Detecting nanoparticles in tissue using an optical iterative technique

Inbar Yariv,1 Gilad Rahamim,1 Elad Shliselberg,1 Hamootal Duadi,1 Anat Lipovsky1
Rachel Lubart,2 and Dror Fixler1,∗
1 Faculty of Engineering and the Institute of Nanotechnology and Advanced Materials, Bar Ilan University, Ramat Gan 5290002, Israel
2 Physics and Chemistry Department, Bar-Ilan University, Ramat-Gan 5290002, Israel
Dror.Fixler@biu.ac.il

Abstract: Determining the physical penetration depth of nanoparticles (NPs) into tissues is a challenge that many researchers have been facing in recent years. This paper presents a new noninvasive method for detecting NPs in tissue using an optical iterative technique based on the Gerchberg-Saxton (G-S) algorithm. At the end of this algorithm the reduced scattering coefficient (µs′), of a given substance, can be estimated from the standard deviation (STD) of the retrieved phase of the remitted light. Presented in this paper are the results of a tissue simulation which indicate a linear ratio between the STD and the scattering components. A linear ratio was also observed in the tissue-like phantoms and in ex vivo experiments with and without NPs (Gold nanorods and nano Methylene Blue). The proposed technique is the first step towards determining the physical penetration depth of NPs.

©2014 Optical Society of America

OCIS codes: (160.4236) Nanomaterial; (120.5820) Scattering measurements; (190.4710) Optical nonlinearities in organic materials.

References and links
1. R. W. Gerchberg and W. O. Saxton, “A practical algorithm for the determination of phase image and diffraction plane pictures,” Optik (Stuttg.) 35, 237–246 (1972).
2. W. F. Cheong, S. A. Prahl, and A. J. Welch, “A review of the optical properties of biological tissues,” IEEE J. Quantum Electron. 26(12), 2166–2185 (1990).
3. T. Lister, P. A. Wright, and P. H. Chappell, “Optical properties of human skin,” J. Biomed. Opt. 17(9), 090901 (2012).
4. S. A. Prahl, I. A. Vitkin, U. Bruggemann, B. C. Wilson, and R. R. Anderson, “Determination of optical properties of turbid media using pulsed photothermal radiometry,” Phys. Med. Biol. 37(6), 1203–1217 (1992).
5. J. W. Pickering, S. A. Prahl, N. van Wieringen, J. F. Beek, H. J. Sterenborg, and M. J. van Gemert, “Double-integrating-sphere system for measuring the optical properties of tissue,” Appl. Opt. 32(4), 399–410 (1993).
6. M. Johns, C. Giller, D. German, and H. Liu, “Determination of reduced scattering coefficient of biological tissue from a needle-like probe,” Opt. Express 13(13), 4828–4842 (2005).
7. A. Kim and B. C. Wilson, “Measurement of ex vivo and in vivo tissue optical properties: methods and theories,” in Optical-Thermal Response of Laser-Irradiated Tissue (Springer, ed. 2011).
8. H. Levy, D. Ringuette, and O. Levi, “Rapid monitoring of cerebral ischemia dynamics using laser-based optical imaging of blood oxygenation and flow,” Biomed. Opt. Express 3(4), 777–791 (2012).
9. I. Sigal, R. Gad, A. M. Caravaca-Aguirre, Y. Atchia, D. B. Conkey, R. Piestun, and O. Levi, “Laser speckle contrast imaging with extended depth of field for in-vivo tissue imaging,” Biomed. Opt. Express 5(1), 123–135 (2014).
10. J. R. Fienup, “Phase retrieval algorithms: a comparison,” Appl. Opt. 21(15), 2758–2769 (1982).
11. Z. Zalevsky, D. Mendlovic, and R. G. Dorsch, “Gerchberg-Saxton algorithm applied in the fractional Fourier or the Fresnel domain,” Opt. Lett. 21(12), 842–844 (1996).
12. D. Misell, “A method for the solution of the phase problem in electron microscopy,” J. Phys. D Appl. Phys. 6(1), L6 (1973).
13. R. Gerchberg, “Super-resolution through error energy reduction,” J. Mod. Opt. 21, 709–720 (1974).
14. D. Sazbon, Z. Zalevsky, and E. Rivlin, “Qualitative real-time range extraction for preplanned scene partitioning using laser beam coding,” Pattern Recognit. Lett. 26(11), 1772–1781 (2005).
15. E. Grossman, R. Tzioni, A. Gur, E. Gur, and Z. Zalevsky, “Optical through-turbulence imaging configuration: experimental validation,” Opt. Lett. 35(4), 453–455 (2010).
16. E. Gur, and Z. Zalevsky, “Image deblurring through static or time-varying random perturbation medium,” J. Electron. Imaging 18, 033016 (2009).
17. D. Mendlovic, Z. Zalevsky, and N. Konforti, “Computation considerations and fast algorithms for calculating the diffraction integral,” J. Mod. Opt. 44(2), 407–414 (1997).
18. H. Duadi, D. Fixler, and R. Popovtzer, “Dependence of light scattering profile in tissue on blood vessel diameter and distribution: a computer simulation study,” J. Biomed. Opt. 18(11), 111408 (2013).
19. H. Duadi, I. Fedor, and D. Fixler, “Linear dependency of full scattering profile isobaric point on tissue diameter,” J. Biomed. Opt. 19(2), 026007 (2014).
20. L. Wang, S. L. Jacques, and L. Zheng, “MCML–Monte Carlo modeling of light transport in multi-layered tissues,” Comput. Methods Programs Biomed. 47(2), 131–146 (1995).
21. A. Welch, M. J. van Gemert, W. M. Star, and B. C. Wilson, “Definitions and overview of tissue optics,” in Optical-thermal response of laser-irradiated tissue (Springer, 1995), pp. 15–46.
22. B. Nikoobakhht and M. A. El-Sayed, “Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method,” Chem. Mater. 15(10), 1957–1962 (2003).
23. R. Anki, H. Duadi, M. Motiei, and D. Fixler, “In-vivo Tumor detection using diffusion reflection measurements of targeted gold nanorods - a quantitative study,” J. Biophotonics 5(3), 263–273 (2012).
24. R. Ankri, V. Peretz, M. Motiei, R. Popovtzer, and D. Fixler, “A new method for cancer detection based on diffusion reflection measurements of targeted gold nanorods,” Int. J. Nanomedicine 7, 449–455 (2012).
25. D. Fixler and R. Ankri, “Subcutaneous gold nanorods detection with diffusion reflection measurement,” J. Biomed. Opt. 18(6), 061226 (2013).
26. J. S. Dam, C. B. Pedersen, T. Dalggaard, P. E. Fabricius, P. Aruna, and S. Andersson-Engels, “Fiber-optic probe for noninvasive real-time determination of tissue optical properties at multiple wavelengths,” Appl. Opt. 40(7), 1155–1164 (2001).
27. R. Cubeddu, A. Pifferi, P. Taroni, A. Torricelli, and G. Valentini, “A solid tissue phantom for photon migration studies,” Phys. Med. Biol. 42(10), 1971–1979 (1997).
28. S. T. Flock, S. L. Jacques, B. C. Wilson, W. M. Star, and M. J. van Gemert, “Optical properties of Intralipid: a phantom medium for light propagation studies,” Lasers Surg. Med. 12(5), 510–519 (1992).
29. S. L. Jacques, “Optical properties of biological tissues: a review,” Phys. Med. Biol. 58(11), R37–R61 (2013).
30. J. S. Maier, S. A. Walker, S. Fantini, M. A. Franceschini, and E. Gratton, “Possible correlation between blood glucose concentration and the reduced scattering coefficient of tissues in the near infrared,” Opt. Lett. 19(24), 2062–2064 (1994).
31. D. Fixler and Z. Zalevsky, “In vivo tumor detection using polarization and wavelength reflection characteristics of gold nanorods,” Nano Lett. 13(12), 6292–6296 (2013).
32. R. Ankri, D. Leshem-Lev, D. Fixler, R. Popovtzer, M. Motiei, R. Komowski, E. Hochhauser, and E. I. Lev, “Gold nanorods as absorption contrast agents for the noninvasive detection of arterial vascular disorders based on diffusion reflection measurements,” Nano Lett. 14(5), 2681–2687 (2014).

1. Introduction

The ability to infiltrate materials into human tissues and determine their physical penetration depth is a great challenge which many researchers are facing. In order to achieve this very distant goal, the initial step was the adding scattering and absorption components into tissues and detecting them by the proposed method. The detection of nanoparticles (NPs) within a tissue has a tremendous research and commercial potential which has not been fully utilized yet. This work combines a technique which is based on an optical image reconstruction method, the Gerchberg-Saxton (G-S) algorithm [1], with the synthesis of NPs from different materials in order to examine their identification within tissues. When light is projected and propagated through a tissue, it is affected by the tissue’s optical properties [2, 3]. Two of these properties are the scattering coefficient ($\mu_s$) and absorption coefficient ($\mu_a$). The scattering coefficient describes the change of light phase when it penetrates the tissue. The optical penetration depth of the light ($\delta$) depends on the anisotropy factor ($g$) and both absorption and scattering coefficient ($\mu_s$, $\mu_a$ respectively), as shown in Eq. (1):

$$\delta = \frac{1}{\sqrt{3\mu_a}} \cdot \frac{1}{\mu_a + \mu_s (1 - g)} = \frac{1}{\sqrt{3\mu_a}} \cdot \frac{1}{\mu_a + \mu_s'},$$

where $\mu_s'$ is the reduced scattering coefficient. Characterizing the optical properties of a tissue, and specifically the reduced scattering coefficient $\mu_s'$, by looking at the phase
distortions is nothing new and various techniques have been developed over the years [4–7] some of them based on laser speckles [8,9]. Each technique has its own advantages depending on the application. The technique for extracting $\mu_s'$ presented in this work is based on G-S algorithm [1]. The G-S algorithm is a well-known iterative method for phase retrieval [10] and beam shaping [11] for image reconstruction. This algorithm uses two known intensity planes in order to reconstruct the missing phase by propagating the light back and forth between the two planes. Since the original G-S algorithm, several modifications were made in [11]. For example, the two plane G-S algorithm described in [11–13] uses Fresnel Transform (FrT) instead of Fourier transform. Furthermore, a multiple plane (more then 2) method was suggested [14] in order to find the true phase and not a local minimum.

In this work we suggest using a multiple measurement G-S algorithm in order to extract the optical properties of an examined tissue sample that contains NPs. This data will be extracted from the standard deviation (STD) of the reconstructed phase given the thickness of the sample.

In order to estimate how deep NPs penetrate a tissue the change in the scattered light is measured. The technique presented here combines an experimental setup and iterative optical G-S algorithm for estimating the reduced scattering coefficient of a substance and detecting changes in the scattered light as a result of the added NPs.

The paper is constructed as follows: the algorithm for retrieving the reduced scattering coefficient based on the G-S algorithm is presented in section 2. Section 3 describes the experimental setup and the materials preparation. Section 4 presents the simulation and the experimental results for the extraction of the light phase’s STD. The discussion is described in section 5.

2. Theory

2.1 Multiple measurement G-S algorithm

An improvement for the G-S algorithm was suggested in [14–16] where the propagation of light back and forth between N planes instead of only 2 planes was used. In this research we used such a multiple measurement G-S algorithm to reconstruct the light phase created by the tissue. However we will not need this phase but rather its STD. After simulating the influence of the tissue thickness and optical properties on the STD we will be able to estimate the value of $\mu_s'$. Figure 1(a) presents the N planes used for the algorithm. Figure 1(b) presents a schematic sketch of G-S algorithm for N planes. The algorithm uses recorded image intensities $I_i$ at N planes ($P_1, P_2, ..., P_N$), as shown in Fig. 1(a), and an imposed initial random phase $\phi_i$ at the first plane $P_1$ in order to calculate the actual estimated phase at $P_f$. The relation between an intensity and the field’s amplitude in plane $i$ is $I_i = |A_i|^2$, where $A_i$ is the amplitude in plane $P_i$. 
The algorithm shown in Fig. 1(b) can be described as follows:

1. Set the input electric field to $P_i = A_i e^{i\phi_i}$.
2. For planes $j = 1...N-1$:
   2.1. Propagate the light from $P_i$ a distance of $d_i$ using the Fresnel free space propagation integral (FSP) [17] to the next plane, resulting in a new electrical field $\tilde{P}_{i+1} = \tilde{A}_{i+1} e^{i\phi_{i+1}}$.
   2.2. Impose the magnitude of this field to be $A_{i+1}$ (the measured amplitude at $P_{i+1}$) and keep the received phase $\phi_{i+1}$ results in $P_{i+1} = A_{i+1} e^{i\phi_{i+1}}$.
3. The field $P_N$ is then back free space propagated all the way to the first plane, a distance of $D$ (e.g., performing an inverse Fresnel transform) and together with the imposed magnitude $A_1$ resulting in $P_1 = A_1 e^{i\phi_1}$.
4. Impose the new random phase $\phi_1$ for the next iteration to be $\hat{\phi}_1$, the received phase in the last iteration.

After running $T$ iterations of the algorithm described at the stages above (1-4) the phase $\hat{\phi}_1$ at the last iteration is the received estimated phase.

### 2.2 Tissue calibration simulation

In order to understand the influence of the tissue’s optical properties on the STD of the light phase, a Matlab simulation was performed. The model is based on a more simplistic model [18, 19] where in an event of scattering the light direction is simply change by $\cos^{-1}(g)$. Free space assumes propagation in the $z$ direction, while direction changes are expressed in the phase of the electromagnetic field. While propagating a distance of $dz$, a change in the direction will create an added optical path of $dz/g$. In this simulation the tissue, which has a known thickness of $Z$ is adjacent to the light source from one side and to the detector from the other side, meaning the tissue thickness is also the distance between the light source and the...
detector. The tissue is divided to small slices (dz) and we assume that the light can only scatter in known positions which are separated by a distance dz where the light free space propagates [18, 19]. Since the pixel size of the detector is 6µm, dz had to be smaller than the pixel size, hence we arbitrarily chose dz = 5µm. In the planes where the light can scatter the probability \( p \) of each pixel (within a 1025 x 1025 matrix) to scatter was calculated as following according to [20, 21]:

\[
p = 1 - e^{-\mu_s dz}
\]  

(2)

If the light scatters in a specific pixel, the optical path is now \( dz/g \), where \( g \) is the anisotropy factor and defined as \( g = \cos\phi \). Hence the additional phase due to the scattering is:

\[
\frac{2(1/1)}{dz g} \pi / \lambda
\]

A condition which had to be considered is the relation between the optical penetration depth (\( \delta \)) and \( \mu_s' \) which is shown in Eq. (1). As \( \mu_s' \) of a tissue increases, the optical penetration depth of the light within it decreases down to a point where the light cannot penetrate the tissue any further. This results in restricted \( \mu_s' \) values that can be used for the scattering probability [Eq. (2)] in order to receive an accurate calibration.

Using these conditions and assumptions, the STD for different tissue thicknesses and different reduced scattering coefficients was calculated in order to create a look up table.

2.3 Reduced scattering coefficient reconstruction

Figure 2 described a schematic sketch of the proposed algorithm for \( \mu_s' \) extraction. First a multiple measurement G-S algorithm is used to reconstruct the phase \( \hat{\phi}_1 \) using N intensity images [as describes in section 2.1 and Fig. 1]. Then the STD of the received phase estimation \( \hat{\phi}_1 \) is calculated. Given the tissue thickness \( Z \), the estimated value for the reduced scattering coefficient is taken from a look up table (that built as described in section 2.2).

![Fig. 2. A schematic description of the algorithm for extracting \( \mu_s' \). After running T iterations of the algorithm shown in Fig. 1(b) the estimated phase \( \hat{\phi}_1 \) is retrieved. When the phase’s STD is calculated given the tissue thickness, \( Z \), the \( \mu_s' \) can be extracted from a look up table (that was built as described in section 2.2).](image)

This is only possible if the STD can be measured accurately enough in the first place. The accuracy of the STD depends on the number of iterations, \( T \). After preforming simulations and examining the effect of iterations number on the STD the ratio between \( T \) and number of planes, \( N \) can be described as follows [Eq. (3)]:

\[
T_{opt} = N^2
\]

(3)
where $T_{opt}$ is the optimal number of iterations and $N$ is the number of planes used in the algorithm.

3. Materials and methods

3.1 The experimental setup

A noninvasive optical technique was designed for light intensity measurements and reduced scattering coefficient extraction. The setup was composed of a Helium Neon gas laser with a wavelength of $\lambda = 633\text{nm}$ and power of 3.4mW, the sample, a lens with a focal length of $f = 50\text{mm}$ in order to focus the light beam and a CMOS camera (Firefly MV FMVU-03MTC, Point Grey, Canada) which was set on a micrometer plate (PT1 150-811ST, Thorlabs, Japan) for recording the light intensity image at different distances as shown in Fig. 3.

Fig. 3. The experimental setup for light intensity measurements and reduced scattering coefficient extraction. The distance between the laser and the sample, $l_1$, is 6cm. The distance between the sample and the camera, $l_2$, is 30cm. The camera records images at 7 planes along $l_3 = 1.524\text{cm}$ with equal intervals between them.

For each sample, the intensity images were recorded at 7 planes, where the distance between each two points was the same. The distance between the laser and the sample is $l_1 = 6\text{cm}$ and the distance between the sample and the camera is $l_2 = 30\text{cm}$. Those distances were chosen due to the following arguments: First, as we looked for the light phase's changes we wished for as many pixels as possible that will represent the detected light beam, so we will be able to observe these changes. Second, the distance between the sample and the lens had to answer the imaging condition. The images were recorded at 7 planes, starting at the plane closest to the lens and advancing 0.254cm to the next plane along $l_3 = 1.524\text{cm}$ as described in Fig. 3. The recorded intensity images (1025 x1025 pixels) were then processed by the algorithm, as explained in section 2, using MATLAB in order to receive the STD of the sample. The signal-to-noise ratio (SNR) was three orders of magnitude (more than 600).

3.2 Nanoparticles fabrication

Two different types of nanoparticles were used in this research: gold nanorods (GNRs) and Methylene Blue (MB) NPs. GNRs were synthesized using the seed mediated growth method [22]. Their size, shape, and uniformity were characterized using transmission electron microscopy (TEM) and the resultant shape was 25 nm X 65 nm, with narrow size distribution (10%) [23–25]. A solution of GNRs suspended in cetyltrimethylammonium bromide (CTAB) (SigmaAldrich, Usa) was centrifuged at 11,000 g for 10 min, decanted and resuspended in water to remove excess CTAB. The MB (SigmaAldrich, Usa) organic NPs (ONPs) were fabricated according to a known technique.

3.3 Liquid phantom preparation

Liquid phantoms with different reduced scattering coefficient were prepared in order to simulate tissues with different optical properties [26]. The phantoms were prepared using varying concentrations of IntraLipid (IL) (Lipofundin MCT/LCT 20%, B. Braun Melsungen AG, Germany) as a scattering component [27]. The reduced scattering coefficient of each phantom was calculated according to [27], where for a solution with 1% of IL the received $\mu'_s$ was 1.14mm$^{-1}$. Ten different phantoms were prepared in cylindrical vials (2ml, diameter of 7mm). The liquid phantoms together with the scattering components were added to a vial
(2ml and diameter of 0.7cm) which was placed on a holder in the same position through the experiments and was cleaned between experiments. The different IL concentrations and the reduced scattering coefficients are presented in Table 1.

| IL concentration [%] | Reduced scattering coefficient, $\mu_s'$ [mm$^{-1}$] | Scattering coefficient, $\mu_s$ [mm$^{-1}$] |
|----------------------|-----------------------------------------------|----------------------------------|
| 0.4                  | 0.46                                          | 2.29                             |
| 0.6                  | 0.69                                          | 3.43                             |
| 0.8                  | 0.91                                          | 4.57                             |
| 1                    | 1.14                                          | 5.71                             |
| 1.2                  | 1.37                                          | 6.89                             |
| 1.4                  | 1.59                                          | 7.99                             |
| 1.6                  | 1.83                                          | 9.14                             |
| 1.8                  | 2.06                                          | 10.28                            |
| 2                    | 2.29                                          | 11.43                            |
| 2.5                  | 2.86                                          | 14.28                            |

Since we measure the reemitted light we had to use values of $\mu_s'$ such that will result with optical penetration depth $\delta$ higher than the tissue thickness $Z$. From Eq. (1) we can extract the condition on $\mu_s'$ as shown in Eq. (4).

$$\mu_s' \leq \frac{1}{3 \mu_a} \cdot \frac{1}{z} - \mu_a \approx \frac{1}{3 \mu_a} \cdot \frac{1}{z}$$

(4)

The $\mu_a$ of liquid phantom is significantly lower than $\mu_s'$ and can vary between 0.0001-0.0015 mm$^{-1}$ [28]. Using these values we can estimate that for $Z = 0.7$cm the limitation is $\mu_s' < 4.5$mm$^{-1}$. This $\mu_s'$ value range has also been reported in human tissues in [29].

3.4 Skin preparation

After examining the effect of the ONPs on tissue-like phantoms, experiments with chicken skin were performed. First, the hairs on the chicken skin were removed then it was sliced to small pieces of 2x5cm with 0.5mm thickness. On each skin slice, 10µL of ONPs were applied on an area of 1x1cm on the external surface of the skin. The slices were laid down for 2 minutes so that the ONPs will be absorbed through the skin. The chicken skin slices were then stretched on a holder with the ONPs' area facing the laser. Each sample was tested in three different spots within the applied area, by moving them in different positions.

4. Results

4.1 Simulation results

Simulation of the light intensity measurements for different tissue lengths and reduced scattering coefficients were performed according to the description in paragraph 2.1 above. The simulation was carried out for a system with anisotropy factor of $g = 0.8$, 0.7cm tissue thickness and a distance of 0.254cm between the images. The STD of the light phase is shown in Fig. 4(a). The STD in Fig. 4(a) is presented for different optical lengths which were calculated from the tissue thickness and $\mu_s'$ according to [30].

The simulation results in Fig. 4(a) present the STD increase with relation to $\mu_s'$. As the substance is more scattering the STD reaches higher values and it has greater optical path. It can be observed from the black line in Fig. 4(a), where $\mu_s'$ equals zero, that the optical length is exactly the tissue thickness (0.7cm). Furthermore, the propagation of light in free space itself increases the STD, however this can be neglected comparing to the reduced scattering coefficient effect on the light's phase. Examination of how different scattering anisotropy ($g$) affects our method was performed, while the scattering coefficient was set to be 5mm$^{-1}$. The
results presented in Fig. 4(b) indicate the STD's sensitivity to the scattering anisotropy (g) hence all our experiments were performed for g = 0.8.

![Image](image_url)

Fig. 4. The STD obtained in the simulation, for (a) different μ’s (g = 0.8) as a function of the optical length and (as presented in the legends) with g = 0.8, (b) Different g (μs = 5mm⁻¹) as a function of the optical length (as presented in the legends). The simulation calibration was done for a system with a tissue thickness of 0.7cm, l₁ = 6cm, l₂ = 30cm and 7 recorded intensity images with equal intervals between them (0.254cm).

4.2 Liquid phantom calibration experiments

The STD of the retrieved phase of the reemitted light from ten different phantoms was measured using the experimental setup described in paragraph 2.2. Figure 5, presents the change in the STD as a result of the reduced scattering coefficient. The STD obtained in the simulation versus the reduced scattering coefficient is presented at Fig. 5 (red squares). It can be observed that the STD increases linearly with the increase of the reduced scattering coefficient. The experiment results in Fig. 5 (green triangles) present the STD for phantoms with different reduced scattering coefficients. The results indicate a linear ratio between the STD of the retrieved phase of the reemitted light and μ’s, as was expected from the simulation. For each sample, 3 repetitions were conducted with a small error of up to 2.4%. The STD for free space propagation was measured and found to be around 0.2 [a.u.]. The simulation, in comparison to the experiment, simulates a system where the total propagation distance is the tissue thickness whereas in the experiments the total propagation distance is much longer as described in 2.2 and 3.1 respectively. This is the reason for the difference in the dynamic range between the simulation and the experiments.
4.3 Gold nanorods

In order to examine the effect of NPs on the reemitted light phase, GNRs were added to the phantom solutions. Different GNR concentrations were added to the same IL concentration in order to investigate the effect of GNRs on the STD. The phantoms used for these experiments had a 1.4% IL concentration and an initial STD value of 0.23[a.u.]. The GNRs were added to the phantom at 6 different concentrations (1.5µM, 15µM, 30µM, 60µM, 75µM, 90µM). The STD versus the added GNRs concentration is presented in Fig. 6. The results indicate the effect of the GNRs on the STD. The GNRs within the phantom created a new scattering component in addition to the scattering phantom which results in the increase of the STD along with the GNR concentration. The experiment was conducted 3 times for each sample, and as in the liquid phantom experiments, we did not move the vial but rather cleaned it between experiments. The error observed in these experiments was up to 2.98%.

4.4 Methylene Blue

The effect of ONPs on the reemitted light phase was examined by adding MB to the phantom solutions. These experiments were done with a MB solution as control and with the MB ONPs and 3 repetitions for each sample where the vial was cleaned between the experiments. Different MB concentrations were added to the same IL concentration in order to examine the effect of MB ONPs on the STD. The MB was added to the phantom at 6 different concentrations (2µM, 4µM, 6µM, 8µM, 10µM, 12µM). The phantoms used for these experiments had 1% IL concentration and an initial STD of 0.212[a.u]. The experimental results that are presented at Fig. 7 show the STD value for the additional MB concentrations
for MB solution (blue rhombus) and for MB ONPs (orange triangle) with a small maximum error of 1.95%. The results present a linearly ratio between the STD and the MB concentrations. The additional MB concentrations add a scattering component to the already scattered phantoms, so that the scattering component increases along with the MB concentration which results in a higher STD. As was predicted in the simulation, the STD increases linearly with the increase of the scattering component. The change in the STD when using MB ONPs (orange triangles) can be easily observed from Fig. 7. Using MB ONPs created a greater change in the reemitted light phase in comparison to the MB solution.

![Graph showing STD vs MB concentration](image)

**Fig. 7.** The STD that was obtained for phantoms with different MB concentrations. The experiments were done for phantoms with 1% IL concentration, an initial STD value of 0.212 [a.u.] (red square) and different MB concentrations (2µM, 4µM, 6µM, 8µM, 10µM, 12µM). Blue rhombuses represent MB solution and orange triangles MB ONPs. The error observed in these experiments with three repetitions was up to 1.95%.

### 4.5 Ex vivo measurements

The next step after examining the effect of ONPs on the reemitted light phase in tissue-like phantoms is to test the new method on real tissues such as animal skin. The experiments were performed with MB solution as control and with MB ONPs, 3 repetitions for each sample. In order to examine the effect of MB intact and ONPs on the STD, 5 different MB concentrations (0.01mM, 0.1mM, 1mM, 5mM, 10mM) were applied on the skin samples. The skin used for these experiments had an initial STD value of 0.177 [a.u.]. Figure 8 presents the effect of the additional MB concentrations, as a solution (red squares) and ONPs (green triangles), on the STD with an error value less than 5%. It can be observed from the graph that the additional MB concentrations result with the STD linearly increase as was received in the simulation as well as in the phantoms experiments. However, most interesting is the change in the STD as a result of the MB ONPs in comparison to the MB solution. This change indicates that using MB ONPs causes a greater change in the STD of the reemitted light phase.
Fig. 8. The STD that was obtained for a 0.5mm thick chicken skin with different MB concentrations. The experiments were performed with different MB concentrations (0.01mM, 0.1mM, 1mM, 5mM, 10mM) and the initial STD value was 0.177[a.u.] (blue rhombus). Red squares represent MB solution and green triangles MB ONPs. The error observed in these experiments with three repetitions was up to 5%.

5. Discussion

In this study we presented a new optical technique based on an iterative algorithm which detects NPs in tissue based on phase changes of the reemitted light. This technique uses the multiple plane G-S algorithm in order to retrieve the phase of the light propagated through a tissue. Adding the NPs to the tissue-like phantoms created a new and higher scattering component which increased the scattering of the light meaning increasing the reemitted light phase's STD. A linear ratio between the scattering component and the STD was observed in the simulations as well as in the experiments.

Since we recorded the intensity images behind the sample, only thin samples were used. In order to measure thicker samples and higher scattering components we have to record images where the light beam has not yet been scattered, meaning recording images within the tissue or use light reflectance methods. The absorption coefficient, $\mu_a$, which describes the ability of a substance to absorb light, was not discussed in this work. As the technique presented here is based on the phase changes, the absorption coefficient effect was not investigated assuming it will affect the intensity of the recorded images but not the phase of the propagated light [18, 19]. When focusing on the phase changes, the reduced scattering coefficient is the significant component in the system. The new method will not only allow us to use higher scattering component but to detect NPs more accurately. Knowing how the light phase changes within the tissue can shed some light on the NPs physical penetration depth. This new technique is the first step toward the goal of determining the physical penetration depth of NPs within tissues.

Our proposed technique, in its current stage, can be applied for the assessment of NPs concentration in histological sections. At the end, this noninvasive optical technique can help find the physical penetration depth of NPs within a tissue without any invasive measurements due to the simple setup used here. Its simplicity and accuracy are major advantages in regards to extracting the reduced scattering coefficient of substances and, with some adjustments, the physical penetration depth of NPs in tissues. Finally, a combination of the suggested method with the diffusion reflection measurements, which have been conducted in [23–25, 31, 32], will constitute sufficient ground for in vivo measurements.