of solid transparent polymeric elastic material with scattering and absorbing particles uniformly distributed in the matrix material. Creating those phantoms requires management methods of controlling its optical properties during manufacture [7]. To set scattering and absorbance coefficients and scattering anisotropy factor of phantom the special concentration and size of the scattering particles, such as TiO2, ZnO is used [8]. Thus, it is possible to create a phantom with the process of light diffusion which is identical to the process occurring in tissues.

Research on the therapeutic thermal effects of laser radiation on biological tissue, as well as plasmonic photothermal therapy[9] require both the identity of the optical properties of phantom and biological tissues, and the equivalence of the thermal properties[10]. However, it must be mentioned that the thermal properties of phantoms are slightly different from those of real skin: the value of skin thermal conductivity—for human epidermis, 0.209 Wm⁻¹K⁻¹; dermis, 0.293–0.322 Wm⁻¹K⁻¹ [11-13].
The purpose of this article is to provide a method for creating a phantom of biological tissue containing various nanostructures such as gold nanorods (AuNRs), upconversion nanoparticles (UCNPs) based on NaYF4: Yb, Er, and CdSe/ZnS quantum dots (QDs). And also to study optical properties, characterization and well whether thermolysis samples are suitable.

2. Materials and methods

2.1. Materials and equipment
Trisodium citrate dihydrate (ACS reagent, ≥99.0%), HAuCl₄ (ACS reagent, ≥99.0%), hexadecyltrimethylammonium bromide (CTAB) solution (ACS reagent, ≥99.0%), NaBH₄ (ACS reagent, ≥99.0%), solution of polyvinylpyrrolidone (PVP) (10 kDa, ACS reagent, ≥99.0%), isopropyl alcohol, aqueous ammonia (25%), tetraethoxysilane (TEOS-SH) (ACS reagent, ≥99.0%) were applied for synthesis of nanoparticles.

Spectrometer Ocean QEPro(Ocean Optics, USA), laboratory centrifuge (Eppendorf 5804, Germany), thermal analyzer Q500 (TA instruments, USA), and scanning electron microscopy (Mira II LMU, Tescan, UK) were used for characterization of the nanoparticles and phantoms with them.

2.2. Synthesis of nanoparticles

2.2.1. Synthesis of NaYF₄:Yb, Er
Nanoparticles NaYF₄:Yb, Er were synthesized by hydrothermal method using trisodium citrate as a capping agent. In relation to Er the Yb concentration was 30 mol% Yb and Er one was 5 mol%. The synthesized particles were rinsed several times with a dilute solution of trisodium citrate. After rinsing, the particles were heated in air for 1 h at 500°C.

2.2.2. Synthesis of gold nanorods
Synthesis of AuNRs was done according to early described protocol [14]. Absorbance spectrum and TEM image of synthesized AuNRs solution are shown in Figure 2.
2.2.3. Coating of gold nanorods

Obtained phantoms have a uniform distribution of the nanoparticles only if the nanoparticles are stable in acetone solution, thus it is needed to coat AuNRs by silica shell. Initial nanorods solution (50 mL) was centrifuged for 60 min at RCF equals to 12000g. The upper phase was discarded and the residue was left (1.5 mL). A solution of PVP of 30 mg per 50 mL of AuNRs was prepared. Solution PVP added to nanorods precipitate, made up to 100 mL in volume, was left for 12 min to take place the reaction. Then solution is centrifuged for 60 min at RCF equals to 12000g. After centrifugation the residue was left (4.5 mL).

Five PVP solutions were prepared by serial dilution (concentration of 8, 4, 2, 0.5, and 0.25 mg to 3g of isopropyl alcohol). In each solution was added 0.75 mL of concentrated aqueous nanorods coated with PVP and 0.5 mL ammonia. In 5 min after the addition of ammonia, 0.25 mL of TEOS-SH was added and kept for 40 min. Over time, whitening solution was observed. Then solution is centrifuged during 5 min at RCF 5000g and dissolved in ethanol, then centrifuged for 2 min at RCF 2000g and dissolved in ethanol, then centrifuged during 1 min at RCF 1000g, two cycles 1 min at RCF 2000g and 2 min at RCF 2000g. Finally result was ethanol solution of coating gold nanorods.

2.3. Fabrication of tissue phantoms

The components are optical epoxy casting compounds (PEO-90, Laboratory of polymer’s physics, Saint-Petersburg, Russia), acetone, isopropyl alcohol, nanoparticle aqueous solutions.

The method of fabrication consists in follows. Firstly, 0.2 mL of acetone is added to 1.2 mL of epoxy and heated to 55°C. Secondly, 0.2 mL of isopropyl alcohol is added. Thirdly, 0.3 mL of thickener is added. Fourthly, 0.2 mL of aqueous solution of nanoparticles is added. After adding of each new component mixture is mixed by magnetic mixer. Finally, the solution is heated to 75°C and keep day. If nanoparticles are stable in acetone they will be distributed regularly in the phantoms.

Figure 2. (a) – absorbance spectrum of AuNRs solution, (b) – TEM image of AuNRs.
3. Results and discussions
Luminescent properties of obtained samples were investigated. The sample made without nanoparticles has own weak luminescence spectrum, where the wavelength of maximum luminescence is 450 nm. The CdSe/ZnS quantum dots have a maximum of luminescence at 590 nm. The epoxy sample with CdSe/ZnS Nps shown luminescence in green region and have two maximums of luminescence (at 470 nm and 585nm). Created epoxy sample with CdSe / ZnS QDs causes distortion of the luminescence spectra of quantum dots (Figure 4).

Absorbance spectrum of AuNRs and luminescence spectrum of UCNPs are shown in Figure 5. According to these results, we can conclude that AuNRs distort the true spectrum of luminescence upconversion nanoparticles.

![Scheme of preparation of tissue phantoms containing nanoparticles.](image)

Figure 3. Scheme of preparation of tissue phantoms containing nanoparticles.

![Experimental luminescence spectra.](image)

Figure 4. The experimental luminescence spectra of CdSe/ZnS, polymer, polymer with CdSe/ZnS.

![Absorbance spectrum of AuNRs and luminescence spectrum of UCNPs.](image)

Figure 5. Absorbance spectrum of AuNRs and luminescence spectrum of UCNPs.

We have studied the thermal stability of the phantoms. A sample of gold nanorods was subjected to thermal gravimetric analysis (TGA). Operating method: the sample was heated to 450°C with 1°C per min step size. The temperature of phase transition to the gaseous state was determined.
4. Conclusion

Using solid phantoms provide repeatability of the initial experimental conditions, the statistical validity of the results, and no degradation of the test system under the influence of external factors that allows carry out a series of experiments on the same sample. The optical properties of the phantoms can be easily customised for any type of biological tissue by varying the concentration of the scattering particles. Thermal properties of phantoms play a key role in carrying out the experiments on laser thermolysis.

According to our experiments, creating phantom distorts the luminescence of the quantum dots. Own weak luminescence of the sample causes small distortions in the measurement. Phantoms containing gold and upconversion nanoparticles well suited for experiments on laser thermolysis. Received phantoms have high temperature of degradation and resistant to thermal stresses. Thus, these samples can be used as biological tissue phantoms for experiments on laser thermolysis.

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Figure 6. Results of TGA of phantom sample loaded with gold nanorods.