

Spectral, spatial and temporal properties of multilayered epithelial tissue in vivo in presence of metal nanoparticles in multimodal spectroscopy

Maria Kholodtsova

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Spectral, spatial and temporal properties of multilayered epithelial tissue in vivo in presence of metal nanoparticles in multimodal spectroscopy

THESIS

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Mis en page avec la classe thesul.

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Whenever you find yourself on the side of the majority, it is time to pause and reflect.

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General Introduction

The important thing is not to stop questioning. Curiosity has its own reason for existing.

Albert Einstein

This thesis represents the work which was done in collaboration between two laboratories: The Laser Biospectroscopy Lab. of A.M. Prokhorov General Physics Institute (Moscow, Russia) and the Research Center on Automatic Control of Nancy of University of Lorraine and National Research Scientific Center (Nancy, France).

The aim of this thesis is to improve optical diagnosis abilities of pre-cancer states of epithelial tissue by means of metal nanoparticles and understand the connection occur between a molecule of a photosensitizer and a nanoparticle.

1 Framework and Objectives of the thesis

The strong non-invasive, non-radiative and real-time potentials of biophotonics approaches and tools to address the question of *in vivo* biological tissue characterization is at stake in current clinical application issues such as tumor border delineation (surgical guiding) or healthy *vs.* pathological tissue optical property estimation with acceptable error. Furthermore, recent years progresses through interdisciplinary collaborations between chemistry, biology, medicine and physics allowed to synthesize nanoparticles of acceptable size distribution and to use them with bio-conjugated molecules to increase the abilities of photodiagnosis. The aim of the present thesis was to investigate the influence of nanoparticles embedded into biological tissues on their optical properties in order to provide deeper and/or more precise probing. To do so, the integral spectroscopic parameters and lifetime of fluorophore in vicinity of metal nanoparticles were analyzed theoretically and experimentally. Another part of the study was to propose new algorithmic solutions for improving the performance of the estimation process of the optical properties values from spatially resolved spectroscopic measurements.

2 Main Contributions

2.1 Experimental modeling/characterization of nanoparticles optical properties

We started from an idea of enhancing or quenching fluorescence signal by means of nanoparticles, this could be done by changing the optical properties on certain wavelength. Indeed, if a nanoparticle has its Local Plasmon Resonance (LPR) at the absorption and emission wavelength of a fluorophore, then energy transfer could occur and tissue might be probed deeper. To prove this, firstly, the optical properties of nanoparticles were obtained through an approach combining experimental measurements and theoretical modeling. Experimentally, extinction spectra of Gold NanoSPheres (GNSP) and NanoStars (GNS) were obtained by means of spectrophotometry. The size of GNSP was determined by dynamic light scattering measurements (goniophotometry), and was compared to available up-to-date semi-empirical models of size and concentration dependencies on optical density of nanoparticles derived from theoretical and experimental data. Thus for the experiment GNSP of 14 nm in diameter and GNS of 50 nm in diameter were chosen. The extinction maximum for GNSP was 523 nm (close to the absorption band of ProtoProphyrine IX (PPIX)), and 700 nm for GNS (close to the emission band of PPIX). Theoretically, optical properties of nanospheres of various diameters and different materials embedded in dielectric media were modeled based on experimental data of Johnson and Christy Johnson and Christy, 1972] and on theoretical approaches of several teams which took into account both interband and intraband transitions, namely the Drude model of free electrons and the bounded electrons mode, respectively. The dielectric permittivity data for several materials (gold, silver, ferric oxide (II)) served for the numerical analysis using both the Mie theory and the Finite-Difference Frequency-Domain (FDFD) method.

2.2 Influence of nanoparticles on the optical properties of biological tissues

The influence of nanoparticles on the spectroscopic macroparameters was investigated using the numerical simulation approaches complementary to the phantom-based experiments, using spatially resolved spectroscopic Diffuse-Reflected Signals (DRS), Fluorescence Signals (FS) and Fluorescence Indices (FI). We were interested in colloidal nanoparticles, not connected to photosensitizers by spacers or being attached directly to a surface of nanoparticles, to investigate the difference in the mentioned above signals and their enhancement or quenching by nanoparticles at variable concentration. Hereby, the multi-layered tissue phantoms were made to analyze the influence of the presence of nanoparticles on the measurements performed by spatially-resolved UV-Visible spectroscopy, i. e. DRS, the FS and the FI. The phantoms were made of two layers mimicking epithelium (top layer) and dermis (bottom layer) optical properties. The top layer was liquid with variable thickness, while the second layer was made solid to avoid mixing between both layers during fabrication and measurements. Bottom layer had variable concentrations of nanoparticles and a constant fluorophore concentration. The spectra were collected by the multifiber optical probe, developed for spectroscopic analysis of biological tissues. Such preliminary analysis showed that for 2 mg/kg concentration of the nanoparticles the fluorescence intensity signal increases twice. More detailed analysis was needed to explain enhancement and quenching of fluorescence integral signal and of fluorescence index as well (See II.d).

2.3 Inverse problem solving to estimate the optical properties of tissues from spatially resolved spectroscopic measurements

Precise and robust estimation of biological tissue optical properties is a key factor in the development of optical spectroscopic and imaging methods and in their applicability to non-invasive diagnosis in clinics. Promising techniques, such as UV-Vis-NIR, optical coherence tomography, and confocal laser-scanning microscopy have already proved significant results in biological tissue characterization and diagnosis. Among these methods, Spatially-resolved Diffuse Reflectance (SDR) spectroscopy has been investigated for about two decades as a valuable optical biopsy tool. Complex media, like biological tissues, provide a possibility to characterize precancerous and early cancerous changes arising in it before evolving towards to malignant lesions. The question of tissue optical properties estimation is in solving an inverse problem which is classically ill-posed (different sets of parameters may lead to close similar data set) and ill-conditioned (numerical instability of the solutions due to finite precision and errors in the data). In this framework, firstly, a mathematical modeling of the physical phenomena light-tissue interaction has to be chosen to represent the forward problem. A new methodological approach to estimate more precisely the values of the optical properties of biological tissue from spatially-resolved spectroscopic measurements was developed. Firstly a population-based stochastic approach (Particle Swarm Optimization, PSO) was applied in the frame of the inverse problem solving as an optimization algorithm dealing with the statistical noise inherent to MC simulated data and with their wavelength-resolved dimension. An in-line regularization-like correction was then proposed to improve the optimization scheme performance by implementing a fitting algorithm over wavelengths. This algorithm involves an *a priori* wavelength curve shape fitting (here exponential decay) of two points $(\mu_s; \mu_a)$, having the lowest error at the moment. The consequent acceleration of all the groups of particles to the 'best' curve leads to error decrease in the optical properties of biological tissue estimation. The study considers analysis of estimated optical properties error dependence on cost-function error. This approach led to errors less than 6% between optimized and ground truth scattering and absorption coefficient values. Known spectral shapes of other components such as oxygenated and de-oxygenated hemoglobin can be implemented.

2.4 Influence of the presence of nanoparticles on the fluorescence lifetime

The theoretical models taking into account different types of coupling between nanoparticles and fluorophores were considered. Field enhancement due to plasmonic resonance was theoretically calculated for different separations between nanoparticle dimers of different materials; the geometry, the sizes of particles and the distance between centers were variable. The media influence on enhancement was also analyzed. The field enhancement occurred on the smallest and on the closest distances from nanoparticles and the resonant peak was red-shifted with increase of medium refractive index. The experiments on lifetime measurements were held by means of a picosecond laser and with a streak-scope as a detector. During these experiments, by decreasing the average statistical distance between the nanoparticle (increasing the concentration of nanoparticles) and the fluorescent molecules of PPIX a short fluorescent life-time component was detected from fluorescent signal time-resolved measurements and analyzed. The experiment conducted revealed decrease of the picosecond component, which appeared due to the energy transfer between the photosensitizer molecules themselves and, in the vicinity of the nanoparticles, was influenced by them. The Gersten-Nitzan and the NanoSurface Energy Transfer models, both taking into account size-dependence of the dielectric function due to nano size of a metal, were considered. Moreover we were able to resolve 2 isomers of PPIX photosensitizer during our integral fluorescence measurements and to reveal the kinetics of PPIX isomers fluorescence life-time in the vicinity of gold nanoparticles. On the certain concentration, the 3^{rd} ultra-short () component occurs, which is due to strong connection between nanoparticle and fluorescent molecule, resulting in fluorescence quenching, i. e. reducing of the PPIX dipole moment by nanoparticle's charges.

3 List of scientific publications (2011-2016)

3.1 International journals

- IJ1 M.N. Kholodtsova, P.V. Grachev, T.A. Savelieva, N.A. Kalyagina, W.C.P.M. Blondel and V.B. Loschenov (2014) Scattered and Fluorescent Photon Track Reconstruction in a Biological Tissue, International Journal of Photoenergy, 2014:517510,doi:10.1155/2014/517510 In preparation:
- IJ2 M.N. Kholodtsova, V. Loschenov, C. Daul and W.C.P.M. Blondel (2015-2016) Spatially and spectrally resolved particle swarm optimization approach to increase precision in optical properties estimation by means of tissular diffuse-reflectance spectroscopy, Optics Express (accepted, April 2016)
- IJ3 M.N. Kholodtsova, P.V. Grachev, W.C.P.M. Blondel, P.A. Zelenkov, A.A. Potapov and V.B. Loschenov (2015-2016), Spatially-resolved optical spectroscopy abilities of stereotactic biopsy cannula verified by optical multi-fiber probe on two-layered phantoms containing metal nanoparticles for fluorescence enhancement or quenching, Journal of Biomedical Optics (submission targeted by middle of 2016)
- IJ4 M.N. Kholodtsova, I.R. Romanishkin, W. C.P.M. Blondel, and V.B. Loschenov (2016), Picosecond lifetime of fluorophore investigation due to presence of plasmonic metal nanoparticles, Biomedical Optics Express (submission targeted by middle of 2016)

3.2 International conferences

- IC1 M.N. Kholodtsova, V.I. Makarov, I.D. Romanishkin, W.C.P.M. Blondel, V.B. Loschenov (2016) Colloidal gold nanoparticles change energy transfer standard scheme of a photosensitizer, St. Petersburg, Laser Optics, 27 June-1 July (submitted)
- IC2 M.N. Kholodtsova, W. C.P.M. Blondel, Ch. Daul and V.B. Loschenov (2015) Particle swarm optimization combined to spectral fitting approach improves precision for parametric estimation of tissue optical properties, In Proceedings of the SPIE Biophotonics Japan International Conference, Tokyo, October 27 – 28 (oral)
- IC3 M.N. Kholodtsova, I.S. Samsonova, W. Blondel and V.B. Loschenov (2015) Metal nanoparticles of different shapes influence on optical properties of multilayered biological tissues, In SPIE Photonics Europe: Medical Laser Applications and Laser-Tissue Interactions VII, 9542, pp.954, doi:10.1117/12.2182575 (oral)
- IC4 M.N. Kholodtsova, V.B. Loschenov, Ch. Daul and W.C.P.M. Blondel (2014) Preprocessing method to improve optical parameters estimation in Monte Carlo-based inverse problem solving, In SPIE Photonics Europe 2014 – Biophotonics: Photonic Solutions for Better Health Care, Brussels, Belgium, April 14-17, 9129, pp.1–9, https://hal.archivesouvertes.fr/hal-00968891 (oral)
- IC5 M.N. Kholodtsova, V.B. Loschenov, Ch. Daul and W. Blondel (2014) Particle swarm optimization algorithm for Monte Carlo-based inverse problem solving, In LO 2014 – 16th Laser Optics International Conference 2014, 3rd International Symposium on Lasers in Medicine, St. Petersburg, Russia, June 30 – July 4 (oral)

- IC6 M.N. Kholodtsova, V.V. Volkov, V.B. Loschenov, A.Yu. Titov and E.E. Zharkov (2012) Coagulation zone determination in blood-filled biological tissue, In 19th International Laser Physics Workshop (LPHYS)'12, Canada, Calgary, 21-29 July (oral)
- IC7 T.A. Savelieva, N.A. Kalyagina, M.N. Kholodtsova, V.B. Loschenov, S.AG. Goryajnov, A.A. Potapov (2012) Numerical Modeling and in vivo analysis of fluorescent and laser light backscattered from glial brain tumors, Biomedical Applications Of Light Scattering VI, SPIE Proc. 2012 doi:/10.1117/12.907444 (conference proceedings). (oral)
- IC8 T.A. Savelieva, M.N. Kholodtsova, V.B. Loschenov, S.A. Goryajnov, P.V. Zelenkov, A.A. Potapov (2012) Combined spectroscopy guided brain tumor resection: physical basis and clinical experience, In Laser Optics 2012, 3rd International Symposium on Lasers in Medicine, St Petersburg, Russia, June, 25-28.
- IC9 P.V. Grachev, M.N. Holodtcova, K.G. Lin'kov, A.N. Okushko, E.V. Filonenko (2012) Light and heat propagation in biological tissues. Methods of their control and evaluation of tissue critical states, In Laser Optics 2012, 3rd International Symposium on Lasers in Medicine, St Petersburg, Russia, June, 25-28. (poster)
- IC10 M.N. Kholodtsova and P.V. Grachev (2011) Determining of the optical sampling depth in brain tissue using different approaches to light distribution modeling, In 20th International Laser PhysicsWorkshop (LPHYS'11), Sarajevo, Bosnia and Herzegovina, July 11-15 (oral)
- IC11 T.A. Savelieva, V.B. Loschenov, M.N. Kholodtsova, A.A. Potapov, P.V. Zelenkov, G.A. Goryanov (2011) Quantification of Neural Tissue Properties with Multimodal Spectroscopic Measurements and Multispectral Visualization, In 20th International Laser PhysicsWorkshop (LPHYS'11), Sarajevo, Bosnia and Herzegovina, July 11-15 (oral)

3.3 National journals (Russian)

- NJ1 K.O. Aslanyan, M.N. Kholodtsova, I.A. Apolikhina (2014) Physical aspects of PDT use during chronic VVC, J. of Gynecology, Vol. 16(3), pp. 42-46.
- NJ2 K.O. Aslanyan, M.N. Kholodtsova, I.A. Apolikhina (2013) Use of Alasens for PDT to cure VVC, J. Success in Medicine Mycology, Vol. 11. pp. 68-70.
- NJ3 I.A. Apolikhina, K.O. Aslanyan, T.A. Teterina, M.N. Kholodtsova, D.Yu. Trophimov, A.S. Ankirskaya, (2013) Abilities of PhotoDynamic Therapy to cure VVC, Bulletin of Postgraduate Medical Education, Vol. 1, p.54
- NJ4 K.O. Aslanyan, M.N. Kholodtsova, I.A. Apolikhina, T.A. Teterina, (2013) Own results of PDT with Alasens for VVC, BV, NV, Photodynamic Therapy and Diagnosis, Vol. 3, pp. 67-68
- NJ5 A.A. Potapov, S.A. Goryainov, V.B. Loschenov, T.A. Savelieva, A.G. Gavrilov, V.A. Okhlopkov, V.A. Zhukov, P.V. Zelenkov, D.A. Golbin, L.V. Shishkina, P.V. Grachev, M.N. Kholodtsova, S.G. Kuzmin, G.N. Vorozhtsov, A.P. Chumakova (2013) Intraoperational combined spectroscopy (optical biopsy) of neuro glioma, J. Questions of neurosurgery, Vol. 77(2), pp. 3-10

3.4 National conferences (French and Russian)

- NC1 M.N. Kholodtsova, W. Blondel and V. Loschenov (2015) Theoretical calculation of field enhancement in system of two ferric oxide (II) particles, In National Photodynamic Conference, Saint Petersburg, September 25-26 (abstract)
- NC2 M.N. Kholodtsova, Ch. Daul, M. Amouroux, V. Loschenov and W. Blondel (2014) Global minimum search in Monte Carlo-based inverse problem of in vivo tissue optical properties estimation, In OPT-DIAG 2014 - Diagnostic et Imagerie Optique en Médecine et Biologie Biophotonique 9ème colloque national, Institut de Physique du Globe, Paris V, 14-16 Mai (poster+ abstract)
- NC3 M.N. Kholodtsova, V.B. Loschenov and W. C.P.M. Blondel (2013) Method of preprocessing for inverse problem of Monte Carlo in order to estimate optical properties of biotissue, In Nowadays questions of photodynamic therapy and photodiagnosis, Vol. 3, p.36. (presentation + abstract)
- NC4 K.O. Aslanyan, M.N. Kholodtsova, I.A. Apolokhina (2013) Statistical analysis of PDT with Alasens for VVC, In Nowadays questions of photodynamic therapy and photodiagnosis, (presentation + abstract)
- NC5 M.N. Kholodtsova, V.B. Loschenov, A.Yu. Titov, E.E. Zharkov, M.V. Abritsova (2012) Evaluation of optical parameters for laser-induced coagulation of biological tissues with a high content of hemoglobin, In Conference on Medical Physics in Troitsk – 5, June, 4-9 (poster + abstract)
- NC6 T.A. Saveleva, M.N. Kholodtsova, L.V. Shishkina, V.B. Loschenov, A.A.Potapov (2012) Cluster analysis of fluorescence spectroscopy and optical diffusion in neurooncology, In Conference on Medical Physics in Troitsk – 5, June, 4-9 (presentation + abstract)
- NC7 A.A. Potapov, V.N. Kornienko, I.N. Pronin, A.G. Gavriolov, A.E. Podpiroga, L.V. Shiskhina, S.A. Goryanov, V.A.Shurhay, V.B. Loschenov, T.A. Saveleva and M.N. Kholodtsova (2012) Results and prospects of application of modern methods of navigation and Biospectroscopy in neurosurgery, In Conference on Medical Physics in Troitsk – 5, June, 4-9 (presentation + abstract)
- NC8 M.N. Kholodtsova, P.V. Grachev (2012) Optical probing depth of biological tissue using laser and fluorescent radiation, Journal Laser Medicine, In Materials of scientific practical conference with international participation "Innovative technologies in laser medicine, p.114 (presentation+ abstract)
- NC9 M.N. Kholodtsova, P.V. Grachev, V.B. Loschenov (2011) The method of the optical probing depth of brain tumor determination, In Scientific session MEPhI'2011, collection of scientific papers, Vol.1228-9 (presentation + abstract)

3.5 Manuscript

M1 A.A. Potapov, Konovalov, S.A. Goryainov, M.N. Kholodtsova, T.A. Saveleva, V.B. Loschenov, Yusubalieva G. (2012) Metabolic Navigation, In Modern technologies and clinic researches in neuroscience, Vol.1, pp.85-105.

3.6 Patents

- P1 Intraoperative combined spectroscopic diagnostic technique for cerebral and spinal tumours, RU2497558-C1, Gavrilov A.G., Golbin D.A., Goryaynov S.A., Grachev P.V., Kholodtsova M.N., Kobyakov G. L., Kuzmin S.G., Loshchenov V.B., Okhlopkov V.A., Potapov A.A., Saveleva T.A., Shishkina L.V., Shurkhai V.A., Vorozhtsov G.N., Zelenkov P.V., Zhukov V.Yu.
- P2 Parenchymal organ biopsy and spectroscopic inspection device, comprises laser light source, light guide connected thereto, biopsy cannula connected to Luer adapter through second input of which optical fiber, RU2529629-C1, Potapov A.A., Loshchenov V.B., Gavrilov A.G., Maryashev S.A., Goryaynov S.A., Golbin D.A., Zhukov V.Yu., Kisarev S.A., Nazarov V.V., Saveleva T.A., Kholodtsova M.N., Grachev P.V., Zelenkov P.V.
- P3 Method for treating hemorrhoids, involve determining size of hemorrhoidal bolus by measuring scattered laser radiation of reflected disperse, and desarterization of internal hemorrhoidal bolus is controlled by ultrasonic Doppler flow meters, RU2012121407-A, Loshchenov V.B., Titov A.Yu., Cherner V.A., Shel, Shelygin Yu.A., Zharkov E.E., Abritsova M.V., Kholodtsova M.N., Grachev P.V.

General Introduction

Chapter 1

Photodiagnosis framework of the thesis and objectives

Ipse se nihil scire id unum sciat

Socratic paradox

This chapter discusses some drawbacks of the present situation in the field of biomedical spectroscopic diagnosis and opens the paths to be filled in by the present research. One of the crucial questions nowadays is fast and reliable analysis of biological tissue in order to earlier detect cancerogenous deceases. The present study opens possibilities to new method of *in vivo* non-ionizing diagnosis in cancer research. The thesis is based on experimental and theoretical research although the aim was not to involve the method developed directly into clinics. The idea was rather to develop and test the results on biological tissue models and give possibilities to use them further in clinics and understand the concept of the proposed method by theoretical modeling and calculations, thus to recover the processes which are undergoing inside biological tissue. The type of tissue to model was chosen to be epithelial, as it is present in many organs and covers surfaces and cavities throughout the body. Epithelial tissue is considered as multilayered thus it becomes more complicated from the modeling and experimental sight and more interesting from the theoretical and experimental research point of view. The main concept is to develop a new method of multilayered tissue analysis according to the last findings and the latest technologies, opening the new paths to preliminary diagnosis.

Firstly, to explain the biophysical processes characterizing the propagation of light through a biological tissue, some basic principles of electromagnetic wave propagation in absorbing and highly diffuse medium consisting of electrons, ions and their combinations, i. e. obstacles, are described.

Secondly, some existing detection methods of medium optical parameters are described and among them one is chosen due its interest for this thesis, i. e. spatially-resolved fiber spectroscopy.

Thirdly, the state-of-the-art of tissular point spectroscopic methods and their applications in biomedical diagnosis are given.

Fourthly, the mathematical and statistical description of light-tissue interaction models is given and the state-of-the-art is presented in a short overview of the existing solutions of their application to biomedical diagnosis *in vivo*.

Finally, the aim of the thesis is described in terms of recent approaches in nanoparticles application advantages for multilayered tissue models.

1.1 Photophysics basics of light-tissue interactions

The interaction of light or electromagnetic waves with a biological tissue can be described by several processes: reflection, refraction, scattering or absorption, and fluorescence. The general description of these processes is given below.

1.1.1 Refraction

A plane electromagnetic wave approaching biological tissue could be reflected according to the refractive index of media, n (dimensionless) and the incident angle θ_1 , i. e. the angle between the incident ray and the line perpendicular to the interface between the two media. The Snell's law describing this process is (see Eq. 1.1, Fig. 1.1):

$$n_1 \sin \theta_1 = n_2 \sin \theta_2 \tag{1.1a}$$

$$\theta_c = \frac{n_2 \sin \theta_1}{n_1} \tag{1.1b}$$

If an index of refraction is higher for the first medium, then refracted ray will go through the second media with a lower angle than the one of the incident ray ($\theta_2 < theta_1$). Usually, the

Δ



Figure 1.1: Light at an optically smooth interface between two media with different refractive indices: a) refraction; b) critical angle; c) reflection

refractive index is considered to be a real number, however in general case it is represented as defined as a complex number with k and p the respective real and imaginary parts (see Eq. 1.2):

$$n = k + ip \tag{1.2}$$



Figure 1.2: Diffuse reflection on irregular surface

For example, water has a complex component, however for the first approximation this could be neglected as in optical region it is less than $0.5 \cdot 10^{-8}$, while for some materials the complex component has a higher value and could not be ignored. The effects occur in the latter case will be described in chapter 2.

An interface is considered to be optically rough when Eq. 1.3 is fulfilled:

$$hcos\theta \ll \lambda$$
 (1.3)

with h - the height of the irregularities, λ - the wavelength of the incident light and θ - the angle between the line perpendicular to surface and the incident ray. If this condition is not met then on such surface diffuse reflection occurs (Fig. 1.2) thus the light is scattered in all directions. The angle between incident and reflected light differs from point to point.

The value of the refractive index depends on the nature of medium and on its temperature and density. It reflection index also slightly changes in optical region of wavelengths. All these reflection index variations should be taken into account when developing methods and models based on Diffuse Reflectance Spectroscopy (DRS). The model which describes the spatial dependence of DR in tissues was developed by Farrell et al [Farrell et al., 1992]. Until now for steady-state spatially resolved reflectance the error in optical properties estimation was found to be considerably smaller than for the commonly used solutions [Kienle and Patterson, 1997]. Optical diagnosis based on reflection analysis is a promising solution for analysis of cervical cancer [Arifler et al., 2006]. Analytical model of light reflectance for extraction of the optical properties in small volumes of turbid media was presented by Reif et al [Reif et al., 2007]. To recover tissue optical parameters a model based on DRS data was proposed by Fredriksson et al [Fredriksson et al., 2012]. Diagnosis of dysplasia is based on superficial tissue analysis, thus it is needed to restrict light propagation to deeper layers. The method limiting depth of light propagation was developed using DRS data [Gomes and Backman, 2012]. Another interesting work should be mentioned about investigating a surface of tissue by means of elliptically polarized light. The group of prof. Dinten presented experimental and modeling results on linear relation between the backscattered light and the depth extention of the volume being probed [Rehn et al., 2013].

1.1.2 Absorption



Figure 1.3: Geometrical representation of the light absorption by a single chromophore

Generally speaking, the process of absorption corresponds to an energy loss of the light propagating in a medium and its conversion to other types of energy. The main process associated with absorption is atom collision between each other while energy is transferred from one atom to another in non-radiative way. The main reason why absorption occurs is that tissue consists of electrons and positrons, i. e. discrete charges. These charges could form any obstacle in tissue and are affected by an electromagnetic wave which makes them oscillate [Bohren and Huffman, 1998].

The effectiveness of this process is described by the parameter which is called absorption coefficient, μ_a . The objects absorbing light in biological tissue are called chromophores and represent molecules, which can absorb light via electronic transitions. Absorption coefficient describes a medium, which contains chromophores at certain concentration. Thus it is considered as a product of volume density of chromophores, $\rho_a \ [cm^{-3}]$, and effective cross-section, $\sigma_a \ [cm^{-2}]$, which is itself a product of absorption efficiency, $Q_a \ [dimensionless]$, and geometrical size of the chromophore, $A \ [cm^2]$. Thus, the absorption coefficient sketched in Fig. 1.3 is (see Eq. 1.4:

$$\mu_a = \rho_a A Q_a \tag{1.4}$$

The light intensity loss due to light absorption is described by the Beer-Lambert law (or Beer–Lambert–Bouguer), i. e. the probability of photon survival:

$$I = I_0 e^{-\mu_a d} \tag{1.5}$$

4

where d is the thickness of the media through which the light propagates, I_0 is the initial intensity of light and I is the intensity after the thickness d. Usually it is considered that the absorption coefficient (μ_a) is the thickness of medium where the light reduces its energy e times. Its value depends on the wavelength and material of media.

Absorption is one of the main light-tissue interaction effects in biomedical optics because when the light is absorbed it gives either terapeuthtical effect, i. e. heating tissue till temperature of blood coagulation, proteins denaturation, etc [Tuchin, 2010]. Absorption limits the depth of light propagation. The main absorbers are water, blood and melanin.

In a spectra registration setup, when light is detected through a thin medium of a known thickness, absorption wavelength-resolved spectra allow for analyzing the constituents of a medium under investigation. However, for biomedical optics *in vivo* source and detector should be on the same side from the object, the configuration for transmission measurements is used for analysis of solutions and thin media in laboratory's practice. Such a configuration will be described and used further in this work.

1.1.3 Scattering



Figure 1.4: Geometrical representation of light scattering by a single diffuser. Part of light meets a particle and scattered according to the form, size and material of a particle, the light which is not scattered is called transmitted.

One of the first evidences in scattering differences between tumorous and healthy tissues was pointed by Mourant et al [Mourant et al., 1998] and related to growth of scattering centers. Scattering is used for spectroscopic measurements as well as for imaging. Particles concentration influence on scattering properties is discussed by Bashkatov et al [Bashkatov et al., 2000b]. Thus, minimal changes in scattering properties of biological tissue could be detected with spectroscopic methods.

Scattering is a process of light deviation from its initial direction while propagating inside a

medium. It occurs when the medium through which light propagates is optically inhomogeneous, i. e. when the refractive index varies spatially throughout the medium or there are particles in the medium which are optically different from the medium's matrix. Second order incoherent waves appear after interaction of light with these particles which are inhomogeneously distributed and moving in media due to thermal effects, thus differences in optical paths appear leading to incoherence [Tuchin, 2010].

Scattering could be induced by continuous changes or discrete particles, which make the light deflect from the incident direction. In biophotonics photons are scattered by the constituents of tissue which are about the dimension (size) of the photon's wavelength. Multiscale structure of tissue consists of small molecules (like chromophores) and finishes up to big elements like cells. Depending on the size of structural components different theories are used to describe the process of light scattering:

- 1. Mie theory (when the diffuser size is of the same order as photon's wavelength: e.g., cell organelles to the cell nucleus),
- 2. Rayleigh scattering (when the diffuser dimension is much smaller than photon's wavelength: e.g. from molecular level until collagen fibers)



Figure 1.5: Representation of a scattering event, illustrating a deflection of a photon trajectory. An azimuthal angle of scattering is considered such that in these directions light is scattered in the same way, as the medium under consideration is isotropic

Scattering coefficient denoted by μ_s , is a cross-sectional area per unit volume of medium. It is defined as the product of the volume density of scattering particles concentration, $\rho_s \ [cm^{-3}]$, by the cross-sectional area, $\sigma_s \ [cm^2]$, which in turn is the product of the scattering efficiency, $Q_s \ [dimensionless]$, by the geometrical size of diffuser, $A_s \ [cm^2]$, gives the mathematical formulation of the principle sketched in Fig. 1.4 (see Eq. 1.6):

$$u_s = \rho_s A Q_s \tag{1.6}$$

The probability $T \in [0,1]$ of the photon's transmission without redirection after the path length L is given by Eq. 1.7:

$$T = e^{-\mu_s L} \tag{1.7}$$

If the size of a diffuser is lower than the incident light wavelength, then the intensity of the scattered light has a distribution which is symmetrical to the direction of incident light and to the line which is perpendicular to it. The curve which is in the plane of the incident light direction and of observation direction is called indicatrix. If the incident light is non-polarized, then the equation of the indicatrix (or scattering phase function) is defined as follows (see Eq. 1.8):

$$P(\theta) = I_0(1 + \cos^2\theta), \tag{1.8}$$

where $P(\theta)$ is the intensity of light scattered at an angle θ to the direction of the incident light, I_0 is the intensity of the incident light. The scattering phase function in terms of the differential scattering cross-section is defined in Eq. 1.9:

$$P(\hat{\mathbf{s}}, \hat{\mathbf{s}}') = \frac{4\pi}{\sigma_s + \sigma_a} \frac{d\sigma_s}{d\Omega} (\hat{\mathbf{s}}, \hat{\mathbf{s}}'), \tag{1.9}$$

where $\hat{\mathbf{s}} \cdot \hat{\mathbf{s}}' = \cos \theta$, s and s' are the directions of the incident and scattered light, respectively. The angular dependence of scattering is called the scattering phase function, $P(\theta)$. It describes the polarizability of a photon scattering into a unit solid angle, which is oriented at an angle θ , mentioned above. Usually is it considered that there is no dependence on azimuthal angle, as the medium is considered as anisotropic. The expected or the mean value for $\cos\theta$ is given by Eq. 1.10 (see also Fig. 1.5):

$$g = \int_0^{\pi} P(\theta) \cos\theta 2\pi \sin\theta d\theta = <\cos\theta>, \qquad (1.10)$$

where $4\pi \int_0^{\pi} P(s, s') 2\pi \sin \theta d\theta = 1.$

The closer is the size of a diffuser to the incident wavelength, the closer to unity is the anisotropy factor.

If a diffuser of a size r is much smaller than an incident wavelength, $\lambda (r \ll \lambda)$, then a so-called Rayleigh scattering occurs, when anisotropy equals to 0, that means that light is isotropically scattered and phase function equals to $\frac{1}{4\pi}$. If size of a diffuser is about wavelength $(r < \lambda)$ then Mie scattering occurs [Mie, 1908]. The Mie scattering theory was specially developed for spherical particles, however it is applied to spherical and nonspherical cellular structures: mitochondria, collagen fibers, nuclei, etc.

An analytical function usually used to model light scattering in biological tissue is derived by Henyey and Greenstein (HG-function [Henyey and Greenstein, 1941]) back to 1941 (This equation is given in chapter 3 in Eq. 2.17).

However, depending on the phase function modeling of light-tissue interaction could differ up to 60 % [Cihan and Arifler, 2011]. For example there was even a work which was dealing with fractal sizes distribution of diffusers [Passos et al., 2005]. The disadvantage of this fractal method is that it is unlikely to be able to reproduce tissue optical properties over a broad range of wavelengths simultaneously.

Thus there is a link between particle size and direction of scattering, which is defined by scattering coefficient μ_s and for anisotropy g.

Scattering analysis allows detecting changes undergoing in turbid, i. e. highly scattering, medium. Through analysis of transmitted or backscattered light distribution of sizes could be detected.

Usually, biomedical optics is dealing with both scattering and absorbing media, in such case analysis of media constituents and their size distributions is a non-trivial task. For a first-step



Figure 1.6: Jablonski diagram of luminescence. After an atom, molecule or ion absorbed a photon of a sufficient energy, the whole system is excited from the ground state (S_0) to a higher energetic level (here S_n). Partly system could relax non-radiatively and then could fluoresce at a longer wavelength. The other processes undergo are internal conversion, which goes through non-radiant way, and phosphorescence.

approximation, by considering single-scattering event, it is possible to use the Beer-Lambert-Bouger's law (See Eq. 1.5) either with sum of scattering and absorption coefficients in exponential power law or by considering absorption negligible to scattering or *vice versa*. For the multiple scattering media this consideration is not allowed, as in transmitted light not only ballistic, i. e. not scattered, photons could be detected, but also those, which were multiply scattered in a medium. However, it is possible to detect only ballistic photons in time-resolved measurements. Main methods used for the case of multiple scattering and absorbing media will be considered further in this work.

1.1.4 Fluorescence

Luminescence is a process of light emission from a medium which was illuminated by an electromagnetic wave. It is divided into phosphorescence and fluorescence depending on the nature of the excited state [Lakowicz, 2006b]. Fluorescence is a process of light absorption and its consequent re-emission at longer wavelength and at lower energy corresponding to return of atom, molecule or ion either from the excited state to the ground state S_0 or by transitions to the level of higher energy (See Fig. 1.6).

The excited electrons in case of a fluorescent molecule could relax down to the underlying levels of a system in non-radiant way. Intensity of fluorescence decreases with time following Eq. refeq:lt:

$$I_t = I_0 e^{-\frac{\iota}{\tau}} \tag{1.11}$$

where τ is the lifetime of fluorescence, I_t stands for the intensity of fluorescence at time t and I_0 is the intensity of fluorescence when excitation stops. The ratio between the number of photons


Figure 1.7: Representation of light-tissue interaction processes. R_f - reflectance, R_d - diffused reflectance, T_c - collimated transmittance, T_d - diffused transmittance. ET - Energy Transfer.

absorbed and the number of photons emitted is called quantum yield of fluorescence γ (see Eq. 1.12):

$$\gamma = \frac{number \quad of \quad photons \quad emmitted}{number \quad of \quad photons \quad absorbed}.$$
(1.12)

Thus the most efficient fluorophores are those, which have quantum yield close to one and have a potential to be used in biomedical diagnosis. Quantum yield could be affected by a medium surrounding the fluorophore or by another particle connected to it, for example a nanoparticle. This thesis is also partly dedicated to the theoretical and experimental investigation of fluorophore lifetime and quantum yield changes due to the nanoparticle presence.

Biomedical optics fluorescence is of interest as it allows for determining tissue state which is in accordance to the concentration of fluorophores. In absence of quenchers lifetime of fluorescence, τ does not depend on the medium, i. e. scattering and asorption parameters [Lakowicz, 2006c]. Swartling et al [Swartling et al., 2005] presented a research on fluorescence-guided deep lesions detection by ratio of two wavelength bands fluorescence signal. Cicchi et al [Cicchi et al., 2007] introduced a method of combined morphological and lifetime analysis of *ex vivo* skin samples. They distinguished between benign and malignant tissues, thus offering a reliable, non-invasive tool for the *in vivo* analysis of inflammatory and neoplastic skin lesions.

Finally, all the processes described above are represented in Fig. 1.7.

There is also a nanoparticle in the figure, but the processes, which occur in medium containing nanoparticles, are discussed in chapter 3 and are the part of the research work done in the thesis.

The life-time of an excited state is around 10^{-4} seconds, fluorescence life-time is about several nanoseconds, however it could decrease to several picoseconds depending on the freedom of a

molecule affected by electromagnetic of other particle. The question of energy transfer between nanoparticle and fluorophore and of lifetime changes is considered in the last part of the thesis (Chap. 5).

1.2 Criteria for statistical characterizing of an optical diagnosis method efficiency

The performance of a lesion detection method is expressed by its specificity, sensitivity, positive and negative predictive factors. Let's imagine a diagnosis method of a disease among a group of people. The results of the detection test can be as follows:

- True Positive: disease is correctly identified (TP),
- False Positive: disease is detected although a subject is healthy (FP),
- True Negative: healthy subject was identified as healthy (TN),
- False Negative: a subject with a lesion was identified as healthy (FN).

1.2.1 Sensitivity

Sensitivity Se is a probability of correctly identifying a disease. It is mathematically expressed by Eq. 1.13:

$$Se = \frac{Number \ of \ true \ positives}{Number \ of \ true \ positives + Number \ of \ false \ negatives} \times 100 \ \%$$
(1.13a)

$$Se = \frac{TP}{TP + FN} \times 100 \quad \% \tag{1.13b}$$

If sensitivity is high, i. e. close to 100 %, a negative result allows identifying subjects who are healthy, since such a negative test result will statistically exclude the disease. However, considering only a positive result is not a guarantee for correct lesion identification, even for a high sensitivity, since this criterion does not take false positive results into account.

1.2.2 Specificity

Specificity Sp is a measure indicating whether a test correctly identifies a person without a disease or not. Mathematically it is expressed by Eq. 1.14:

$$Sp = \frac{Number \ of \ true \ negative}{Number \ of \ true \ negative + Number \ of \ false \ positive} 100 \ \%$$
(1.14a)
$$Sp = \frac{TN}{TN + FP} \times 100 \ \%$$
(1.14b)

If specificity is high, i. e. close to 100 %, a positive result in a test will exclude disease in all healthy patients. Thus a positive result defines a high probability of the presence of disease. However, even for high S_p values, the specificity taken as unique criterion is not a guarantee for excluding a disease because it does not take false negative results into account.

To fully characterize the lesion detection performance, sensitivity and specificity should be taken into account simultaneously. Indeed, a method having for example high Sp of 97% is not reliable for a low S_e value of 40% for instance.

1.2.3 Positive and negative predictive factors

Ensuring best performance for a detection method implies the highest rates of sensitivity and specificity, i. e. 100 % for both Se and Sp. The confidence in sensitivity and specificity is quantified by two additional criteria: the positive predictive value and the negative predictive value respectively. These two values are used to identify the probability of subjects to have a decease in case of positive test (see Eq. 1.15):

$$Positive \ predictive \ value = PPV = \frac{TP}{TP + FP}100 \tag{1.15}$$

and to be healthy in case of negative test (see Eq. 1.16):

Positive predictive value =
$$NPV = \frac{TN}{TN + FN} \times 100 \%$$
 (1.16)

1.3 The state-of-the-art in cancer optical diagnosis

Skin cancer worldwide is the most frequent cancer in the Caucasian population worldwide. Early diagnosis of this disease is a key step to higher survival rates and faster recovery. Incidence of Basal Cell Carcinoma (BCC), Squamous Cell Carcinoma (SCC) and Malignant Melanoma (MM) is still increasing. Besides standard physical examination and common questions about the health history of a patient and his family, the first and most common assessment of cancer is obtained by the histopathological analysis of a tissue removal (biopsy). It means that a piece of suspicious tissue is removed from skin and then examined under microscopy. Dermatologists may also use dermoscopes (dermoscopy is also called EpiLuminescence Microscopy (ELM) or surface microscopy) to see more clearly spots on the skin. This tool consists of a magnifying lens and a light source which allows for preventing biopsy and reassuring presence of cancer in particular spot.

Examination	Se	Sp	PPF	References		
Clinical	40 - 92	NA-64	36 - 78	[Nagao et al., 2000, Epstein		
				et al., 2003]		
ME	95	90	NA	[Andrea and Dias, 1999, Gyn-		
				ther et al., 2000]		
ESS	72–98	68 - 75	NA	[Bigio and Bown, 2004, Swin-		
				son et al., 2006, Upile et al.,		
				2012, Evers et al., 2012 , Can-		
				polat et al., 2013]		
AF	81-86	94 - 100	93	[Betz et al., 2002, Stelzle		
				et al., 2013]		
RS	85-91	75–91	76	[Calin et al., 2013, Devpura		
				et al., 2014]		
OCT	79–94	83–96	27	[Korde et al., 2007, Mogensen		
				et al., 2009, Sun et al., 2014]		

Table 1.1: Sensitivity, Se, specificity, Sp, and Positive Predictive Factor PPF for common optical diagnosis methods of skin cancer.

With the development of non-invasive therapies for BCC such as PhotoDynamic Therapy (PDT) and immune therapies, non-invasive diagnosis becomes relevant. Optical methods such as AutoFluorescence (AF), Raman Spectroscopy (RS), Elastic Light Spectroscopy (ELS), MicroEndoscopy (ME) and Optical Coherence Tomography (OCT) are among the most studied because of their ability to local, non-invasive and atraumatic measurements. Some other methods, like Life-time Imaging Fluorescence Spectroscopy (LIFS), involve special agents as fluorophores or nanoparticles which are injected to patient to improve the Se or Sp values of the method. In 2012, a group of scientists developed a method of BCC subtypes in vivo diagnosis using Reflectance Confocal Microscopy (RCM) and conventional OCT. The method implies High Definition OCT with a help in vivo diagnosis of BCC and has micrometer resolution in both transversal and axial directions, enabling visualization of individual cells up to a depth of around 570 μ m [Boone et al., 2012]. Table 1.1 shows the most recent results obtained in terms of sensitivity Se, specificity Sp and positive predictive factor PPF for different types of cancer examination.

Each year cancer diagnosis reaches promising results with the development of detection techniques and by using the combinations of the existing methods. in Table 1.2 some recent approaches with their advantages and drawbacks are presented in terms of epithelial cancer diagnosis.

Optical tech- niques	Advantages	Drawbacks	Feasibility barring unex- pected break- through in next 2 years
Micro-endoscopy	In situ, cheap, auto- clavable, intra-operative use, endoscopic use, real-time magnification, can be used to guide simple surveillance of large areas of suspect mucosa, could provide the state of the <i>in situ</i> epithelial margin when excising squamous cell carcinoma	User dependent Shallow learning curve, difficult to interpret, subjective in sampling strategy	Poor, specialist center use
Elastic scattering spectroscopy	In situ, less user de- pendent, cheap, fiber auto-clavable, endo- scopic use, objective in analysis, most ev- idence from <i>ex vivo</i> experiments, almost real-time	Processing dependent, requires physicist and statistician, machine dependent, subjective in sampling strategy, expensive fibers	Promising

	• • •			
Optical tech- niques	Advantages	Drawbacks	Feasibility barring unex- pected break- through in next 2 years	
Optical coherence tomography	In situ, real time	User dependent Shal- low learning curve, dif- ficult to interpret, re- quires special probes and expensive imaging processing, subjective in sampling strategy and requires pathologist	Promising	
Fluorescence	In situ, can guide resec- tion, real-time, autoflu- orescence	Subjective, reaction to fluorophores if not auto-fluorescent, detec- tors initial high capital outlay, subjective in sampling strategy, ex- ogenous fluorophores have to be authorized for clinics	Very promising	
Raman spec- troscopy	Detailed single cell physiology	Ex vivo	Promising, spe- cialist centre use but hand held device now available	
Narrow band imaging	In situ	Expensive	Unproven	
Infra red spec- troscopy	In situ	Expensive, few applica- tions	Unproven	

Table 1.2: Main features of *in vivo* optical diagnosis technique[Swinson et al., 2006, Jerjes et al., 2004, Jerjes et al., 2011].

1.4 Tissue optical properties change due to pathological modifications

When tissue is subjected to alter due to precancer, absorption, scattering and intrinsic fluorescence change, which could be reflected in observed by means of optical techniques spectra or images. Thus, pathological changes in tissue are in direct connection with scattering, absorption, refractive index and fluorescence, so they consequently will be visible in detected spectroscopic signals. From a wavelength-resolved intensity it is possible to compute morphological and structural changes within tissues since, beside scattering by cells, intrinsic (inside cells, by their organellas) scattering takes place [Mourant et al., 1998]. Scattering process is due to gradients in the optical index of refraction resulting from differences in densities that occur at cellular and sub-cellular levels on nucleus, chromatin, and sub-cellular organelles, i. e. mitochondria, Goldji apparatus [Badizadegan et al., 2004, Jerjes et al., 2004, Bigio and Bown, 2004, Jerjes et al., 2005, Lovat et al., 2006]. At a tissue level, scattering modifications are related to changes in morphology of epithelial surface texture and thickness as well as disorganization of epithelial cell orientations or architecture. These later criteria are used by histopathologists, when they look for malignancy within a tissue, and can be used as the basis for diagnosis instead of histopathology analysis. Although in the case of precancerous or very early pathological states of tissue, those changes are hard to identify by histopathology, however they affect and may therefore be detected by fluorescence and DR spectrum.

To come closer to the main problems to solve, which are connected to tissue photodiagnosis, some drawbacks will be considered below. Precise delineation of the pathological tissue border – is one of the challenges for clinicians. Generally speaking, suspicious tissue has to be taken in order not to let a pathology spread again, but healthy tissue needs to be left untouched. PhotoDynamicDiagnosis (PDD) is considered as a noninvasive (although some fluorophores and markers could be introduced or applied to an object of investigation) promising method for cutaneous malignancy detection [Allison and Sibata, 2008]. It consists in the administration of a photosensitizer (for example, Amino-Levulinic Acid-5 - ALA-5), which enhances the fluorescence of malignant lesions by increasing Protoporphyrine IX (PPIX) accumulation. One of the improvements could be connected with nanoparticles application. Thereby it is needed to spot the type of analysis which is spatially resolved, so some concepts of optical diagnosis probes are presented below.

1.5 Probes for optical diagnosis

1.5.1 Probe configurations for *in vivo* applications

In recent years, optical techniques for biological tissue diagnosis have proved their usefulness in tremendous amount of applications [Keiser et al., 2014]. Optical techniques are non-invasive, inexpensive and allow for analyzing biological tissue in real time. For example, it is possible to detect oxygenation, as well as first changes in tissue due to change in size of nuclei [Allison and Sibata, 2008, Upile et al., 2012, Calin et al., 2013].

Basically, to detect the light outcoming from tissue a spectrophotometer is needed. Main concepts of its configuration could be found in the Biomedical Photonics Handbook [Vo-Dinh, 2003]. To deliver light from lasers to tissue optical guides are used. Their configuration highly depend on the type of lasers used, type of tissue under investigation, its accessibility, etc. In the following sections some optical point and imaging probes recently developed for biomedical diagnosis and therapy are discussed, but not taking into account those features of probes which depend on accessibility of tissue as it is beyond the scope of this thesis.

1.5.2 Point-approach techniques

Geometry and composition of fluorescence and DR probes affect measurements: Source-Detector Separations (SDS), numerical aperture (NA), their diameter (D), material of core and cladding, form of the distal tip, the amount of sources and detectors. The basic parameters of an optical fiber construction are:

1. the numerical aperture NA is the range of angles over which system can accept or emit light, usually around 0.22 to 0.8,



Figure 1.8: Representation of fiber standard geometry and numerical aperture NA sence.

- 2. the diameter of core D is usually around 100-600 μm for optical spectroscopy,
- 3. the materials of a core n_{core} and a cladding n_{clad} are usually quartz and polymer, respectively.

The numerical aperture depends on the refractive cladding as shown in Eq. 1.17 (see Fig. 1.8):

$$NA = \sqrt{n_{core}^2 - n_{clad}^2}.$$
(1.17)

In Eq. 1.17 n_{core} is the refractive index of the core, n_{clad} stands for the refractive index of the cladding.

Pfefer et al [Pfefer et al., 2001, Pfefer et al., 2002, Pfefer et al., 2004] and Zhu et al [Zhu et al., 2003] investigated Monte-Carlo based models and conducted experiments with different SDS and with variable apertures on detection while collecting fluorescence and DR signals. Their researches showed that to selectively detect fluorescence from different layers of epithelial tissue it is necessary to have numerical apertures in the range of 0.22 to 0.37. As well it was shown that higher diameter or higher fiber-tissue spacing led from selectivity of superficial fluorophores to a more homogeneous probing depth. Full up to date information on optical fibers available for biomedical optics applications can be found in the review paper by Keiser et al [Keiser et al., 2014]. Another interesting approach for non-contact diffuse-reflectance spectroscopy proposed by Dinten et al [Sorgato et al., 2015] consists of the algorithm which compensates the effect of scattering. However, it is not used in the thesis, it should be mentioned, that compression of a fiber on tissue affects the estimated optical properties. This effect was investigated by several research groups [Chan et al., 1996b, Reif et al., 2008].

1.5.3 In vivo imaging techniques

Optical imaging systems can be classified in the following way:

- 1. continuous wave measurement system,
- 2. frequency domain based system and
- 3. time domain based system.

The modes of measurements are planar, reflectance and cylindrical. The reviews by Solomon et al. [Solomon et al., 2011] and Keiser et al [Keiser et al., 2014] discuss the state-of-the-art on imaging techniques currently available.

Diffuse Optical Tomography (DOT) is considered as the most established imaging techniques which allows for determining spectrally dependent scattering and absorption coefficients as well as fluorophores concentrations which in turn are associated with change of endogenous and exogenous fluorophores concentration.

A more complex technique than DOT is Diffuse Optical Imaging (DOI) which acquires threedimensional images of tissue. It is a combination of measurements from multiple sources and detectors with modeling of light-tissue interaction (the latter will be discussed further in this chapter). The group of prof. Dinten investigated the spatial depth resolution for time-resolved Diffuse Optical Tomography (DOT) for short source-detector separation with small inclusion [Puszka et al., 2014].

Imaging systems are allowing to investigate a broad region of tissue and are thus able to follow the changes in wider field. To my knowledge the latest review on imaging techniques is given by Fass et al [Fass, 2008]. This contribution was followed by a review on preclinical imaging techniques [Cunha et al., 2014].

1.5.4 Spatially-resolved probe used in the thesis



Figure 1.9: Multi-fiber probe used in this thesis: a) profile of a single fiber, b) distal end of a single fiber, c) the multi-fiber probe geometry. NA of a single fiber = 0.22, diameter D equals 200 μ m. The colored fibers are those which were used in the experiments presented in this thesis

The fiber used in this thesis was specially developed for multimodal spectroscopy for elasticscattering (or DR) and AF for biological tissue *in vivo* and *in vitro* characterization [Pery, 2007]. Its configuration combines the principles of DOS and DOI to provide data with spatial, spectral and depth resolutions associated to light-tissue interaction models and giving a simple approach to biomedical optics.

The fiber geometry and main properties are shown in Fig. 1.9. According to the measurements performed with power meter (JSC Biospec), the light losses in the multi-fiber probe were no more than 10 %, however in thesis of Pery [Pery, 2007] the . Is is explained by the difference in light sources used and their output power, for this thesis, the maximum output power was 30 mW, losses - less than 3 mW. Also, only 6 SDS shown in the Fig.1.9 were used. The distances between the used source-detector pairs are:

- 260.8 μ m for the pair number 10-3,
- 343.6 μ m for the pair number 31-15,
- 499.6 μ m for the pair number 31-6,
- 777.6 μ m for the pair number 31-1,
- 1040.5 μ m for the pair number 31-3 and
- 1289.5 μ m for the pair number 31-10.

The widest SDS of 1341.0 μm (between the fibers with numbers 31 and 22) was not used because of insufficient intensity of captured light and the consequent high error - hereby the multi-fiber probe used in the thesis is described. To understand the way light propagates to biological tissue, the theoretical models relating to propagation of photons inside the tissue and outcomes from it after multiple scattering as well as fluorescent events are discussed in the following chapter.

1.6 The state-of-the-art in light-tissue interaction modeling

Several modeling approaches have been developed to describe light-tissue interactions:

- 1. Numerical modeling approaches:
 - RTE : Multi-flux method [Welch et al., 1987], P_n method [Storchi, 1984], Method of discrete coordinates [Liou, 1973], Adding-Doubling method [Prahl, 1995].
 - Maxwell's equations: Finite-Difference Time-Domain (FDTD) and Frequency-Domain (FDFD)
- 2. Approximation approaches:
 - Physical models: Diffusion Approximation (DA) [Takatani and Graham, 1979, Contini et al., 1997]
 - Phenomenological model: Kubelka-Munk (KM) theory[Kubelka and Munk, 1931, Vargas, 2002].
- 3. Numerical simulation approaches: Monte Carlo (MC) modeling [Prahl et al., 1989, Wang et al., 1995, Pery et al., 2009, Sassaroli, 2011];
- 4. Hybrid approaches:
 - MC + DA [Wang, 1998].

The most commonly-used in biomedical optics are the Mie theory, the DA of the RTE and Adding-Doubling (AD) – as other way of the RTE solution, Finite-Difference Time-Domain (FDTD), Kubelka-Munk theory and Semi-Empirical Models (SEM), Monte Carlo (MC) simulations of light propagation into tissue. Wilson et al [Wilson and Mycek, 2011] has given an overview of DA to the RTE, Monte Carlo simulations, SEM applied to to reflectance and endogenous fluorescence detection for cancer diagnosis in biological tissues. The usefulness of these methods and some other methods according the application is presented in Table 1.3.

\mathbf{Method}	Advantages and dis-	Recent works		
	advantages			
Mie	Scattering by spherical	[Lee and Lu, 2012 , Pu et al.,		
	particles	2014]		
Diffusion	Mostly used for simple	[Wilson and Mycek, 2011,		
Approxi-	setup geometries and far	Zhang et al., 2014, Grabtchak		
mation	SDS distances	et al., 2014]		
Adding-	For the slabs	[Saager et al., 2010, Zamora-		
doubling		Rojas et al., 2013a, Zamora-		
		Rojas et al., $2013b$]		
FDTD	Time and spatially	[L'Huillier and Humeau, 2004,		
	resolved light tissue-	Kortun et al., 2008, Asllanaj		
	interaction	and Fumeron, 2012]		
Kubelka-	For the slabs	[Vyas et al., 2013, Vasefi		
Munk		et al., 2013, Sandoval and		
		Kim , 2014]		
SEM	Suitable for special	[Reif et al., 2007, Wilson		
	cases (i. e. recover-	et al., 2009, Wilson and		
	ing known absorber	Mycek, 2011]		
	$\operatorname{concentration})$			
MC	Quite precise, but time-	[Wilson and Mycek, 2011,		
	consuming. Used for	Zhang et al., 2014, Yan-Fei		
	short distances.	et al., 2014, van Leeuwen-van		
		Zaane et al., 2014]		

Table 1.3: The state-of-the-art for methods of light-tissue interaction modeling.

1.6.1 Radiative Transport Equation (RTE)

The fundamental equation, which describes light of special intensity dynamics in medium, is referred to as the RTE and is also known as the Boltzmann equation [Vo-Dinh, 2003]. Light is considered as a collection of localized, incoherent photons (due to scattering the initial polarization is lost, however it is remaining the same if the light was reflected). Let's consider a small package of light energy defined by its position, $\mathbf{r}(t)$, and direction of propagation $\hat{\mathbf{s}}$ at time t. The package flows along the direction $\hat{\mathbf{s}}$ and losses its energy due to scattering and absorption, respectively quantified by coefficients μ_s and μ_a . It also gains energy which comes from other directions into the $\hat{\mathbf{s}}$ direction from the source of light at $\mathbf{r}(t)$. These processes are described by integro-differential equation known as the radiation transport relation (see Eq. 1.18):

$$\frac{1}{c_m} \frac{d}{dt} I(\mathbf{r}(t), \mathbf{s}, \hat{\mathbf{s}}, t) =$$

$$(\mu_a + \mu_s) I(\mathbf{r}(t), \hat{\mathbf{s}}, t) + \frac{\mu_a + \mu_s}{4\pi} \int P(\hat{\mathbf{s}}, \hat{\mathbf{s}}') I(\mathbf{r}(t'), \hat{\mathbf{s}}', t) d\Omega' + Q(\mathbf{r}(t), \hat{\mathbf{s}}),$$

$$(1.18)$$

where c_m is the speed of light in the medium, $Q(\mathbf{r}(t), \hat{\mathbf{s}}, t)$ stands for a source term. In this form the Eq. 1.18 describes the dynamic change of $I(\mathbf{r}(t), \hat{\mathbf{s}}, t)$ with time. The term $(\mu_a + \mu_s)I(\mathbf{r}(t), \hat{\mathbf{s}}, t))$ decreases the value of $I(\mathbf{r}(t)$ as it corresponds to scattering (μ_s) and absorption $(\mu_a$. The integral term $I(\mathbf{r}(t))$ always increases, as it is 'collecting' all the scattered light in the direction of cone $\hat{\mathbf{s}}$. The total time derivative $\frac{d}{dt}$ is reduced to partial derivatives: $\frac{\partial}{\partial t} + c\hat{\mathbf{s}} \cdot \vec{\nabla}$ if we fix the coordinate system from the package of photons to the medium. Thus, the time-dependent RTE equation takes the form (see Eq. 1.19):

$$\frac{1}{c_m}\frac{\partial}{\partial t}I(\mathbf{r},\hat{\mathbf{s}},t) = (-\hat{\mathbf{s}}\cdot\overrightarrow{\nabla} - (\mu_a + \mu_s))I(\mathbf{r},\hat{\mathbf{s}},t) + \frac{\mu_a + \mu_s}{4\pi}\int_{4\pi} P(\hat{\mathbf{s}},\hat{\mathbf{s}'})I(\mathbf{r},\hat{\mathbf{s}'},t)d\Omega' + Q(\mathbf{r},\hat{\mathbf{s}},t), \quad (1.19)$$

Then we apply the steady-state condition, meaning that the losses and gains at each point **r** of the medium does not change with time, t, and the source is always on, thus the condition $\frac{d}{dt}I(\mathbf{r}(t), \hat{\mathbf{s}}, t) = 0$ is fulfilled and Eq. 1.18 in a source-free region takes the form (see Eq. 1.20):

$$\hat{s} \cdot \overrightarrow{\nabla} I(\mathbf{r}, \hat{\mathbf{s}}) = -(\mu_a + \mu_s)I(\mathbf{r}, \hat{\mathbf{s}}) + \frac{\mu_a + \mu_s}{4\pi} \int_{4\pi} P(\hat{\mathbf{s}}, \hat{\mathbf{s}}')I(\mathbf{r}, \hat{\mathbf{s}}')d\Omega'$$
(1.20)

1.6.2 Analytical solutions for particular cases

In the straight-forward solution of the RTE, complication always arises with dealing with different boundary conditions, various types of sources and different geometry of biological tissue. When absorption is low enough to permit the diffusion of photons into the biological tissue, and scattering is considered as a dominant process, then this limit is known as diffusion limit. This is an important condition to be fulfilled for biomedical optical diagnosis, as photons are able to penetrate tissue even if strong scattering occurs, thus by back-scattered signal it is possible to analyze tissue. Hereafter we will consider the scattering-dominant limit as it appears for biological tissue which is considered as a turbid medium [Vo-Dinh, 2003].

Solution of the RTE for DR could be found analytically using DA [Wang et al., 2009, Ishimaru, 1991].

Incident and Diffuse light

The intensity of the light penetrating in the medium from an external source is described by Eq. 1.21

$$I(\mathbf{r}, \hat{\mathbf{s}}, t) = I_c(\mathbf{r}, \hat{\mathbf{s}}, t) + I_d(\mathbf{r}, \hat{\mathbf{s}}, t), \qquad (1.21)$$

where I_c is the unscattered component, if the light source is coherent it is the collimated component and I_d is the scattered or the diffused component. Thus the RTE Eq. 1.18 could be split into two parts. The first one part corresponds to coherent light component which losses its energy (see Eq. 1.22):

$$\frac{1}{c_m} \frac{\partial I_c(\mathbf{r}, \hat{\mathbf{s}}, t)}{\partial t} + \hat{\mathbf{s}} \cdot \overrightarrow{\nabla} I_c(\mathbf{r}, \hat{\mathbf{s}}, t) = -(\mu_a + \mu_s) I_c(\mathbf{r}, \hat{\mathbf{s}}, t)$$
(1.22)

and the second one considers both coherent and incoherent components, which respectively receives and retains energy (see Eq. 1.23):

$$\frac{1}{c_m} \frac{\partial I_c(\mathbf{r}, \hat{\mathbf{s}}, t)}{\partial t} + \hat{\mathbf{s}} \cdot \overrightarrow{\nabla} I_c(\mathbf{r}, \hat{\mathbf{s}}, t) = -(\mu_a + \mu_s) I_c(\mathbf{r}, \hat{\mathbf{s}}, t) \\
+ \frac{\mu_a + \mu_s}{4\pi} \int_{4\pi} P(\hat{\mathbf{s}}, \hat{\mathbf{s}}') I_d(\mathbf{r}, \hat{\mathbf{s}}', t) d\Omega' \\
+ \frac{\mu_a + \mu_s}{4\pi} \int_{4\pi} P(\hat{\mathbf{s}}, \hat{\mathbf{s}}') I_c(\mathbf{r}, \hat{\mathbf{s}}', t) d\Omega'$$
(1.23)

The diffuse light explains the phenomena of diffuse-reflected light, which contains information about the slab from which it arise after multiple scattering and absorption events. The diffusely reflected light contains information about the biological tissue through which it was transmitted to the surface.

Diffusion Approximation (DA)

While propagating into the tissue, photons diffuse through it in a series of steps of random length and direction. This diffusion process is called random walk. When the number of photons propagating into tissues is large, the continuous photon density function according to the Diffusion Equation (DE) is described by Eq. 1.24 which is represented in the form of angular dependence of the specific intensity and is well approximated by the first-order expansion in the unit vector \hat{s} :

$$I_d(\mathbf{r}, \hat{\mathbf{s}}, t) \cong \frac{1}{4\pi} \Phi_d(\mathbf{r}, t) + \frac{3}{4\pi} F_d(\mathbf{r}, t) \hat{\mathbf{s}} \cdot \hat{\mathbf{s}}, \qquad (1.24)$$

where $\Phi_d(\mathbf{r}, t) = \int_{4\pi} I_d(\mathbf{r}, \hat{\mathbf{s}}, t) d\Omega$ is the total intensity at the point \mathbf{r} or fluence rate, and $F_d(\mathbf{r}, t) = F_d(\mathbf{r}, t) \cdot \hat{\mathbf{s}}_f = \int_{4\pi} I_d(\mathbf{r}, \hat{\mathbf{s}}, t) \hat{\mathbf{s}} d\Omega$ is the net intensity vector. By integrating all the terms of the RTE over 4π we derive Eq. 1.25

$$\frac{1}{c_m}\frac{\partial}{\partial t}\Phi(\mathbf{r},t) + \nabla \cdot F_d(\mathbf{r},t) = -\mu_a \Phi_d(\mathbf{r},t) + Q_c + Q_s, \qquad (1.25)$$

where Q_s and Q_c are the source terms of local sources and coherent field. Equation 1.25 is an expression of divergence F_d in terms of the total intensity Φ_d .

$$I_d(\mathbf{r}, \hat{\mathbf{s}}', t) \cong \frac{1}{4\pi} \Phi_d(\mathbf{r}, t)$$
(1.26)

By integrating eq. 1.25 over the scattering phase function (see Eq. 1.9) we obtain Eq. 1.27:

$$\frac{\mu_a + \mu_s}{4\pi} \int \int_{4\pi} P(\hat{\mathbf{s}}, \hat{\mathbf{s}}') I_d(\mathbf{r}, \hat{\mathbf{s}}', t) d\Omega' d\Omega =$$

$$\frac{\mu_a + \mu_s}{4\pi} \int_{4\pi} \left[\int_{4\pi} P(\hat{\mathbf{s}}, \hat{\mathbf{s}}') d\Omega \right] I_d(\mathbf{r}, \hat{\mathbf{s}}', t) d\Omega' =$$

$$\mu_s \int_{4\pi} I_d(\mathbf{r}, \hat{\mathbf{s}}', t) d\Omega' = \mu_s \Phi_d(\mathbf{r}, r).$$
(1.27)

This term allows for canceling out the component relating to scattering loss contribution in Eq. 1.19. To define relationship between F_d and Φ_d Flick's law is used for the lowest-order approximation (see Eq. 1.28):

$$c_m F_d(\mathbf{r}, t) = -D \overrightarrow{\nabla} \Phi_d(\mathbf{r}, t), \qquad (1.28)$$

where $D = \frac{1}{3(\mu_a + \mu_s(1-g))}$ stands for diffusion coefficient. The Flick's law postulates that the photon density decreases along the light distribution direction. Substitution of Eq. 1.28 to 1.25 yields to the DE (see Eq. 1.29):

$$\frac{\partial}{\partial t}\Phi_d(\mathbf{r},t) = D \bigtriangleup \Phi_d(\mathbf{r},t) - \mu_a c_m \Phi_d(\mathbf{r},t) + Q_c + Q_s \tag{1.29}$$

Boundary conditions

Since most biomedical optics applications involve delivery and collection of light going through interface between air and biological tissue, boundaries are becoming important [Jacques and Pogue, 2008]. The method of images is used to model boundary conditions. It is adapted to solve the relationship between fluence rate at the surface and flux escaping the biological tissue surface. Several assumptions are made to obtain the steady-state DA. For such steady-state, the DA provides simple solution of the DE with the limit of low absorption $(\frac{\mu'_s}{\mu_a} >> 1)$ and for the distances $(r > \frac{1}{\mu'_s})$. However, there are improved and higher order equations for closer SDS where the photon paths cannot be approximated as being diffuse. [Yoo et al., 1990, Farrell et al., 1992, Patterson and Pogue, 1994, Kienle and Patterson, 1997, Zevallos et al., 1998, Star et al., 1988, Makropoulou et al., 2007 provide an analytical solution to the RTE for simple geometries under conditions that the absorption of the tissue is negligible compared to the scattering and that the delivery and detection of light occur far enough apart (in space and in time) such that the photon trajectories can be treated as diffuse. Higher-order versions of the DA (such as the P_3 and P_N approximations [Hull and Foster, 2001, Phillips and Jacques, 2009]) offer increased accuracy for short SDS; however the mathematical expressions are more complicated. The air tissue-boundary is replaced by an infinite tissue with negative source located outside the original tissue boundary and mimics the loss of light outcoming from the tissue (see Fig. 1.10).

On average, each photon travels the distance $L = 2 \triangle z$ for every $\triangle z$ movement along the z-axis. Let's consider a test point at the surface, i. e. z = 0. Thus the flux at this point will result in the contribution of both I and J fluxes and is twice the value of flux passing along the z-axis: $\Phi(0) = 2(I + J)$. As the light J reaches the surface it is reflected with probability r_i , thus the flux inside the tissue is $r_i J$, so that $\Phi(0) = 2(1 + r_i)J$. The flux escaping the tissue is the observed reflectance which is $R = (1 - r_i)J$, and the fluence rate at the surface is:

$$\Phi(0) = 2(I+J) = 2R\frac{1+r_i}{1-r_i}.$$
(1.30)

Then the escaping flux R is represented by Eq. 1.31:

$$R = -D\frac{\Phi(z_0)}{|z_b|}.$$
(1.31)

The gradient from the tissue surface to the virtual surface symmetry at z_b is describing the boundary condition (Eq.1.32):

$$R = -D\frac{\partial\Phi}{\partial z} = D\frac{\Phi(0)}{z_b}.$$
(1.32)

The combination of Eqs. 1.30 and 1.32 and elimination of R gives Eq. 1.33:

$$z_b = -2\frac{1+r_i}{1-r_i}D.$$
 (1.33)



Figure 1.10: Scheme of the mismatched boundary condition. A - The photons diffuse in a J flux towards the surface and in an I flux away from surface, traveling on average the distance $L = 2 \triangle z$ per $\triangle z$ along the z-axis, the internal reflectance r_i at the surface is then $I = r_i J$. The escaping reflectance $R = (1 - r_i)J$. Thus the fluence rate at the surface is Eq. 1.30. B - the 1-D fluence rate distribution due to a planar source at $z = z_s$ and an air-tissue boundary at z_0 . z_b is the surface of symmetry. If the positive source is at z_s , then the negative one is calculated as $z_i = 2z_b - z_s$ and is placed outside the tissue. A flux out of the surface is guided by the gradient of light between z_0 and z_b , thus R is represented in Eq. 1.31

Thus the value of internal reflectance r_i specifies the value of z_b and can be derived with Eq. 1.34:

$$r_i = \int_{\theta=0}^{\frac{\pi}{2}} R_{Fresnel}(\theta) 2\pi \sin \theta d\theta, \qquad (1.34)$$

where $R_{Fresnel}$ is the Fresnel reflectance as the function of the angle of incidence θ relative to surface normal and the refractive index mismatch at the boundary. For an air/tissue interface the internal reflectance is well approximated as follows [Egan and Hilgeman, 1979] in Eq. 1.35:

$$r_i = 0.6681 + 0.0636n + \frac{0.7099}{n} - \frac{1.4399}{n^2}, \tag{1.35}$$

where n - is tissue refractive index. This equation is an accurate approximation for the range 1.0 < n < 2.2. For biological tissue $n \approx 1.4$ leads to $r_i \approx 0.5$. Hereby $z_b \approx -2z_s$, so that the negative source position is $z_i \approx -5z_s$. The net fluence rate in the tissue is the superposition of two sources (see Eq. 1.36):

$$\Phi(r,z) = \frac{e^{\frac{-r_1}{\sqrt{D/\mu_a}}}}{4\pi Dr_1} - \frac{e^{\frac{-r_2}{\sqrt{D/\mu_a}}}}{4\pi Dr_2},$$
with
$$\begin{cases} r_1 = \sqrt{(r-r_s)^2 + (z-z_s)^2} \\ r_2 = \sqrt{(r-r_s)^2 + (z-z_i)^2}. \end{cases}$$
(1.36)

Finally, as formulated in Eq. 1.36 the gradient of the fluence rate is the escaping reflectance as

a function of radial position is (see Eq. 1.37):

$$R(r) = -D\frac{\partial\Phi}{\partial z}\Big|_{z=0} = \frac{1}{4\pi}z_s\Big(\frac{1}{\delta} + \frac{1}{r_1}\Big)\frac{exp(-|r_1|/\delta)}{4\pi Dr_1^2} + (z_s + 2z_b)\Big(\frac{1}{\delta} + \frac{1}{r_2}\Big)\frac{exp(-|r_1|/\delta)}{4\pi Dr_2^2} \quad (1.37)$$
with
$$\begin{cases} \delta = \sqrt{D/\mu_a} & \text{is the optical penetration depth} \\ \mu'_t = \mu_a + \mu_s(1-g) & \text{is the diffusion length} \\ D = \frac{1}{3\mu'_t} & \text{is the reduced transport coefficient,} \end{cases}$$

The values of absorption and reduced scattering coefficients μ_a and μ'_s of biological tissue can be estimated from fitting of Eq. 1.37 to the reflectance spectrum acquired with a fiber-optic probe with different SDS. Even if the method is fast and seems to be simple, it has some restrictions which limits its distances to be shorter than one mean free path $(1/\mu'_t)$. Thus, with the multifiber probe used in the thesis work, it is possible to take this equation as first approximation with the advantage of reduced computation time. The concept of its application instead of MC simulations will be discussed later on in this thesis.

1.6.3 Adding-doubling method

Two methods, discrete coordinates and adding-doubling, allow for finding accurate solution of the RTE for anisotropic scattering and mismatched boundaries. The adding-doubling method generates fast and accurate solutions of light distributions in biological tissue. It is developed by Van de Hulst [van de Hulst, 1980a, van de Hulst, 1980b]. Since this fast and accurate model is intended for light propagation in biological tissues, the particular features associated with this medium must be incorporated in the model:

- 1. There should be no restriction on the ratio of scattering to absorption $\frac{\mu_s}{\mu_a}$ since this ratio varies from nearly zero in the both the ultraviolet (due to protein absorption) and the mid-infrared (due to water absorption) to large values in the therapeutic window in the red and near-infrared (NIR).
- 2. There should be no restriction on the scattering anisotropy, g, since tissue scattering tends to be strongly forward-peaked, which corresponds to anisotropy factor values $g \in [0.7-0.99]$.
- 3. Internal reflection at boundaries should be included in the model since air-tissue interfaces are common.

The doubling method assumes knowledge of the reflection and the transmission properties for a single thin homogeneous layer [Prahl et al., 1993]. The reflection and the transmission of a slab twice as thick as found by just posing two identical slabs and summing the contributions from the two slabs. The reflection and transmission for an arbitrarily thick slab are obtained by repeatedly doubling until the desired thickness is reached. The adding method extends the doubling method to dissimilar slabs, thereby allowing one to simulate media with different layers and/or internal reflection at boundaries. Adding-doubling works naturally with layered media and yields reflection and transmission readily, while discrete ordinates generates internal fluencies easily.

Unfortunately, the adding-doubling method is not suitable for *in vivo* diagnosis as it operates with slabs, thus when measuring the optical properties of a sample, the values needed are the total reflection and transmission of the sample.

1.6.4 Finite Difference, Finite Element and Boundary Element Methods

There are several basic approaches allowing to solve DE numerically: Finite Difference (FDM), Finite Elements (FEM) and Boundary Element (BEM) Methods [Jacques and Pogue, 2008].

The FDM is based on the Maxwell's equations applied to the discrete Cartesian mesh, where the volume is arbitrary. The volume should be with a fine discreteness to take into account smoothed and curved boundaries. It allows modeling time- and spatially-resolved light-tissue interactions. Time-domain DE has a major limitation - computational time due to iterative approach to converge to solution [Tanev, 2010, Tu et al., 2014, Chao and Sung, 2010]. However, the FDM is used for highly complex problems as test solution, mainly because it is easy to construct and to sample the volume.

The FEM is based on solving partially differential equations which are integrated on arbitrary discrete elements, called basis functions ψ_i , which map pieces of the entire domain between discrete points, called nodes. This method is also called Galerkin method and results in linear systems of equations, which can be solved by matrix methods. This approach has computational cost limitations depending on the sampling of an arbitrary volume of the problem to solve.

The BEM is quite useful for solving the problems in the case of medium modeled with homogeneous properties of the medium interior moreover the exterior of the medium can have an arbitrary shape. This method allows for solving the DE under homogeneity conditions with simplified Helmholtz equation. It is best implemented when the boundaries are known from some structural multimodal imaging *a priori* data. Its main drawback lies in the use of full matrices. The BEM was applied to study Asian and Caucasian skin and gave more precise results compared to the MC method and the FDM [Ansari and Massudi, 2009]. Even if its computational cost is said lower, several other methods allowing computational time reduction by means of parallel computing for MC method, will be discussed later in this chapter. Moreover since a biomedical optical spectroscopy diagnosis problem will be more complex, in terms of defining several SDS, increasing number of photons launched, adding more complexity to the tissue structure, the computational time will increase. Thus the computational time increase makes the BEM method unsuitable for the geometry of our multi-fiber probe, however not limiting the advances of this model application for imaging.

There are also works combining these methods in order to gain precision and to decrease computational time. The work of Paulsen et al [Srinivasan et al., 2010] coupling FEM and BEM for modeling DE in 3D for multi-modality optical imaging. This coupled solution showed better computational time than single FEM and was comparable to stand-alone BEM.

Even though all these methods give suitable results for reflectance analysis, they become more complex and require much more computational time for well-defined geometry of the experiment.

1.6.5 Semi-empirical models

Several groups have recently developed semi-empirical models to extract absorption and scattering parameters from reflectance measurements on human tissue. These models basically have two main characteristics: they are based on simple analytical functions of the tissue absorption and scattering coefficients, and they implicitly account for the geometry of the fiber-optic probe. The combination of these two characteristics makes semi-empirical models useful, because they can be more accurate than DA models while avoiding the high computational times associated with MC simulations.

For a setup with small SDS, Wilson et al [Reif et al., 2007, Reif et al., 2008] developed a forward semi-empirical model of reflectance from a homogeneous tissue slab. These authors used

the inverse model to study the effect of probe pressure on reflectance measurements acquired *in vivo* from thigh muscles of mice. The forward model was based on three assumptions:

- 1. the reflectance is proportional to the reduced scattering coefficient,
- 2. the attenuation of the reflectance can be described by a Beer-Lambert law $e^{-\mu_a < L>}$, where < L > is the mean total path length of a photon in the tissue, and
- 3. the mean total path length $\langle L \rangle$ of a photon in the tissue is inversely proportional to both the absorption coefficient and the reduced scattering coefficient.

The resulting semi-empirical reflectance Eq. 1.38 is:

$$R_{emp} = a\mu'_{s}(\lambda)exp\Big[-\frac{(C_{corr}\mu_{a}(\lambda)b)}{\left[\left[C_{corr}\mu_{a}(\lambda)\mu'_{s}(\lambda)\right]^{c}\right]}\Big].$$
(1.38)

The analytical model to describe the turbid tissue reflectance R was developed for small volumes by Bigio et al [Wilson et al., 2009] to detect pancreatic cancer.

$$R(\mu_a) = (a\mu'_s + a_0)e^{-\mu_a \frac{\delta}{(\mu_a \mu'_s)^c}},$$
(1.39)

where b and c are fitting coefficients, values a and a_0 are derived from $R(0) = a\mu'_s + a_0$, while the absorption is neglected. In Eq. 1.39, the factor C_{corr} accounts for the confinement of blood into cylindrical blood vessels. The values of a, b, and c are related to the properties of the fiber-optic probe and can be determined from MC simulations or reflectance measurements on tissue phantoms.

Zonios and Dimou [Zonios and Dimou, 2006] developed a forward semi-empirical model of reflectance from a homogeneous tissue and used the inverse model to extract absorption and scattering properties of normal skin and melanocytic nevi from *in vivo* reflectance measurements.

Marchesini et al [Marchesini et al., 2009] employed an inverse semi-empirical, homogeneousslab model to assess the melanin content of skin lesions (including 288 melanomas and 424 dysplastic nevi) from multispectral images acquired *in vivo*.

Abnormalities in melanin productions cause disorders in skin pigmentation, the model recovering absorption of this chromophore was developed by Hani et al. [Hani et al., 2012].

We will not focus on these solutions more, as the present research work of this thesis aims at using more universal forward model, than available by SEM.

1.6.6 Monte Carlo simulations

The MC method is a quite flexible and precise approach for simulating light propagation in tissues, but it is also time consuming method. The simulation is based on the random walk description of the photons as they travel through tissues. The random walks are chosen by statistically sampling the probability distributions for step size and angular deflection per scattering event. After propagating many photons, the net distribution of all the photon paths yields an accurate approximation to reality. There is a variety of ways to implement MC simulations of light transport in biological tissue. One approach is to predict steady-state light distributions. The most popular one is a steady-state model of MC for Multi-Layered media (MCML) that is well developed and documented and has been used by many groups.

The algorithm used for light-tissue interaction is shortly described hereafter. Basically, MC simulation is a stochastic propagation of photons into tissue, which optical properties being

represented by the probabilities for photons to be scattered, and to be eventually converted to fluorescence after absorption.

The Monte Carlo forward simulations code used in this thesis is written in the C++ language and uses CUDA parallel computing library.

As described in subsection 1.5.4, the packages of photons are launched through the sourcefiber and are detected with several fibers located at 6 different SDS which for higher sensitivity and for saving computational time are transformed into 6 rings surrounding the source-fiber.

The package of photons is going out of the fiber-source with predefined NA and diameter d similar to the multi-fiber probe configuration. The photon free pathway is defined by Eq. 1.40:

$$\delta s = \frac{-\log\xi}{\mu_t},\tag{1.40}$$

where ξ is a random value uniformly distributed in the interval [0, 1]. The probability of photon to be internally reflected is defined by the Fresnel reflection coefficient $R(\theta_i)$ (see Eq. 1.41):

$$R(\theta_i) = \frac{1}{2} \left(\frac{\sin^2(\theta_i - \theta_t)}{\sin^2(\theta_i + \theta_t)} + \frac{\tan^2(\theta_i - \theta_t)}{\tan^2(\theta_i + \theta_t)} \right), \tag{1.41}$$

where θ_i is the angle of incidence at the boundary and θ_t the transmission angle, which is defined by the Snell's law (see Eq. 1.1). A random number ξ is simulated to decide whether the photon is transmitted ($\xi < R(\theta_i)$) or internally reflected ($\xi \ge R(\theta_i)$). The same procedure is done for the photon reaching the interface between two layers. If the photon left the medium, then the reflectance is recorded. If the photon is internally reflected it is traveling the distance δs from (x,y,z) in the direction (η_x, η_y, η_z) to (x', y', z') defined by (see Eq. 1.42):

$$\begin{aligned} x' &= x + \eta_x \delta s, \\ y' &= y + \eta_y \delta s, \\ z' &= z + \eta_z \delta s. \end{aligned}$$
(1.42)

The technique of implicit capture assigns a weight to the photon package, then it is reduced step by step after each propagation: part is absorbed, part continues to move. The fraction absorbed (f_{abs}) is defined by Eq. 1.43:

$$f_{abs} = \frac{\mu_a}{\mu_s + \mu_a} = 1 - \frac{\mu_s}{\mu_s + \mu_a},\tag{1.43}$$

The photon direction is changed according to the phase function (see Eq. 2.17). The function generating the frequently used HG-function is defined by Eq. 1.44:

$$\cos\theta = \frac{1}{2g} \left(1 + g^2 - \left[\frac{1 - g^2}{1 - g + 2g\xi} \right]^2 \right)$$
(1.44)

If the scattering is isotropic (case of fluorescence), then another equation is used:

$$\cos\theta = 2\xi - 1 \tag{1.45}$$

If a photon is scattered at an angle (θ, ϕ) from the direction (η_x, η_y, η_z) , the new direction $(\eta'_x, \eta'_y, \eta'_z)$ is defined by Eq. 1.46:

$$\begin{cases} \eta'_x &= \frac{\sin\theta}{\sqrt{1-\eta_z^2}} (\eta_x \eta_z \cos\phi) - \eta_y \sin\psi) + \eta_x \cos\theta, \\ \eta'_y &= \frac{\sin\theta}{\sqrt{1-\eta_z^2}} (\eta_y \eta_z \cos\phi) + \eta_x \sin\psi) + \eta_y \cos\theta, \\ \eta'_z &= -\sin\theta \cos\psi\sqrt{1-\eta_z^2} + \eta_z \cos\theta. \end{cases}$$
(1.46)



Figure 1.11: Flowchart for the MCML model. The photon packet is initialized, i. e. number of photons and their weights are defined. Then random walk is calculated and the photon package is moved to the medium. If the photon package has left the medium and/or the layer, the possibility of the internal reflection is checked. If the photon left the medium, then the absorption and photon weight are updated, reflection is updated too. If the weight is too small, the possibility of photon survival is checked. If the weight is too small, the new photon is launched.

If the angle is $|\eta_i| > 0.99999$ almost normal to the current direction then the new direction is defined by Eq. 1.47:

$$\begin{cases} \eta'_x = \sin\theta\cos\psi, \\ \eta'_y = \sin\theta\sin\psi, \\ \eta'_z = \frac{\eta_z}{|\eta_z|}\cos\psi. \end{cases}$$
(1.47)

If the photon reaches one of the fiber-detectors, its direction has to be checked whether it get into NA of the fiber or not.

The global MCML scheme is presented in Fig. 1.11.

The other approach is to predict time-resolved light distributions [Prahl et al., 1989, Wang et al., 1995]. Drezek et al [Drezek et al., 2001] implemented a fluorescent MC model in layered slab geometry to distinguish between normal tissue and squamous epithelial lesions in an *in vivo* study in cervix. Vishwanath and Mycek [Vishwanath and Mycek, 2004] developed layered MC simulations to characterize the effect of tissue and probe properties on the time-resolved fluorescence measured *in vivo* from normal and precancerous human colon tissues. Pavlova et al [Pavlova et al., 2009, Pavlova et al., 2008] used layered MC models to distinguish between precancerous, neoplastic, and normal oral tissue *in vivo*.

1.7 Models of light-tissue interactions used in the thesis

Two methods were chosen as forward modeling for light tissue interactions simulation in the specific geometrical configuration of the multi-fiber probe described in section 1.5.4. The MC method was chosen as the main algorithm and DA to the RTE as an auxiliary one with noise corresponding to Monte Carlo simulations.

The last acceleration algorithms made MC simulations quite fast, which opens possibilities to further use them in real time what is crucial for clinical implementation of spectroscopic diagnosis. To increase speed of the MC simulations the CUDA MCML is used to increase speed of MC simulations through parallel simulations involving multi-core processing on Graphic card [Alerstam et al., 2008]. In 2010, next-generation acceleration and code optimization for light transport in turbid media using GPUs was presented followed by several publications which successfully used this algorithm [Alerstam et al., 2010, Sassaroli, 2011, Cai and He, 2012, Hennig et al., 2013].

Recent studies demonstrated that MC simulations enable quantitative description of light transport in turbid media with multiple layers, containing different fluorophores in each layer and allow for simulating any source and detector of photons close to real one optical fiber, i. e. precise numerical aperture, defining its refraction index, diameter. These advantages are making forward model based on MC simulations suitable for this research.

As MC simulation is a stochastic method, the number of photons launched, the mismatched boundary conditions, the presence of one or several fluorophores and other improvements make the model more complex and increase computational cost.

We have used an MC model and a DA as forward models for the inverse problem developed hereafter in the following chapters.

1.8 Thesis aim and objectives

A review on the emerging field on nanoparticles for *in vitro* diagnosis was published in 2015 by Gao et al [Gao et al., 2015]. It opens numerous possibilities in imaging and spectroscopic biomedical diagnosis. The aim of this research was in understanding the concept of the nanoparticle application for optical properties of biological tissues more specifically, epithelial tissue, as it covers many organs of the human body and is multilayered. In this frame the problem of light-tissue modeling becomes more complicated. For example, gold nanoparticles were used to manipulate fluorescence of the molecules inside the tissue [Kang et al., 2011]. The aim is to derive the optimal parameters of nanoparticle-fluorophore combination and experimentally confirm the applicability of theory on biological tissue phantoms with such combinations. To do this, it is needed to cover several gaps and answer several questions, which will be asked at the beginning of each chapter to give understanding to the main question: how nanoparticles can affect optical properties of biological multi-layered tissue on the example of epithelial tissue.

The further objective of this thesis lies in detection of cancerous and precancerous states of multilayered biological tissues. Carcinomas are the most common type of cancer (about 85% of all cancers) that begins in the superficial layer of skin (with a thickness typically of 100–500 μ m) or in tissues that line or cover internal organs. Thus, the measurement techniques should be sensitive to the superficial layers, what is considered both as a drawback for biomedical optical diagnosis and as an advantage to diagnose disease on the superficial layers. The penetration depth of the collected photons depends on the fiber probe geometry, as well as on the wavelength range. But it should be taken into account that tissue undergoes a lot of changes which result in

optical properties modifications, thus modifying in turn the penetration depth, which may allow for the optical probing depth extending beyond the epithelial layer[Zhu et al., 2003, Nieman et al., 2004, Wang et al., 2005, Liu and Ramanujam, 2006].

Keeping in mind that the thesis aim is in improving the recovering procedure of biological tissues optical parameters and understanding the influence of nanoparticles on intrinsic properties of the system, hereafter we present the general plan for the conducted research.

Firstly, to start the research, the nanoparticles suitable for optical diagnosis were chosen and characterized experimentally and theoretically [Jennings and Strouse, 2007]. Thus the theoretical and experimental research on nanoparticles to estimate their optical properties is conducted in chapter 2.

Secondly, to modify optical properties of biological tissue with chosen nanoparticles. For this purpose it is needed to model biological tissue. In this thesis it is epithelial tissue, more precisely - skin. In chapter 3 a special experimental setup was made using the multi-fibre optical probe, parameters of which are described previously and more precise in the thesis of Pery [Pery, 2007]. Some conclusions of the nanoparticle concentration influence on the spectra obtained are given at the end. This chapter answers a preliminary question - how nanoparticles can influence the optical properties by experimental modeling. Thus, chapters 2 and 3 are forming the first aim.

Thirdly, to spread more light on the processes undergoing in tissue containing nanoparticles and a fluorophore under laser exposure, it is needed to recover optical properties by means of a so-called inverse problem solving or parameter estimation. The inverse problem in biomedical optical spectroscopy is based on comparison of modeled and experimental spectra. The optimal parameters are obtained when a similarity function, called cost-function, reaches a global minimum. Thus, a dedicated inverse problem solving algorithm is developed and presented in chapter 4. It is based on the forward Monte Carlo simulations to model DR spectra close enough to the experimental ones. This optimization algorithm solves some present problems of inverse problem in biomedical diagnosis. Its features and capabilities are explained and discussed in same chapter 4 and correspond to the second aim.

Then, to detect any changes in fluorescence life-time of the photosensitizer, used to model intrinsic fluorescence in epithelial tissue, a model of nanoparticle-fluorophore interaction was theoretically calculated in chapter 5). Thus, forming the third aim this chapter answers a main question: how the nanoparticles under electromagnetic field excitation transfer the energy to a fluorophore and how do they couple? Afterwards, a life-time measurements experiment with picosecond excitation is made for the solution of colloidal nanoparticles and photosensitizer.

Finally, the conclusion is given and the consequent perspectives are discussed in the last chapter 6.

Chapter 2

Modeling of nanoparticles optical properties

2.1 Nanoparticles: from one to many

The most recent book on plasmonics edited by Geddes[Geddes, 2015] and published in February 2015. A recent review by Chen et al [Gao et al., 2015] highlighted the use of gold nanoparticles for *in vitro* diagnosis. The effects occur in gold nanopaticles could be applied to some other noble metals. In recent years materials having the largest dimension less than 100 nm have been deeply investigated and reached outstanding facilities in preparation procedures of nanoparticles [Champion et al., 2007], their use in many areas of nowadays life[Weibo et al., 2008]. Among all the nanoparticles practical usages and the theoretical investigations there is a one quite prominent, which is connected with inner properties of a material from which nanoparticles are made of. Some materials have abilities to resonantly absorb and scatter light, these nanoparticles are called plasmonic nanoparticles. Applied to photodiagnosis of biological tissue, this kind of nanoparticles seems to be leading to the new approaches in fluorescence diagnosis

Many questions arise: what should be the geometry of a nanoparticle? Will the nanoparticles allow to "go deeper" into tissue? Should they more scatter of absorb the incident light? How the energy is transferred between a nanoparticle and a photosensitizer? To spread some light on these questions, we will try to solve them in this and the following chapters.

One of the most-used models of light-spherical particles interaction is Mie theory of light scattering [Mie, 1908]. The most well-known up-to-date theoretical models of light interaction with small particles were described by Bohren and Huffman in their book of *Absorption and scattering of light by small particles* and many other authors earlier in 1950-70s [Bohren and Huffman, 1998]. Since then these models were applied to many materials of different shapes and compositions: from spherical monomaterial to coated polymaterial rods, spheroids, disks, etc [Ishimaru, 1991, Bashkatov et al., 2000b, Kelly et al., 2003, Ehrhold et al., 2008].

In this chapter the theoretical and the experimental component of the light-nanoparticles interaction are being discussed. Firstly, the dielectric function of metals and its change due to small size of objects (less than 100 nm) under investigation are presented. Then the theory of nanoparticles is argued, beginning from the optical properties of one single nanoparticle, depending on the media it is embedded to and the geometry of a nanoparticle. Thirdly, the local field change due to presence of two nanoparticles close enough to each other is modeled. Fourthly, some colloids of nanoparticles are investigated experimentally to figure out the nanoparticles of interest for optical diagnosis. Finally, according to the results several types of nanoparticles are chosen to investigate their influence on the optical properties of biological tissues.

2.2 Drude-Sommerfeld theory of metals optical properties for free electrons

The material properties are calculated by using solid-state theory. According to a simple Drude theory free electrons around nanoparticle oscillate 180^{0} out of phase relative to the incident electric field [Novotny and B.Hecht, 2006]. The Drude-Sommerfeld theory assumes free electrons oscillate without any interactions between each other with common relaxation time, τ . Each collision leads to directionality loss, what results in random value of electron velocity. For such materials as gold and silver free electrons lay in the 6s and 5s states, correspondingly. Let's apply this model to take into account free electrons on the surface of the metal in an equation of motion 2.1.

$$m_e \frac{\partial^2 \mathbf{r}}{\partial^2 t} + m_e \gamma \frac{\partial \mathbf{r}}{\partial t} = e E_0 e^{-i\omega t}, \qquad (2.1)$$

where e and m_e are the charge and the effective mass of free electrons, $\gamma = 1/\tau$ stands for the frequency of electrons collision (τ is the relaxation time). E_0 and ω are the amplitude and the frequency of the applied electric field.

This equation of motion does not contain the restoring force, as the electrons are considered as free. Solution of Eq. 2.1 with ansatz $\mathbf{r}(t) = \mathbf{r}_0 e^{-i\omega t}$ leads to a modified dielectric function which takes into account free electrons (see Eq. 2.2):

$$\varepsilon_{Drude}(\omega) = 1 - \frac{\omega_p^2}{\omega^2 + i\gamma\omega},\tag{2.2}$$

where $\omega_p = \sqrt{ne^2/(m_e\varepsilon_0)}$ is the plasma frequency and γ is the damping constant and related to the Fermi-velocity (v_F) and the free electron mean path (l) as v_F/l [Kawata, 2001].

Thus, light can penetrate a metal only to a very very small extent, as the imaginary dielectric function is negative and leads to strong refractive index $(n = \sqrt{\varepsilon})$, the effect occurs is called skin-effect and characterized by the skin-depth $\delta = \sqrt{2\rho/(\omega\mu)}$, where μ stands for the relative permeability of the medium (gold and silver are considered as efficient conductors, thus $\mu =$ 1), ω is the frequency of the incident light, ρ is the resistance of the medium ($\rho_{Au} = 2.44 \cdot$ $10^{-8}\Omega m, \rho_{Ag} = 1.62 \cdot 10^{-8}\Omega m$), $\omega = 2\pi c/\lambda$. Thus, the skin-depth of gold and silver in the optical region ($\lambda \approx 300$ -900 nm) is about 1-1.7 nm and 0.8-1.4 nm, correspondingly. The imaginary part describes the energy dissipation which is associated to the movement of electrons near a metal surface.

For the noble metals like gold and silver in the optical region the ion cell influence increase. However, this theory fails at the optical frequencies, as it does not take into account the interband transition. Its contribution becomes valuable for the high frequencies ($\omega \gg \omega_p$), thus for the wavelengths lower than 550 nm it is convenient to use the following dielectric function, which takes into account a bulk dielectric function:

$$\varepsilon(\omega) = \varepsilon_{bulk} - \frac{\omega_p^2}{\omega^2 + i\gamma\omega}$$
(2.3)

2.3 Dielectric function of metals with bounded electrons contribution

The imaginary part of the dielectric function increases much more strongly in case of bounded electrons, this is because higher-energy photons can promote electrons of the lower-lying bands into the conduction band. Those bound electrons lay in the lower shells of metal atoms. For gold and silver it's not possible to ignore the interband transitions: $5d \rightarrow 6sp$ and $4d \rightarrow 5sp$, respectively. For gold the interband transition lies in the visible range, so that's why we see its color. However for silver this transition lies on the UV region it results in the absorption in the visible range.

For bound electrons the dielectric function is calculated using the interband transitions mentioned above [Rosei et al., 1973]. Scaffard et al [Scaffard and Tocho, 2006] used the simplified equation from Inoyue et al [Inouye et al., 1998] of the bound-electrons contribution (see Eq. 2.4):

$$\varepsilon_{IB}(\omega) = Q_{Bulk} \int_{\omega_g}^{\infty} \frac{\sqrt{x - \omega_g}}{x} \frac{[1 - F(x, E_F, T)]}{x^2 - \omega^2 + \gamma_b^2 - 2i\omega\gamma_b} dx, \qquad (2.4)$$

where $h\omega_g$ is the gap energy E_g ; $F(x, E_F, T)$ is the Fermi energy distribution function fro conduction electron of energy hx at the temperature T with the Fermi energy E_F ; γ_b is the damping constant for the interband transitions and Q_{bulk} is a proportionality factor.

2.4 Size-dependent dielectric function

For nanoparticles sized less than 20 nm the dielectric function of their bulk material becomes size-dependent, thus it is needed to take into account the intraband transitions described by the Drude theory and the interband transitions described by bound electrons. For large wavelengths the free-electrons contribution dominates, for short wavelengths the bounded electrons component is major. For example, when metal nanoparticles are smaller than the skin-depth of their corresponding metals, then the electron's free mean path can be of the same order as the size of nanoparticle, *a*. Hereafter we consider the nanoparticles bigger than 5 nm in size, as it was shown that small nanoparticles quench fluorescence, however big nanoparticles drastically increase it. A resent review on fluorescence quenching and enhancement is presented by Achilefu et al [Kang et al., 2011]. Fluorescence enhancement mostly occurs in the vicinity of bigger nanoparticles and this question is described in the last chapter.

Considering the dielectric function changes due to the size of a nanoparticle comparable with the skin-depth we first take into account conduction electrons. Thus, the scattering of conduction electrons on a surface of a nanoparticle leads to higher frequency of collisions, thus the relaxation time decreases (see Eq. 2.5):

$$\Gamma = \tau_{surface}^{-1} = \tau^{-1} + A \frac{v_F}{a} = \gamma + A \frac{v_F}{a}, \qquad (2.5)$$

where v_F is the Fermi velocity, A is the effective parameter which describes the coherency loss on the surface (for spheres this parameters equals 1), for comparison with the experimental data we still account for this value taken for spheres, as the experiment was held on the colloids of nanoparticles, thus the surface roughness of the nanoparticles is negligible. Scaffaerdi et al [Mendoza Herrera et al., 2014] calculated the dielectric function for the spherical nanoparticles from 3 to 50 nm in diameter.

To take into account that bound electrons contribution is size-dependent the metal nanoparticle dielectric function of both gold and silver is calculated, thus, we follow the work of Scaffard [Scaffard and Tocho, 2006] by considering Eq. 2.4 with the size-dependent parameters. The gap energy E_g , which was chosen being size-dependent according to the research of Logunov et al [Logunov et al., 1997] suggesting different density of states for different sizes of nanoparticles, however it concerns only nanoparticles of skin-depth size, which is not our case. However, this work proposed the size-dependent proportionality factor $Q(a) = Q_{Bulk}[1 - exp(-a/a_0)]$, where a is a nanoparticle radius, and a_0 is a scale factor representing the range where the density of states can be considered to reach that for a bulk metal.

The plasma frequency ω_p and the Fermi velocity v_F , the relaxation constant τ , for gold and silver are presented in the following Table 2.1 [Sonnichsen, 2001]:

Metal	ω_p, Hz	$\gamma_{free}, \mathrm{Hz}$	$\gamma_{bound}, \mathrm{Hz}$	E_g , eV	E_F, eV	$v_F,\mathrm{m/s}$	a_0, m	$Q_{bulk}, cm^{\frac{3}{2}}$
Gold	13.23	$12.6 \cdot 10^{13}$	$2.4 \cdot 10^{14}$	2.1	2.5	$14.1 \cdot 10^5$	0.35	$0.43 \cdot 10^{24}$
	$\cdot 10^{15}$						$\cdot 10^{-9}$	
Silver	13.75	$3.12 \cdot 10^{13}$	$1.5 \cdot 10^{14}$	1.9	4.12	$14.1 \cdot 10^5$	0.35	$0.5 \cdot 10^{24}$
	$\cdot 10^{15}$						$\cdot 10^{-9}$	

Table 2.1: Parameters (the Fermi velocity, v_F ; the relaxation constant, τ ; the plasma frequency, ω_p) for gold and silver used to calculate the size-dependent dielectric function (see Eq. 2.6) [Johnson and Christy, 1972].

Johnson and Christy [Johnson and Christy, 1972] obtained the dielectric functions of bulk gold and silver (see Fig. 2.1).



Figure 2.1: Real and imaginary parts of the dielectric function ($\varepsilon(\lambda)$) for gold and silver nanoparticles in the optical region (350-1000 nm) obtained by [Johnson and Christy, 1972]

In the work of Scaffard et al[Scaffard and Tocho, 2006] the size-dependency is taken into

account for free electrons by adding the size-dependent frequency $\gamma_{free} = \gamma_{bulk} + aV_F/r$ and for bounded electrons by exchanging the Q_{bulk} to the $Q_{bulk}(1 - e^{-a/a_0})$ (see Eq. 2.6):

$$\varepsilon^{MNP}(\lambda, a) = \varepsilon_{\infty} + \varepsilon^{Drude}(\lambda) + \varepsilon^{IB}(\lambda, a) = 7 - \frac{\omega_p^2}{\omega^2 + i(\gamma + Av_F/a)\omega} + Q_{Bulk}(1 - exp(-a/a_0)) \int_{\omega_g}^{\inf} \frac{\sqrt{x - \omega_g}}{x} \frac{[1 - F(x, E_F, T)]}{x^2 - \omega^2 + \gamma_b^2 - 2i\omega\gamma_b} dx,$$
(2.6)

where $\varepsilon_{\infty} = 6$ is the offset value which accounts for the integrated effect of all the higher-energy interband transitions [Novotny and B.Hecht, 2006]. The contribution of the Drude electrons accounting for the size of nanoparticles is given on Figs. 2.2 and ??.



Figure 2.2: Contribution to the real and the imaginary parts of free (Drude) electrons of the size-dependent dielectric function ($\varepsilon^{Free}(\lambda, a)$) of gold nanoparticles in optical region (350-900 nm) calculated for the following radii: 7±1 and 25±1 nm

The bigger a nanoparticle is, the closer its dielectric function to one corresponding to its bulk material. The contribution of bounded electrons taking into account the size of nanoparticles is shown on Fig. 2.3.

2.5 Mie theory applied to nanoparticles

The response of a metal nanosphere on electromagnetic field excitation can be calculated by solving the Maxwell's equations. Briefly, these fundamental equation describe the evolution of the electromagnetic waves in present of charges, current and polarizable media. To define field quantities in the media we denote the local polarizability \vec{P} and the local magnetisation \vec{M} , then the properties of the free space: permeability (μ_0) and permittivity (ε_0), thus we have:

$$\overrightarrow{D} = \varepsilon_0 \overrightarrow{E} + \overrightarrow{P} \tag{2.7a}$$

$$\vec{H} = \mu_0^{-1} \vec{B} - \vec{M} \tag{2.7b}$$



Figure 2.3: Contribution to the real and the imaginary parts of bounded electrons of the sizedependent dielectric function ($\varepsilon^{InterBand}(\lambda, a)$) of gold nanoparticles in optical region (350-900 nm) calculated for the following radii: 7±1 and 25±1 nm

The optical properties of gold are due to valence (5d) and conduction (6sp) electrons. The outermost d and s electrons of the constituent atoms must be treated together leading to six bands: five of them are fairly flat, lying a few eV below the Fermi level, and are usually denoted as d bands; the sixth one, which is almost free-electron like is known as sp-band or the conduction band [Lin et al., 2007]. An analytical solution, which exist for an uncharged sphere belongs to Mie [Mie, 1908] and also was described by Bohren and Huffman [Bohren and Huffman, 1998]. The spherical symmetry suggest the use of a multipole extension of the fields, which is numbered by n. The scattering and absorption cross-sections are calculated by 2.8:

$$\sigma_{ext} = \frac{2\pi r^2}{x^2} (2n+1) Re(a_n + b_n)$$
(2.8a)

$$\sigma_{abs} = \frac{2\pi r^2}{x^2} (2n+1)(|a_n|^2 + |b_n|^2), \qquad (2.8b)$$

where $x = kr = \frac{\hbar \omega n_{medium}}{\hbar c}$, k - wavevector, r - particle radius, n_{medium} - refractive index of media, a_n and b_n the Mie coefficients, which are calculated by 2.9:

$$a_n = \frac{m\psi_n(mx)\psi'_n(x) - \psi_n(x)\psi'_n(mx)}{m\psi_n(mx)\xi'_n(x) - \xi_n(x)\psi'_n(mx)}$$
(2.9a)

$$b_n = \frac{\psi_n(mx)\psi'_n(x) - m\psi_n(x)\psi'_n(mx)}{\psi_n(mx\xi'_n(x) - m\xi_n(x)\psi'_n(mx)}$$
(2.9b)

$$m = \sqrt{\varepsilon_r} = \sqrt{\frac{\varepsilon_{particle}}{\varepsilon_{medium}}},$$
(2.9c)

where $\varepsilon_{particle}$ is as considered above; ψ and ξ - the Ricatti-Bessel functions [Bohren and Huffman, 1998]. Using these equations it is essential to calculate optical properties of nanoparticles with wavelength resolution. The bulk dielectric function was obtained from data of Christy and Johnson [Johnson and Christy, 1972]. In Fig. ?? and 2.2 size-dependent extinction coefficients $(Im(\varepsilon(\lambda, r)))$ and refractive indices $(Re(\varepsilon(\lambda, r)))$ for silver and gold are represented. The calculations were made in *Wolfram Mathematica 8*.

2.6 Modeling of optical properties for nonspehrical nanoparticles and nanoshells

There are some interesting effects occur due to non-spherical form of nanoparticles, i. e. field enhancement can occur due to longitude resonances and is quite different if the orientation of nanoparticles to the excitation field direction (which is defined by \vec{k} is the direction of electromagnetic field and \vec{E} is the electromagnetic field).

For calculating the response of a nanoparticle on electromagnetic field it is needed to know the polarization of a nanoparticle. This type of interaction os well known as the solution for electromagnetic field diffraction in terms of quasi-static theory of excitation for solving the Maxwell's equations [Voytovich et al., 1977, Klimov, 2009]. Quasi-static theory does not account for delay effects which occur in ellipsoid, but with enough precision gives the results comparable to experimental data. The advantage of this theory is in plasmon modes oscillations calculation moreover the magnetic fields are not taken into account, thus magnetic permittivity equals one.

For calculation of optical parameters of nanoparticles, i. e. scattering and absorption crosssection it is needed to know the dielectric permittivity of the materials and geometry of the system. Let's consider a nanoobject consisting of two layers in homogeneous electromagnetic field: flat electromagnetic wave propagates in z-direction with wavevector $\vec{\mathbf{k}}$, which is defined by dielectric permittivity of the host medium ε_h , frequency $\omega = 2\pi n_/ \lambda$, where $n_h = \varepsilon_h^2$ is refractive index of medium. Geometrical parameters of the object are its semi-axes a, b, c and its coat thickness d. Considering the far filed of such an object it is a dipole's field and the polarizability of a particle has a diagonal representation:

$$\Pi = \begin{bmatrix} \Pi_x & \dots & \dots \\ \dots & \Pi_y & \dots \\ \dots & \dots & \Pi_z \end{bmatrix}$$
(2.10)

Its components for coated nanoparticle:

$$\Pi_{j} = \frac{(a+d)(b+d)(c+d)}{3} \frac{(\varepsilon_{sh} - \varepsilon_{h})[\varepsilon_{sh} + (\varepsilon_{sh} - \varepsilon_{c})(L_{i} - fL'_{i})] + f\varepsilon_{sh}(\varepsilon_{h} - \varepsilon_{c}))}{[\varepsilon_{sh} + (\varepsilon_{sh} - \varepsilon_{c})(L_{i} - fL'_{i})][\varepsilon_{h} + (\varepsilon_{h} - \varepsilon_{sh})L'_{i}] + fL'_{i}\varepsilon_{sh}(\varepsilon_{c} - \varepsilon_{sh})},$$
(2.11)

where $f = \frac{abc}{(a+d)(b+d)(c+d)}$ is the ratio of coat and sphere volumes, $L_i = \frac{abc}{3}I_i$ is geometrical factor, $I_i = \int_0^{\inf} \frac{du}{(x+a)R(x)}$, $R(x) = \prod_{m=1}^3 \sqrt{x-a_i}$, $a_i = a, b, c$ are the semi-axes or a + d, b + d, c + d for the coat, indices sh, c and h correspond to shell, core and host medium, respectively.

A simple and the most studied is the mode whose potential is linear according to the coordinates in the volume of a nanoparticle. For those mode are corresponding to their eigenvalues, which could be found by geometrical factor L_i connected with the values if the linear potentials $L_i = 1 - \varepsilon_i / \varepsilon_h$.

If the polarizability is known, then the scattering and the absorption cross-sections could be



Figure 2.4: Coated nanoparticle in dielectric medium, \mathbf{k} is the direction of electromagnetic field, a, b and c are the semi-axes, d stands for the thickness of the coat. The coordinate system is indicated by x, y and z.

found:

$$\sigma_{abs} = 4\pi k (\frac{\Pi_x}{3} + \frac{\Pi_y}{3} + \frac{\Pi_z}{3})$$
(2.12a)

$$\sigma_{sca} = \frac{8\pi k^3}{3} \frac{|\Pi_x|^2}{3} + \frac{|\Pi_y|^2}{3} + \frac{|\Pi_z|^2}{3}$$
(2.12b)

(2.12c)

The whole algorithm to calculate scattering and absorption cross-section is represented in Annex B.

2.7 Simulation results and discussion

By means of *MATLAB R2015a* (the MathWorks, Inc.) the optical properties of gold and silver spherical nanoparticles were modeled, the media was chosen to be water, the refractive index is n = 1.33 (Figures 2.5 and 2.6).

The change of resonant wavelength for gold nanoparticles is shown in Fig. 2.7.

For media with refractive index n = 1.33, which is water, the absorption and scattering crosssections were estimated for the resonant wavelengths of gold nanoparticles in the range of r = 5.50nm. Consequently, for small radius absorption dominates scattering, then at the certain point, around r = 40 nm, scattering equals absorption, then scattering starts to dominate 2.8. The shapes of cross-sections are not smooth due to experimental data errors in dielectric functions.



Figure 2.5: Normalized scattering of the gold nanoparticles calculated with the analytical Mie solution of the Maxwell's equations, r = 5, 10, 20, 30, 40, 50 nm. The figure shows the red-shift for nanoparticles with radii growth.



Figure 2.6: Normalized absorption of gold nanoparticles calculated with analytical Mie solution of the Maxwell's equations, r = 5, 10, 20, 30, 40, 50 nm. The figure shows the red-shift for nanoparticles with radii growth.



Figure 2.7: Dependence of resonant scattering, absorption and extinction wavelengths on gold nanoparticles radius. The calculations show that the resonant wavelengths are different for scattering and absorption, thus extinction cross-section resonant wavelength is lying between absorption and scattering maxima.



Figure 2.8: Dependence of absorption, scattering and extinction cross-sections dependence on the gold nanoparticles radii. The figure shows the difference between cross-section value for scattering and absorption, what changes the resonant wavelength of the extinction coefficient.

2.8 Expanding Mie theory to Finite-Difference Frequency-Domain method

Implementation of analytical solution of the Maxwell's equations by Mie to the Finite-Difference Frequency-Domain method is described further. The FDFD method is a numerical solution method for electromagnetic problems. The FDFD is based on finite-difference approximations of the derivative operators in the differential equation being solved. This method is implemented by use of a Yee grid [Schneider and Kruhlak, 2001], which (1) implicitly satisfies the zero divergence conditions to avoid spurious solutions, (2) it naturally handles physical boundary conditions, and (3) it provides a very elegant and compact way of approximating the curl equations with finite-differences. The example of a Yee's grid is presented in Fig. 2.9. Let's define the geometry



Figure 2.9: An example of a Yee's grid and electromagnetic vectors positions on the border in 3D domain. A Yee's grid is used to calculate the electromagnetic field distribution in a medium.

of nanoparticles and the medium it is embedded to: we are considering a metal gold nanoparticle of a radius r in a dielectric medium (water) with n = 1.33. Due to the symmetry of the problem posed it is possible to consider a quarter of the media, meshed with different types of blocks: layers of certain thickness and tetrahedrons to fit physical conditions (see Fig. 2.10). The time of calculations depends on the size of the domains, which in turn results in the solution accuracy. The following conditions are the trade-off between precision and computational time:

$$\delta_{maxmeshelement} = \lambda/6$$

 $\delta_{PML} = \delta_{medium} = \lambda/2.$

Initial conditions were as follows:

$$E_0 = 1V/m$$

$$\lambda = 350: 2: 850 nm$$
$$r = 5: 5: 50 nm$$

. The light is co-directional to the z-axis, $E = E_0 e^{-ikx}$. Due to the symmetry and the direction of the electromagnetic wave, the XZ plane is considered to be a Perfect Magnetic Conductor (PMC), $\overrightarrow{n} \times \overrightarrow{H} = 0$, and the XY plane as a Perfect Electric Conductor (PEC), $\overrightarrow{n} \times \overrightarrow{\mathbf{E}} = 0$, the magnetic field:

$$\vec{\mathbf{B}} = \mu_0 \mu_r \vec{\mathbf{H}}.\tag{2.13}$$



Figure 2.10: Sample of the geometry and the meshing of a nanoparticle and a medium. PML is perfectly matched layer which is used to calculate the scattered far filed resulting in the infinite media is truncation and consequent reduce of the computational time. Dielectric media - is the media where the nanoparticle is placed, it is characterized by n_{medium} , while the nanoparticle domain is the nanoparticle itself characterized by its refractive index and its extinction coefficient

The scattering boundary condition is used to calculate the scattered far field and to use truncated media, considered as infinite, which allows time gaining:

$$\mathbf{n} \times (\nabla \times (\overrightarrow{\mathbf{E}} + \overrightarrow{\mathbf{E}}_b)) - (jk + \frac{1}{r})\overrightarrow{\mathbf{n}} \times (\overrightarrow{\mathbf{E}} \times \overrightarrow{\mathbf{n}}) = 0$$
(2.14)

Wave equation:

$$\nabla \times \mu_r^{-1} (\nabla \times \overrightarrow{\mathbf{E}}) - k_0^2 (\varepsilon_r - \frac{j\sigma}{\omega\varepsilon_0}) = 0.$$
(2.15)

2.8.1 Results

The calculations results are presented in Fig. 2.11 and 2.12.

Scattering and absorption resonance picks are red-shifted with growth of nanoparticle size.



Figure 2.11: Normalized absorption cross-sections for gold nanoparticles, radius, r: 10, 20, 30, 40 and 50 nm. The graph shows red-shift of resonant wavelength with growth of nanoparticle radius.



Figure 2.12: Normalized scattering cross-sections for gold nanoparticles, radius, r: 20, 30, 40 and 50 nm. The graph shows red-shift of resonant wavelength with growth of nanoparticle radius.

2.9 Experimental estimation of nanoparticles optical properties

Spectrophotometer is a device for determining optical properties of a turbid or reflecting media. To estimate the optical properties of nanoparticles, the Hitachi U-3400 spectrophotometer was used. Spectra were taken in the wavelength scanning mode, the range of wavelengths scanned was 350-850 nm. The double beam scheme allows for determining the extinction coefficient.

2.9.1 Double beam scheme

The spectrophotometer Hitachi U-3400 allows for determining the light attenuation in a turbid media by measuring the light transmitted through two cuvettes: first contains a solution of nanoparticles and the second - a solvent. In the double beam scheme, the energy from the light source is divided for two beams by means of the half mirror, the light passes the same distance from the source to each cuvette, absorbed and scattered in a sample and in a reference solution (solvent) and then is collected by photomultipliers. The principle scheme is presented in the Fig. 2.13. Thus, the extinction spectrum is estimated by taking into account the solvent optical properties suchwise avoiding its influence on a sample extinction. The cuvettes were 10 mm width, which is the optical way passed by light, l. In this scheme the collimated transmission was measured, T_c , which in first approximation equals to the whole transmission, which is usually measured with the sample in front of the sphere, as soon as the nanoparticles in this experiment mostly absorb light. The measurements were taken for several types of nanoparticles:



Figure 2.13: Double-beam scheme of the spectrofotometer is used to measure transmission, T, by comparison of the solution under investigation and its solvent.

gold nanospheres and gold nanostars. The gold nanostars were obtained from Calin Hrelescu (Institute of Applied physics and Johannes Kepler University, Austria). The procedure of the
nanoparticle synthesis could be found elsewhere [Hrelescu et al., 2011]. Individual gold nanostars show different effects according to the polarization of incident light to a nanostar.

2.9.2 Integrating sphere measurements

Integrating sphere accessory mounted in the spectophotometer Hitachi U-3400 allows for determining the absorption coefficient of the same solution. The scheme is presented in Fig. 2.14. The sample was made in the following way: the solution of the nanopaticles in distilled water was placed between two thin round glasses which are separated by silicon ring of thickness 0.5 mm, behind the glass the white paper was placed to avoid absorption and to fulfill the condition of diffuse reflection. The reference sample was made in the same way. Thereby the light is traveling double distance between the two glasses, 1 mm, before it is scattered to the integrating sphere. Thus reflectance, R, was measured. According to the theoretical calculations, it was



Figure 2.14: Integrating scheme of the spectrophotometer used to measure reflection, R, of the solution under investigation by comparison with its solvent in the reference beam. If the solution is placed at the entrance of the integrating scheme, then the collimated Transmission is measured, T_c .

estimated, that small nanoparticles are scattering much less than they do absorb, so the collimated transmission equals to transmission, thus the collimated transmission was not measured, because scattering could be neglected in comparison to absorption.

2.10 Honiophotometry

Phase function was obtained with Dynamic Light Scattering (DLS) system (Photocor, Ltd.) which geometry is sketched in the Fig. 2.15. The sample of the nanoparticles solution in distilled

water was placed in a round glass test-tube, the temperature was kept stable by thermostat (20 0 C), the solvent was distilled water, which was taken into account while taking the measurements. The system allows for angle-resolved measurements in the 40-130 degrees range. The step was 5 degrees, each point was measured during 60 seconds. Light source was the He-Ne laser with 632.8 nm output wavelength. To measure the anisotropy coefficient the solution of the particles under investigation should fit the condition of one-event scattering, i. e. the light should be scattered only once before it being detected by a photon counter. To calculate this the following equation was used (2.16)[Du Le et al., 2014]:

$$N = \frac{6x}{y\pi d^3},\tag{2.16}$$

where x is the concentration, g/ml, y is the density of the particle's material, g/ml and d stands for the diameter of the particle, μm . The measured phase function was fitted with modified



Figure 2.15: Dynamic light scattering scheme was used to measure the size of nanoparticles at the angle of 30^{0} , the phase function was measured from 30 degrees to 140.

Heyney-Greenstein function (see Eq. (2.17)) by the least squares method.

$$P(\theta) = \frac{1}{4\pi} (b + (1 - b)) \frac{1 - g^2}{(1 + g^2 - 2g\cos\theta)^{\frac{3}{2}}},$$
(2.17)

where b is the fraction of isotropic scattering. The knowledge of an anisotropy factor allows for determining scattering reduced coefficient, μ'_s , from the following relation (see Eq. (2.18)):

$$\mu'_s = \mu_s (1 - g). \tag{2.18}$$

2.11 Summary

The optical density of the gold nanospheres and nanostars measured by means of the double-beam scheme and the integration sphere are presented in Fig. 2.16.



Figure 2.16: Relative absorption of the gold nanospheres and nanostars measured by means of the two-beam scheme

2.11.1 Nanoparticle size measurements with Dynamic Light Scattering

To measure the nanoparticles size, the Photon-Correlation Spectroscopy (PCS) technique was used. The Photocor System (Photocor, Ltd.) is also designed to measure sub-micron particle sizes. The measurements were made with the same sample, following the steps of the previous section 2.10, the only difference was that the angle of the honiophotometer was fixed on a single angle of 30 degrees and signal was collected during 2 minutes. The PCS method is based on determining the velocity distribution of the particles movement. It measures dynamic fluctuations of a scattered light intensity. The disperse particles were suspended in water and undergone Brownian motion, which causes the fluctuations of the local concentration of the particles, resulting in local inhomogeneities of the refractive index. This in turn results in fluctuations of intensity of the scattered light. The linewidth of the light scattered spectrum Γ (defined as the full-width at half-maximum) is proportional to the diffusion coefficient of the particles D (see Eq. (2.19)):

$$\Gamma = D_t \left(\frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right)\right)^2,\tag{2.19}$$

where λ is the He-Ne laser wavelength, 632.8 nm, *n* is the refractive index of water, programdefined according to temperature, θ is the scattering angle, 30⁰. The diffusion coefficient D_t of the particles is inversely proportional to the decay time, τ , of light scattering fluctuations. The decay time is obtained from the time-dependent correlation function of the scattered light, then the mean radius of the particles in the solution, assuming they are spherical and non-interacting, is obtained by Einstein-Strokes equation ((2.20)):

$$R = \frac{k_B T}{6\pi\eta D},\tag{2.20}$$

where k_B is the Boltzmann constant, T is the temperature of the solution and η stands for the shear viscosity of the solvent. All the coefficients are predefined by the program according to the chosen media and the set temperature (T = 20 ⁰C). The inner diameter of the glass round cuvette was 10 mm, 3.5 μ l of nanoparticles were added to 3 ml of water, gold density is 19.3 g/ml. Then, according to Eq. (2.16) number of particles per cubic μ m was estimated 3.24 10^{-3} . The measured diameter of the particles was (14.0 ± 1.2) nm.

2.11.2 Experimental results

The group of scientists [Haiss et al., 2007] defined the nanoparticle diameter d using the experimental extinction spectra according to the following equation:

$$d = e^{B_1 \frac{A_{spr}}{A_{450}} - B_2},\tag{2.21}$$

where B_1 and B_2 are the experimentally defined coefficients, $B_1 = 3.00$ and $B_2 = 2.20$, giving the error in diameter determination no more than 11%, A_{spr} is the extinction coefficient on the maximum of the surface plasmon resonance and A_{450} stands for the extinction coefficient on 450 nm wavelength.

According to their results the nanoparticle diameter was determined as (13.0 ± 1.4) nm, what corresponds with our result of the DLS measurements, which gave (14.0 ± 1.2) nm diameter.

2.12 Local field enhancement in dimer of nanoparticles

In the system of two nanoparticles energy migration is possible due to electrons interaction. A group of professor Kik modeled the cascaded field enhancement in the system of two silver nanoparticles. They showed that the light interaction with two silver nanospheres of different volume ratio results in the local field enhancement on a smallest of the nanoparticles, depending on a geometry of a dimer: distance between nanospheres and their volumes ratio. For a silver dimer in a host with refractive index of 1.5, the maximum internal field enhancement of $2.9 \cdot 10^3$ was obtained, which is 75 times larger than that of an isolated silver nanoparticle. Following their theoretical calculations, a system of two nanospheres was modeled as interacting dipoles even though they have finite sizes [Toroghi and Kik, 2012b, Toroghi and Kik, 2012a]. These theoretical considerations can be used for other types of nanoparticles made of gold in case of this thesis. Each nanosphere has a polarizability (see Eq. 2.22):

$$\alpha = 3\varepsilon_m \varepsilon_p V \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m},\tag{2.22}$$

where ε_m is the dielectric function of media, ε_p is the dielectric function of a metal particle and V_i stands for the volume of a nanoparticle. By the superposition rule, the dipole moment of a nanoparticle at the position \mathbf{r}_i is a sum of an incident field $\mathbf{E}_0(\mathbf{r}_i)$ and a field of other electric fields at the same point \mathbf{r}_i :

$$\mathbf{E}_{np}(\mathbf{r}_i) = \mathbf{E}_0(\mathbf{r}_i) - \Sigma_j A_{ij} \mathbf{p}_j, \qquad (2.23)$$

where A_{ij} is the dipole interaction matrix. If the incident field is polarized parallel to the dimer axis, then the matrix is given by:

$$\mathbf{A}_{12} = \mathbf{A}_{21} = \frac{e^{ikd}}{2\pi\varepsilon_0\varepsilon_m} \Big(\frac{ik}{d^2} - \frac{1}{d^3}\Big),\tag{2.24}$$

where d is the distance between the particles and \overrightarrow{k} is the wave vector in a medium. The dipole moments of the nanoparticles are as described by Eq. 2.25:

$$\mathbf{p}_1 = \alpha_1 (E_0 - A_{12} p_2) \tag{2.25a}$$

$$\mathbf{p}_2 = \alpha_1 (E_0 - A_{12} p_1). \tag{2.25b}$$

Solution for one particle is as follows (see Eq. 2.26):

$$p_1 = \alpha_1 \frac{1 - \alpha_2 A_{12}}{1 - \alpha_1 \alpha_2 A_{12}^2} E_0 \tag{2.26}$$

Then, taking $E_{np,1} = p_1/\alpha_1$ it is possible to obtain the internal field enhancement $\eta_{in,1} = E_{in,1}/E_0$ and the relation $E_{in}/E_{np} = 3\varepsilon_m/(\varepsilon_p + 2\varepsilon_m)$ defined by Eq. 2.27:

$$\eta_{in,1} = \frac{E_{in,1}}{E_0} = \frac{3\varepsilon_m}{\varepsilon_p + 2\varepsilon_m} \frac{1 - \alpha_2 A_{12}}{1 - \alpha_1 \alpha_2 A_{12}^2}.$$
(2.27)

The corresponding external field is then (see Eq. 2.28:

$$\eta_{ext,1} = \frac{E_{in,1}}{E_0} = \frac{3\varepsilon_p}{\varepsilon_p + 2\varepsilon_m} \frac{1 - \alpha_2 A_{12}}{1 - \alpha_1 \alpha_2 A_{12}^2}.$$
(2.28)

The dielectric function for gold, silver and ferric dioxide(II) nanoparticles was taken as calculated before in Eq. 2.6.

The calculations were performed for the wavelength range 350-1000 nm and different refractive indices corresponding to air $n_{air} = 1$, to water $n_{H_20} = 1.33$, to a biological tissue $n_{tissue} =$ 1.44 and to quartz $n_{quartz} = 1.54$. The following figures show the descent of field enhancement in the nanoparticles dimers due to the interparticle distance growth (see Figs. 2.18, 2.19, 2.20). As we were using the gold nanospheres with r = 7 nm and the nanostars with r = 25, their possible local field enhancement were calculated and the results are presented in Fig. 2.17.

The field enhancement can be calculated for any other nanoparticles dimers with known dielectric function dependence on wavelength using the code presented in Appendix A.1, however the applicability of this theory for other particles should be checked experimentally.

It could be noticed, that for both gold and silver nanoparticles the local field enhancement on the smallest of the nanoparticles grows with the refractive index of media, however for the ferric oxide dimer, the linear regression is observed. This is due to dielectric function of ferric oxide, which imaginary part is lower than the medium refractive index, thus the opposite effect occur.

The local filed enhancement influence the energy migration path from nanoparticle to fluorophore in vicinity of nanoparticle dimer. After the certain distance the coefficient $\alpha_1 \alpha_2 A_{1,2}^2$ becomes neglectful and the enhancement becomes the same as for a single nanoparticle.



Figure 2.17: Field enhancement on the smallest nanoparticle from the dimers of the gold nanoparticles of 7 nm and 25 nm in radius depending on medium: air, water or biotissue



Figure 2.18: Field enhancement on the smallest nanoparticle from the dimer of gold nanoparticles in a - air, b - water, c - biological tissue



Figure 2.19: Field enhancement on the smallest nanoparticle from the dimer of silver nanoparticles in a - air, b - water, c - biological tissue



Figure 2.20: Field enhancement on the smallest nanoparticle from the dimer of ferric oxide nanoparticles in a - air, b - water, c - biological tissue

Chapter 3

Nanoparticles influence on optical properties of biotissue

3.1 Nanoparticles use for biomedical diagnosis

The use of plasmonic nanoparticles to improve the optical properties of biological tissues is of a certain interest for the biomedical diagnosis, because of resonant absorption and/or scattering of nanoparticles may significantly contribute to drastic increase of the signal intensity detected and thus potentially improve the capability of distinguishing between healthy and pathological tissues. A nanoparticle can be functionalized with different transport agents, i. e. biomolecules, to accumulate in a precancerous tissue, thus increasing the spectroscopic signal, as nanoparticles can be tuned to have the enhanced optical properties, i. e. scattering and/or absorption, in a certain wavelength range.

The effect of resonant scattering and/or absorption of the incident light in presence of a nanoparticle was used for changing the optical properties of biological tissues [Cihan and Arifler, 2011]. For this purpose the experiment on the special plasmonic nanoparticles influence on the optical parameters of epithelial multilayered tissue was conducted.

This chapter represents some experiments to answer the question posed in the previous chapter 2 about the light interaction with the multilayered tissue model due to the nanoparticles presence. Firstly, the phantoms modeling optical properties of multilayered epithelial tissue are presented. A photosensitizer was Protoporphyrine IX which is usually used for the fluorescence diagnosis as 5-AminoLevulinic Acid (5-ALA) which is enhancing the PPIX accumulation due to the enhanced methabolic activity in lesions [Krammer and Plaetzer, 2008, Rick et al., 1997]. Secondly, the experimental setup using the optical probe described above in subsec. 1.5.4 was proposed to reveal changes arise in the presence of the nanoparticles characterized previously. Thirdly, the processing of the obtained data was preformed according to the properties of the experimental setup and to unify the results of the experiment for other applications. Finally, the first results and a preliminary analysis of the spectra are presented at the end of this chapter.

3.2 The state-of-the-art for epithelial tissue modeling

The two main concepts of the biological tissue modeling should be fulfilled: a model should be realistic enough to make the results obtained on phantoms be applicable to a real biological tissue and it should be universal enough not to limit the applicability to a single case of a single disease. Recent reviews on biotissue modeling for optical spectroscopy, imaging and dosimetry have been published by several groups [Pogue and Patterson, 2006, Lister et al., 2012]. One of the research groups has been dealing with a fractal size distribution of particles in a solution [Passos et al., 2005]. The multilayered silicon phantoms with interchangable layers for imaging have been developed by a group of Durkin et al [Saager et al., 2010]. A group of Zaccanti has been working with India Ink as an absorber which is close to melanin [Ninni et al., 2010]. This group as well has suggested Intralipid 20% as an optical standard for preparing phantoms [Ninni et al., 2011].

Our models of biological tissues were made of two layers: epidermis and dermis. All the phantoms were prepared in $30\times30\times30$ mm plastic cuvette with transparent walls. The bottom layer mimicking dermis was made solid with a purpose to have a distinguishable border between two layers, thus the holding component was gelatin 5%. The main absorber in the second layer was Protoporphyrine IX and the diffuser - Intralipid 10% (10% here is a concentration of a bulk turbid solution). The first superficial layer had a variable thickness (0:100:400 μ m) and was liquid, it consisted of Intralipid 10% and Indian Ink.

3.2.1 Components' optical properties verification

All the optical properties were verified for each component by measurements on Hitachi Spectrophotometer [Corp.,] for Intralipid 10% - scattering coefficient, India Ink and PPIX absorption coefficients and anisotropy factor for Intralipid 10% using DLS [Instruments,]. The gelatin absorption coefficient was also verified to avoid errors in phantom absorption coefficient. For these measurements it was sufficient to use two-beam scheme described in Subsec. 2.9.1 in order to measure separated components of the future phantoms [Bashkatov et al., 2011]. Intralipid is a brandname (Kabivitrum Inc., California and Stockholm) for an aqueous suspension of lipid droplets that is sterile and suitable for intravenous feeding of patients. However Intralipid is not an optical standard it is usually used as a scattering media with negligible absorption, as there are deviations from bottle to bottle each time it is needed to verify the optical properties of Intralipid before using it. However there are some publicaitons claming the use of Intralipid as an optical standard [Ninni et al., 2011]. Both layers of the phantom were made from Intralipid 10% in different concentrations of 2 and 3 % for epidermis and dermis, correspondingly (see Fig. 3.1).

However gelatin has a slight absorption in the visible wavelength range, it was chosen as a holder for the second layer to make it solid and avoid diffusion of the two layers. The structure of gelatin is described in the PhD thesis of Lipgens [Lipgens, 1997]. A group of professor Tuchin [Bashkatov et al., 2000b] has made experiments on quantification of optical properties of Intralipid in different concentrations and gelatin in concentration 10%. Several types of gelatin were studied by Cole et al [Cole and Roberts, 1997].

India Ink's absorption is similar to melanin, which is one of the main absorbers in epithelium, so it was chosen to mimic it. A huge investigation of different types of Indian Ink was held by Zaccanti group [Ninni et al., 2010], a group of professor Ramanujam [Liu et al., 2003] suggested linear dependence of ink's absorption on concentration, what facilitates the absorbance estimation, thus the once optical properties were measured for one concentration of ink they could be linearly approximated to other concentration. The same procedure was made for gelatin. As soon as there are a lot of producers of India ink and gelatin, moreover both components can change their optical properties with time and conditions of conservation, there are differences between the data obtained experimentally and found in literature (Figure 3.3).

The main epithelial tissue model absorber which optical properties were supposed to be changed by the chosen nanoparticles was PPIX in concentration 1 mg/kg. This concentration



Figure 3.1: Comparison between literature data and experiment of scattering coefficients for Intralipid 2 and 3 %. 2 % Intralipid was used as the diffuser of the first layer, 3 % Intralipid for the second layer. (1) - [Bashkatov et al., 2000b]

was chosen as the average amount of a photosensitizer which is accumulated in normal tissues. The absorption and the emission spectra of PPIX are shown in Fig.3.4. In pure state PPIX has the main absorbance peak at 410 nm which is called Soret band, 4 much smaller so-called Q-band peaks (510, 545, 580 and 630 nm) and 2 emission peaks at 630 and 700 nm, however due to change of pH in tissue's phantoms, as well as in biological tissues, the spectra have red shift of 5 nm [Kim et al., 2010]. In Fig. 3.4 only the absorption of PPIX 1 mg/kg in DyMethyl SulfOxide (DMSO) is presented. The fluorescence peak is also red shifted for 5 nm for PPIX being in a biological tissue, thus the main fluorescence peak from 630 nm moves to 635 nm, and the second peak, which is detected while exciting in the band of 635 nm, is shifted from 700 to 705 nm.

3.2.2 Tissue and phantoms optical properties fitting

After the optical properties of components were verified, the experimental data was compared with tissue optical properties found in literature. The main absorber in epidermis is melanin. The study of prof. Tuchin's group [Bashkatov et al., 2000b] had shown, that natural melanin has much lower absorption rate according to a synthetic one. Thus, the absorption baseline of epidermis is a non-melanin skin, which could be considered as white caucausian skin[Jacques and McAuliffe, 1991]. The skin baseline absorption equation was taken from PhD thesis of Saidi [Saidi, 1992], who estimated data (450-750 nm) for *in vitro* neonatal skin samples using a scheme based on an integrating sphere after accounting for excess absorption due to residual hemoglobin and bilirubin in the samples. The skin baseline absorption according to his data is:

$$\mu_a^{baseline} = 7.8410^8 (\lambda [nm]^{-3.255}) [cm^{-1}]. \tag{3.1}$$

55



Figure 3.2: Comparison between literature data and experiment of absorption coefficients for Gelatin 5%. Gelatin 5% was used as fixing component for the second layer, nethertheless its optical properties should be taken into account as it has a slight absorption. (1) - [Lipgens, 1997]; (2) - [Cole and Roberts, 1997]



Figure 3.3: Comparison between literature data and the experiment for the absorption coefficients of India Ink 0,02%. India Ink was used as absorber for the first layer. (2) - [Ninni et al., 2010]



Figure 3.4: Comparison between the literature data and the experiment for the absorption coefficient of PPIX in concentration 1 mg/kg. PPIX was used as the fluorophore in the second layer.(1) - [Kim et al., 2010]

Then a slight content of melanin was added according to the equation 3.2a, derived by group of prof. Jacques [Jacques and McAuliffe, 1991], the fraction of melanin, f_{mel} , was chosen to be 1% by volume to show the range of absorption coefficients for epidermis, taking into account the slight change of real melanin according to synthetic one. Finally, the absorption coefficient of epidermis, μ_a^{epi} , is represented as the sum of skin baseline and the absorption of melanin by Eq. (3.2a):

$$\mu_a^{mel} = 6,610^{11}\lambda(nm)^{-3,33} \tag{3.2a}$$

$$\mu_a^{epi} = f_{mel}\mu_a^{mel} + (1 - f_{mel})\mu_a^{baseline}.$$
 (3.2b)

Thus, the volume fraction of India Ink was 0.02% and the derived absorption is laying closer to skin baseline absorption (Figure 3.5). The main absorber in dermis is hemoglobin, but for this study we do not consider blood presence, as the main question is to reveal the influence of nanoparticles on photosensitizer's optical properties in the first place. Thus, to mimic epidermis the skin baseline absorbance was considered, which is sufficient for the first approximation. Nevertheless, the PPIX absorbance should be taken into account. Figure 3.6 represents the sum of absorption coefficients for gelatin 5% and PPIX in 1 mg/kg concentration and their separated spectra. The spectral range shown is 450-800 nm and corresponds to the spectrum of the second layer of the phantom compared to dermis absorption spectrum.

The scattering properties of both layers were modeled with Intralipid 10%, but in different concentrations: for epidermis it was 2% Intralipid and for dermis - 3% Intralipid. The derived scattering coefficients are compared to known data from literature (Figs. 3.7 and 3.8). The



Figure 3.5: Epidermis absorption coefficients. Comparison of theory ([OMLC] - [Jacques and McAuliffe, 1991, Saidi, 1992]) and experiment.



Figure 3.6: PPIX 1 mg/kg and gelatin 5% absorption coefficients, experimental data and data from literature. [OMLC] - [Jacques and McAuliffe, 1991]

chose of the concentrations was made this way to justify one of the possible concentrations to the scattering range of both layers and to make scattering properties of epidermis and dermis different.



Figure 3.7: Comparison of the epidermis scattering coefficient from the literature and those of phantoms. (1) - [Sterenborg et al., 1994], (2) - [Lilge et al., 1993], (3) - [Jacques and McAuliffe, 1991]

The third one optical property vital for phantom preparation is anisotropy factor, g, which affects the light distribution field in tissue. Experimentally it was obtained by DLS, the procedure could be found elsewhere [Kholodtsova et al., 2014a]. As for both layers the same scattering solution was used, the anisotropy factor stays the same for both layers. According to Flock[Flock et al., 1992] investigations, anisotropy factor of 10% Intralipid could be expressed in the following way:

$$g = 2.25\lambda^{-0.155}.$$
 (3.3)

The other data by Staveren [van Staveren et al., 1991] suggests anisotropyfactor according to wavelength to be expressed by the Mie theory as:

$$g = 1.1 - 0.58 \ 10^{-3}\lambda \tag{3.4}$$

3.2.3 Preparation of phantoms

The phantoms were made semi-liquid/semi-solid to avoid diffusion between two layers. Thus if the first layer was prepared by simple mixing of distilled water, Intralipid 10 % and India Ink, the second layer needed some preparations:



Figure 3.8: Comparison of the dermis scatteringcoefficient from the literature and those of phantoms. (1) - [McDonald et al., 2004], (2) - [Lilge et al., 1993], (3)- [Pogue et al., 1997]



Figure 3.9: The anisotropy factor for Intralipid 10%. (1) - [Flock et al., 1992], (2) - [van Staveren et al., 1991], (3) - [Kholodtsova et al., 2014a]

- 1. Weigh 1 mg of the PPIX powder (P562-9, Frontier Science) with electronic scales;
- 2. Mix the PPIX with 1 ml of DMSO in an Eppendorf tube to obtain the concentration of 1 mg/ml);
- 3. Weigh the gelatin (A1693, AppliChem) with the electronic scales;
- 4. Stir with a magnetic stirring bar at a constant speed until the gelatin dissolves completely and the solution turns transparent (temperature did not exceed 300 K, otherwise there would be differences in the optical properteis[Lai et al., 2014]);
- 5. Add the appropriate amount of Intralipid 10%;
- 6. Add the appropriate amount of the PPIX to get 1 mg/kg concentration;
- 7. Take out 10 ml of the solution to the first cuvette;
- 8. Add the nanoparticles to the mixing solution in concentration 1 mg/kg;
- 9. Take out 10 ml of the solution to the second cuvette;
- 10. Add nanoparticles to get 2 mg/kg concentration;
- 11. Take out 10 ml of the solution to the third cuvette;
- 12. Add nanoparticles to get 4 mg/kg concentration;
- 13. Take out 10 ml of the solution to the fourth cuvette;
- 14. Leave the phantoms in a fridge for 20-30 minutes to become stable.

The set of phantoms was done in approximately 30 minutes. All the weight measurements for the PPIX and the gelatin were held with the electronic scales with 0.1 mg precision. Finally, the concentration of the components and the geometry of each layer are presented in the Table 3.1:

T_{-} [1] 9 1. O_{-} [4] [4]		4 1	1 6	1-44:
Table 5.1: Content descr	ption of epitnemal	tissue phantoms used	i for macro parameters	detection

Layer	Epidermis	Dermis
Thickness	0-100-400 μm	Semi-infinite $(> 3 \text{ cm})$
Absorber	India Ink: 0.02%	Gelatin: 5%; PPIX: 1 mg/kg
Diffuser	Intralipid: 2%	Intralipid 3%
Nanoparticles	NA	Au: 0, 1, 2, 4 mg/kg

3.3 Experimental setup used for spectrally- and spatially-resolved measurements

As the small gold nanoparticles (14 nm in diameter) described in previous chapter 2.1 have the maximum of absorption peak around 530 nm, the fluorescence of PPIX was excited by green diode laser (532 nm, 30 mW, Biospec) to the absorption peak and then by HeNe laser (632.8

nm, 30 mW, Biospec) aside the absorbance peak of nanoparticles. The big gold nanoparticles (50 nm in diameter) have the maximum of absorption at 700 nm, what corresponds to the maximum of the fluorescence second peak of PPIX. Both lasers fall to the absorbance Q-bands of PPIX. Registration of signal was made with Laser Electron Spectra Analyzer (LESA-01-Biospec). The multi-fiber probe[Pery, 2007] was used for detecting the back scattered light. For 532 nm excitation the detection range was 500-850 nm, for 632.8 nm - 600-850 nm. The setup is presented in Fig. 3.10, it has detailed description of the multi-fiber probe and its parameters.



Figure 3.10: The experimental setup used to measure the optical response of the modeled tissue. The lasers are connected to the multi-fiber probe [Pery, 2007] with 6 different SDS. The diffuse-reflected light is collected to the receiving fibers, then transferred to spectrophotometer LESA and analyzed by the UnoMomento software. The model of the tissue is described elsewhere 3.2

3.4 Spectra post-processing

The variability of equipment for spectral measurements requires post-processing of the obtained spectra. Although, there are several methods to do it, no one is still approved to be a golden standard [Lakowicz, 2006a]. As soon as in the post processed data we used the areas under the laser component and the fluorescence component, it was needed to prove that the fluorescence spectra are not due to Wood's anomalies on diffraction gratings and to avoid instrumental function. The procedure is described below. As soon as a spectrum depends on the diffraction gratings, for example resolution - the number of gratings per mm, firstly, white light spectrum, $I(\lambda)$, obtained as the reflection from $BaSO_4$ solid tablet, was approximated with the black body function, $I_{bb}(\lambda)$, which is:

$$I_{bb}(\lambda) = \frac{2hc^2}{\lambda^5} \frac{1}{e^{\frac{hc}{\lambda kT}} - 1},\tag{3.5}$$

where h is the Plank constant, $6.6260957(29) \times 10^{-34}$, J, k is the Boltzmann constant, 1.3806488(13)

 10^{-23} , $\frac{J}{K}$, c stands for the velocity of light in vacuum, 2.99792458 10^8 , $\frac{m}{s}$. As a correction coefficient the following function was taken:

$$K(\lambda) = \frac{I(\lambda)}{I_{bb}(\lambda)}.$$
(3.6)

Then, the corrected spectra, $I_c(\lambda)$, was calculated as:

$$I_c(\lambda) = I_{in}(\lambda)K(\lambda). \tag{3.7}$$

Finally, it should be mentioned, that the white light spectrum was taken without a filter. This was made with purpose to leave the fluorescence on the same level as backscattered laser light, thus leaving the effect of the filter already used while measurements and avoiding the Wood's anomalies on the diffraction grating. The shape of the white light spectra obtained with spectrometer and fitted with least squares is presented in Fig. 3.11.



Figure 3.11: The real spectrum of Halogen lamp and the black body correction with approximated temperature of 3700 K

The correction function has the form of the filter optical density to cut the 532 nm excitation (Fig. 3.12).

For each of the lasers special filters were used (see Fig. 3.13).

For 632.8 nm excitation the Wood's anomalies could not be seen clearly on the spectra obtained, but for the 532 nm excitation after correction we avoid Wood anomalies, thus having the corrected spectrum on Fig. 3.14.

The laser intensity was chosen at the very beginning and was not changed till the end of the experiment. The output power on the tip of the fiber varies for no more than 1-2 % from the laser output, which is 30 mW for both excitation wavelengths, exposition time was 500 ms.



Figure 3.12: Correction function, $K(\lambda)$ to unify the spectra obtained during out measurements



Figure 3.13: The filters optical density dependence on the wavelength for the 532 nm and 632.8 nm lasers



Figure 3.14: Non- and post processed emission spectra for 532 nm excitation (PPIX 1 mg/kg, Au NP 1 mg/kg, dermis phantom), normalized by area under the curves

3.5 Results and analysis

The spectra obtained from all the phantoms are separated in different groups which form 3D surfaces with X and Y axes:

- 1. the thickness of the epidermal layer (0:100:400 μ m) and the concentration of nanoparticles (0, 0.5, 1, 2, 4 mg/kg);
- 2. the SDS (200:200:1200 μ m) and the concentration of nanoparticles (0, 0.5, 1, 2, 4 mg/kg).

Each group was analyzed separately by the following parameters:

- 1. the Laser component, L = S(M1..M2) (see 3.5.1));
- 2. the Fluorescence component F = S(M3..M4) (see 3.5.2);
- 3. the Fluorescence Index $FI = \frac{S(M3..M4)}{S(M1..M2)}$ (see 3.5.3);

where M1...M4 are the numbered markers which are placed ± 10 nm from the laser and the fluorescence maximum peaks, S(Mi...Mj) is the area under the spectrum curve and between the markers Mi and Mj. The graphic interpretation is shown in Fig. 3.15.

It should be noticed that concentration of 1 mg/kg gives 4-5 nanoparticles per $100 \times 100 \times 100$ cubic μm^3 and PPIX concentration of 1 mg/kg - 1.07×10^9 particles per same volume (see Fig. 3.16).



Figure 3.15: Graphic interpretation of the analyzed components (L - Laser, F - Fluorescence, FI - Fluorescence Index) for the spectra obtained in the experiments. M1 - M4 are the markers placed ± 10 nm from the laser and fluorescence maximum peaks

3.5.1 Laser component

The laser components plotted according to the SDS and the concentration are presented in Fig. 3.17. Firstly, it should be noted, that the DR light of 632.8 nm is lower for both nanoparticle types than the one for 532 nm excitation. This is explained according to the optical properties of the tissue and higher penetration depth of longer wavelength. For the gold nanospheres (14 nm in diameter) it appeared, that with growth of nanoparticles' concentration without the first layer firstly DR signal is the lowest then it grows with SDS. The decay of the DR light with nanoparticles concentration is expected, as they absorb much stronger than they scatter the light for 532 nm excitation. For the other epidermal layer thickness DR light is slightly decaying. The experiment was held in parallel with calculations, thus it was decided not to measure higher concentration of the phantoms containing the gold nanostars it was decided to stop on 2 mg/kg concentration of the nanostars, as the effect was seen on longer wavelength excitation.

The laser components plotted according to the thickness of layer and concentration are presented in Fig. 3.18. The situation with lower DR light for 632.8 nm excitation for gold nanospheres remains in this representation. From the figures it could be noted that with growth of layer thickness detection ability, i. e. DR light, is going lower for the concentration of gold nanospheres 1 mg/kg and then grows up for approximately 10 % or remains the same. Then with growth of the top-layer thickness and the concentration of the nanospheres the DR light of 532 nm is detected better than there are no nanoparticles at all, the same is going on with 632.8 nm excitation. For the gold nanostars the DR measurements did not show any improvement in probing depth, as dependence of DR signal on SDS remains linear.



Figure 3.16: Graphic representation of the nanoparticles concentration of 1 mg/kg in the volume of a phantom, which has 100 μm epidermal layer thickness and 3 cm dermal layer depth and exposed through the fiber with 200 μm diameter



Figure 3.17: Influence of concentration and SDS on laser component of obtained signal



Figure 3.18: Influence of concentration and epidermal layer thickness on laser component of obtained signal

It could be concluded that if the tissue containing the gold nanospheres will be excited by 532 nm, we will observe higher response than for 632.8 nm, where it would be a dark spot in contrast with the green diffusely-reflected light. Thus the higher concentration of the gold nanospheres is, the brighter is the spot if tissue is excited with 532 nm wavelength and vice versa with 632.8 nm excitation, where the "dark" spot should be observed.

3.5.2 Fluorescence component

The fluorescent components plotted according to the SDS and the concentration are presented in Fig. 3.19. As it was with the laser component for 632.8 nm excitation of the gold nanostars, fluorescence excited by the same wavelength gives the lower response than one excited by 532 nm wavelength. As it is observed from the data on the figure, the fluorescent component increase with the concentration growth and then getting lower for 532 nm. With the increase of the SDS the fluorescent component firstly decays and then grows up, with the concentration growth it raises up and then decays, thus at a certain point fluorescent response is the highest, which is explained by the higher fluorescent probing depth at the second SDS. In our proceedings paper[Kholodtsova et al., 2014a] it was obtained, that the fluorescent probing depth grows with SDS increase, but the signal detected as it is seen from the experiment is higher 2-3 times at the concentration of nanoparticles 1-2 mg/kg. The gold nanostars did not show significant increase in fluorescence rate. The fluorescent components plotted according to the epidermal layer thickness and the concentration of the nanoparticles are presented in Fig. 3.20. What concerns the gold nanospheres, firstly, it should be noticed that the thicker is the epidermal layer, the lower is the fluorescent component, which is explained by lower volume excited by the source-fiber. The most effective concentration of the gold nanospheres appeared to be 2 mg/kg for each layer thickness. This effect could be explained by the excitation light absorption by the nanoparticles and more effective transfer to the PPIX molecules surrounding the nanoparticle. The dependence of the fluorescence life-time on nanoparticles concentration will be discussed in the following chapter 5.



Figure 3.19: Influence of the concentration and the SDS on the fluorescent component of the obtained signal



Figure 3.20: Influence of the concentration and the epidermal layer thickness on the fluorescent component of the obtained signal

3.5.3 Fluorescence index

The Fluorescence Index FI plotted against the SDS and the concentration of the gold nanoparticles is presented in Fig. 3.21. As it is seen from the data the nanospheres concentration of 2 mg/kg remains the most effective due to effective energy transfer from nanoshpere to PPIX, however for 632.8 nm excitation the FI is still from 2 to 10 times lower than one for 532 nm excitation, which is explained by the lower absorption of longer wavelengths by the gold nanospheres, thus longer wavelengths are preferably absorbed by PPIX. With growth of SDS the FI is raising, as the difference between laser and fluorescent component is getting higher. Gold nanostars remain to give lower rates of FI in comparison with gold nanospheres. The fluorescence indices plotted according to the SDS and the concentration are presented in Fig. 3.22. The form of the surfaces is quite similar to each other. With growth of the epidermal layer thickness it is getting harder to reach the dermal layer and to excite PPIX molecules present in it.



Figure 3.21: Influence of the gold nanoparticles concentration and the SDS on the fluorescence index FI



Figure 3.22: Influence of the concentration and the epidermal layer thickness on the fluorescence index FI

3.5.4 Preliminary analysis of spectra

The rate of the fluorescent photons detected is in the direct ratio to the epidermal layer thickness and depends on the dermal volume excited by diffusively propagating photons: the deeper probing depth is the higher is excited volume. The gold nanospheres concentration of 2 mg/kg appeared to be the most effective in energy transfer from a nanosphere to a PPIX molecule when it is excited to extinction peak of the gold nanospheres and PPIX simultaneously. For the gold nanoparticles concentration of 2mg/kg the 3D surfaces of the same components plotted according to SDS and layer thickness are considered separately (see Fig. 3.23).



Figure 3.23: Influence of the SDS and the epidermal layer thickness on the laser, the fluorescence component and the fluorescence index FI

The laser component does not change more than 16% with the thickness growth, but the fluorescence does increase, thus the fluorescence index increases too. The gold nanostars did not show any effect on the fluorescence component as expected due to the local field enhancement effects on the sharp edges of the nanostars. Finally, the main conclusions are made for the 532 nm excitation of the phantoms containing the gold nanospheres, as they appeared to be more effective:

- 1. the gold nanospheres concentration of 2 mg/kg in accordance with 1 mg/kg of PPIX is the most effective in the range investigated, as it increases the fluorescence rate twice;
- 2. however the laser and the fluorescence components decrease with the SDS growth, the character of the decrease is different (the fluorescent component decreases slower, than the laser one), thus the fluorescence index FI, i. e. contrast, appears to be the highest 8.9 for the longest SDS compared to the phantom without nanoparticles, where FI was obtained to be 4.3.
- although the longer wavelength, i. e. 632.8 nm, allows for probing deeper layers, the presence of the nanoparticles in the second layer gives better results for the shorter wavelength, i. e. 532 nm, as it corresponds to the maximum of the extinction of the nanospheres.

These spectra obtained can be used to estimate optical properties of multilayered bio-tissue containing nanoparticles and fluorophores using inverse problem solution described elsewhere (see sec. 4.2.1).

Chapter 4

Spatially and spectrally resolved approach to increase precision in optical properties estimation using diffuse-reflectance spectroscopy

To reveal changes arising due to a precancerous state of epithelial tissue, the inverse problem of biomedical diagnosis should be accurately solved.

This chapter presents a new approach to estimate the optical parameters (here μ_a and μ_s) of a biological tissue from spatially-resolved spectroscopy measurements. Firstly, some basics of inverse problems in mathematics are described. Secondly, the inverse problem solutions the state-of-the-art is given. Finally, the results of the modeling work are discussed, including the analysis of the estimated optical properties error dependence on cost-function error.

4.1 Basics of inverse problem solutions

Generally speaking, inverse problem is finding the parameters of a model according to the experimental data observed. All the linear inverse problems have a solution, but the non-linear are frequently considered as ill-posed if the number of arguments to find exceeds the number of observed parameters. The inverse problem is usually expressed as:

$$y = G^{-1}(x), (4.1)$$

where x is the model parameters to be found, y is the observed parameters of system, i. e. back-scattered light intensity, G is the operator, which describes the relationship between the observed data y and the model parameters x and is called forward problem, i. e. FDTD, FDFD, MC method, RTE, DA to RTE, etc, $^{-1}$ in the G-operator indicates the inverse operation.

Precise and robust estimation of the optical properties of biological tissues is a key issue in the development of optical spectroscopic and imaging methods and in their applicability to non invasive diagnosis in clinics. Promising UV-Vis-NIR spectroscopy and imaging techniques have led to significant results in biological tissue characterisation and diagnosis [Serafetinides et al., 2008]. Among these methods Spatially-resolved Diffuse Reflectance Spectroscopy (SDRS) has been investigated for about two decades and proved to be a valuable optical biopsy tool [Canpolat and Judith, 2000, Bigio and Bown, 2004, Badizadegan et al., 2004, Murphy et al.,



Figure 4.1: A) Principle of SDRS (source, probe, tissue, spectrum); B) Scheme tissue/simula-tion/model; C) Inverse problem general scheme.

2005, Arifler et al., 2006, Allison and Sibata, 2008, Borisova et al., 2008, Evers et al., 2012, Calin et al., 2013, Cunha et al., 2014]. The principle of SDRS (Fig. 4.1 a) is to use a multi-fiber optical probe in gentle contact with a tissue to be probed. One excitation fiber of the probe conducts the light of a wide wavelength range source down to the tissue inside which it is absorbed and multiply scattered. Several receiving fibers collect the backscattered photons at known Source-Detector Separations (SDS) in the probe which are then counted by a spectrometer to measure experimental reflectance intensity spectra $I_{exp}(\lambda, \mathbf{r})$ as a function of wavelength $\lambda =$ $[\lambda_1, ..., \lambda_k, ..., \lambda_K]$ (vector of K discrete number of wavelengths depending on the spectral range and resolution of the system) and distance $\mathbf{r} = [r_1, ..., r_m, ..., r_M]$ (vector of M discrete number of SDS depending on the probe features). Taking into account the complexity of biological tissues, the *in vivo* quantification, at various depths, of their optical properties (Fig. 4.1 b) such as absorption, scattering coefficients $\mu_s(\lambda)$, $\mu_a(\lambda)$ and anisotropy factor $g(\lambda)$, is intended to provide new key features for characterising pathological changes arising before evolving towards malignant lesions [Calin et al., 2013, Dong et al., 2014, Rogalla and Contag, 2015].

The estimation of the optical property values of biological tissues consists in solving an inverse problem which is classically ill-posed (different sets of parameters may lead to close similar results) and ill-conditioned (numerical instability of the solutions due to finite precision and errors in the data). In this inverse problem framework sketched in Fig. 4.1c, firstly, a mathematical and numerical modeling of the physical phenomena of light-tissue interaction has to be chosen to describe the forward problem, i. e. to provide modeled reflectance intensity spectra $I_{mod}(\lambda, \mathbf{r}; \mathbf{p}(\lambda))$ simulated with the tissue optical parameter set $\mathbf{p}(\lambda)$ (for instance $\mathbf{p}(\lambda) = [\mu_s(\lambda), \mu_a(\lambda), g(\lambda), z(\lambda), n(\lambda)])$). Secondly, an optimization procedure is applied with the aim of maximizing or minimizing an objective function (also called energy- or cost-function, usually quantifying a distance between experimental data and modeled data) by iteratively varying the values of the parameter set. Indeed, the choice of the function and of the optimization method to optimize that function has a direct impact on the precision and robustness of the overall estimation scheme.

Thus, the steps of inverse problem solution basically involve:

- 1. simulation of output with initial data;
- 2. calculation of error between simulated data and experimental;
- 3. optimization algorithm and new initial data.

4.2 The state-of-the-art of the inverse problem solutions in biomedical diagnosis

Optimization is widely used in many areas like economics, engineering, physics, etc. If the optimization algorithm is iterative, then the principle of an inverse problem applied to skin cancer optical diagnosis is to step by step find the input parameters of the forward problem or to define the properties of an experiment with an appropriate model, which simulates the most real conditions of an experiment conducted. This model is called forward problem. Algorithm of an inverse problem solution is chosen according to its forward problem description and type of parameters to estimate. The experimental data is gathered by means of optical spectroscopy and will be described in Section 4.2.1. The experimental data give first constraints to the inverse problem algorithm's elements. The principal scheme in case of optical diagnosis is as follows on Figure 4.2. According to the scheme above there are multiple choices to be done: type of



Figure 4.2: The principal scheme of inverse problem for multi modal spatially-resolved optical diagnosis. This scheme is representing the global optimization process however for our research we optimized only scattering and absorption coefficients, fixing other optical properties

forward problem, an optimization algorithm 4.2.1, regularization method, initial data, also the

data range could be fixed. In the following sections the elements' choice for the inverse problem will be discussed.

4.2.1 Inverse problem for diffuse reflectance spectrosopy

A DRS is a very sensitive optical biopsy techniques used to probe the optical properties of biological tissues non-invasively [Hallacoglu et al., 2013]. The principle consists in collecting photons back reflected from tissue after multiple scattering and absorption of an incident large wavelength band light source (typically 360-750 nm). DR intensity spectra are collected through a multiple optical fiber probe in the case of Spatially Resolved DRS (SRDRS), with various possible geometrical configurations and associated conditions of measurements. To analyze biological tissue at various depths, several SDS are used [Gomes and Backman, 2012, Fang et al., 2011]. Although the measurement technique is rather simple and the instrumentation cost limited, the estimation of the optical properties of the tissue, namely scattering and absorption coefficients, from the DR spectra collected is still a modeling challenge in terms of speed, robustness and precision [Fredriksson et al., 2012, Arifler et al., 2006, Liu and Ramanujam, 2006]. Based on the data set of spatially and spectrally resolved measurements provided by DRS, these optical properties can be recovered by solving an inverse problem. This inverse problem is usually illposed because of the limited number of input parameters and the complexity of the biological medium and more difficult to solve when implementing statistical simulation approach, i. e. MC to model the forward problem [Alerstam et al., 2010]. The latter is well appropriate to take into account the complex geometry of the tissues and the geometrical characteristics of the optical probe, especially for short SDS. The main drawback of MC simulations is the statistical noise on data which increases with SDS, for the DA to RTE - it is specificity of the analytical function which reflects in the variation space shape where local minima occur (Fig. 4.7, C). The resolution of this inverse problem is under investigation based on different algorithms [Fredriksson et al., 2012, Darne et al., 2014, Chen et al., 2013, Kortun et al., 2008, Sharma et al., 2013, Hani et al., 2012] of global minimum search by minimising cost-functions [Fredriksson et al., 2012, Darne et al., 2014, Kortun et al., 2008, Liu et al., 2012, which could also be of different types. The convergence of the algorithm highly depends on the nature of the forward problem as well as on the type of normalisation of simulated or experimental data. Also the cost-function should take into account the wavelength range of spectrum where the algorithm is used, the shape of DR signal or the absorption peaks present in the spectrum. Then if several SDS are used it is crucial to take into account the increase of noise with SDS associated to MC simulations. In our experimental set up 4.3 we have 6 output values – dependence of number of photons from distance $I(\lambda, r_{i,i=1:6})$; it is needed to estimate the optical properties of the phantoms.

 $I(\lambda, r_{i,i=1:6})$ depending on SDS and on wavelength. To model the same conditions MC method was chosen, as soon as it quite well represents actual conditions of the experiment and the error, if 10⁸ photons will be launched, is less than 10%.

Robust, precise and fast estimation of optical parameters (absorption, scattering and anisotropy coefficients) from tissular spectroscopic data is still under investigation for providing reliable non invasive and real time characterisation of a tissue status (optical biopsy). Over the past two decades several methods of light-tissue interaction were employed taking into account biologically significant parameters. They are the DA, semi-empirical models, Monte Carlo (MC) simulations, photon migration and look-up table-based models [Gemert et al., 1989, Wilson and Mycek, 2011]. In comparison with other methods the implementation of MC simulations for light propagation to biological tissues gives acceptable error rate depending on the number of photons launched and the complexity of the model [Arifler et al., 2006, Alerstam et al., 2008, Alerstam et al., 2010].


Figure 4.3: Setup geometrical configuration for numerical simulations. The excitation light source is applied to tissue through fiber F_0 of the multifiber probe [Pery, 2007] with tissue incident light intensity, $I_0(\lambda)$. DR intensity spectra $(I_{exp}(\lambda, r))$ are collected at 6 different SDS (261, 344, 500, 778, 1041, 1290 μm) corresponding to distances $r_{1..6} = [261, 344, 500, 778, 1041, 1290] \ \mu m$, respectively.



Figure 4.4: Intensity dependence on distance for 10^8 photons

However its application for inverse problem solutions gives high error rate as MC simulation is a stochastic method, thus error function between experimental and modeled data to be minimized gives higher rates and obstruct the convergence process. Thus the first main drawback of the existing solutions to inverse experimental modeling which use stochastic methods as forward model is a probable convergence to local minima.

Several recently developed spectroscopic methods allow for solving the inverse problem of optical spectroscopy diagnosis with considerably low error in optical properties estimation. Furthermore if a stochastic method is used as forward model, it affects computation time, as during each optimization iteration derivatives are calculated [Fredriksson et al., 2012]. These are the drawbacks for inverse problem based on Levenberg-Marquardt Algorithm (LMA) [Kholodtsova et al., 2014b].

Among the various works addressing this problem, Ramanujam et al [Liu et al., 2012] applied inverse MC technique[Liu and Ramanujam, 2006] to a turbid media with single and multiple fluorophores over a wide wavelength range using the LMA-based optimization. The errors of the final values estimated were around 20 and 10 % for absorption and scattering coefficients respectively. But it was shown, that the LMA could converge to local minima according to the starting point [Kholodtsova et al., 2014b]. Recently developed Look-Up Table-Based algorithm[Sharma et al., 2013, Zhong et al., 2014] is based on the data simulated for the specific fiber configuration used in optical diagnosis. It is a ready-to-use solution useful for clinicians and certain cases represented by phantoms, however to fulfill all the medical cases and consider multilayered tissue the huge amount of new phantoms should be investigated. The second problem is to choose of the algorithm according to the forward problem. In the case of MC simulation, it means that there is no equation, just a set of input parameters and several output values. The main problem of choosing the algorithm to use is to find one which will converge to the real values and reduce time of calculations, i. e. function calls. Thus in the following sections several commonly used algorithms and types of algorithms will be considered.

4.2.2 A word on cost-function and constraints

One of the criteria of an inverse problem convergence is a value of a cost-function. Usually, the squared sum of difference between experimental and modeled data is used, χ^2 , or the difference between one and ratio of modeled data to experimental, $(1 - \frac{X_{model}}{X_{exper}})^2$. The cost-function is calculated after each iteration and the value calculated is approaching zero. As soon as the cost-function value reaches the predefined by user limit, it is considered as the convergence of the inverse problem. Usually, properties to estimate also depend on another internal parameter, for optical diagnosis it could be wavelength or SDS dependence. Thus, cost-function evaluates in a sum of squares by wavelength and/or SDS with correction coefficients which depend on number of wavelengths and SDS. The above way is a direct method for the convergence criteria for an inverse problem. If there are any constraints on the range of estimated properties, or, what is more complicated, on their behaviour, cost-function becomes complexed. Some interesting approaches on the form of the cost-function will be described with methods they were implemented to in the following subsections.

Constraints in optimization could be of two types: inequality and equality. For the present case the inequality constraints are used, i. e. there are restrictions on the range of optical parameters of the system:

$$\mu_a = 0.01 - 10cm^{-1}$$
$$\mu_s = 100 - 1000cm^{-1}$$

q = 0.7 - 0.99

Thus, if the parameter estimated is out of range, it is assigned the closest value from allowed range of data.

4.2.3 Levenberg-Marquardt optimization algorithm

Classically used algorithm is Levenberg-Marquardt optimization. It is based on the Gauss-Newton algorithm and the method of gradient descent. It chooses the one of the above mentioned methods according to the damping non negative parameter, λ , which is changed for each iteration. The algorithm is attended in finding the first local minimum, which is the closest to the the initial point. Thus, in the case of multiple minima, the convergence procedure will be finished, but this does not mean that the minimum found is the global one. In case of MC simulations as a forward problem, algorithm is calculating numerical derivatives, thus evaluating the function. Moreover errors occur because of noise in MC simulations and the algorithm became more time consuming too. The LMA optimization is as shown in Fig. 4.5.



Figure 4.5: LMA scheme

The research group of Ramanujam et al [Liu and Ramanujam, 2006, Liu et al., 2012] has also applied MC technique, developed by them earlier [Liu and Ramanujam, 2006], as a forward problem modeling to a turbid media with single and multiple fluorophores over a wide wavelength range using the LMA based optimization by applying the following quadratic error function (4.2) over the wavelengths, λ to determine the thickness of the top layer (z), scattering (μ_s) and absorption (μ_a) coefficients of the bottom layer:

$$f(I_{ref}, I_{model}) = \Sigma (I_{ref}(\lambda, r_m) - I_{model}(\lambda, r_m; \mathbf{p}(\lambda)))^2,$$
(4.2)

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where $\mathbf{p}(\lambda) = [\mu_s(\lambda), \mu_a(\lambda), z]$ with fixed g, n. The errors of the final values estimated were around 20 and 15 % for absorption μ_a and scattering μ_s coefficients respectively for two-layered model, the top-layer thickness was defined with 20 % error.

For their inverse problem they used least square optimization, the cost-function was standard, $f(I_{ref}, I_{model})$, but with calibration factor (ratio of the theoretical lamp spectrum to a tungsten calibration lamp standard (Optronic Laboratories Inc., Orlando, FL)) for measured spectrum. Firstly, they defined optical properties of the phantoms. Then, the single and multiple fluorophore phantoms were investigated. The errors between expected and estimated values vary from $9.93\% \pm 7.94\%$ to $21.81\% \pm 14.52\%$.

4.2.4 Look-Up table-based approaches

Recent methods developed concern look-up table-based algorithm[Sharma et al., 2013, Zhong et al., 2014]. It is based on the data simulated for specific multi-fiber configuration. It allows to search the most closest solution without simulating new data. Algorithm was shown to be efficient and ready to use in clinics for optical properties analysis, but there is a lack of number of phantoms, as well as there is a difference between phantoms and real skin. Therefore, for the wider spectrum range and other multi-fiber probes configurations new set of simulations should be performed. The method allows determining the values of absorption coefficient (μ_a) and the optimal value of the latter was obtained with an error of 10% compared to the exact known value. The reduced scattering coefficient was determined with an average error 15% and the absorption coefficient error was estimated to be from 12 to 25% with highest error for maximum thickness 550 μm . The inverse problem solution was based on the LMA optimization. As a cost-function the following mean squared percent error between the measured SDR spectrum over discrete wavelengths λ , $I_{ref}(\lambda, r_m)$, and the modeled one, $I_{model}(\lambda, r_m; \mathbf{p}(\lambda))$, was used (4.3):

$$f(I_{ref}, I_{model}) = \frac{1}{MK} \Sigma_{\lambda} \Sigma_{m=1}^{M} \left(1 - \frac{I_{model}(\lambda, r_m; \mathbf{p}(\lambda))}{I_{ref}(\lambda, r_m)} \right)^2, \tag{4.3}$$

where *m* stands for Source-Detector Separations (SDS) number with maximum value M = 2, corresponding to the two SDS 370 and 740 μm , *K* - number of wavelengths, $\mathbf{p}(\lambda) = [\mu_s(\lambda), \mu_a(\lambda)]$ with fixed *n*, *z*, *g*. A research conducted by group of prof. Dinten based on this method is representing the first results obtained for the DRS measurements of skin redness [Koenig et al., 2015].

However it is a ready-to-use solution which is useful for clinicians and certain cases which phantoms represent.

4.2.5 Inverse Adding-Doubling

The Inverse Adding-Doubling (IAD) method provides a tool for the rapid and accurate solution of inverse scattering problem [Prahl et al., 1993, Pickering et al., 1993, Qu et al., 1994, Chan et al., 1996b, Chan et al., 1996a, de Vries et al., 1999, Sardar et al., 2001, Chen et al., 2005, Chandra et al., 2006, Mahadevan-Jansen et al., 2006, Sardar et al., 2004, Bashkatov et al., 2004, Bashkatov et al., 2005] [Sardar et al., 2005, Sardar et al., 2007, Bashkatov et al., 2010, Zamora-Rojas et al., 2013a]. It is based on the general method for the solution of the transport equation for plane-parallel layers suggested by van de Hulst and introduced to tissue optics by Prahl [Prahl et al., 1993, Sardar and Levy, 1998]. An important advantage of the IAD method when applied to tissue optics is the possibility of rapidly obtaining iterative solutions with the aid of up-to-date microcomputers; moreover it is flexible enough to take into account anisotropy of scattering and the internal refection from the sample boundaries. The method includes the following steps:

- 1. The choice of optical parameters to be measured;
- 2. Counting reflections and transmissions;
- 3. Comparison of calculated and measured reflectance and transmittance;
- 4. Repetition of the procedure until the estimated and measured values coincides with the desired accuracy.

In principle, the method allows any intended accuracy to be achieved for all the parameters being measured, provided the necessary computer time is available. An error of 3% or less is considered acceptable. Also, the method may be used to directly correct experimental bindings obtained with the aid of integrating spheres. The term "doubling" in the name of the method means that the reflection and transmission estimates for a layer at certain ingoing and outgoing light angles may be used to calculate both the transmittance and reflectance for a layer twice as thick by means of superimposing one upon the other and summing the contributions of each layer to the total reflectance and transmittance. Reflection and transmission in a layer having an arbitrary thickness are calculated in consecutive order, first for the thin layer with the same optical characteristics (single scattering), then by consecutive doubling of the thickness, for any selected layer. The term "adding" indicates that the doubling procedure may be extended to heterogeneous layers for modeling multilayer tissues or taking into account internal reflections related to abrupt change in refractive index.

4.2.6 Inverse Monte Carlo

Scientists from Sweden in 2012 [Fredriksson et al., 2012] developed inverse MC algorithm fir three layered tissue and 2 SDS. The two main conclusions are: a homogeneous model can not describe light propagation in tissue and knowledge of relative intensities between detectors is sufficient for the inverse algorithm. Inverse MC simulations of fluorescence have also been used for cancer diagnosis in human tissues. In 2012 scientists used inverse MC to distinguish between melanin types. Result shows that the concentrations of eumelanin and pheomelanin of all melanoma lesions are higher than concentration of eumelanin and pheomelanin of normal skin, resulting in higher light photon absorption. It can be inferred that the developed inverse model is able to determine relative concentrations of eumelanin and pheomelanin [Hani et al., 2012].

4.2.7 Analytical methods

Inverse semi-empirical models of reflectance and fluorescence have been developed for cancer detection in human pancreatic tissues [Wilson et al., 2009, Wilson et al., 2010]. In order to study the reflectance and fluorescence spectra of human pancreatic tissues, Chandra et al [Chandra et al., 2006, Chandra et al., 2007, Chandra et al., 2010] developed prototype clinical instrumentation to acquire 96 pairs of reflectance and fluorescence spectra from 50 sites on pancreatic tissues (within 30 minutes of excision) from nine patients undergoing pancreatic surgery. A Photon-Tissue Interaction (PTI) algorithm developed by Wilson et al [Wilson et al., 2009, Wilson et al., 2010] was employed to mathematically model the measured reflectance and fluorescence spectra to extract biophysically-relevant parameters related to tissue morphology and biochemistry from normal pancreatic tissue, chronic pancreatitis, and adenocaricnoma. The PTI reflectance model [Wilson et al., 2009, Wilson et al., 2010] used a semi-analytical, homogeneous-slab reflectance equation developed by Reif et al [Reif et al., 2007, Reif et al., 2008] to extract information about the tissue absorption (attributed to haemoglobin) and scattering (attributed to collagen fibers and cell nuclei). The PTI fluorescence model corrected the measured fluorescence spectra for attenuation artifacts and then fit the resulting "intrinsic" fluorescence spectra to a linear combination of collagen, NADH, and FAD fluorescence spectra to obtain the relative contributions of extracellular components (collagen) and intracellular components (NADH, FAD) to the intrinsic fluorescence [Wilson et al., 2009, Wilson et al., 2010]. The fractional contribution of extracellular collagen to the intrinsic fluorescence was significantly larger in adenocarcinoma than in normal pancreatic tissue or chronic pancreatitis [Wilson et al., 2009]. These results suggest that semi-empirical models of reflectance and fluorescence have the potential to extract biomedically-relevant parameters for distinguishing pancreatic adenocarcinoma from other pancreatic tissue types and for skin lesions. Bigio, Reif and A'Amar investigated 4 fiber probes, on different distances to each other and angles according to surface. They derived reflectance as the function of reduced scattering coefficient, when absorption is negligible, and developed inverse analytical algorithm. It is applicable on the small SDS and is using common for bio tissue g-factor which equals to 0.9. Hence, the model is a simplification of light transport in a turbid medium. Further studies could implement other values of g-factor and could be applied to wider range of tissues [Reif et al., 2007].

4.2.8 Other approaches on inverse problem solving

Other recent approaches on that inverse problem solving, to determine the values of the optical parameters for a 2-layered tissue model, proposed the use of one or several neural networks [Wang et al., 2012, Juger et al., 2013]. The application of several neural networks leads to the error in the thickness estimation of 12-42 % depending on the absorption rate: the higher it is, the higher is the estimated error in values of optical properties. The cost-function for this method was the same as mentioned above (see Eq. 4.2).

In 2012, another work of Fredrikson et al [Fredrikson et al., 2012] used a merit function not only based on difference between modeled and experimental DR intensity spectra, but also taking into account relative differences according to distances by assigning lower weight to longer SDS (see Eq. 4.4):

$$f(I_{ref}, I_{model}) = \Sigma_{m=1}^{M} \Sigma_{\lambda} \left(\frac{I_{ref}(\lambda, r_m) - I_{model}(\lambda, r_m; \mathbf{p}(\lambda))}{\sigma_s} \right)^2 \\ + \left(\frac{\phi(I_{ref, 0.4}(\lambda; \mathbf{p}(\lambda))) - \phi(I_{model, 0.4}(\lambda))}{2} \right)^2 \\ + \left(\frac{\phi(I_{ref, 1.2}(\lambda; \mathbf{p}(\lambda))) - \phi(I_{model, 1.2}(\lambda))}{0.5} \right)^2,$$
(4.4)

where σ_s is influenced both by absolute and relative difference of intensities. Moreover this costfunction (see Eq. 4.4) is also assigning additional regularisation term $\phi(I)$, adding importance to the shape of the spectrum by taking into account double-peak of oxygenated haemoglobin.

The principal difference between the above mentioned cost-functions is that the Eq. 4.3 is taking into account the intensities on different SDS with the same weight, i. e. relative difference however the Eqs. 4.2 and 4.4 do take into account the weight at each SDS as the measured DR intensity decreases with larger SDS, thereby they are calculating absolute difference between measured and modeled spectra. If the forward problem is based on a stochastic method the increasing with SDS statistical noise is affecting the cost-function value at longer SDS.

4.3 Initial data analysis

The first step of algorithm could be performed on initial data, i. e. experimental data and output of a forward model. In the previous work [Kholodtsova et al., 2014b] this was made by smoothing the initial data with local regression weighted second degree polynomial and then fitting the data with double exponential decay function decreases the probability of the inverse algorithm to converge to local minima close to the initial point of first guess. In this work the impact of noise on variation space was highlighted, then difference between DR curves for two close values of absorption coefficient was increased with reducing noise with the method mentioned above. The developed method allows essential decreasing of errors occur during inverse problem solving.

For the preliminary analysis the variation space of the cost function was built to investigate the algorithm convergence. This space is represented by three-dimensional surface with x and y axes corresponding to (μ_s, μ_a) and the z axis corresponding to the cost function's value. Thus, for the discretisation of the (μ_s, μ_a) plane, scattering and absorption parameters' values were chosen respectively in the range (10-1000 with 10 cm⁻¹ step) and (1-10 by 0.1 cm⁻¹ step). The first pairs of parameters (μ_s, μ_a) cover the range of optical properties common for biological tissues like skin [19, 20]. The last g-factor of 0.8 is used because for each pair of (μ_s, g) which has the same μ'_s diffuse reflectance keeps the same value [21]. Here I_0 corresponds to the reference point mentioned earlier $(\mu_s, \mu_a) = (500, 5)$ cm⁻¹ to which the algorithm should converge. After delimiting the range of values for the variation space, 500 simulations for I_0 were performed. For each of these 500 simulations, the minimum of the variation space was found. For all combinations of the edge and middle values of the $(\mu_s, \mu_a) = (100, 500, 1000; 0.1, 5, 10)$ cm⁻¹, ten simulations with 10⁷ photon (per each of the 9 combination pairs) were performed and standard deviation was calculated.

4.3.1 Preliminary analysis of the variation space of the cost function

The statistical noise in I(r) data due to MC simulations, which increases with distance as shown in Fig. 4.6, results in the presence of noise in the cost function's variation space as it can be seen in Fig. 4.7 (A and C). Noise level increases with scattering and absorption coefficients values, because the number of photons detected decreases, also far distance results in higher Standard Deviation (SD). The related number of photons was represented in the log-scale to show the increase of standard deviation with distance. With photon number growth the calculation time increases, so $N_{ph} = 10^7$ photons were chosen as good compromise between error level and calculation time needed.

The variation spaces represented are log-scaled for the z-axis. Along μ_s -axis, the cost-function value varies in wide region for both types of the forward models. For μ_a -axis this variation is much smaller, it is represented in Fig. 4.7 (C, D) where y-axis is the minimum of the variation space for each value of absorption μ_a . For the MC based forward modeling, local minima and global one can hardly be distinguished, thus optimization could converge to any of local or global minima (Fig. 4.7, C). On Figure 4.7(A), X and O correspond to expected and estimated minimum for one forward MC simulation, respectively. For the DEA based modeling, local minima exist, the global minimum (zero value of the cost function is not seen on Figure 4.7 (B, D) because of log-scale) still does correspond to the predefined reference values of (μ_s, μ_a). Thus it can be seen that even with an analytical forward model (DEA) the optimization algorithm may converge to a local minimum. For the MC simulations, 500 global minima of the cost function's variation space were obtained (Fig. 4.8) considering (μ_s, μ_a) = (500, 5) cm⁻¹ as expected (known) global



Figure 4.6: Normalized log-scale intensity dependence on source-detector separation with SD errors for 10 simulations for some extreme values of (μ_s, μ_a) , $N_{ph} = 10^7$



Figure 4.7: Log-scaled variation spaces for MC (A) and DEA (B), log-scaled minimum values of the variation spaces along μ_a -axis (C – for MC simulations, D – for DEA simulations)

minimum.



Figure 4.8: Distribution of 500 global minima found for the variation spaces calculated for values of scattering and absorption coefficients in the ranges 100-1000 cm⁻¹ and 0.1-10 cm⁻¹, correspondingly, and considering the known global minimum at $(\mu_s, \mu_a) = (500, 5)$ cm⁻¹ with SD $(\sigma_{x,y})$, median $(\langle x \rangle, \langle y \rangle)$, minimum (x_{min}, y_{min}) and maximum (x_{max}, y_{max}) values

The distribution is not concentrated close to the expected point, thus the global minimum could be obtained for each pair of (μ_s, μ_a) along the curve-shape area plotted. So the probable estimated range of data obtained for μ_s is 490.0±89.8 cm⁻¹ and for μ_a is 5.3±1.95 cm⁻¹, i. e. maximum standard deviation in percent for scattering estimation is 20 % and for absorption 45 %.

4.3.2 Solution proposed to improve convergence procedure

To decrease SD values, we tried to reduce noise on the model output data $(I_{mod}(r)$ curves calculated from forward simulations) using smoothing filters and fitting procedures, alone or combined: moving average, polynomial fittings, local regression, splines, piecewise cubic interpolation. As the data noise increases with distance a weighting solution was applied to take account of that. The weights were defined in inverse proportion to the source-detector separation such as w = 1000/r. The fitting and smoothing functions applied were chosen according to the shape of data. None of the procedures mentioned above lead to proper results when applied alone. Therefore, the following smoothing filters were applied for comparison: moving average (moving) with windows size (WS) 5 and weight w, local regression (loess) with a second order polynomial with WS equals 5, Savitsky-Golay filter (sgolay) with second degree polynomial and with WS - 5,

Table 4.1: Results of smoothing and fitting functions application to 500 curves simulated for $(\mu_s, \mu_a) = (500, 5) \text{ cm}^{-1}$ and the whole variation space in range of $(\mu_s, \mu_a) = (100:10:1000, 0.1:0.1:10) \text{ cm}^{-1}$. The most efficient result was achieved by application of exp2 fitting and Loess filter, indicated by bold text.

Filter	exp	02	cubicir	nterp	smoothing spline	
	μ_s, cm^{-1}	$\mid \mu_a, \mathrm{cm}^{-1}$	μ_s, cm^{-1}	μ_a, cm^{-1}	μ_s, cm^{-1}	μ_a, cm^{-1}
Moving	500.0 ± 134.4	$5.10{\pm}2.09$	470.0 ± 102.0	$5.40{\pm}2.01$	470.0 ± 104.0	5.5 ± 2.05
Loess	$500.0{\pm}87.4$	$5.00{\pm}1.85$	490.0 ± 90.6	$5.10{\pm}1.81$	490.0 ± 82.6	$5.20{\pm}1.76$
Sgolay	520.0 ± 152.5	$4.80{\pm}2.13$	490.0 ± 90.5	$5.10{\pm}1.83$	490.0 ± 89.2	$5.20{\pm}1.84$
Rloess	500.0 ± 160.8	$4.90{\pm}2.29$	490.0 ± 125.7	$5.10{\pm}1.99$	980.0 ± 490.0	5.25 ± 2.04

robust fit (loess) with second degree polynomial with weight w. After that, the smoothed data was fitted with one of the following functions: double exponential decay; $ae^{bx} + ce^{dx}$ (exp2); piecewise cubic interpolation (cubicinterp); smoothing spline (smoothingspline) (Table 4.1). The same 500 curves with the same reference couple, $(\mu_s, \mu_a) = (500, 5) \text{ cm}^{-1}$, were smoothed and fitted with the same functions as well as the data obtained for the wide ranges of (μ_s, μ_a) = (100:10:1000, 0.1:0.1:10) cm⁻¹. Then for 500 variation spaces' minima were found and the distributions were analyzed. The minima of the variation spaces were projected on the (μ_s, μ_a) plane. They are distributed along the exponential decay curve. All the values of (μ_s, μ_a) along this curve are the probable correct optical properties, derived for the curve simulated with same optical properties. After we found out the best pair of function to concentrate the correct optical properties near its real values (Table 4.1). By the values of standard deviation and median smoothing with weighted local regression and fitting with double exponential decay were chosen as optimal. Application of these types of smoothing and fitting to each curve before starting the LMA procedure gives correct optical properties, (μ_s, μ_a) .

Thus for scattering coefficient the error was reduced and the ranges of probable estimated OP appeared to be in the following ranges: $\mu_s = 500.0\pm87.4 \text{ cm}^{-1} (17.5 \%)$ and for absorption - $\mu_a = 5.00\pm1.85 \text{ cm}^{-1} (37 \%)$. The suggested preprocessing method allows reducing the level of error on the estimation of both optical parameter values without any additional changes inside the inverse problem algorithm itself. By smoothing the initial data with local regression weighted second degree polynomial and then fitting the data with double exponential decay function decreases the probability of the inverse algorithm to converge to local minima close to the initial point of first guess.

Consequently the preprocessing method of spatially-resolved DR intensity smoothing with moving average and fitting with double exponential decay function allowed decreasing the probability of local minima convergence without change of the inverse algorithm itself. Although the error was reduced by 17 % for absorption and by 12.5 % for scattering coefficients it is quite fast preliminary step to estimate global minimum.

4.4 New approach proposed - Particle Swarm Optimization

Hereafter we propose a new approach for reducing the error between the optimized and ground truth values by considering a multidimensional approach of the inverse problem solving for SDR spectroscopy. The PSO is a population based stochastic technique which leads to faster solution in complex multidimensional problems [Eberhart et al., 1996]. A first promising advantage of this algorithm is that it does not calculate the derivatives, thus reducing computation cost. Another advantage lies in the directivity of the algorithm, that is its ability to choose the direction of the next iteration with new starting points based on the previous experience [Kholodtsova et al., 2014b, Zhang et al., 2003. The method consists of initialising the optimization process with a population of random solutions, called particles, and searching for optima by updating generations while progressing throughout the space of probable solutions. PSO stores the coordinates of each particle related to the personal best solution obtained as far by each particle (personal best) and the global best value among all the particles (global best value or gBest). At each iteration step, the acceleration of each particle towards grBest and gBest locations is changed by respective weighting random terms. In section 4.4, the description of the Particle Swarm Optimization (PSO) method and its features are presented for spatially-resolved simple configuration, i. e. 'point' single wavelength approach. Section 4.4 shows how the algorithm is trained for Wavelength-Resolved (WR) spectra. This WR-PSO approach is then refined with a WR exponential decay fitting of the WR-PSO (FWR-PSO) and trained again to exclude difference in convergence algorithm. In order to save computing time during the training procedure and moreover to take into account the statistical noise features of the MC-based simulations the application of the Diffusion Approximation (DA) with white Gaussian noise according to the number of photons is proposed. The cost-function proposed is based on Eq. 4.3 but is averaged inside each group of particles, will be explained later, thus avoiding local minima convergence, while estimating optical properties over the wide space of probable optical parameters. In 4.4.2presents the results of the experimental validation performed on numerical data, obtained with MC simulations performed for a configuration of multi-fiber probe used in our clinical and experimental research works [Pery, 2007, Pery et al., 2009, Kholodtsova et al., 2015]. the results which allows avoiding local minima convergence and the cost-function value minimisation for optical properties estimation not only with higher precision, i. e. lower error, but with significant reduction of iterations number, leading to reduced computational cost. Particle swarm optimization(PSO) [Zhang et al., 2003] idea is based on collective behaviour of insect colonies and other animal societies observation. Generally speaking, the algorithm is moving several particles or groups of particles in the n-dimensional space of variables, accelerating each group or each particle to the best particle's group's of particles of global solution with certain speed:

$$x_{i+1}^n = x_i^n + v_{i+1}^n \tag{4.5a}$$

$$x_{i+1}^n = \omega x_i^n + c_1 rand_1 (pBest - x_i^n) + c_2 rand_2 (gBest - x_i^n)$$

$$(4.5b)$$

$$\omega = W_{max} - (W_{max} - W_{min})\frac{\iota}{N},\tag{4.5c}$$

where ω - the weight, which varies with each iteration, coming from the global search, W_{max} , to local, W_{min} , pBest and gBest - personal and global best values, determined by the lowest χ^2 value for each particle, c_1 - the speed with which algorithm approaches pBest, c_2 - the speed with which algorithm approaches gBest.

The principal scheme of the PSO is shown in Fig. 4.9.

PSO point approach

It is suggested instead of *personal* best values use *group* best values (grBest) and instead of *global* best value leave the *bestfromallthegroupbest* values (gBest) for MC forward problem. As soon as MC has an error, it could be reduced by finding out the group of particles with smallest cost-function value, which was averaged inside the group, *grBest*. Afterwards, each particle is assigned global best value, *gBest* and the process of speed and coordinate calculation is repeated N times.



Figure 4.9: Particle swarm optimization principal scheme

Next step to tune this algorithm is to find appropriate number of particles' groups and number of particles inside the group.

For the following ranges the algorithm was tested using data simulated with DA to RTE and forward problem based on DA to converge to the global minimum :

$$c_1 = (0.1 : 0.1 : 3)$$

$$c_2 = (0.1 : 0.1 : 3)$$

$$W_{min} = (0.6 : 0.1 : 3)$$

$$W_{max} = (0.9 : 0.1 : 3)$$

The values of speeds and weights were found to be $c_1 = 0.3$, $c_2 = 2.1$, $W_{min} = 0.8$, $W_{max} = 0.9$ with error in $\chi^2 = 10\%$ (χ^2 is calculated according to Eq. 4.3) to obtain error 20 and 20 % in scattering and absorption coefficient determination. In comparison to the LMA, PSO either converges to global minimum or reaches maximum iterations, and LMA could converge to local minimum, depending from the starting point.

Characteristics of the numerical model

White light source is a halogen lamp with maximum temperature 3700 K, spectrometer was tuned for the range 400-900 nm with resolution of 1 nm. For our numerical study we took the wavelength range 500-600 nm with 10 nm step (however the results can be applied to any wavelength range in optical region). The geometrical configuration and features of the present numerical study are



Figure 4.10: MC simulation noise level (standard deviation in %) as a function of SDS (r) and photon number N_f launched : crosses for $N_f = 10^7$, triangles for $N_f = 10^8$ and squares for $N_f = 10^9$. The continuous, dashed and dotted curves drawn are respective exponential fitting (with corresponding equations listed in the legend) for each N_f

based on the ones for our experimental SDR spectroscopy setup, implemented in the previous studies (see details in [Kholodtsova et al., 2014b, Kholodtsova et al., 2014a, Kholodtsova et al., 2015]).

As sketched on Fig. 4.3, our multi-fiber probe has M = 6 different SDS $r_{1..6} = [261, 344, 500, 778, 1041, 1290] \mu m$, with each fiber having core's refractive index n = 1.54 and diameter $d = 200 \ \mu m$ [Kholodtsova et al., 2015]. The choice of the appropriate ranges of the optical parameters to be adjusted is discussed in section 4.4.

PSO wide range approach

Exchange of the MC method on the DA to the RTE with Gaussian noise Before training the PSO algorithm and conducting our numerical study we have been searching for a reduced computation time approach (compared to MC but with similar features in terms of statistical noise. The idea is to study the convergence of our inverse problem optimization process where the forward problem equation has the same noise rates thus noise was chosen according to the number of photons simulated by MC method. The MC method description could be found elsewhere[Prahl et al., 1989, Wang et al., 1995, Zhu et al., 2003, Arifler et al., 2006]. For each of the following number of photons, $N_f = 10^7$, 10^8 , 10^9 , MC simulations were run 10 times for the configuration described above (Fig. 3.10). The obtained probability distributions were analyzed as Gaussian and the noise levels were revealed according to SDS (r), and N_f (Fig. 4.10). The latter were then applied to the SDR intensity spectra $I_{mod}(\lambda, r)$ calculated by DA equation in the range $\lambda = 500:10:600$ nm and defined by Eq. 1.37 hereafter: The DA equation used to model spatially resolved DR $(I(\lambda, r_m))$ is (see Eq. 4.6):

$$I(\lambda, r) = \frac{1}{\mu_t'(\lambda)} \Big(\mu_{eff}(\lambda) + \frac{1}{r_1(\lambda, r)} \Big) \frac{e^{-\mu_{eff}(\lambda)r_1(\lambda, r)}}{r_1^2(\lambda, r)} + \Big(\frac{1}{\mu_t'(\lambda)} + 2z_b(\lambda) \Big) \Big(\mu_{eff}(\lambda) + \frac{1}{r_2(\lambda, r)} \Big) \frac{e^{\mu_{eff}(\lambda)r_2(\lambda, r)}}{r_2^2(\lambda, r)},$$

$$(4.6)$$

where $\mu_{eff}(\lambda) = \sqrt{\frac{\mu_a(\lambda)}{D(\lambda)}}$; $D(\lambda) = \frac{1}{3\mu'_t(\lambda)}$ - diffusion coefficient; $\mu'_t(\lambda) = \mu_a(\lambda) + \mu_s(\lambda)(1 - g(\lambda))$ - transport coefficient; $r_1(\lambda, r) = \sqrt{\frac{1}{\mu'_t(\lambda)} + r^2}$; $r_2(\lambda, r) = \sqrt{2\left(\frac{1}{\mu'_t(\lambda)} + 2z_b(\lambda)\right)^2 + r^2}$; $z_b(\lambda) = 2D(\lambda)$ - extended boundary [Feng et al., 1995].

PSO-based algorithm applied to spatially resolved data In this subsection we describe the basic scheme of the PSO algorithm adapted to our inverse problem solving by taking into account the spatial resolution of the spectroscopic data (spectral dimension being added in the next subsection).

In our case, a particle corresponds to a point in the (μ_a, μ_s) variation space of the cost-function as μ_a and μ_s are the two parameters to be estimated. Considering the dimension D = 2 of the problem, our approach was to consider $C = (D+1)^2 = 9$ clusters of P = D + 1 = 3 particles. In the rest of the thesis, any cluster will be denoted c while a specific cluster will be noticed c_j with j = [1:C] the cluster index and any particle will be denoted p while a specific particle in a cluster c_j will be denoted $p_{i,j}$ with i = [1:P] the particle index in a cluster. At start, these 9 clusters were spread all over the solution space and the 3 particles of each group were place close to each other with less than 5 % difference in their coordinates $X = (\mu_a, \mu_s)$ in the space of variables. Given the data found in literature on epithelial tissue optical properties [Gemert et al., 1989, Bashkatov et al., 2005, Borisova et al., 2008, Bashkatov et al., 2011, Jacques, 2013], the limits chosen for the solution space of μ_s and μ_a values to be estimated were respectively $100:1000 \text{ cm}^{-1}$ and $1:10 \text{ cm}^{-1}$. The "error of a cluster c_j " corresponds to the averaged value of the cost-function χ^2 calculated for the 3 particles in this group (see Eq. 4.7):

$$\chi_j^2 = \frac{1}{C} \Sigma_{j=1}^C \left(1 - \frac{I_{mod}(\lambda, r_m; \mathbf{p}_j(\lambda))}{I_{exp}(\lambda, r_m)} \right)^2, \tag{4.7}$$

where $\mathbf{p}(\lambda) = [\mu_s, \mu_a]$ - optical parameters to be estimated.

In combination, the optical parameters are averaged inside each group thus giving, for each group, $grBest_{c_j} = [\chi_j^2, \mu_s^j, \mu_a^j]$. The *gBest* is defined as minimal value of χ_p^2 among all the $(D+1)^2$ groups, $gBest = min(grBest_p)$. The use of grBest (averaged value of optical parameters inside each group) contributes to limit the risk of convergence to local minima.

Thus, after calculating the cost-function, $\chi^2_{c_j}$ we move all the *c* clusters of particles to one which has the global 'best' properties, i. e. μ^{gBest}_s , μ^{gBest}_a , and each cluster to its averaged 'best' properties, i. e. $\mu^{grBest,j}_s$, $\mu^{grBest,j}_a$.

Thus going from iteration k to k+1 each particle with coordinates $X^k(\mu_s^k, \mu_a^k)$ is moved with the speed, V^{k+1} , to next position X^{k+1} in the space of variables (μ_s, μ_a) . Their speed V^{k+i} and coordinate X^{k+i} are defined in the following way (see Eq. 4.8):

$$V^{k+1} = wV^k + a_1 rand_1 (grBest - X^k) + a_2 rand_2 (gBest - X^k),$$
(4.8a)

$$X^{k+1} = X^k + V^{k+1}, (4.8b)$$

where w is a weighting function $w = w_{Max} - (w_{Max} - w_{Min})\frac{iter}{maxIter}$, with w_{Max} and w_{Min} - initial and final weights, *iter* and *maxIter* - the current and maximum numbers of iterations, rand₁ and rand₂ - random values distributed in [0, 1], a_1 and a_2 are coefficients weighting the acceleration of particles to the *gBest* and *grBest*, correspondingly. To accelerate each cluster simultaneously in the same direction rand₁ and rand₂ are fixed inside each cluster.

Thus, at first iterations the weighting function assists to global search and at the end facilitates local search, i. e. decreasing w forces the algorithm to go from global to local search. It is worth to mention that the number of clusters was chosen as a trade-off between the computational cost during one iteration step and the number of iterations. Thus the higher is the number of clusters the longer is each iteration step and the higher is the probability of clusters to find the proper solution. We fixed the number of clusters to $(D+1)^2$ as it was mentions above. The values of the parameters: w_{Max} , w_{Min} , a_1 , a_2 , maxIter are estimated through training process of the algorithm.

WR-PSO approach and preliminary results

The objective here is to estimate the values of absorption μ_a and scattering μ_s coefficients over several wavelengths using PSO-based inverse problem solving on spatially resolved data.

In the following numerical simulations we chose the wavelength range 500:10:600 nm, though the algorithm could be run with other limits and step corresponding to the spectroscopic measurements obtained from experiment.

Definition of algorithm transformation from global search to local is defined from the border conditions of (μ_s, μ_a) space, thus maximum steps are:

$$L_{\mu_s} = w(a_1 + a_2) \frac{\mu_s^{max} - \mu_s^{min}}{10}$$

$$L_{\mu_a} = w(a_1 + a_2) \frac{\mu_a^{max} - \mu_a^{min}}{10}.$$
(4.9)

By doing so the space is 'divided' in rectangles with sides 90 and 9 cm^{-1} , thus the number of squares is 100. By starting $(D + 1)^2 = 9$ groups of particles, first several iteration steps the weight w is reduced to make each group 'choose' the square of local search. Thereby after the 10^{th} iteration, it was decided to keep weight w constant as change in weight from maximum to minimum depends on number of maximum iterations (Eqs. 4.5) and by increasing the number of maximum iterations, maxIter, we just slow down the transformation of global search to local, thus maxIter = 10, but i_{max} was 30. Suchwise, after 10^{th} simulation the global search is transformed to local, and during no more than 20 iterations the algorithm should converge to reference data.

Thus, after ten iterations weighting function w is permanently fixed to local search till the algorithm converges.

The parameters of the WR-PSO were determined through training the algorithm in the following way:

1. Initialise the tissue optical model with g = 0.8, n = 1.44 for the 4.10):

$$a_{1} = (0.1:0.1:3)$$

$$a_{2} = (0.1:0.1:3)$$

$$w_{Min} = (0.1:0.1:2.9)$$

$$w_{Max} = (0.2:0.1:3)(w_{Max} > w_{Min});$$
(4.10)

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2. the best PSO parameters were chosen according to the minimum error as (see Eq. 4.11):

$$\chi^{2}(\lambda) = \Sigma_{\lambda} \Sigma_{m=1}^{6} \left(1 - \frac{I_{mod}(\lambda, r_{m}; \mathbf{p}(\lambda))}{I_{exp}(\lambda, r_{m})} \right)^{2}, \tag{4.11}$$

where $\mathbf{p} = [\mu_s(\lambda), \mu_a(\lambda)];$

- 3. to have sufficient statistical error 50 simulations were run for each error in a range $\chi^2 = 5-10$ % with step 1%
- 4. the following performance criteria were calculated:
 - error in optical parameters estimation, i. e. error in scattering ϵ_{μ_s} and absorption ϵ_{μ_a} coefficients:

$$\epsilon_{p_i} = \left(\frac{p_i^{ref} - p_i^{final}}{p_i^{ref}}\right) * 100\%$$
(4.12)

- success rate in percent (number of successful simulations divided by overall number of simulations 50) number of simulations when convergence is achieved before reaching the maximum number of iterations, i_{max} ;
- average < I > and maximum I_{max} number of iterations before convergence is achieved.

The results are presented in Table 4.2. It can be observed that the lowest error in estimating scattering and absorption coefficients are $\epsilon_{\mu_s} = 10$ % and $\epsilon_{\mu_a} = 9$ % with the following PSO properties:

$$a_1$$
 a_2
 $w_{Min}w_{Max}\chi^2$, Success
 $I_{max} <$
 ϵ_{μ_s} , ϵ_{μ_a} ,

 $\%$
 rate, $\%$
 $I >$
 $\%$
 $\%$
 0.3
 0.8
 0.1
 0.6
 10
 100
 29
 13
 18.5
 37.6
 0.1
 0.6
 0.4
 0.7
 10
 52
 21
 10
 25.3
 19.8
 0.3
 1
 0.6
 0.8
 10
 42
 23
 12
 21.4
 34.7
 0.3
 0.8
 0.1
 0.6
 9
 28
 25
 11
 22.6
 39.3
 0.1
 0.6
 0.4
 0.7
 9
 50
 18
 10
 24.7
 20.6
 0.1
 0.6
 0.4
 0.7
 7
 18
 24
 14
 9.8
 8.8
 0.1
 0.6
 0.4
 0.7
 6
 20
 26
 13
 12.8
 15.0
 0.1
 0.6
 0.4
 0.7
 5
 10
 16

$$a_1 = 0.1, a_2 = 0.6, w_{min} = 0.4, w_{max} = 0.7, \chi^2 = 7\%.$$

Table 4.2: Particle Swarm Optimization parameters: accelerations of the particles to the global best (gBest) - 1_1 and group best (grBest) - 1_2 , initial (w_{Max}) and final (w_{Min}) weights; and results of inverse problem WR solution: rate of success (%), maximum (I_{max}) and average $(\langle I \rangle)$ number of iterations, error in scattering $(\epsilon_{\mu_s}, \%)$ and absorption $(\epsilon_{\mu_a}, \%)$ coefficients estimation.

However even if the errors in optical properties estimation are comparatively small, the algorithm will converge only once out of five runs of the WR-PSO algorithm, thus further refinements were needed. In the next subsection 4.4.1 the complementary approach of the WR-PSO algorithm extension is proposed.

4.4.1 Fitting WR-PSO-based approach with spectral fitting

To improve convergence of WR-PSO-based approach modified with spectral Fitting the FWR-PSO algorithm is proposed. Our idea is to take advantage of these spectral data to bring new coherence constraint to the choice of scattering μ_s and absorption μ_a values during the optimization process. To do so, an *a priori* spectral shape is considered for each optical parameter. Then at each iteration step and for each parameter, its values are calculated independently at each wavelength and then are slightly modified to fit to the *a priori* known spectral shape.

Optical parameters to fit, i. e. scattering, μ_s , and absorption, μ_a , coefficients, were chosen to be exponential dependent on wavelength (Fig. 4.12) as it is the most common shape for tissue optical properties in the wide range such as absorption of melanin and scattering by small particles compared with wavelength according to Rayleigh and Mie theories [Mie, 1908, Gemert et al., 1989, Ishimaru, 1991, Bashkatov et al., 2000a, Sardar et al., 2001]. However the PSO-based algorithm could be trained for other spectral shapes such as water, fat, hemoglobin (oxy- and deoxy-), photosensitizers, etc[Sardar et al., 2001, Lister et al., 2012, Jacques, 2013].

This refining approach is implemented through additional steps (steps 3 to 4) to the previous WR-PSO algorithm:

- 1. calculate for each particle χ^2 and take mean values inside the group;
- 2. find two minimal $\chi^2_{gBest}(\lambda)$;
- 3. fit two gBest with exponential decay function, $x_{fit}(\lambda) = f_x(\lambda) = a_x e^{-b_x \lambda}$, where $x_{fit}(\lambda)$ parameter is one from $\mathbf{p} = [\mu_s(\lambda), \mu_a(\lambda), g(\lambda), n(\lambda)];$
- 4. calculate $I_{mod}^{fit}(\lambda, r_m; \mathbf{p}_{fit})$ with new fitted parameters of optical properties;
- 5. for each particle inside the group calculate $\chi^2_{fit}(\lambda) = \sum_{m=1}^{M} \sum_{\lambda} \chi^2_{fit}(\lambda);$
- 6. if previous χ^2_{sim} is higher than the new one χ^2_{fit} , exchange all the parameters with fitted ones, i. e. apply $gBest = [\chi^2_{fit}(\lambda), \mu^{fit}_s(\lambda), \mu^{fit}_a(\lambda), g^{fit}(\lambda), n^{fit}(\lambda), z^{fit}]$, however save the values of grBest.
- 7. repeat the process till convergence is achieved.

For this new step the FWR-PSO-based algorithm was trained again following the one presented in Fig. 4.11.

The training process was done among the same ranges of values as for WR-PSO (4.10) for a_1, a_2, w_{Min} and w_{Max} fixing the weight w to local after 10th iteration.

Also in this FWR-PSO approach we fix anisotropy (g = 0.8), refractive index (n = 1.44) too and do all the simulations for one layer numerical model, so z = 5 cm, scattering and absorption coefficients are presented in Fig. 4.12.

The parameters of FWR-PSO were found to be with maximum number of iterations -31 (the first try is 0 and from 1^{st} to 30^{th} iteration).

The dependence of error between data obtained and experimental on optical parameters estimation and rate of success as well as on the number of iterations was revealed. The algorithm was started for several error rates in data: $\chi^2 = 5{-}10$ % with step 1 %. The simulations were performed 50 times per each set of PSO parameters, then the best were chosen according to error



Figure 4.11: The final algorithm of optical properties estimation while solving inverse problem based on MC forward simulations by PSO

in scattering and absorption properties thus the same procedure was repeated for the algorithm with fitting approach. The *a priori* data: scattering (μ_s) and absorption (μ_a) coefficients; was chosen the same as for WR-PSO approach (Fig. 4.12).

4.4.2 Results and discussion for FWR-PSO approach

The results of the FWR-PSO approach are presented in Table4.3.



Figure 4.12: Scattering μ_s^{ref} and absorption μ_a^{ref} coefficients dependencies on wavelength λ which were used as the reference data to be estimated by WR-PSO and FWR-PSO algorithms

The best balance between the following parameters was found to define which parameters of the PSO algorithm to use:

- 1. number of iterations which affects time of inverse problem solution (Fig. 4.13);
- 2. error $(\epsilon_{\mu_s} \text{ and } \epsilon_{\mu_a})$ in optical properties estimation, %, which depends on chosen χ^2 -value (Fig. 4.15);
- 3. success rate (%) indicating that the number of maximum iterations was not overcome by the algorithm (Fig. 4.14).

a_1	a_2	w_{Mi}	$m w_{Ma}$	$_{ix}\chi^2$,	Success	Imax	$_{r}$ <	$\epsilon_{\mu_s},$	$\epsilon_{\mu_a},$
				%	rate, $\%$		I >	%	%
0.2	0.8	0.2	0.6	10	92	16	4	19	15
0.3	2.1	0.8	0.9	9	52	16	3	13.6	6.3
0.6	1.7	0.8	1.3	8	36	28	6	5.9	11.1
0.8	1	0.7	0.9	8	72	20	4	13.6	8.9
0.6	1.7	0.8	1.3	7	38	28	6	4.8	7.7

a_1	a_2	w_{Mi}	$a_n w_{Ma}$	$\chi^2,$ %	Success rate, %	I _{max}	x < I >	$\epsilon_{\mu_s}, \ \%$	$\overset{\epsilon_{\mu_a}}{\%},$
0.3	2.1	0.8	0.9	6	54	29	7	3.3	8.3
0.6	1.7	0.8	1.3	6	34	29	6	4.8	4.9
1.6	1.2	0.1	0.5	6	60	28	3	6.2	6.6
1.6	1.2	0.1	0.8	5	48	29	18	5.8	4.9
0.3	1	0.6	0.8	5	34	30	21	2.5	4.01

Chapter 4. Approach to increase precision in optical properties estimation

Table 4.3: Columns 1-4: PSO parameters for with exponential fitting approach (FWR-PSO) showing accelerations of the particles to the global best (gBest) - a_1 and group best (grBest) - a_2 , initial (w_{Max}) and final (w_{Min}) weights; rate of success (%), maximum (I_{max}) and average $(\langle I \rangle)$ number of iterations, error in scattering $(\epsilon_{\mu_s}, \%)$ and absorption $(\epsilon_{\mu_a}, \%)$ coefficients estimation.

As it is seen from Fig. 4.15, error between modeled and experimental data is in direct ratio to error in optical properties estimation, i. e. $\chi^2 \sim \epsilon(p_i)$. Thus it could be concluded that error between modeled and reference data should be 5-7 % to have an error in optical properties less than 10%. According to the average $\langle I \rangle$ and maximum number I_{max} of simulations it was chosen to use 6 % error to reduce computational time (rate of success is 60 % and average number of iterations until algorithm converges is 3), thus error in optical parameters estimation was - $\epsilon_{\mu_s} = 6.2\%$ and $\epsilon_{\mu_a} = 6.6\%$ for scattering and absorption coefficients, respectively.



Figure 4.13: Dependence of average $\langle I \rangle$ and maximum (I_{max}) number of iterations on χ^2 for WR and FWR PSO-based approaches, solid curves correspond for fitting functions indicated in the legend



Figure 4.14: Dependence of WR-PSO and FWR-PSO convergence success rates (%) on χ^2 value



Figure 4.15: Dependence on χ^2 value of estimated optical parameters error in scattering (ϵ_{μ_s} , %) and absorption (ϵ_{μ_a} , %) values during WR-PSO (section 4.4) and FWR-PSO (section 4.4.1) algorithms convergence

Thus the FWR PSO-based algorithm parameters are: $a_1 = 1.6$, $a_2 = 1.2$, $w_{Min} = 0.1$, $w_{Max} = 0.5$, $\chi^2 = 6$ %. The lower success rate of 48 % with higher average number of simulations ($\langle I \rangle = 29$) increasing computational time which is drastically if MC simulations are used as forward model, could give lower error of less than 5 % for both scattering (μ_s) and absorption (μ_s) values, thus FWR-PSO algorithm properties are: $a_1 = 0.6$, $a_2 = 1.7$, $w_{Min} = 0.8$, $w_{Max} = 1.3$, $\chi^2 = 6$ %.

Results of WR ad FWR PSO-based approaches are compared below. The FWR PSO-based approach allowed decreasing the rate of successful convergences 3 times in average. As for scattering (μ_s) and absorption (μ_a) coefficients, errors in its' determination ϵ_{μ_s} , %) and ϵ_{μ_a} , %) were decreased 2 and 2.4 times in average, accordingly. Comparison of the best results given by both approaches, showed that the fitting approach decreased error in optical properties determination to 6 and 5 % for scattering (μ_s) and absorption (μ_a) coefficients, respectively, however for simple WR-PSO approach minimum errors are $\epsilon_{\mu_s} = 15$ % for scattering and ϵ_{μ_a} = 12 % for absorption coefficients (from the approximated data on figures). Average number of iterations ($\langle I \rangle$) was also decreased from 14 to 6 and 3, which is in direct ratio with time of simulations, i. e. if the MC would be used to simulate the forward model, the computation time of the inverse problem till convergence would be decreased by a factor of 3.

4.5 Conclusion

The proposed and analyzed FWR-PSO method allows determining optical properties which have a certain dependence on wavelength. For each type of curve the algorithm should be trained on newly simulated curves, which is taking the minimal amount of acquisition time, as the DA was used instead of MC method. The method presented allows reducing the number of iterations and simplifies convergence process, avoids the possibility of confusing local minima with the global one, as the area occupied by each cluster reduces with each iteration step with acceleration a_2 , at the same time the area where the cluster can be moved is reduced with acceleration a_1 , beyond that the value of optical parameters $\mathbf{p}(\lambda)$ is averaged in each cluster. The use of two best points from the whole spectrum of optical properties (algorithm could be extended for wavelength dependent anisotropy factor $g(\lambda)$, refractive index $n(\lambda)$, and to multilayer model, thus with different thickness of layers z_i , where i - number of layer) allows avoiding additional steps during convergence process and moves all the particles moving in the space of probable solutions, leaving the possibility to find the lower error, thus the global minimum. This method is giving the smooth result for optical properties allowing its' determination with acceptable error of less than 5 %. Moreover adding the complexity to the forward problem, for example apply it to two-layer model, can lead to faster solution in comparison with widely used methods, i. e. LMA. Finally, the FWR-PSO method could be used with any forward model, though having the same space of optical properties, as group and individual speeds depend on this range and have to be redefined if they are changed. Further this algorithm could be applied to other functions of optical properties on wavelength, such as absorption of oxy- and deoxyhaemoglobin with Protoporphyrine IX and for multilayered model.

Chapter 5

Fluorescence life-time change in presence of nanoparticles

Never replicate a successful experiment.

Fett's Law of the Lab

5.1 The state-of-the-art

Professor Novotny et al [Anger et al., 2006] showed that the local field enhancement leads to an enhanced excitation rate however non-radiative energy transfer to the particle leads to a decrease of the quantum yield, i. e. quenching.

The last step of the present thesis was to understand the process of the energy transfer between a nanoparticle and a fluorophore in case of the PPIX molecule and the gold nanosphere in order to go deeper in the understanding of the undergoing processes.

As it is known, energy transfer between a nanoparticle and a fluorophore goes through two ways: non-radiant and radiant. The type of energy transfer depends on the center to center separation:

- 1. Dexter type correspond to the distances less than 1 nm,
- 2. Forster type correspond for the distances from 1 nm to 10 nm,
- 3. The range more than 10 nm to micrometers is under discussion.

Hereafter the above mentioned types of the energy transfer are being discussed. The Dexter type energy or electron transfer is a mechanism of fluorescence quenching, which occurs on the short distances lower than 1 nm and requires a wave-function overlap and is presented in the form of spectral overlap, thus the energy transfer rate is:

$$k_D = \int f_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda e^{\frac{2r}{L}}, \qquad (5.1)$$

where L is the sum of Van der Waals radii of the donor D and the acceptor A and r stands for the separation between donor and acceptor.

The Forster energy transfer occurs between two fluorescent molecules of point-size and can not be applied to a system of two particles where one has a finite size according to a molecule which could be considered as a point dipole. This type of energy transfer is explained through dipole-dipole energy transfer. The fluorescence life-time highly depends on the distance between fluorophores. The principal scheme of the energy transfer is sketched in Fig. 5.1.



Figure 5.1: Principal scheme of energy transfer between a nanoparticle and a fluorescent molecule

The theory for the last range is still under discussion and will be applied to the case of the colloidal nanoparticles in a solution of a fluorophore. There were several works suggesting different types of energy transfer between nanoparticle and fluorophore:

- 1. CPS-Kuhn model[Chance et al., 1975],
- 2. Gersten-Nitsan model (GN-model)[Gersten and Nitzan, 1981, Dulkeith et al., 2005],
- 3. Nano-Surface Energy Transfer (NSET)[Chance et al., 1978, Persson and Lang, 1982, Yun et al., 2005, Jennings et al., 2006, Singh and Strouse, 2010, Algar et al., 2014]
- Dipole to Metal Particle Energy Transfer (DMPET)[Carminati et al., 2006, Pons et al., 2007].

Hereafter these theories will be considered and some of them will be applied to the case of colloidal nanoparticles with photosensitizer PPIX used in previous experiments. To evaluate the validity of these energy transfer theoretical models life-time measurements experiments were conducted. To compare these theories it is needed to compare three terms:

- 1. R_0 distance on which quenching efficiency reaches 50 %,
- 2. the power law(N) for quenching efficiency (E);
- 3. total character of quenching (E).

Thus quenching efficiency E is defined (see Eq. 5.2):

$$E(r) = \frac{1}{1 + \left(\frac{r}{R_0}\right)^N},\tag{5.2a}$$

$$E(I) = 1 - \frac{I}{I_0},$$
 (5.2b)

$$E(\tau) = 1 - \frac{\gamma}{\tau_0},\tag{5.2c}$$

where r is the distance from nanoparticle to photosensitizer, I and I_0 are the fluorescence signal respectively with and without quenchers, i. e. nanoparticles, τ and τ_0 stands for the decay time respectively with and without quenchers. For the traditional FRET the power law N is 6, as well as for the GN model, however for the NSET and the CPS-Kuhn the quenching efficiency follows N = 4 distance dependency.

5.2 Theoretical models of energy transfer

5.2.1 CPS-Kuhn model

Chance, Prock, Silbey and Kuhn developed a model (CPS-Kuhn model) of fluorophore to a nanofilm or to a metal surface energy transfer. This model suggests a mirror-like behavior of a metal surface. In case of the CPS-Kuhn model a photosensitizer is represented by a linear harmonic oscillator and has a mirror dipole on the surface of a metal nanoparticle represented by a perfect mirror. This results in both quenching and enhancement depend on the projection of the electric field from the nanoparticle surface. The projection is considered to be size-dependent to account for small nanoparticles of several nanometers in diameter. The $R_0^{CPS-Kuhn}$ is described as (see Eq. 5.3):

$$R_0^{CPS-Kuhn} = \frac{\beta\lambda}{n} (Abs \cdot q)^{\frac{1}{4}} \left[\frac{Re(n_{NP})}{2n} \left(1 + \frac{Re(\varepsilon_{NP})^2}{|Im(\varepsilon_{NP})|^2} \right) \right]^{\frac{1}{4}},\tag{5.3}$$

where $\beta = \frac{1}{4\pi}9^{\frac{1}{4}}$, of dipole is oriented perpendicularly to the metal surface either $\beta/2^{\frac{1}{4}}$, when dipole is parallel to the metal surface, ε_{NP} , Abs - absorptivity of the thin metal film, and $n_{NP} = p_{NP} + ik_{NP}$ is the dielectric function and the refractive index of the metal film, d is the thickness of the mirror and q stands for the quantum yield of the photosensitizer. This equation is somehow reminds the FRET overlap integral (see Eq. 5.1) if to assume a donor oscillator is coupled to the states of electrons in a metal nanoparticle below the Fermi level. The mirror absorptivity Abs is described as follows:

$$A = \frac{4\pi Im(n_{mirror})d}{\lambda},\tag{5.4}$$

where n_{mirror} is the index of refraction for mirror and d stands for the thickness of the the metal film. The size-dependent behavior of nano-sized films has not been applied here, however as it was mentioned before in chapter 2 if the thickness is below skin depth then the dielectric function becomes size-dependent.

5.2.2 NanoSurface Energy Transfer model

The next model is taking into account a size-dependent dielectric function for the NanoSurface Energy Transfer model (NSET) thus providing changes to the previously described model of CPS-Kuhn by applying instead of the mirror absorptivity absorptivity *Abs* of the metal nanoparticles as follows (see Eq. 5.5):

$$Abs = 10^3 ln(10) \left(\frac{\sigma_{ext}(a,\lambda)(2a)^2}{N_a V \delta_{skin}}\right),\tag{5.5}$$

where $\sigma_{ext}(r, lambda)$ is the extinction cross-section, was calculated before and the absorptivity Abs itself was measured as well in the chapter 2. The parameters V and a are the volume and the radius of a nanoparticle respectively in cm³ and cm, N_A is the Avogadro number. Thus taking into account both scattering and absorption, influence on energy transfer is treated in the correct manner [Breshike et al., 2013]. By doing so the NSET model considers coupling between a point dipole of a photosensitizer molecule and a nanoparticle instead of thin film, thus describing the 2-dimensional local surface plasmon resonance at a surface of a nanoparticle. This consideration takes into account the Drude model of free electrons which predicts strong coupling between metal oscillators. The NSET model applied by Strouse et al [Breshike et al., 2013] allowed for describing the interaction of an oscillator which was verified by several fluorescent molecules on different fixed distance from the gold nanoparticles of radius less than 20 nm. This group of scientist observed experimentally the R^{-4} distance dependence quenching for gold nanoparticles 0.945-8.25 nm in radius and several fluorophores, which emission spectra overlap the extinction of the nanoparticles.

5.2.3 Gersten-Nitzan model

The Gersten-Nitzan (GN) model assumes that nanoparticle has a strong field, which affects a photosensitizer molecule modeled as a single-point dipole which is placed close to a nanoparticle. The dipole presence results in the radiant and the non-radiant decay rates change as the dipole is coupled to the nanoparticle's local electric field. The value of R_0^{GN} for a metal nanoparticles is described by:

$$R_0^{GN} = \left(2.25 \frac{c^3}{\omega_{ph}^3} q a^3 \frac{(Re(\varepsilon + NP) + 2)^2 + Im(\varepsilon + NP)^2}{Im(\varepsilon + NP)}\right)^{\frac{1}{6}},\tag{5.6}$$

where ω_{ph} is the absorption frequency of a photosensitizer. This theory assumes no enhancement effects and no change in the radiative rate of a photosensitizer.

5.2.4 Theoretical results

Hereafter the theoretical results of the GN (see Eq. 5.6) and the NSET (calculated by substitution of Eq. 5.5 to the CPS-Kuhn model (see Eq. 5.3)) models are presented (see Fig. 5.2). The calculations were done for the gold and the silver nanoparticles of 7 and 25 nanometers in radius to compare the efficiencies of energy transfer and the distances of half-quenching, R_0 (see Eq. 5.2a).

As it is seen from the data on Fig. 5.2 the half-quenching distance for the GN-model is higher, and for the gold nanoparticles in the GN model the R_0 is smaller, however in the NSET model gold nanoparticles has shorter R_0 .

In the next experiment specially synthesized gold nanoparticles were used to check the models presented here for the energy transfer between a PPIX molecule and the synthesized gold nanoparticle.



Figure 5.2: (A) Theoretical energy transfer distance of half-quenching, R_0 , depending on nanoparticle's radius under 637 nm excitation and (B) energy transfer efficiency dependence on distance between a PPIX molecule and a gold and a silver nanoparticles of radius 7 and 25 nm.

5.3 Experimental section

5.3.1 Phantoms preparation for life-time measurements

The phantoms for the lifetime measurements were made of PPIX as a fluorophore and of the gold nanoparticles, which were synthesized by Turkewich method [Wuithschick et al., 2015]. Distilled water solution of 0.25 mM of $HAuCl_4$ was heated on the magnetic mixer to 90^oC temperature, then 250 μl of Na_3Cit was added. During the redox reaction citrate is redacting gold to Au^0 , the gold seeds start to appear and the nanoparticles start to grow around these seeds. During the 20 minutes synthesis the color of the solution is changing: when citrate is added, the solution becomes dark gray, this means that gold seeds appeared, then the color is changed to violet, finally it becomes wine-red, meaning that the synthesis is completed. Then the solution was analyzed by the sprectrophotometer Hitachi U-3400 [Corp.,] to obtain the size and concentration as it was done before in the chapter 2 with double-beam scheme. The maximum absorptivity was 0.3242 at 520.1 nm, at 450 nm absorptivity was 0.2182. The final concentration was calculated by the following Eq. 2.21[Haiss et al., 2007], according to the authors estimated error is 11 %. Thus, the diameter of synthesized gold nanoparticles is (13.2 ± 1.5) nm. The number density was estimated according to the same research paper as $3.17 \cdot 10^{10}$.

The phantoms were made in a way that no backscattering occurs while the integral fluorescence spectra measurements by the LESA-Biospec spectrometer, the optical probe has one excitation fiber and 6 detecting fibers on the same fixed distance from the source-fiber around 212 μm , the core's diameter is 200 μm , NA equals 0.22. The top 0.5 ml Eppendorf was covered with a thin transparent film to avoid scattering by the Eppendorf walls, and closed with a cover which has a specially made hole to keep the thin film strained. An Eppendorf with a phantom was turned upside down so the solution was in a full contact with a thin film, the multi-fiber probe was placed in a tight contact to thin film perpendicularly to its surface. Microspheres of 0.5 μm in diameter were dissolved with 5 mg/kg PPIX in DMSO solution. The microspheres volume fraction of 1 to 50 was chosen experimentally in a way that the laser light is scattered before it reaches the narrow bottom of the Eppendorf to avoid reflection from a 'water lens' which could occur due to surface tension close to narrow end of an Eppendorf.

The fluorescence spectra were taken to find out if the effect of nanoparticles presence could be seen from the spectroscopic steady-state signal. It was found out, that being dissolved by distilled water twice from 10 mg/kg concentration, PPIX molecules first aggregate as the fluorescence signal is decreased and then again separated due to the microspheres presence, this effect does not occur without microspheres, however the fluorescence signal growths to it's maximum faster by use of Intralipid 2% solution. However for the lifetime measurements the use of Intralipid was not convenient, as the backscattered light depends on the angle between the optical probe and phantom's surface, i. e. thin film separating phantom from the optical probe. This is explained by different sizes of the particles (lipid droplets) present in the Intralipid, thus backscattering is averaged among the wide range of those droplets and the backscattered signal could not be controlled. The opposite situation is with 500 nm microspheres, which have tinier size distribution, thus the backscattered light is more stable to the low angle between the optical multi-fiber probe and surface of the phantom.

$C_{PPIX}, \ \mathrm{mg/kg}$	f_{MS}	$f_{NP},~\%$	$f_{DMSO}, \%$
		0	
		10	
5	1:50	20	50

$C_{PPIX},$	f_{MS}	$f_{NP}, \%$	$f_{DMSO},\%$
_mg/kg			
		30	
		40	

Table 5.1: Phantoms set main characteristics prepared for the measurements. C_{PPIX} is the concentration of PPIX, f_{MS} is the volume fraction of 0.5 μm microspheres, C_{NP} is the concentration of gold nanoparticles and f_{DMSO} stands for the fraction of DMSO in a phantom.

The repeatability of the results was checked in the following way: the phantoms were kept at the 2 ${}^{0}C$ for 2 days and their fluorescence spectra under 532 nm and 632.8 nm excitation were taken, the measurements were repeated in one hour after the phantoms being kept at the room temperature 20⁰C. The comparison of the obtained spectra showed that the fluorescence is quenched after the phantoms were frozen and then returns to the same level as before freezing. Also, a just prepared phantom solution has a decreased fluorescent signal, thus the life-time measurements should be taken in one hour after a phantom was made.

5.3.2 Experimental setup for life-time measurements

The analysis of lifetime components allows to estimate the character of energy transfer between nanoparticle and a photosensitizer in a medium. By Vigoureux et al [Carminati et al., 2006] it was shown that in a close proximity of a nanoparticle, the non-radiative decay rates increases, thus the radiative rates should decrease linearly with a distance between a nanoparticle and a photosensitizer, if the later emits at the plasmon resonance wavelength of this nanoparticle. For the life-time measurements in pico- and nanosecond range the Hamamatsu Streak-camera C10627-13 was used. The measurements were performed on a system of registration with 15 picosecond resolution of streak-scope which is synchronized with the picosecond laser with 637 nm wavelength maximum and FWHM 65 ps (Fig. 5.3).

Each measurement was started with exposition 50-80 ms, repetition rate 16 MHz, with full time of measurements around 1 hour.

The phantoms were excited through an optical fiber (NA = 0.22, D = 200 μ m) and collected with 6 surrounding fibers at the SDS 261 μ m. The multi-fiber probe was placed little bit aside from perpendicular position to a 1 ml Eppendorf with phantom to avoid laser reflection from the wall of an Eppendorf. An interference filter which cuts 50 % of intensity on 660 nm was used to avoid influence of laser on fluorescence spectra.

The experiment was conducted on the set of phantoms with gold nanoparticles in different concentrations presented in Table 5.1, the mean distance between gold nanoparticles and PPIX molecules is shown in Fig. 5.5.

The pH of the phantoms was measured as well, it changed from 7.4 to 7.6, so this range does not affect fluorescence life-time.

While changing the concentration of nanoparticles, the fluorescence signal was measured with spectrophotometer LESA, the same as was used before in our experiments.

The spectra were analyzed in the same way as it is described in previous chapter, i. e. the spectral shape was corrected with white light signal. By such measurements, it's possible to obtain the concentration which is quenching the fluorescence signal twice.



Figure 5.3: Lifetime measurements setup: A - the principal scheme of the setup, B - the photo of the setup, C - the optical fiber bandle endings from the left to the right: the single mode fiber used for excitation, the 6 fibers collecting fluorescent signal are aligned in one line to transfer signal to the polychromator



Figure 5.4: Transmission of filters used for the lifetime measurements



Figure 5.5: Dependence of mean distance between gold nanoparticle and PPIX molecule on concentration of nanoparticles

5.4 Results of fluorescence quenching and enhancement experiment

5.4.1 Influence of colloidal gold nanoparticles presence on PPIX fluorescence signal

Here we present the results of gold nanoparticles influence on integral fluorescence of PPIX according to Eq. 5.2b the $R_0(I)$ distance is where fluorescence is quenching twice. From the data represented in Fig. 5.5 it is possible calculate the concentration of nanoparticles, C_{NP} to obtain the approximate experimental R_0 . However the knowledge of mean calculated distance is not allowing to judge about the real fluorophore-nanoparticle separation, as they could be coupled at completely different distances, which are under influence of nanoparticle local field.

For 532 nm excitation the concentration of nanoparticles was around 75 ± 2 mg/kg for PPIX concentration of 2 mg/kg and for 1 mg/kg it was needed to extrapolate the data to higher concentrations, thus we obtained 71 ± 2 mg/kg.

The fluorescence spectra were taken after time-resolved experiment, it is clearly seen that after around one hour of measurements, photo-products appeared: chlorine-1, chlorine-2 and derivative of PPIX, which have fluorescent maximum at 675, 678 and 700 nm, respectively. The obtained signals are shown in Fig. 5.6.

It is seen that the signal is not clearly Gaussian, but has two components, this means that the two derivatives of PPIX could be resolved. Thus, the fluorescent spectra were deconvoluted to 2 components, each corresponding to one of the PPIX's isomers. The example of the deconvoluted spectra is shown in Fig. 5.7. The adjusted R^2 was more than 0.99 for all the fits obtained.

After the life-time measurements the fluorescence integral measurements were repeated.



Figure 5.6: Fluorescence signal of phantoms derived by integral spectra measurements before starting the life-time measurements



Figure 5.7: Fluorescence signal of 150 μl solution of NS in DMSO with 5 mg/kg PPIX and deconvolution to two components of the PPIX isomers, excitation by 532 nm diode laser.



Figure 5.8: Fluorescence integral signal quenching, $F(700 \pm 10nm)$, due to nanoparticles presence. Orange point indicates no nanoparticles in the phantom, red point - half quenching of the fluorescence integral signal.

5.4.2 Influence of colloidal gold nanoparticles on the kinetics of PPIX fluorescence life-time

The obtained fluorescence time-resolved data was deconvoluted to two or three time components, taking into account laser pulse with FWHM around 65.0 ± 7.5 ps:

$$I(t) = L(t) \otimes F(t), \tag{5.7}$$

where F(t) is the sample's decay curve and L(t) stands for the laser pulse.

The fitting was done after integrating by wavelength the data obtained, which is represented by spectrally-time-resolved signals (see Fig. 5.9)

From the fitting results the time components and the percentage of each component were estimated (Figures 5.11 and 5.12). The adjusted R_{adj}^2 was 1.04 ± 0.03 for all the deconvoluted data.

Each life-time component is in accordance with the PPIX molecules degrees of freedom. The life-time components are changing under influence of the additional electromagnetic field induced by gold nanoparticles. In this region of wavelengths the gold nanoparticles used in the experiment have strong enhancement, thus the nanoparticle's dipole moment, which is enhanced due to the picosecond laser excitation, is compensating the dipole moment of a PPIX molecule. For the PPIX isomer fluorescing at the 670-690 nm region, the ultra-short component occurs when the volume fraction of the gold nanoparticles reaches 30%, for the second one isomer, the ultra-short component occurs at the volume fraction of 40 %. Both ultra-short components are about hundred picoseconds and both of them comprise about one half of the fluorescence decay curve, meaning that half of the PPIX molecules dipoles are compensated by nanoparticles strong field. If to come back to fluorescence integral signal change, which occurs due to nanoparticles



Figure 5.9: Analyzed image from the streak camera representing a time- and spectrally-resolved signal. With colored rectangles integrated within chosen wavelength ranges normalized signals are shown. At the bottom the sum is made by time, at the right the sum is made by wavelength, all the spectra are normalized by maximum.


Figure 5.10: Fluorescence decay within 20 ns range. Colored lines - the obtained fit, dots - the experimental data.

presence, the highest enhancement is reached at the volume fraction of 20 %, then it decreases with increase of nanoparticles concentration.

It was revealed, that by means of the life-time measurements it is possible to judge about the compensation of the PPIX molecule's dipole moment with the free electrons surrounding the gold nanoparticles, as the strong coupling field occurs due to the nanoparticles enhanced electromagnetic field, which is described by the picosecond component, which increase for both isomers with the growth of the nanoparticles coupling with PPIX molecules, i. e. increase of the nanoparticles concentration.

The appearance of the picosecond lifetime component means that the probability of the intersystem conversion to the triplet level of PPIX, which can not be excited directly, but only through relaxation from upper levels, is drastically reduced and the consequent transfer of the energy to molecular oxygen becomes less probable. As the gold nanoparticles seems to be bio-compatible, the change in energy transfer mechanism could be used after a PhotoDynamic Therapy (PDT) by applying superficially nanoparticles considered in our work to 'block' singlet oxygen formation and consequent cells death, thus reducing photo-toxicity of skin.



Figure 5.11: A - Fluorescence life-time long, short and ultra-short components for the isomer on 670-690 nm wavelength range calculated after deconvolution of the fluorescence signal. B -Percentage of components. Ultra-short component occurs at the NP volume concentration of 30, 40 and 50 % for derivative of PPIX detected at the wavelengths 670-690 nm.



Figure 5.12: A - Fluorescence life-time: the long, the short the and ultra-short components for the isomer on the 690-710 nm wavelength range calculated after deconvolution of the fluorescence signal. B - Percentage of components. The ultra-short component occurs at the NP volume concentration of 40 and 50 % for derivative of PPIX detected at the wavelengths 690-710 nm.

Chapter 6

Conclusion and perspectives

Maintenant JE SAIS, JE SAIS QU'ON NE SAIT JAMAIS !

La vie, l'amour, l'argent, les amis et les roses

On ne sait jamais le bruit ni la couleur des choses

C'est tout c'que j'sais ! Mais ça, j'le SAIS...

Chanson (1974) interprétée par Jean Gabin

The main objectives of the work were spectral, spatial and temporal properties of multilayered tissue *in vivo*, especially epithelial tissue, spatially resolved spectroscopy improvement with the nanoparticles application and the inverse problem solution for biomedical optical diagnosis.

The electromagnetic field enhancement by a single nanoparticle was analyzed theoretically by means of the Mie theory and the FDFD simulations. The enhancement for the gold, silver and ferric oxide (II) nanoparticle dimers were investigated theoretically by the dipole-dipole interaction model.

The fluorescence integral signal enhancement and quenching were revealed during the spatiallyand spectrally-resolved measurements on the multilayered phantoms of epithelial tissue, containing the photosensitizer and the nanoparticles in different concentrations - the gold 14 nm nanospheres and the gold 50 nm nanostars.

The FWR-PSO method was proposed for determining optical properties of biological tissue numerical model which have a certain dependence on wavelength. The method presented allows reducing the number of iterations and simplifies convergence process, avoids the possibility of confusing local minima with the global one, as the area occupied by each cluster reduces with each iteration step with acceleration a_2 , at the same time the area where the cluster can be moved is reduced with acceleration a_1 , beyond that the value of optical parameters $\mathbf{p}(\lambda)$ is averaged in each cluster. The use of two best points from the whole spectrum of optical properties (algorithm could be extended for wavelength dependent anisotropy factor $g(\lambda)$, refractive index $n(\lambda)$, and to multilayer model, thus with different thickness of layers z_i , where i - number of layer) allows avoiding additional steps during convergence process and moves all the particles moving in the space of probable solutions, leaving the possibility to find the lower error, thus the global minimum. This method is giving the smooth result for optical properties allowing its' determination with acceptable error of less than 5 %. Moreover adding the complexity to the forward problem can lead to the faster solution in comparison with widely used methods, i. e. LMA. Another one advantage of the FWR-PSO method is that is could be used with any forward model, though having the same dependence of optical properties on wavelength. The group and the individual speeds obtained depend on the range of applied optical parameters and have to be redefined if they are changed. The algorithm proposed opens wide possibilities for other non-linear functions of optical parameters on wavelength, such as absorption of oxy- and deoxy-hemoglobin, Protoporphyrine IX and other photosensitizers and for multilayered model as well.

As the last step, the kinetics of PPIX fluorescence integral signal and fluorescence coupling of PPIX with the gold nanoparticles was investigated. The gold nanoparticles were synthesized with maximum of extinction on 523 nm. Two life-time components were resolved without nanoparticles in the solution, their consequent change with growth of nanoparticle's concentration was analyzed.

The critical point for the isomer which fluoresces in 670-690 nm range is when the concentration of nanoparticles is 30 %, for the other one isomer, this point occurs when the concentration of nanoparticles is 40 % of phantom's volume. The life-time measurements showed that the colloidal gold nanoparticles change the energy transfer scheme of the photosensitizer PPIX, thus the energy transfer to the triplet level of the PPIX molecule is partly blocked. Such study suggests a potential application in biomedical optics for fluorescence life-time sensing for uptake of nanoparticles by biological tissue. The other one effect could be achieved by applying nanoparticles superficially, thus avoiding phototoxicity of skin, as nanoparticles will block the energy transfer to oxygen.

Appendix A

MATLAB code for dimers

A.1 Main part of the code

```
1 clear all; clc
2 global Eps0
3 Eps0 = 8.854*10^(-12)/10^9; % F/nm Vacuum permittivity
4 lambda = 349:1000; % wavelength, nm
5 %% set values of Ag, Au and Fe_20_3 refractive index real
6 % and imaginary parts from Johnson and Christy, 1972, either from Eq. ...
      \ref{eq:epsdrr}
7 % NRe = [];
8 % NIm = [];
9 Quartz =1.544;
10 H2O =1.33;
11 Air =1.029;
12 Nmed = [1.00 \ 1.33 \ 1.44];
13
  88
14 % r1 = 5:5:50;
15 % r2 = 5:5:50;
16 delta = [0.1:0.1:0.9 1:30]; % distance between nanoparticles' surfaces
17 r1 = [0.01:0.01:0.1 0.2:0.1:0.9 1:30];
18 r2 = 5;
  \circ = 0;
19
   for m = 1:length(Nmed)
20
21
       for i = 1:length(r1)
           for j = 1:length(r2)
22
               for n = 1:length(delta)
23
                   A12 = dipoleMatrix(lambda, Nmed(m), r1(i), r2(j), delta(n));
24
25
                   alfa1 = alfa(r1(i), Nmed(m), NRe, NIm); % 1st nanoparticle
26
                   alfa2 = alfa(r2(j), Nmed(m), NRe, NIm); % 2d nanoparticle
27
                   Enh1(m, i, j, n, :) =...
                   real(3*Nmed(m).^2./((NRe+1i*NIm).^2 + 2*Nmed(m).^2).*...
28
                        (1 - A12.*alfa2)./(1 - alfa1.*alfa2.*A12.^2));
29
   %% Calculate external field near 1st nanoparticle
30
                     Enh2(m, i, j, n, :) =...
31
   8
                    real(3*(NRe+1i*NIm).^2./((NRe+1i*NIm).^2 + 2*Nmed(m)^2).*...
32
   8
                          (1 - A12.*alfa2)./(1 - alfa1.*alfa2.*A12.^2));
33
                   EnhMax1(m, i, j, n) = squeeze(max(Enh1(m, i, j, n, :)));
34
  %% Calculate maximal external field near 1st nanoparticle
35
                     EnhMax2(m, i, j, n) = squeeze(max(Enh2(m, i, j, n, :)));
36
  00
37
                   col = [rand rand rand];
```

```
38
               end
           end
39
       end
40
  end
41
42
43
  88
  for i = 1:length(Nmed)
44
       figure(4), subplot(2, 2, i), contourf(r1', delta', squeeze(EnhMax1(i, :, ...
45
           1, :))')
       xlabel('log(Separation (d)), nm'), ylabel('log(r), nm')
46
       title(['Nmed = ', num2str(Nmed(i)), ', r = ', num2str(r2), ' nm'])
47
       colorbar, set(gca,'xscale','log'), set(gca,'yscale','log')
^{48}
49 %% Output external field of 1st NP
  00
        figure(5), subplot(2, 2, i), contourf(r1', delta', squeeze(EnhMax1(i, ...
50
      :, 1, :))')
51 %
        xlabel('Separation (d), nm'), ylabel('r, nm')
        title(['Nmed = ', num2str(Nmed(i)), ', r = ', num2str(r2), ' nm'])
52
  8
        colorbar, set(gca,'xscale','log'), set(gca,'yscale','log')
53 %
54 end
```

A.2 Dipole interaction matrix

```
1 function A12 = dipoleMatrix(lambda, Nmed, r1, r2, delta)
2 global Eps0
3 A12 =...
4 exp(li*K(lambda, Nmed).*diam(r1, r2, delta)).*(li*K(lambda, Nmed)...
5 ./diam(r1, r2, delta).^2 - 1./diam(r1, r2, delta).^3)./(2*pi*Nmed^2*Eps0);
```

A.3 Polarization of nanoparticle

```
1 function pol = alfa(r, Nmed, NRe, NIm)
2 global Eps0
3 pol = Eps0*Nmed^2*4*pi*r^3*((NRe + 1i*NIm).^2-Nmed^2)./((NRe + 1i*NIm).^2 + ...
2*Nmed^2);
```

A.4 Center-to-center separation of nanoparticles

```
1 function d = diam(r1, r2, delta)
2 d = r1 + r2 + delta;
```

Appendix B

Wolfram Mathematica code

B.1 Code for calculating scattering and absorption cross-sections of nanoshells and nanosheroids

```
1 (*dielectric functions of gold, silver and Ferric oxide (II):
 2 Au, Ag, Fe2O3
 3 lambda := 370:1195 - wavelength, nm
 4 a, b, c, d - 3 semi-axes and shell thickness, nm
 5 AA, BB, CC - angle between ellipsoid a, b, c semi-axes and x, y, z axes,
       respectively, in degrees
 6
 7 MuM, MuC, MuSH - magnetic permittivity of medium, core and shell, respectively
 8 *)
 9 Ncore, Nmed, Nshell *)EpsC[Ncore_, MuC_] := Ncore^2/MuC;
10 EpsM[Nmed_, MuM_] := Nmed^2/ MuM;
11 EpsSH[Nshell_, MuSH_] := Nshell^2 /MuSH;
12
13 Intl1[a_, b_, c_] :=
14
         NIntegrate[
             1/((x + a^2) (x + a^2)^{0.5} (x + b^2)^{0.5} (x + c^2)^{0.5}), \{x, 0, \dots, n\}
15
                Infinity}];
16
17 Int12[a_, b_, c_] :=
         NIntegrate[
18
              19
                 Infinity}];
20
21 Int13[a_, b_, c_] :=
        NIntegrate[
22
23
               24
                  Infinity}];
25
26 L1[a_, b_, c_] := a b c Int11[a, b, c]/2;
27 L2[a_, b_, c_] := a b c Int12[a, b, c]/2;
28 L3[a_, b_, c_] := a b c Int13[a, b, c]/2;
29 Int21[a_, b_, c_, d_] :=
30
         NIntegrate[
31
               1/((x + (a +
                                   d)^2) (x + (a + d)^2)^0.5 (x + (b + d)^2)^0.5 (x + (c + d)^2)^0.
32
                                     d)^2)^0.5), {x, 0, Infinity}];
33
34 Int22[a_, b_, c_, d_] :=
35
        NIntegrate[
            1/((x + (b +
36
```

```
d)^2) (x + (a + d)^2)^{0.5} (x + (b + d)^2)^{0.5} (x + (c + d)^2
37
                                         d)^2)^0.5), {x, 0, Infinity}];
38
        Int23[a_, b_, c_, d_] :=
39
40
             NIntegrate[
41
                 1/((x + (c +
42
                                       d)^2) (x + (a + d)^2)^{0.5} (x + (b + d)^2)^{0.5} (x + (c + d)^2
43
                                         d)^2)^0.5), {x, 0, Infinity}];
44
      Lsh1[a_, b_, c_, d_] := (a + d) ( b + d) ( c + d) Int21[a, b, c, d]/2;
45
       Lsh2[a_, b_, c_, d_] := (a + d) ( b + d) ( c + d) Int22[a, b, c, d]/2;
46
       Lsh3[a_, b_, c_, d_] := (a + d) ( b + d) ( c + d) Int23[a, b, c, d]/2;
47
        f[a_, b_, c_, d_] := a b c/((a + d) ( b + d) ( c + d));
48
49
        Polariz1[Nmed_, Nshell_, Ncore_, MuM_, MuC_, MuSH_, a_, b_, c_,
50
                 d_{]} := ((a + d) (b + d) (c + d))/
51
52
                    3 ((EpsSH[Nshell, MuSH] -
                                 EpsM[Nmed, MuM]) (EpsSH[Nshell,
53
                                    MuSH] + (EpsC[Ncore, MuC] -
54
                                         EpsSH[Nshell, MuSH]) (L1[a, b, c] -
55
                                          f[a, b, c, d] Lsh1[a, b, c, d])) +
56
57
                         f[a, b, c, d] EpsSH[Nshell,
                              MuSH] (EpsC[Ncore, MuC] -
58
                                 EpsSH[Nshell, MuSH]))/((EpsSH[Nshell,
59
                                    MuSH] + (EpsC[Ncore, MuC] -
60
                                         EpsSH[Nshell, MuSH]) (L1[a, b, c] -
61
                                         f[a, b, c, d] Lsh1[a, b, c, d])) (EpsM[Nmed,
62
63
                                    MuM] + (EpsSH[Nshell, MuSH] - EpsM[Nmed, MuM]) Lsh1[a, b, c,
64
                                         d]) + f[a, b, c, d] Lsh1[a, b, c, d] EpsSH[Nshell,
65
                               MuSH] (EpsC[Ncore, MuC] - EpsSH[Nshell, MuSH]));
66
        Polariz2[Nmed_, Nshell_, Ncore_, MuM_, MuC_, MuSH_, a_, b_, c_,
67
                d_{]} := ((a + d) (b + d) (c + d))/
                    3 ((EpsSH[Nshell, MuSH] -
68
                                 EpsM[Nmed, MuM]) (EpsSH[Nshell,
69
                                    MuSH] + (EpsC[Ncore, MuC] -
70
                                         EpsSH[Nshell, MuSH]) (L2[a, b, c] -
71
                                          f[a, b, c, d] Lsh2[a, b, c, d])) +
72
                         f[a, b, c, d] EpsSH[Nshell,
73
74
                              MuSH] (EpsC[Ncore, MuC] -
                                 EpsSH[Nshell, MuSH]))/((EpsSH[Nshell,
75
                                    MuSH] + (EpsC[Ncore, MuC] -
76
                                         EpsSH[Nshell, MuSH]) (L2[a, b, c] -
77
78
                                          f[a, b, c, d] Lsh2[a, b, c, d])) (EpsM[Nmed,
79
                                    MuM] + (EpsSH[Nshell, MuSH] - EpsM[Nmed, MuM]) Lsh2[a, b, c,
80
                                          d]) + f[a, b, c, d] Lsh2[a, b, c, d] EpsSH[Nshell,
                              MuSH] (EpsC[Ncore, MuC] - EpsSH[Nshell, MuSH]));
81
        Polariz3[Nmed_, Nshell_, Ncore_, MuM_, MuC_, MuSH_, a_, b_, c_,
82
                 d_{]} := ((a + d) (b + d) (c + d))/
83
                    3 ((EpsSH[Nshell, MuSH] -
84
                                 EpsM[Nmed, MuM]) (EpsSH[Nshell,
85
                                    MuSH] + (EpsC[Ncore, MuC] -
86
                                          EpsSH[Nshell, MuSH]) (L3[a, b, c] -
87
                                          f[a, b, c, d] Lsh3[a, b, c, d])) +
88
                         f[a, b, c, d] EpsSH[Nshell,
89
                              MuSH] (EpsC[Ncore, MuC] -
90
                                 EpsSH[Nshell, MuSH]))/((EpsSH[Nshell,
91
                                    MuSH] + (EpsC[Ncore, MuC] -
92
93
                                         EpsSH[Nshell, MuSH]) (L3[a, b, c] -
                                         f[a, b, c, d] Lsh3[a, b, c, d])) (EpsM[Nmed,
94
                                   MuM] + (EpsSH[Nshell, MuSH] - EpsM[Nmed, MuM]) Lsh3[a, b, c,
95
```

```
d]) + f[a, b, c, d] Lsh3[a, b, c, d] EpsSH[Nshell,
96
            MuSH] (EpsC[Ncore, MuC] - EpsSH[Nshell, MuSH]));
97
   k[lambda_, Nmed_] := Nmed 2 Pi/lambda ;
98
99
   CosA[AA_, BB_, CC_] :=
100
101
     Cos[AA/360 2 Pi]/(Cos[AA/360 2 Pi]<sup>2</sup> + Cos[BB/360 2 Pi]<sup>2</sup> +
102
          Cos[CC/360 2 Pi]^2)^0.5;
103
    CosB[AA_, BB_, CC_] :=
      Cos[BB/360 2 Pi]/(Cos[AA/360 2 Pi]^2 + Cos[BB/360 2 Pi]^2 +
104
105
          Cos[CC/360 2 Pi]^2)^0.5;
106
   CosC[AA_, BB_, CC_] :=
      Cos[CC/360 2 Pi]/(Cos[AA/360 2 Pi]^2 + Cos[BB/360 2 Pi]^2 +
107
          Cos[CC/360 2 Pi]^2)^0.5;
108
109
1110
   Qsca[lambda_, Nmed_, Nshell_, Ncore_, MuM_, MuC_, MuSH_, a_, b_, c_,
111
       d_, AA_, BB_, CC_] :=
      8 Pi (k[lambda, Nmed])^4 (
112
         CosA[AA, BB, CC]^2 (
113
             Abs[Polariz1[Nmed, Nshell, Ncore, MuM, MuC, MuSH, a, b, c,
114
115
               d]])^2 +
116
          CosB[AA, BB,
             CC]^2 (Abs[
117
              Polariz2[Nmed, Nshell, Ncore, MuM, MuC, MuSH, a, b, c,
118
               d]])^2 +
119
          CosC[AA, BB,
120
             CC]^2 (Abs[
121
122
              Polariz3[Nmed, Nshell, Ncore, MuM, MuC, MuSH, a, b, c,
123
               d]])^2)/3 ;
124
    Qabs[lambda_, Nmed_, Nshell_, Ncore_, MuM_, MuC_, MuSH_, a_, b_, c_,
125
      d_, AA_, BB_, CC_] :=
      4 Pi k[lambda, Nmed] Im[
126
        CosA[AA, BB, CC]^2 Polariz1[Nmed, Nshell, Ncore, MuM, MuC, MuSH,
127
128
           a, b, c, d] +
         CosB[AA, BB, CC]^2 Polariz2[Nmed, Nshell, Ncore, MuM, MuC, MuSH,
129
130
           a, b, c, d] +
         CosC[AA, BB, CC]^2 Polariz3[Nmed, Nshell, Ncore, MuM, MuC, MuSH,
131
           a, b, c, d]];
132
```

B.2 Create manipulated plot

```
Manipulate[{
1
     If[tick == True, AA := 180/Pi ArcCos[1/3^0.5];
2
3
     BB := 180/Pi ArcCos[1/3^0.5]; CC := 180/Pi ArcCos[1/3^0.5];];
     If[tick2 == True, AA2 := 180/Pi ArcCos[1/3^0.5];
4
5
     BB2 := 180/Pi ArcCos[1/3^0.5]; CC2 := 180/Pi ArcCos[1/3^0.5]];
6
     Manipulate[
      ListLinePlot[{Transpose[{lambda,
7
          Qsca[lambda, Nmed, Nshell, Ncore, MuM, MuC, MuSH, a, b, c, d,
8
           AA, BB, CC]}],
9
10
        Transpose[{lambda,
          Qsca[lambda, Nmed2, Nshell2, Ncore2, MuM, MuC, MuSH, a2, b2,
11
           c2, d2, AA, BB, CC]}]},
12
13
       AxesLabel -> {"Wavelength, nm",
         "\!\(\*SubscriptBox[\(\[Sigma]\), \(sca\)]\), \
14
15 \!\(\*SuperscriptBox[\(nm\), \(2\)]\)"},
```

```
PlotStyle -> {{Red, Thick}, {Orange, Thick}},
16
       PlotRange -> {Ymin, Ymax},
17
       AxesOrigin \rightarrow {385, 0},
18
       PlotLabel -> "Scattering cross-section",
19
20
       BaseStyle -> {FontSize -> 14},
21
       ImageSize -> 450], "Control maximum and minimum of graph",
22
      {{Ymin, 0,
         "Axis \!\(\*SubscriptBox[\(\[Sigma]\), \(abs\)]\), Ymin"}, 0,
23
24
       100000, Appearance -> "Open"},
25
       {{Ymax, Full,
         "Axis \!\(\*SubscriptBox[\(\[Sigma]\), \(abs\)]\), Ymax"}, 0,
26
       100000, Appearance -> "Open"},
27
      ControlPlacement -> Bottom, ControlType -> Manipulator],
28
     Manipulate[
29
      ListLinePlot[{Transpose[{lambda,
30
31
          Qabs[lambda, Nmed, Nshell, Ncore, MuM, MuC, MuSH, a, b, c, d,
32
           AA, BB, CC]}],
33
        Transpose[{lambda,
          Qabs[lambda, Nmed2, Nshell2, Ncore2, MuM, MuC, MuSH, a2, b2,
34
           c2, d2, AA, BB, CC]}]},
35
36
       AxesLabel -> {"Wavelength, nm",
         "\!\(\*SubscriptBox[\(\[Sigma]\), \(abs\)]\), \
37
   \low  (\*SuperscriptBox[\(nm\), \(2\)]\)"},
38
       PlotStyle -> {{Red, Thick}, {Orange, Thick}},
39
       PlotRange -> {Ymin2, Ymax2},
40
       AxesOrigin -> {385, 0},
41
       PlotLabel -> "Absorption cross-section",
42
       BaseStyle -> {FontSize -> 14},
43
44
       ImageSize -> 450],
45
      "Control maximum and minimum of graph",
46
      {{Ymin2, 0,
         "Axis \!\(\*SubscriptBox[\(\[Sigma]\), \(sca\)]\), Ymin"}, {0,
47
        100000}, Appearance -> "Open"},
48
      {{Ymax2, Full,
49
         "Axis \!\(\*SubscriptBox[\(\[Sigma]\), \(sca\)]\), Ymax"}, {0,
50
        100000}, Appearance \rightarrow "Open"},
51
      ControlPlacement -> Bottom, ControlType -> Manipulator]},
52
    Delimiter,
53
    Style["Scattering and absorption cross-sections for elipsoid, \
54
  nonpolarized e/m wave is following z-direction", 14, Bold],
55
    Delimiter,
56
57
    Style["Control properties of the red graph", 13, Bold, Red],
58
    Style["Geometry of core and shell", 12, Bold],
    {{a, 40, "Semi-axis a, nm"}, 1, 500, Appearance -> "Open"},
59
    {{b, 40, "Semi-axis b, nm"}, 1, 500, Appearance -> "Open"},
60
    {{c, 40, "Semi-axis c, nm"}, 1, 500, Appearance -> "Open"},
61
    {{d, 3, "Shell thickness, nm"}, 0, 50, Appearance -> "Open"},
62
    Delimiter, Style["Magnetic permittivity", 12, Bold],
63
    {{MuM, 1, "Magnetic permittivity of medium"}, 0, 100},
64
    {{MuC, 1, "Magnetic permittivity of core"}, 0, 100},
65
    {{MuSH, 1, "Magnetic permittivity of shell"}, 0, 100},
66
    Delimiter, Style["Refractive indice of medium and shell", 12, Bold],
67
    {{Nmed, 1.33, "Medium refractive index"}, 1, 5, Appearance -> "Open"},
68
    {{Nmed, "Water", "Medium refractive index"}, {1.33 -> "Water",
69
      1.544 -> "Quartz", 1.00029 -> "Air"}},
70
    {{Nshell, Au, "Shell refractive index"}, 1, 5, Appearance -> "Open"},
71
    {{Nshell, Au, "Choose shell refractive index"}, {Au -> "Au",
72
      Fe203 ->
73
74
       "\!\(\*SubscriptBox[\(Fe\), \(2\)]\)\!\(\*SubscriptBox[\(0\), \(3\
```

```
75 \)]\)", Ag -> "Ag"}},
    Delimiter, Style["Core refractive index", 12, Bold],
76
     {{Ncore, 1.72, "Constant value for all wavelengths"}, 1, 5,
77
      Appearance -> "Open" },
78
79
     {{Ncore, 1.72, "Choose material of core"}, {Au -> "Au",
       Fe203 ->
80
81
        "\!\(\*SubscriptBox[\(Fe\), \(2\)]\)\!\(\*SubscriptBox[\(0\), \(3\
    \)]\)", Ag -> "Ag"}},
82
    Delimiter, Style["Elipsoid position accroding to z-axis", 12, Bold],
83
     {{AA, 180/Pi ArcCos[1/3^0.5],
84
       "According to axis X, \left( \left( \times SuperscriptBox[(() (), (0)]) \right), 0, \right)
85
86
      180},
     {{BB, 180/Pi ArcCos[1/3^0.5],
87
       "According to axis Y, \langle \langle x  uperscriptBox[\langle \langle \rangle \rangle, \langle 0 \rangle])"}, 0,
88
89
      180}.
90
     {{CC, 180/Pi ArcCos[1/3^0.5],
       "According to axis Z_{, ||(\times SuperscriptBox[(() ), (0)])"}, 0,
91
92
      180}.
     {{tick, True, "Average position"}, {True, False},
93
      ControlType -> Checkbox },
94
95
    Style["If chosen Average position, then the angles stay constant",
96
     10, Red],
    Delimiter,
97
    Style["Control the parameters of orange graph", 13, Bold, Orange],
98
     Style["Geometry of core and shell", 12, Bold],
99
100
     {{a2, 40, "Semi-axis a, nm"}, 1, 500, Appearance -> "Open"},
101
     {{b2, 40, "Semi-axis b, nm"}, 1, 500, Appearance -> "Open"},
102
     {{c2, 40, "Semi-axis c, nm"}, 1, 500, Appearance -> "Open"},
103
     {{d2, 5, "Shell thickness, nm"}, 0, 50, Appearance -> "Open"},
     Delimiter, Style["Magnetic permittivity", 12, Bold],
104
     {{MuM2, 1, "Magnetic permittivity of medium"}, 0, 100},
105
     {{MuC2, 1, "Magnetic permittivity of core"}, 0, 100},
106
     {{MuSH2, 1, "Magnetic permittivity of shell"}, 0, 100},
107
    Delimiter, Style["Refractive indice of medium and shell", 12, Bold],
108
    {{Nmed2, 1.33, "Medium refractive index"}, 1, 5,
109
     Appearance -> "Open" },
110
     {{Nmed2, 1.33, "Choose medium refractive index"}, {1.33 -> "Water",
111
       1.544 -> "Quartz", 1.00029 -> "Air"}},
112
     {{Nshell2, Au, "Shell refractive index"}, 1, 5, Appearance -> "Open"},
113
114
     {{Nshell, Au, "Choose shell refractive index"}, {Au -> "Au",
115
       Fe203 ->
116
        "\!\(\*SubscriptBox[\(Fe\), \(2\)]\)\!\(\*SubscriptBox[\(0\), \(3\
117
    \)]\)", Ag -> "Ag"}},
118
    Delimiter, Style["Core refractive index", 12, Bold],
    {{Ncore2, 1.72, "Constant value for all wavelengths"}, 1, 5,
119
      Appearance -> "Open" },
120
121
     {{Ncore2, 1.72, "Choose material of core"}, {Au -> "Au",
122
       Fe203 ->
123
        "\!\(\*SubscriptBox[\(Fe\), \(2\)]\)\!\(\*SubscriptBox[\(0\), \(3\
124
    \)]\)", Ag -> "Ag"}},
     Delimiter, Style["Elipsoid position accroding to z-axis", 12, Bold],
125
126
     {{AA2, 180/Pi ArcCos[1/3^0.5],
127
       "According to axis X, \langle \langle x \rangle
128
      180},
129
     {{BB2, 180/Pi ArcCos[1/3^0.5],
       "According to axis Y, \langle \cdot \rangle (\*SuperscriptBox[\(\\\ \), \(O\)]\)"}, 0,
130
131
     180},
132
     {{CC2, 180/Pi ArcCos[1/3^0.5],
133
       "According to axis Z_{, || (| \times SuperscriptBox[| (|| |), |(0|)]) "}, 0,
```

Appendix B. Wolfram Mathematica code

```
134 180},
135 {{tick2, True, "Average position"}, {True, False},
136 ControlType -> Checkbox },
137 Style["If chosen Average position, then the angles stay constant",
138 10, Red]]
```

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'Classic' - a book which people praise and do not read.' Mark Twain

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Résumé

Le travail de thèse est consacrée aux interactions spatio, temporo- et spectro- résolue de laser avec des tissus biologiques. L'objectif de cette thèse était d'étudier l'influence des nanoparticules colloïdales embarqués dans les tissus biologiques multicouches sur leurs propriétés optiques afin de fournir plus profond et / ou plus précise de sondage. Pour ce faire, les paramètres spectroscopiques intégrales et durée de vie de fluorophore dans les environs de nanoparticules métalliques ont été analysées théoriquement et experimentalement. L'autre partie de l'étude était de proposer de nouvelles solutions algorithmiques pour l'amélioration de la performance du processus d'estimation des valeurs des propriétés optiques de la résolution spatiale des mesures spectroscopiques. La dernière partie de la thèse est la modélisation expérimentale et théorique de la cinétique de fluorophores en présence des nanoparticules d'or colloïdales. La composante ultra-courte picoseconde (environ 100 ps) a été résolue et a été corrélée à champ dipolaire forte des nanoparticules qui compense le dipôle de la molécule.

Mots-clés: tissu épithélial, spectroscopie optique, nanoparticules, composante picosecond, fluorophore, probleme inverse

Abstract

The thesis work is devoted to spatially-, temporally- and spectrally- resolved laser and biological tissue interactions. The aim of the present thesis was to investigate the influence of colloidal nanoparticles embedded into multilayered biological tissues on their optical properties in order to provide deeper and/or more precise probing. To do so, the integral spectroscopic parameters and lifetime of fluorophore in vicinity of metal nanoparticles were analyzed theoretically and experimentally. Another part of the study was to propose new algorithmic solutions for improving the performance of the estimation process of the optical properties values from spatially resolved spectroscopic measurements. The last part of the thesis was the experimental and theoretical modeling of fluorophore's kinetics in presence of colloidal gold nanoparticles. The ultra-short picosecond component (around 100 ps) was resolved and correlated to strong nanoparticles dipole field which is compensating the molecule's dipole.

Keywords: epithelial tissue, optical spectroscopy, nanoparticles, picosecond component, fluorophore, inverse problem