Summary in English

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The main reason prompting me to undertake the research topic described in this dissertation was the lack of efficient inorganic nanoscale luminescent agents which would be suitable for use in three-dimensional imaging techniques and simultaneously in therapy. The emergence of nanotechnology has offered an opportunity to draw diagnostics and therapy closer. However, in most cases nanoparticles based imaging has been investigated separately from therapy. In this work I wanted to design, synthesize and characterize, on many stages, a nanoagent which would allow imaging to be performed not only before or after, but also during a treatment regimen. I am hoping that the nanoagent created by me will bring in the future both imaging and therapy closer to each other, ultimately, the boundary between them will be blurred.

During my work I was manipulating the matter which had at least one dimension sized from 1 to 100 nanometers, thus this work belonged to the field of science that is called nanotechnology. The origin of nanotechnology concept can be found in the talk presented by a renowned physicist Richard Feynman in 1959 entitled: "There's Plenty of Room at the Bottom", in which he described the possibility of synthesis via direct manipulation of atoms. In 1974 Norio Taniguchi used for the first time a term "nano-technology", which since that time has been widely known and used. The "bottom-up" and the "top-down" approaches are the main ways of building of materials and devices used in nanotechnology. Nowadays, there is huge interest and big debate about future implications of nanotechnology. It is believed that this field of science will have an enormous impact in creation new materials and devices with a vast range of applications: in medicine, biomaterials, energy conversion, date storage, electronics, and consumer products. Some of them we can already see on the market. On the other hand, we should remember that nanotechnology struggles with the same problems as any other technology, such as: toxicity, environmental impact, production efficiency as well as the causes of disasters, economical and ecological. Much work has to be done in order to solve these problems.

One of the branches of nanotechnology is nanoplasmonics which is the study of optical phenomena on the nanoscale, in vicinity of metal surfaces. In 2011 Mark Strockman wrote

in a futuristic article "The field of nanoplasmonics in young but rich in phenomena that inspire practical use in physics, biomedicine, environmental monitoring, and national security".

Coming back to the past, since Middle Ages people have been interested (although they were not aware of the actual physical details of the effect) in the optical effects that arise from resonant properties of plasmonic metal nanoparticles which absorb and scatter light at optical frequencies visible for the naked eye, this property was used to decorate e.g. the famous Lycurgus cup, which can be seen in the British Museum. Another example of the use of nanoparticles is stained glasses, giving the magnificent appearance in Paris in sunny days of the Sainte Chapelle.

Nowadays, the most commonly exploited effect of surface plasmon resonance is the shift in the plasmon band caused by aggregation of metal nanoparticles, used in sensing. Other applications use the enhancement of local optical fields on tiny metal particles due to the large number of electrons that coherently contribute to surface plasmon resonance. In many cases we are looking for such applications of nanoparticles that may greatly benefit humankind. One of the most important potential uses of gold nanoparticles is the thermal phototherapy. Because of the compatibility with human cells and low toxicity, the most commonly used nanoparticles are gold nanoshells (NSs) composed of a dielectric core coated with a gold shell, where the extinction band can be easily shifted into the near-infrared region where biological tissues do not absorb light and are not damaged by the irradiation. NSs can be passively accumulated in cancer cells, or they can be targeted to cancer antigens. Due to strong absorption of the near-infrared light, they generate heat that kills tumor cells but leaves healthy tissue unharmed. Phototherapy based on the nanoshells is currently undergoing clinical trials on human patients. Because of smaller sizes and better surface plasmon band tunability, gold nanorods (NRs) are also seen as having a big potential in theranostics application. The ratio of absorption to scattering is bigger here than for nanoshells thus nanorods are more efficient in converting light to heat than NSs.

Before the decision which type of theranostics nanoparticles are to be used, it is necessary to carefully examine all factors, starting from the synthesis of a variety of sizes and shapes of nanoparticles, like NRs and NSs, purification of the samples, in order to obtain mostly uniform samples. It is necessary to analyze the possible modification of properties when the nanoparticles start to self-assembly and "hot-spots" are created in spaces between nanoparticles which may have further use in sensing by SERS techniques. The characterization of the nanoparticles should be followed by determination of their linear and nonlinear optical properties. This is an important part of the present Thesis because there are very few publications providing quantitative data about third-order NLO properties of plasmonic nanoparticles. In particular there is little information on those properties determined in wide wavelength range with the use of low repetition rate laser systems, which is necessary in order to eliminate the effect of heat accumulation. Additionally, it can also enrich our knowledge if the obtained data on two-photon absorption cross sections of nanoparticles are compared with other efficient nonlinear absorbers e.g. organic dyes. And finally, one needs to explore the use of the nanoparticles, possessing chosen size and shape for biofunctionalization and application in theranostic treatment.

This dissertation shows the experimental results, which I strongly believe prove the possibility of application the proposed bioprobe in theranostics treatment. The advantages and disadvantages of the probe were discussed on the basis of imaging of cancer cells, toxicity and fluorescent efficiency. It is important to mention that the process of synthesis of the biomarker was controlled on each step, starting from the selection of appropriate size and shape of the core, through optical characterization, effective way of biofunctionalization and finally application in cell visualization.

At first, I presented an improved method of separation of distinct shapes of gold nanoparticles from a heterogeneous mixture. The method of centrifugation in a glucose density gradient was applied in order to get homogenous fractions. The procedure of sample preparation, centrifugation and collection of the separated nanoparticles is described. Moreover, I discussed the synthesis with and without Ag^{+} ions added to the growth solution. Synthesis with Ag^{+} enables better control of shape and size and results in homogenous size NRs. I believe that this report may provide valuable information how to prepare morphologically mono-disperse gold nanoparticles. Homogeneity and well-controlled size of the nanoparticles were the base for the next steps of research, dedicated to self-assembly of nanoparticles and studies of NLO properties of gold nanoparticles.

Then, I had a closer look on transferring procedure of the NRs from water into IPA solvent, which induce self-organization of the nanoparticles. Optical characterization as well as recorded ATR spectra gave the foundations to understanding of the assembly process taking place. Additionally the work is enriched with the theoretical calculations indicating that individual self-assembled nanostructures show strong light polarization dependent properties. The electric field localized in the gap between NRs is estimated to be enhanced over 350 fold. These properties can be applied in detection of biomolecules by the SERS technique as well as in building blocks for optical materials.

In the next part of my thesis I have performed a systematic and quantitative description of the interactions of NRs with light (femtosecond laser pulses, 130 fs, 800 nm) in order to characterize the optical properties and design NRs with specific functionalities. In this work I focused on the investigation of structural changes of the NRs and the parameters influencing the reshaping, like surface modification using sodium sulfide, laser power and the position of the longitudinal surface plasmon resonance band (l-SPR) with respect to the laser wavelength. Additionally, a thermogravimetric analysis experiment was performed to examine changes in the composition of the NRs upon heating. The obtained results showed that NRs treated with Na2S were more stable during illumination, compared to NRs with the l-SPR band placed in a similar range, but with unmodified surfaces. When NRs were illuminated with a laser wavelength on the slope of the l-SPR band, the size distribution and the l-SPR bands became narrower, which can also provide an alternative method for obtaining mono-disperse size distribution of nanoparticles. The experiment presents a procedure of obtaining a new type of banana-shaped nanoparticles, based on post-synthesis reshaping. In order to acquire this shape it is necessary to illuminate the sample at the wavelength corresponding to the maximum of the l-SPR band (at 3.6 mJ/cm^2), the fraction constituted approximately 10% of the nanoparticles. Concluding, the results obtained in this experiment confirm the significant contribution of the surface melting in the photoinduced transformation of gold nanorods as well as show the production of new shapes of gold nanoparticles by laser irradiation under controlled conditions. I was also able to calculate the threshold of nanoparticles reshaping, in order to know the energy per nanoparticles below which one should work in order to preserve undisturbed the nanoparticle morphology.

In the next part of the thesis I have quantified the probability of simultaneous absorption of two photons by plasmonic nanoparticles: gold nanorods and gold nanoshells, and by several dye molecules, by using the open-aperture Z-scan technique available in the laboratory at WUT in Poland. The data were taken over a broad wavelength range (530-1600 nm), the two-photon cross-section results being given in Goeppert-Mayer units (1GM = 10-50 cm^4 s $photon^{-1}$ $molecule^{-1}$). At first, I started from fabrication of stable and highly monodisperse NSs suspensions in water, with a varying degree of gold coverage. Then, the NLO properties of the nanoshells were quantified in terms of the two-photon absorption coefficient (α_2), the nonlinear refractive index (n_2), and the saturation intensity for one-photon absorption (I_{sat}), which are extensive quantities. Then I calculated the two-photon absorption cross-section (σ_2) taken per nanoparticle, which was also interpreted in terms of the merit factor σ_2/M (where M is the molar mass of the nanoparticle), the quantity suitable for comparisons with other types of nonlinear absorbers. The obtained results on NSs with various shell thickness, showed that gold nanoshells demonstrate a number of features that may be useful in practical applications, especially those involving the absorption saturation. Curiously, the most advantageous combination of the relevant parameters is obtained for the thinnest gold shells. Then, I made a comparison of the NLO optical properties of colloidal NSs and NRs in water suspensions, determined by the same open- and closed-aperture Z-scan technique. The studies were carried out paying attention to use laser pulse energies well below the threshold of nanoparticle melting, which I estimated in a previous section, so NSs and NRs were stable during laser irradiation. Additionally, the data are enriched with the theoretical studies, giving a view onto the contributions of absorption and scattering to the total extinction spectra, for all synthetized gold nanoparticles. From the performed research one can conclude that the nanorods seem to be more efficient, per unit mass of gold, than the nanoshells as two-photon absorbers. However, due to higher absolute values of σ_2 the nanoshells can still possess an advantage over the much smaller plasmonic nanoparticles with other geometries. Finally, the NLO properties of plasmonics nanoparticles were compared with the ones obtained for new organic dyes, in order to have necessary prerequisites for further research in technological applications making use of interaction of light with matter. It is important to mention that all studied dyes have three distinct structural elements: electron-donating (D) groups, electron-accepting (A) groups and conjugated π-bridges connecting donor and acceptor moieties, which increase the probability of absorption of two photons. Briefly, the main purpose of the investigation described in this chapter was to quantify the NLO properties of the studied samples and then classify them for appropriate applications such as: photonics, nanophotonics, biophotonics, cancer treatment or data storage.

Finally, in the last chapter I have combined the results and knowledge from all previously described experiments in order to propose a new bioprobe. The probe is based on NR functionalized by DNA strand with attached fluorophore. The distance between gold surface and dye is selected in a such way as to maximize the fluorescent emission. The viability tests show low toxicity for cells and high compatibility. I showed that biofunctionalized NRs can provide fluorescent labeling of cancer cells and enable effective photothermal therapy. This is one of the first demonstrations of coupling a bioimaging application to a cancer therapy application using NRs targeted against a clinical relevant biomarker. I hope that the future studies will extend the in vitro concept demonstrated here to in vivo animal experiments.