CREATION AND EVALUATION OF SOLID OPTICAL TISSUE PHANTOMS FOR BIO-MEDICAL OPTICS APPLICATIONS

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Zusammenfassung

Wegen ihrer Veträglichkeit und präzisen Ergebnissen gewinnen biooptische Methoden stetig an Bedeutung in der nichtinvasiven medizinischen Diagnostik und Therapie. Die Grundlage von Anwendungen wie Laser-Doppler-Flussmessungen und Untersuchungen zur Sauerstoffsättigung des Blutes bilden Messungen der optischen Parameter der betrachteten Gewebe. Variationen in diesen optischen Eigenschaften, namentlich dem Absorptionskoeffizienten μ_a, dem Streukoeffizienten μ_s, dem reduzierten Streukoeffizienten $\mu_s' = \mu_s (1 - g)$ und dem Anisotropiefaktor g, ermöglichen das frühzeitige Erkennen von Gewebeveränderungen. Zur Entwicklung und Standardisierung neuer Messgeräte eignet sich die Verwendung optischer Phantome. Dabei erhalten feste Modelle aufgrund ihrer längeren Haltbarkeit, Stabilität hinsichtlich äußeren Einflüssen und sicheren Handhabung den Vorzug gegenüber flüssigen Phantomen. Zudem ermöglichen sie die Herstellung mehrschichtiger Objekte, was die Nachbildung der Gewebe optimiert.

Die vorliegende Studie beinhaltet die Kreation solcher Modelle und deren Erprobung. Sie wurden auf Basis einer gelartigen Substanz (Agar) hergestellt. Als Absorber fungierte eine Mischung aus wasserfester Tinte und Aceton, die dem Agar beigegeben wurde. Streuende Eigenschaften wurden durch die Zugabe von Vasolipid, einer milchigen Flüssigkeit, erreicht. Agar wurden mittels Erhitzen auf 94 °C in deionisiertem Wasser gelöst. Während des Abkühlvorgangs wurden die benannten Stoffe beigemischt. Nach ca. zwei Stunden waren die Phantome fest und vollständig erkaltet.

Zur Bestimmung der optischen Eigenschaften wurden die Modelle mit einem Vibratom in Scheibchen von 0,2 bis 1,1 mm Dicke zerschnitten. Diese wurden in eine mit Wasser gefüllte Küvette gelegt, um nach dem Schneiden entstandene Oberflächenrauhigkeiten zu kompensieren, und von einem Laser durchstrahlt. Gemäß dem Gesetz von Lambert-Beer, $\mu_t = -\frac{1}{L}\ln\frac{P}{P_0}$, wurde aus dem Verhältnis von gemessener Leistung P beim Durchleuchten des Objekts und unabgeschwächter Leistung P_0 des Lasers ohne Objekt und der entsprechenden Schichtdicke L der Schwächungskoeffizient μ_t berechnet. Dieser wurde durch den Vergleich verschieden zusammengesetzter Phantome in μ_a- und μ_s-Anteile gesplittet.

Als Ergebnis der Untersuchung wurde eine Formel zur Herstellung fester optischer Phantome mit vorgegebenen optischen Eigenschaften entwickelt: $\mu_{t_{ph}} = 2500\,cm^{-1} \cdot c_{ink} + 3400\,cm^{-1} \cdot c_{vaso} + 0.91 cm^{-1}$.

Sie ist für Modelle mit einem 1,12 %igen Agar-Anteil gültig.

Abstract

Because of their compatibility and precise results bio-optical methods based on measurements of the optical tissue properties gain importance in non-invasive medical therapy and diagnostic. For development and standardization of medical devices optical phantoms are suitable. The present report handles the creation and evaluation of solid tissue phantoms, made up of Agar, Vasolipid and ink utilizing different mixture ratios. After cutting the models in slices of 0.2 to 1.1 mm thickness the absorption- and scattering coefficient were measured using a collimated laser beam setup. As result of the study a formula for the preparation of solid optical tissue phantoms with desired optical properties was found, that is valid for models containing 1.12 % Agar.

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List of abbreviations and terms

abs	- absorption
agarvaso	- Vasolipid in an Agar-phantom
c; conc	- volume concentration
compo	- phantom composition
constink	- phantoms containing a constant amount of ink
constlip	- phantoms containing a constant amount of Vasolipid
corrfact	- correction factor
eq.	- equation
ext	- extinction
fig.	- figure
ges	- total
green	- laser with wavelength $\lambda = 543$ nm used
h	- height
highconc	- high concentrations
homo	- homogeneity
inksol	- ink solution, consisting of acetone and ink in a mixture ratio of 2:1
L	- sample thickness
lip	- lipid, Vasolipid
liq	- liquid
metros	- Metylrosanilin
mix	- phantom with mixture of Agar, Vasolipid and ink
mod	- modified
μ_a*; mya;*	- absorption coefficient
μ_s*; mys;*	- scattering coefficient
μ_t*; myt;*	- total attenuation coefficient
μ_{ph}*; my_ph*	- attenuation coefficient for the present phantom
P_mess	- measured power of attenuated laser beam
P_null	- power of unattenuated laser beam
r	- radius
red	- laser with wavelength $\lambda = 633$ nm used
regcoeff	- regression coeffient
scat	- scattering
σ, stddev	- standard deviation
sol	- solution
vaso	- Vasolipid
vgl	- compare
V_ink(sol)	- used volume of ink or ink solution, respectively
V_lip	- used volume of Vasolipid
V_water	- used volume of water

1. Introduction

Medical imaging is an important field of diagnostics in modern medicine. As X-rays and Computer Tomography are harmful to the patients, bio-optical methods gain interest. Based on variations in the complex refractive index, a parameter that is more sensitive than that of X-ray- or Ultrasound applications, tissue abnormalities can be detected earlier, for instance. A change of the refractive index causes significant alteration in optical scattering [1]. Furthermore bio-optical techniques require exact knowledge about the optical properties of the tissue, namely the absorption coefficient μ_a, the scattering coefficient μ_s, the reduced scattering coefficient $\mu_s' = \mu_s (1 - g)$ and the anisotropy factor g, respectively [2]. To measure these properties the use of multilayered optical tissue phantoms is suitable as they mimic the tissue, e.g. skin, with good approximation. Therefore bio-optical applications like Laser Doppler- [3], spectroscopy- or microscopy procedures can easily be tested. The methods facilitate the examination of the skin and the layers below. Predictions of skin perfusion [4] or the blood oxygenation in the brain [5] can be taken, as examples. Additionally they mean a great improvement in cancer detection techniques [1; 5].

Using tissue mimicking phantoms has many advantages. Constraints of accessibility, storage of the fresh samples, property changes due to preparation, few identical specimens and therefore poor reproducibility are examples why actual tissue seems impractical [6]. Additionally the optical properties of the phantoms are known. The determination of the tissue optical properties is difficult as it has an inhomogeneous structure. Thus, optical phantoms get more relevant for evaluation of new diagnostic and therapeutic techniques and their calibration and standardization before clinical use [1]. Liquid phantoms are difficult to use because of the measuring procedure and storing. Additionally they lose or change their properties after a few days. On the contrary solid matters are highly reliable during the operation, show no sensitivity to temperature variations, are usually stable with time [3] and can be reproduced without changes in structure and properties. Furthermore they enable the creation of multilayer phantoms which in fact mimic the optical properties of biological tissue much better than unilayer ones as tissue can be regarded as a medium consisting of several layers.

Hence, solid phantoms were preferred in the present project which handles the production of these phantoms and appropriated measurement techniques to define their optical properties.

2. Materials and methods

2.1 Preparation of liquid phantoms

Before starting the preparation of the phantoms it is necessary to get experienced in working with the measurement setup and handling the substances that are to be utilized. Several publications concerning studies of Intralipid®, an intravenous nutrient containing 20% soy oil, show its scattering properties with negligible absorbance [7]. It was recently replaced by Vasolipid® modifying the ingredients a little. Therefore new measurements on the optical properties, namely the scattering coefficient μ_s were necessary.
As ink should be used as absorber due to almost no scattering [8] its absorption coefficient μ_a had to be determined as well.
The procedure of both measurements was similar. Different amounts of Vasolipid (Vasolipid 200mg/ml, B. Braun Medical AB, Bromma, Sweden) and ink (Parker Quink, black resp. blue, Parker Pen Products, Newhaven, England) were mixed with ordinary, distilled or deionised water {A}. The obtained solutions varied in the concentration of the added substances. Thus, their optical properties were diversified and the amount of transmitted light changed, accordingly. Following Beer's law the total attenuation coefficients $\mu_t = \mu_a + \mu_s$ were calculated by Eq. (1)

$$\mu_t = -\frac{1}{L}\ln\frac{P}{P_0} \tag{1}$$

where L is the sample thickness, P_0 is the measured power of the not attenuated beam and P is the power of the transmitted light. As ink can be regarded as an ideal absorber and Vasolipid as a perfect scatterer, μ_t reduces to μ_a for water-ink solutions and to μ_s for water-Vasolipid solutions.

2.2 Creation and handling of solid phantoms

Due to the above mentioned advantages this study has its focus on the creation of solid optical tissue phantoms. Agar (Difco™ Agar, granulated; Becton, Dickinson and Company, Sparks, USA) was chosen as basic substance of the phantoms. Beside its hardening qualities it is considered to have negligible absorption itself. Being made up of

an aqueous solution the final solid product may dry out when exposed to air. This process is eliminated or at least effective decelerated by storing the phantoms wrapped in plastic foil in a refrigerator. Different amounts of Vasolipid were used to vary the scattering coefficient of the phantoms.

A special waterproof ink (Artline xylene free marking ink, black, Shachihata Inc., Malaysia) acted as absorber. As it is not totally dissolvable in the Agar a pre-diluted batch solution was created. It consisted of acetone and ink with a mixture ratio of 2:1. To provide further confusion it is termed as ink from now on.

Deionised water was appropriate as a diluent for the named substances [9].

All ingredients are easy to handle and no special laboratory equipment is needed and the preparation is fast and unproblematic.

As the Agar phantoms consist of almost 99 % water their refracting index was expected to match that of water. This hypothesis could be confirmed by not further documented experiments where the refractive index of both substances was compared. Thus, the influence of surface throatiness after cutting can be eliminated, when putting the slices into water while illuminated with laser light during the measuring period. Furthermore there are no differences in the refractive indexes at solid-solid or solid-liquid interfaces. That submits the manufacture of multilayer phantoms [5]. Distilled or deionised water does not absorb or scatter any light. Therefore it causes no change in the measured properties μ_a and μ_s. It could also be used as an optical coupler between the slices of the multilayer phantom.

Following the descriptions in [5] the tissue phantoms were produced according to the recipe below.

Agar with added scatterer and absorber:

- mix 0.5 g Agar with 44.5 ml deionised water
- heat it up to 94˚C in a microwave oven
- stir it meanwhile so that all granulate solutes
- cool it down to 75˚C
- add the wanted amount of scattering and/or absorbing substance
- stir it up while cooling down to 40˚C
- the phantom is solid after ca. 2 hours

Measuring the optical properties of the phantoms requires cutting them into thin slices to obtain samples of different thickness. Therefore a Vibratome (Vibratome® Series 1000 Sectioning System, Technical Products International Inc., St. Louis, USA, input power: 100 VA, input voltage: 230 V~, frequence: 50/60 Hz) was utilized: a vibrating razor blade is moved forward under water and cuts thin layers off the fixed phantom. With this technique slices with thicknesses between 0.2 and 1.1 mm were created. A very careful handling of the slices is necessary to guarantee that no damages like scratches or ruptures are caused that would falsify the measurement results. The slices were stored on a glass plate wrapped in plastic foil to preserve them from draining.

2.3 Measurement

A typical collimated laser beam arrangement (Fig. 1) was utilized to measure the optical properties of the phantoms at a laser wavelength of λ = 633 nm, preferentially. For the liquid phantoms two different wavelengths were tested: λ = 543 nm and λ = 633 nm.

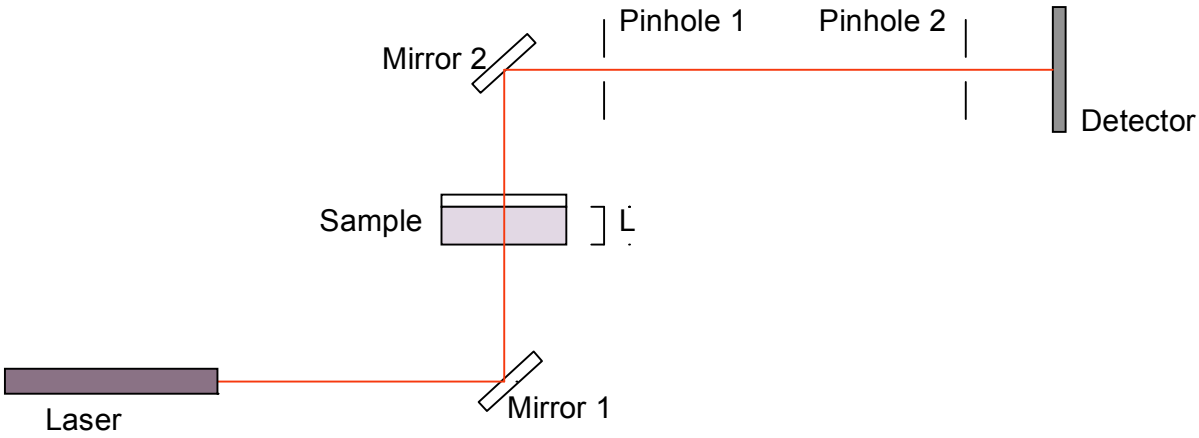

Fig. 1: Measurement setup

Two pinholes were inserted to confine that the scattered light of the transmitted beam only was collected in the forward direction. For the pre-processing measurements the liquid solutions were put into a polystyrene dish (Cell Culture Dish, treated, polystyrene, Corning Incorporated, USA). The slices of the created Agar phantoms were put into the same dish filled with deionised water. The water was used to compensate the surface roughness of the slices after cutting and thus to provide the transmitted light from being scattered at the surface.

2.4 Determination of the total attenuation coefficient μ_t

The power P of the transmitted light T was measured by a detector (Ophir Laserstar, Danvers, USA), (Eq. 2):

$$T = \frac{P}{P_0} = e^{\mu_t L} \tag{2}$$

where P_0 is the power of the not attenuated laser beam (Optlectra, model 1125, no. CL2897, HeNe gas laser head, 632.8 nm, randomly pol, TEMoo, min. 5 mW, Feldkirchen-Westerham, Germany), μ_t is the total attenuation coefficient and L is the sample thickness. After taking the natural logarithm of Eq. (2) it can be shown:

$$\ln(P) = -\mu_t L + \ln(P_0) \tag{3}$$

which is representing a linear equation $y = mx + n$. The optical properties of the phantom embedded in μ_t were calculated from the slope of the straight line that is approximated by inserting all measured pairs of variates L and P into this equation. Therefore the MATLAB®-function polyfit was used. Fig. 2 illustrates this procedure for Agar-ink-phantom {C: Agar_inksol_02.m}.

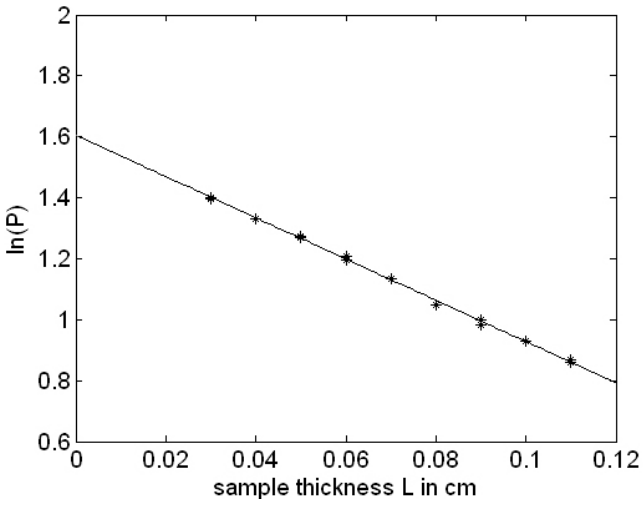

Fig. 2: Determination of μ_t, represented by the slope of this straight line

3. Results and conclusions

3.1 Liquid phantoms

The results of the measurements on the liquid phantoms are shown in appendix {A} and {C}. They were accomplished to determine a reference value for the μ_a and the μ_s of the used substances.

Comparing both measurements in A.1 and A.2 as well as A.4 with A.6 it is obvious that the values of the calculated attenuation coefficients vary significantly. The main reason might be the use of lower concentrations in the first examinations. A high standard deviation for these measurements affirms this surmise. As ordinary water was the solvent to create the solutions lots of additional particles were included that may falsify the results.

A second problem appeared due to different volumes of the solutions in the dish. In A.4 a high deviation from the mean value of the μ_a for low sample volumes is perceivable. Because of more adhesion between the solution and the sides of the dish the sample surface was not planar but appeared concave shaped. Therefore the sample thickness that has direct influence on the final result could not be calculated correctly. This effect also occurred in A.3. For a volume of 2 ml or 3 ml in the dish the measurement values differ much more than for the other volumes.

Two different laser wavelengths were applied. As expected, μ_a and μ_s are dependent on the used wavelength λ. The attenuation coefficients increase with decreasing laser wavelength for the present substances (Fig. 3).

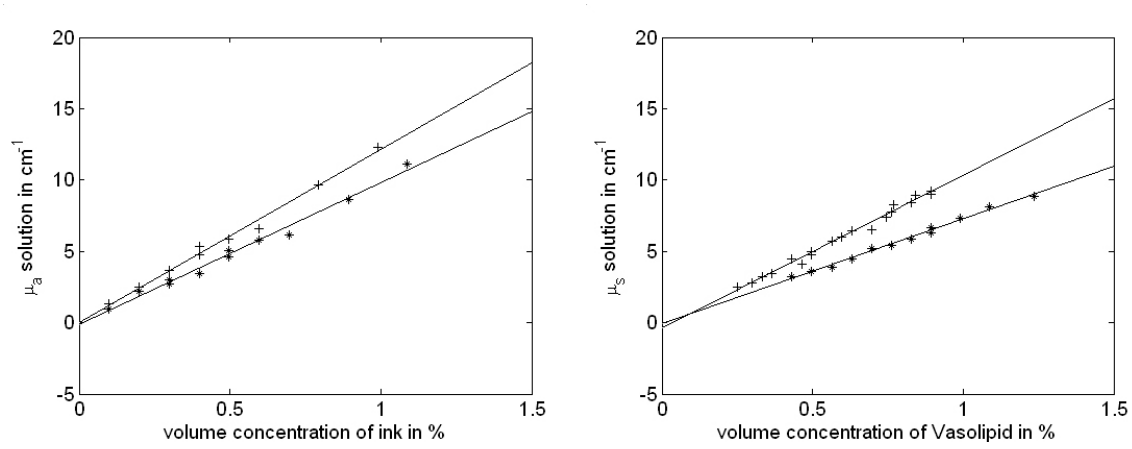

Fig. 3: μ_a of ink and μ_s of Vasolipid at different wavelengths, represented by the slopes of the graphs; (+: λ = 543 nm; *: λ = 633 nm)

3.2 Separation of μ_a and μ_s in solid phantoms

The main hypothesis of the project was that the optical properties, namely μ_a of the ink, μ_s of the Agar and μ_s of the Vasolipid within the phantom would agitate additionally.
First the scattering coefficient μ_s of the Agar was determined. This is identical with its total attenuation μ_t which is calculated as described above. That the absorption of the Agar is negligible is substantiated later in this report.
Thereafter the μ_t of the Agar-ink-phantoms was assigned analogous. As the same amount of Agar was used in all phantoms the value of μ_s for the pure Agar phantom was subtracted from the ink enriched phantom. The conceived $\mu_{a_{ink}} = \mu_{t_{ink,ph}} - \mu_{s_{agar}}$ was plotted in dependence on the volume concentration of the ink in the phantom. Comparing this graph with the appertaining plot of a water-ink mixture of the same concentration no significant difference in the slope of the graphs, which represents the μ_a of pure ink, was ascertainable (Fig. 4).

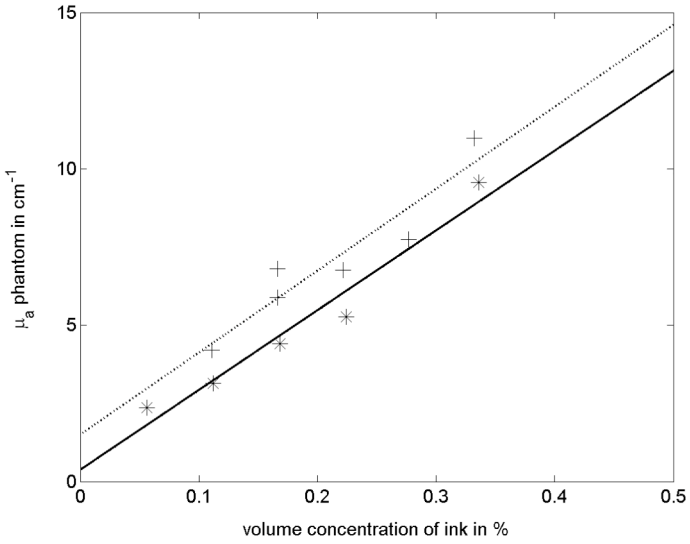

Fig. 4: μ_a of ink in an Agar-ink-phantom (+) and a water-ink-phantom (*), represented by the slopes of the graphs

Therefore it was concluded that the μ_a of the ink and the $\mu_s = \mu_t$ of the Agar based on their volume concentration act additionally to create the μ_t of the phantom according to Eq. (4)

$$\mu_{t_{ph}} = \mu_{a_{ink}} \cdot c_{ink} + \mu_{s_{agar}} \cdot c_{agar} \tag{4}$$

where the sub-subscript *ph* is the abbreviation for phantom and *c* for volume concentration, the latter one without unit. An equivalent comparison was accomplished for Agar-Vasolipid-phantoms and water-Vasolipid solutions. Evaluations do not confirm the hypothesis that $\mu_{s_{vaso}} = \mu_{t_{vaso,ph}} - \mu_{s_{agar}}$ as the value for the calculated μ_s for Vasolipid in water-Vasolipid mixtures rises from ca. 800 cm^{-1} up to ca. 3400 cm^{-1} for Vasolipid in Agar-Vasolipid-phantoms. This difference is obvious in the slopes of the graphs in Fig. 5.

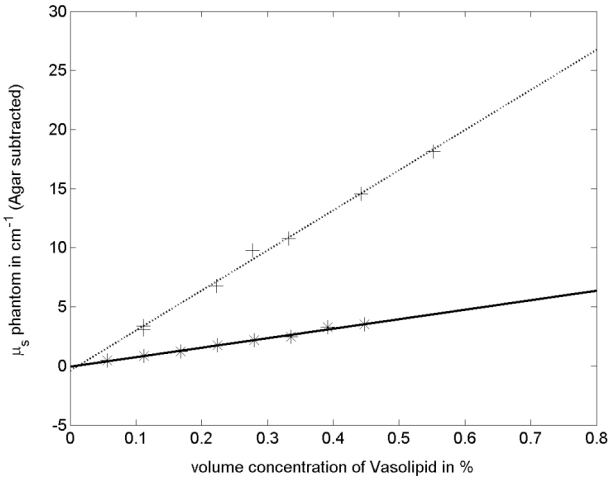

Fig.5: μ_s of Vasolipid in an Agar-Vasolipid-phantom (+) and a water-Vasolipid-phantom (*), represented by the slopes of the graphs

Therefore there does not exist any equation analogous to Eq. (4), what leads to the following conclusion (Eq. 5):

$$\mu_{t_{ph}} \neq \mu_{s_{vaso}} \cdot c_{vaso} + \mu_{s_{agar}} \cdot c_{agar} \tag{5}$$

A kind of chemical reaction with effect on the resulting particle size could be the reason for this phenomenon.

3.3 Final estimation of μ_a and μ_s for phantoms with Agar, ink and Vasolipid

According to the previous results in 3.2, especially Eq. (5), a modified hypothesis was established. The optical properties should follow Eq. (6)

$$\begin{aligned} \mu_{t_{ph}} &= \mu_{a_{ph}} + \mu_{s_{ph}} \\ &= \mu_{a_{ink}} \cdot c_{ink} + \mu_{s_{agarvaso}} \cdot c_{vaso} + \mu_{s_{agar}} \cdot c_{agar} \end{aligned} \tag{6}$$

where the sub-subscript ph stands for phantom, c for volume concentration, and $\mu_{s_{agarvaso}} \cdot c_{vaso}$ means the μ_s for Vasolipid in an Agar-Vasolipid-phantom. As the same amount of Agar was the base of all phantoms the last addend of the equation reduces to a constant value, concrete 0.91 cm^{-1}.

For all phantoms with a mix of all three substances the μ_t was determined applying Eq. (3). Then all pairs of variates L and P as well as the according concentrations were inserted into Eq. (6) and the best fitting values for $\mu_{a_{ink}}$ and $\mu_{s_{agarvaso}}$ were calculated from the attained system of equations. The concerning values match those of the phantoms that were made up only using Agar and ink respective Agar and Vasolipid.

3.4 Creating phantoms with known optical properties

As shown in chapter 3.3 all measurements confirm to the hypothetical Eq. (6). The results lead to a final proposal for a mixture ratio to prepare optically tissue-like phantoms with stated optical properties (Eq. 7). This equation is valid for phantoms containing 1.12 % Agar illuminated with laser light at a wavelength of 633 nm.

$$\mu_{t_{ph}} = 2500\,cm^{-1} \cdot c_{ink} + 3400\,cm^{-1} \cdot c_{vaso} + 0.91 cm^{-1} \tag{7}$$

The μ_a and μ_s of the phantoms are determined as to be seen in Eq. (8) and (9).

$$\mu_{a_{ph}} = 2500\,cm^{-1} \cdot c_{ink} \tag{8}$$

$$\mu_{s_{ph}} = 3400\,cm^{-1} \cdot c_{vaso} + 0.91 cm^{-1} \quad (9)$$

3.5 Verification of negligible absorption of the Agar

Comparing the results of the μ_s in {B.1} and the μ_a in {B.2}, the latter one calculated analogues [10], it is easily comprehensible that the absorption coefficient μ_a = 0.72 cm^{-1} of the Agar is much lower than its scattering coefficient μ_s = 109 cm^{-1}. As the μ_a represents only 0.66 % of the μ_s it can be neglected without appreciable error. A similar result should be found when the content of Agar, used to create the final phantoms, is decreased to 1.12 %.

3.6 Homogeneity

Several phantoms were tested according to their homogeneity. Therefore slices of the same thickness were cut out of the phantoms at diverse positions. The μ_t was determined as described earlier in this report. Fig. 6 represents the derivation of the μ_t for a phantom made up of Agar and Vasolipid {C: Agar_vaso_homo.m; B.10}.

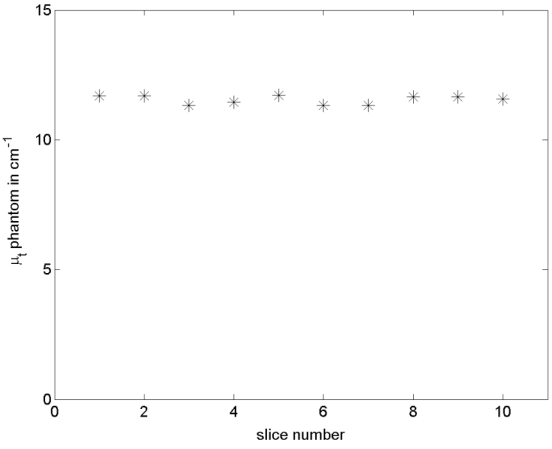

Fig. 6: Derivation of the μ_t in an Agar-Vasolipid-phantom

The standard deviation for the μ_t amounts between 1.4 % and 3.9 %. Thus, the created phantoms can be regarded as homogeneous independent of the cutting position inside the

phantom. The measurements in {B.9} present the same result as the calculated μ_s for matching phantoms fluctuates between 1.6 % resp. 2.2 %.
Homogeneity is an important requirement concerning the reproducibility of the measurements of the phantoms.

3.7 Applicative absorber

While cutting the ink-accumulated phantom into slices the ink (Parker Quink, black, Parker Pen Products, Newhaven, England) was appreciable washed out. The significant differences within a) and b) in {B.7} result from this effect. Therefore another absorbing substance had to be found.
First a second kind of ink (Artline stamp pad ink, black, Shachihata Inc., Malaysia) was tested. As it was more viscous it ought to stay in the mixture. This expectation could not be confirmed. Therefore the results in {C: agar_stamppadink.m} are not usable for further evaluations.
A scientific report [1] shows that dyes have an absorbing character. In a solution containing 0.5 g Sudan III, 25 ml 70% alcohol, 25 ml acetone, an intensive colouring appeared, but the dye did not solute completely. Usually the remaining particles are filtered out. However, after the filtration the concentration is changed to an unknown value. Preparing the phantoms requires exact knowledge of all concentrations to create the wanted optical properties. Therefore Sudan III is an ineligible absorber.
Another substance to be examined was Jodopax (Jodopax Hud & Sår 1%, Cederoth International AB, Upplands Väsby, Sweden). This dye was also washed out when putting the slice back into water as it was necessary for cutting it into slices. Additionally it also reduced its coloring while reheating. No further tests were done.
Trying Metylrosanilin (Märkbläck, metylrosanilin 2%, Apoteket, Umeå, Sweden) the same problem occurred. Being tested directly after cutting the μ_t of the phantom was determined to 4.58 cm^{-1}. Repeating measuring after having kept the slices 2.5 hours in water the value decreased to 3.90 cm^{-1}. After that time it seems to stay constant and the measured value of 3.98 cm^{-1} more than one week later confirms this {B.8.}. However, after that time the correct amount of absorber is not known which is necessary for the determination of the μ_a of the phantom.

Also Fenolrött (0.4% till LSU and 1-Naftoltalein 1%-lösning till LSU), was tested and showed a second disadvantage beside the previous one: the created phantoms were not homogeneous due to sedimentation. In this case it was renounced to cut and test the phantoms.

Following the idea that fat soluble absorbers could work some more experiments were realized. But fatty dyes like jojoba oil and oil paint were not practical as they did not solute in the Agar mixture, even when using special dissolver for the latter one. Powdery toner and graphite acted in the same way.

According to [5] a kind of waterproof ink (Artline xylene free marking ink, black, Shachihata Inc., Malaysia) was used to operate as absorber. Since it does not dissolve in water a pre-diluted batch solution was created, using alcohol. As it destructs the Agar substance it was replaced by acetone. That does not induce this effect, but to achieve good homogeneity of the phantom the amount of Agar had to be reduced. The final Agar concentration was set to 1.12 %.

4. Discussion and perspective

4.1 Measurement setup

The measuring aperture described in 2.3 and shown in Fig.1 is based on the technique of collimated transmission [11]. Placing the polystyrene dish with the liquid substance or the slices between the mirrors the power of the detected laser beam was dependent on the angular position of the dish. After marking the dish and taking the highest value presented by the detector, that error could be reduced to 5 %. However, large variations in the measurement and therefore large standard deviation up to 10-15 % for the calculated values could not be prevented.

The actual technique is furthermore not capable to differentiate between total forward scattered and unscattered light. The latter one is detected as well. This leads to an underestimation of the μ_s because the laser beam is less attenuated.

According to the mentioned observations and advisements the present setup is not totally satisfying due to its lacking accuracy and the utilisation of other measuring methods is wise. A solution might be the use of an integrating sphere [12] or the use of a laser beam with an oblique angle of incidence [13].

4.2 Proposals

As this study concentrates on the measurement of the absorption coefficient μ_a and the scattering coefficient μ_s it is advisable to continue with the determination of the residual optical properties, namely the reduced scattering coefficient $\mu_s' = \mu_s (1 - g)$ and the anisotropy factor g.
Additionally the wavelength dependence of the optical properties could be examined to gain knowledge about their behaviour within the whole spectrum of light.
Thereafter, the creation of multilayered phantoms could start. Undocumented tests during the actual project foresee an easy realization. It is possible to stack several slices in the water-filled dish. Experiments confirm that the μ_t sum up for slices out of the same phantom. Now it could be examined how slices of different phantoms with variable properties agitate.
As lots of earlier studies are based on simulated data, a comparison of the experimental results with theoretical determined values is suggested. Monte Carlo simulations are acknowledged to afford exact data and therefore dedicated for this purpose. Thus, it is possible to appraise the quality of the phantoms and the measuring method.

4.3 Applicability of the phantoms to mimic biological tissue

An optical phantom that shall mimic biological tissue should fulfil several requirements. It should match the geometry and optical parameters of the physical structures that are relevant for the transport of light. The important parameters should be reproducible and predictable from the sample composition. Storage and environmental changes should not influence the physical properties of the phantoms. Furthermore a construction of inhomogeneous samples by stacking phantom slabs should be possible. Not at least the sample preparation should be simple, quick, safe and inexpensive [9].
As described the present method is suitable to create solid optical phantoms with a desired μ_a and μ_s following Eq. (7). Therefore biological tissue, where these optical properties are known, can be mimicked by verifying the sample composition. In addition these parameters are reproducible since the homogeneity of the phantoms could be confirmed in chapter 3.6 and the preparation and measurement procedure was similar for all phantoms. Alteration of their properties can be provided by keeping the phantoms wrapped in plastic

foil in a refrigerator. First tests showed that the prepared samples could be suitable to construct multilayer phantoms by stacking several slices inside the dish using water as an optical coupler. Finally the utilized substances are not harmful to health and easy to supply. Following the mentioned recipe the creation of the phantoms is simple and rapid and the measurements can be realized by common labour equipment.

Since the presented phantoms satisfy all requirements above they should be applicable to mimic biological tissue.

Appendix A.: Protocols for liquid phantom measurements

1. Measurement of the absorption coefficient μ_a of ink depending on its concentration using a Laser of wavelength $\lambda = 633$ nm.

Date: a) 21.10.2004 b) 27.10.2004
Sample Volume: 6000 µl
Diameter dish: 34 mm
Laser Wavelength: $\lambda = 633$ nm
Background light: 0 W
Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 10s
Wavelength: $\lambda = 633$ nm

a)

V_water/ml	**V_ink/µl**	**P_0/mW**	**P/mW**
30	20	6.19	3.29
40	20	6.18	3.87
40	30	6.19	3.17
40	40	6.19	2.84
40	50	6.20	2.26
40	60	6.20	2.08
40	70	6.20	1.91
40	80	6.20	1.64
50	50	6.20	2.57
50	40	6.20	3.06
50	30	6.20	3.98
50	20	6.19	4.15
60	20	6.19	4.14
30	30	6.19	2.90
30	40	6.19	2.35
30	50	6.19	1.98
30	80	6.19	1.19
30	120	6.19	0.62
20	100	6.19	0.30
20	200	6.19	0.05

$\mu_a = 886\ cm^{-1}$ $\sigma = 11.49\ \%$
[C]: liq_abs_red.m

b)

V_water/ml	**V_ink/µl**	**P_0/mW**	**P/µW**
20	20	5.73	3110
20	40	5.78	1440
20	60	5.81	1020
20	80	5.83	650
20	100	5.76	300
20	120	5.77	141
10	30	5.78	845
10	50	5.78	235
10	70	5.79	112
10	90	5.79	23.40
10	110	5.79	4.76

$\mu_a = 966\ cm^{-1}$ $\sigma = 6.81\ \%$
[C]: liq_abs_red_highconc.m

2. Measurement of the absorption coefficient μ_a of ink depending on its concentration using a Laser of wavelength $\lambda = 543$ nm.

Date: a) 26.10.2004 b) 27.10.2004
Sample Volume: 4000 µl
Diameter dish: 34 mm
Laser Wavelength: $\lambda = 543$ nm
Background light: 0 W
Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 10s
Wavelength: $\lambda = 532$ nm

a)

V_water/ml	V_ink/µl	P_0/µW	P/µW
40	20	537	389
40	30	540	337
40	40	540	252
40	50	540	270
40	100	541	153
30	100	541	100
30	50	542	219
30	40	541	262
30	30	542	305
30	20	540	363
20	20	540	286
20	40	540	215
20	60	539	120
20	100	539	57
10	100	538	3.70
80	50	537	365
60	40	530	351
60	60	530	295
60	80	534	250
60	100	535	222

$\mu_a = 1343\ cm^{-1}$ $\sigma = 12.93\ \%$
[C]: liq_abs_green.m

b)

V_water/ml	V_ink/µl	P_0/µW	P/µW
20	20	539	306
20	40	540	189
20	60	541	114
20	80	542	73
20	100	541	45
10	100	539	3.03
10	80	536	9.23
10	60	536	33.30
10	40	532	56.10
10	20	536	213

$\mu_a = 1290\ cm^{-1}$ $\sigma = 6.77\ \%$
[C]: liq_abs_green_highconc.m

3. Measurement of the absorption coefficient μ_a of ink depending on the sample thickness.

Date: 25.10.2004

Diameter dish: 34 mm

Laser Wavelength: $\lambda = 633$ nm

Background light: 0 W

Detector settings: Filter: OUT

Power Range: AUTO

Average Over: 10s

Wavelength: $\lambda = 633$ nm

Volume concentrations:

Measurement-No.	**V_water/ml**	**V_ink/µl**	**Conc_ink/%**
1	200	20	0.01
2	30	20	0.0666
3	20	20	0.1
4	40	20	0.05

Measurements:

Meas.No.							
1	**V_sol/µl**	2000	3000	4000	5000	6000	7000
	P_0/mW	6.17	6.16	6.13	6.14	6.15	6.16
	P/mW	5.48	5.46	5.24	5.34	5.26	5.33
2	**V_sol/µl**	2000	3000	4000	5000	6000	7000
	P_0/mW	6.15	6.16	6.16	6.16	6.17	6.17
	P/mW	4.86	3.87	3.76	3.84	3.44	3.35
3	**V_sol/µl**	2000	3000	4000	5000	6000	7000
	P_0/mW	6.18	6.18	6.18	6.18	6.18	6.18
	P/mW	4.37	3.90	3.79	3.36	3.29	3.03
4	**V_sol/µl**	2000	3000	4000	5000	6000	7000
	P_0/mW	6.18	6.19	6.19	6.19	6.19	6.19
	P/mW	5.18	4.74	4.72	4.47	4.25	4.08

$\mu_a = 679\ cm^{-1}$ $\mu_a = 914\ cm^{-1}$

[C]: liq_abs_2.m

4. Measurement of the scattering coefficient μ_s of Vasolipid depending on its concentration using a Laser of wavelength $\lambda = 633$ nm.

Date: a) 26.10.2004 b) 27.10.2004

Sample Volume: 5000 µl

Diameter dish: 34 mm

Laser Wavelength: $\lambda = 633$ nm

Background light: 0 W

Detector settings:

Filter:	OUT
Power Range:	AUTO
Average Over:	10s
Wavelength:	$\lambda = 633$ nm

a)

V_water/ml	V_lip/µl	P_0/mW	P/µW
40	100	5.54	1561
30	100	5.54	1207
20	100	5.53	340
10	150	5.53	10.33
20	150	5.54	172.50
30	150	5.54	498
40	150	5.54	928
40	180	5.54	652
30	180	5.54	404
20	180	5.54	112.30
20	220	5.54	83
30	220	5.54	310
30	260	5.54	148
20	260	5.54	25.68
20	285	5.54	19.25
20	315	5.54	11.50
20	350	5.54	8.03
20	380	5.54	5.00

$\mu_s = 830\ cm^{-1}$ $\sigma = 11.25\ \%$

[C]: liq_scat_red.m

b)

V_water/ml	V_lip/µl	P_0/mW	P/µW
30	130	4.47	0.800
30	150	4.44	0.670
30	170	4.44	0.565
30	190	4.44	0.410
30	210	4.44	0.281
30	230	4.44	0.255
30	250	4.44	0.201
30	270	4.43	0.160
20	180	4.43	0.130
20	200	4.43	0.091
20	220	4.43	0.059
20	250	4.44	0.041

$\mu_s = 730\ cm^{-1}$ $\sigma = 2.99\ \%$

[C]: liq_scat_red_2.m

5. Measurement of the scattering coefficient μ_s of Vasolipid depending on its concentration using a Laser of wavelength $\lambda = 543$ nm.

Date: 28.10.2004
Sample Volume: 5000 µl
Diameter dish: 34 mm
Laser Wavelength: $\lambda = 543$ nm
Background light: 0 W
Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 10s
Wavelength: $\lambda = 532$ nm

V_water/ml	**V_lip/µl**	**P_0/µW**	**P/µW**
40	100	337	89.80
30	100	331	59.40
20	100	330	26.45
20	170	324	2.81
20	155	317	3.93
30	140	316	36.10
30	180	317	13.15
30	230	322	5.35
30	270	320	2.45
30	250	322	3.67
30	210	314	9.88
30	190	315	10.22
30	170	316	15.29
30	150	314	22.39
30	130	317	29.60
30	110	315	50.90
30	90	318	72
40	360	316	2.68
40	300	318	6.25

$\mu_s = 1000\ cm^{-1}$ $\sigma = 4.81\ \%$

[C]: liq_scat_green.m

6. Measurement of the scattering coefficient μ_s of Vasolipid depending on the sample thickness.

Date: 02.11.2004
Diameter dish: 34 mm
Lip-volume-frac.: 0.005
Laser Wavelength: λ = 633 nm
Background light: 0 W
Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 10s
Wavelength: λ = 633 nm

V_sol/µl	4000	4500	5000	5500	6000	6500	7000	7500
P_0/mW	4.56	4.56	4.55	4.54	4.53	4.52	4.53	4.50
P/mW	0.800	0.650	0.533	0.424	0.340	0.273	0.221	0.182

μ_s = 792 cm^{-1} σ = 0.92 %

[C]: liq_scat_red_length.m

7. Measurement of the absorption coefficient μ_a of blue ink depending on the sample thickness using a Laser of wavelength $\lambda = 633$ nm.

Date: 24.11.2004
Diameter dish: 34 mm
Ink-volume-fract.: 0.001
Laser Wavelength: $\lambda = 633$ nm
Background light: 0 W
Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 10s
Wavelength: $\lambda = 633$ nm

V_sol/µl	4500	5000	5500	6000	6500	7000
P_0/mW	5.93	5.93	5.93	5.93	5.93	5.93
P/mW	2.03	1.76	1.63	1.45	1.23	1.19

$\mu_a = 2210\ cm^{-1}$ $\sigma = 2.34\ \%$

[C]: liq_abs_blueink.m

8. Measurement of the absorption coefficient μ_a of a water-ink phantom with added pre-diluted ink solution at different concentrations.

Date: 07.02.2005

Laser Wavelength: $\lambda = 633$ nm

Background light: 0 W

Detector settings: Filter: OUT

Power Range: AUTO

Average Over: 3s

Wavelength: $\lambda = 633$ nm

Volume concentrations:

Measurement-No.	**V_water/ml**	**V_inksol/µl**	**Conc_ink/%**
1	44.5	25	0.056
2	44.5	50	0.112
3	44.5	75	0.168
4	44.5	100	0.224
5	44.5	150	0.336

Measurements:

Meas.-No.	1	2	3	4	5
V_sol/ µl ↓	**P/mW ↓**	**P/mW ↓**	**P/mW ↓**	**P/mW ↓**	**P/mW ↓**
4000	1.958	1.187	0.612	0.354	0.118
3700	2.107	1.318	0.701	0.416	0.172
3400	2.299	1.471	0.795	0.484	0.230
3400	2.293	1.422	0.788	0.501	0.223
3100	2.445	1.610	0.947	0.588	0.298

$\mu_a = 2550\ cm^{-1}$

[C]: liq_inksol.m

9. Measurement of the scattering coefficient μ_s of the water-Vasolipid phantom with added Vasolipid at different concentrations.

Date: 07.02.2005

Laser Wavelength: $\lambda = 633$ nm

Background light: 0 W

Detector settings: Filter: OUT

Power Range: AUTO

Average Over: 3s

Wavelength: $\lambda = 633$ nm

Volume concentrations:

Measurement-No.	**V_water/ml**	**V_lip/µl**	**Conc_lip/%**
1	44.5	25	0.056
2	44.5	50	0.112
3	44.5	75	0.168
4	44.5	100	0.224
5	44.5	125	0.280
6	44.5	150	0.336
7	44.5	175	0.392
8	44.5	200	0.447

Measurements:

Meas.No.	1	2	3	4	5	6	7	8
V_sol/µl↓	**P/mW↓**	**P/mW↓**	**P/mW↓**	**P/mW↓**	**P/mW↓**	**P/mW↓**	**P/mW↓**	**P/mW↓**
4000	3.120	2.757	2.461	2.044	1.748	1.473	1.128	0.992
5000	3.050	2.600	2.152	1.691	1.433	1.174	0.845	0.693
5000	3.030	2.609	2.139	1.663	1.415	1.182	0.817	0.701
6000	2.831	2.301	1.877	1.398	1.093	0.865	0.556	0.464

$\mu_s = 803\ cm^{-1}$

[C]: liq_vaso_mod.m

Appendix B.: Protocols for solid phantom measurements

1. Measurement of the scattering coefficient μ_s of the Agar-phantom using {1}.

Date: 28.10.2004
Phantom compo: 97 ml deionised water, 2 g Agar
Dish Volume: 5000 µl deionised water
Laser Wavelength: $\lambda = 633$ nm
Background light: 0 W
Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 3s
Wavelength: $\lambda = 633$ nm

L/cm	0.09	0.08	0.07	0.06	0.05	0.04	0.03
P/µW	4.15	4.28	4.36	4.45	4.56	4.67	4.73

μ_s_agar= 109 cm^{-1}
[C]: agar.m

2. Determination of the absorption coefficient μ_a of the Agar-phantom using {1}.

Date: 30.11.2004
Dish Volume: 4000 µl deionised water
Laser Wavelength: $\lambda = 633$ nm
Background light: 0 W
Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 3s
Wavelength: $\lambda = 633$ nm

μ_a_agar = 0.72 cm^{-1} $\sigma = 7.53$ %
[C]: agar_abs.m

3. Measurement of the scattering coefficient μ_s of the Agar-phantom with Agar concentration of final phantoms.

Date: a) 18.01.2005 b) 28.01.2005
c) 01.02.2005 d) 03.02.2005

Phantom compo: 44.5 ml deionised water, 0.5 g Agar

Dish Volume: 5000 µl deionised water

Laser Wavelength: $\lambda = 633$ nm

Background light: 0 W

Detector settings:
Filter: OUT
Power Range: AUTO
Average Over: 3s
Wavelength: $\lambda = 633$ nm

a) **P_0 = 4.86 mW**

L/cm	0.11	0.08	0.07	0.06	0.05	0.04	0.1
P/mW	4.45	4.58	4.63	4.68	4.72	4.75	4.48

μ_s_ph = 0.98 cm^{-1} μ_s_agar = 87.20 cm^{-1}

[C]: agar_01.m

b) **P_0 = 4.635 mW**

L/cm	0.1	0.09	0.07	0.05	0.04	0.03	0.05	0.06	0.07
P/mW	4.30	4.34	4.41	4.48	4.53	4.57	4.48	4.44	4.41

μ_s_ph = 0.85 cm^{-1} μ_s_agar = 75.90 cm^{-1}

[C]: agar_02b.m

c) **P_0 = 4.83 mW**

L/cm	0.11	0.1	0.09	0.08	0.03	0.04	0.05	0.06	0.07	0.1
P/mW	4.43	4.49	4.49	4.56	4.74	4.71	4.66	4.62	4.59	4.49

μ_s_ph = 0.82 cm^{-1} μ_s_agar = 73.05 cm^{-1}

[C]: agar_03b.m

d) **P_0 = 4.845 mW**

L/cm	0.08	0.06	0.05	0.04	0.06	0.07	0.08	0.09	0.1	0.11
P/mW	4.60	4.69	4.73	4.77	4.68	4.65	4.60	4.55	4.50	4.46

μ_s_ph = 0.97 cm^{-1} μ_s_agar = 86.55 cm^{-1}

[C]: agar_04.m

conclusion:

μ_s_ph = 0.91 cm^{-1} determined as mean value of μ_s_ph from a) to d)

μ_s_agar = 80.67 cm^{-1} determined as mean value of μ_s_agar from a) to d)

σ = 8.99 %

[C]: agar_end.m

4. Measurement of the absorption coefficient μ_a of the Agar-phantom with added pre-diluted ink solution at different concentrations.

Date: a) 18.01.2005 b), c), d) 19.01.2005
e), f) 28.01.2005

Phantom compo: 44.5 ml deionised water, 0.5 g Agar, ink:
a) 50 µl b) 100 µl c) 75 µl
d) 125 µl e) 75 µl f) 150 µl

Dish Volume: 5000 µl deionised water

Laser Wavelength: $\lambda = 633$ nm

Background light: 0 W

Detector settings:
Filter: OUT
Power Range: AUTO
Average Over: 3s
Wavelength: $\lambda = 633$ nm

a) **P_0 = 4.88 mW**

L/cm	0.11	0.09	0.08	0.06	0.05	0.03	0.04	0.05	0.06
P/mW	3.13	3.33	3.43	3.83	3.94	4.35	4.17	3.98	3.85

L/cm	0.07	0.08	0.09	0.1	0.11
P/mW	3.72	3.43	3.35	3.23	3.13

μ_a_ph = 3.29 cm^{-1}

[C]: agar_inksol_01.m

b) **P_0 = 4.945 mW**

L/cm	0.11	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03
P/mW	2.38	2.72	2.85	3.11	3.31	3.56	3.79	4.04	4.06

L/cm	0.04	0.05	0.06	0.07	0.09	0.1	0.11
P/mW	3.78	3.58	3.34	3.11	2.67	2.53	2.36

μ_a_ph = 5.86 cm^{-1}

[C]: agar_inksol_02.m

c) **P_0 = 4.80 mW**

L/cm	0.07	0.06	0.05	0.04	0.03	0.08	0.09	0.1	0.11
P/mW	2.95	3.17	3.35	3.66	3.92	2.73	2.61	2.43	2.26

μ_a_ph = 5.89 cm^{-1}

[C]: agar_inksol_03b.m

d) **P_0 = 4.85 mW**

L/cm	0.1	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04
P/mW	2.28	2.66	2.80	3.01	3.36	3.59	3.90	3.93	3.64

L/cm	0.05	0.06	0.07	0.07	0.1	0.11
P/mW	3.38	3.08	2.86	2.87	2.30	2.09

μ_a_ph = 6.84 cm^{-1}

[C]: agar_inksol_04.m

e) **P_0 = 4.47 mW**

L/cm	0.06	0.04	0.03	0.05	0.07	0.08	0.09	0.1	0.11
P/mW	3.23	3.67	3.85	3.42	3.09	2.85	2.72	2.55	2.42

μ_a_ph = 4.98 cm^{-1}

[C]: agar_inksol_05.m

f) **P_0 = 4.53 mW**

L/cm	0.1	0.08	0.07	0.06	0.05	0.03	0.04	0.06	0.07	0.09	0.1
P/mW	1.52	1.84	2.17	2.32	2.60	3.27	2.96	2.33	2.16	1.67	1.53

μ_a_ph = 10.08 cm^{-1}

[C]: agar_inksol_06b.m

conclusion: μ_a_ink = 2600 cm^{-1}

[C]: agar_inksol_conclusion.m

5. Measurement of the scattering coefficient μ_s of the Agar-phantom with added Vasolipid at different concentrations.

Date: a), c), d) 20.01.2005 e), f), g) 21.01.2005
b) 03.02.2005

Phantom compo: 44.5 ml deionised water, 0.5 g Agar, ink:
a) 50 µl b) 50 µl c) 100 µl d) 150 µl
e) 200 µl f) 250 µl g) 125 µl

Dish Volume: 5000 µl deionised water

Laser Wavelength: λ = 633 nm

Background light: 0 W

Detector settings:
Filter: OUT
Power Range: AUTO
Average Over: 3s
Wavelength: λ = 633 nm

a) **P_0 = 4.925 mW**

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.04
P/mW	3.68	3.78	3.81	4.07	4.16	4.24	4.38	4.50	4.40

L/cm	0.05	0.06	0.08	0.1	0.11
P/mW	4.29	4.14	3.95	3.62	3.51

μ_t_ph = 3.10 cm^{-1}

[C]: agar_vaso_01.m

b) **P_0 = 4.82 mW**

L/cm	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04
P/mW	3.54	3.66	3.80	3.97	4.10	4.22	4.38	4.35	4.23

L/cm	0.05	0.06	0.07	0.09	0.1	0.11
P/mW	4.07	3.94	3.82	3.56	3.47	3.35

μ_t_ph = 3.37 cm^{-1}

[C]: agar_vaso_01b.m

c) **P_0 = 4.895 mW**

L/cm	0.11	0.09	0.08	0.07	0.05	0.04	0.03	0.03	0.04	0.05	0.06	0.1	0.11
P/mW	2.30	2.63	2.89	3.06	3.48	3.68	3.96	3.99	3.72	3.48	3.25	2.50	2.29

μ_t_ph = 6.78 cm^{-1}

[C]: agar_vaso_02.m

d) **P_0 = 4.89 mW**

L/cm	0.1	0.09	0.08	0.06	0.05	0.04	0.03	0.05	0.06	0.07	0.09	0.1
P/mW	1.74	1.99	2.17	2.71	3.10	3.43	3.61	3.06	2.80	2.51	1.98	1.76

μ_t_ph = 10.79 cm^{-1}

[C]: agar_vaso_03.m

e) **P_0 = 4.74 mW**

L/cm	0.1	0.09	0.07	0.04	0.03	0.03	0.05	0.06	0.07	0.08	0.09	0.1
P/mW	1.07	1.20	1.61	2.56	2.93	2.91	2.06	1.82	1.58	1.35	1.20	1.05

μ_t_ph = 14.57 cm^{-1}

[C]: agar_vaso_04.m

f) **P_0 = 4.80 mW**

L/cm	0.09	0.08	0.07	0.06	0.05	0.03	0.04	0.05	0.06	0.1
P/mW	1.07	1.22	1.50	1.77	2.09	3.25	2.45	2.02	1.75	0.84

μ_t_ph = 18.15 cm^{-1}

[C]: agar_vaso_05.m

g) **P_0 = 4.76 mW**

L/cm	0.08	0.06	0.05	0.04	0.03	0.04	0.05	0.07	0.08	0.09	0.1	0.11
P/mW	2.28	2.63	3.01	3.23	3.60	3.28	3.02	2.45	2.25	1.98	1.84	1.62

μ_t_ph = 9.80 cm^{-1}

[C]: agar_vaso_06.m

conclusion: μ_s_agarvaso = 3400 cm^{-1}

[C]: agar_vaso_conclusion.m

6. Measurement of the μ_a and the μ_s in Agar-phantoms with added ink solution and Vasolipid at different concentrations.

Date: a), b) 25.01.2005 c), d), e) 26.01.2005
f), g) 28.01.2005 h), i), k), l) 01.02.2005

Phantom compo: 44.5 ml deionised water, 0.5 g Agar, ink, Vasolipid

Dish Volume: 5000 µl deionised water

Laser Wavelength: λ = 633 nm

Background light: 0 W

Detector settings: Filter: OUT
Power Range: AUTO
Average Over: 3s
Wavelength: λ = 633 nm

Meas.-No.	V_water/ml	V_inksol/µl	V_lip/µl	Conc_ink/%	Conc_lip/%
A	44.5	50	50	0.111	0.111
B	44.5	50	100	0.111	0.221
C	44.5	50	150	0.111	0.332
D	44.5	75	50	0.166	0.111
E	44.5	100	50	0.221	0.111
F	44.5	50	125	0.111	0.277
G	44.5	125	50	0.277	0.111
H	44.5	50	75	0.111	0.166
I	44.5	50	150	0.111	0.332
K	44.5	150	50	0.332	0.111
L	44.5	25	50	0.055	0.111

a) **P_0 = 4.715 mW**

L/cm	0.11	0.1	0.09	0.08	0.07	0.06	0.06	0.05	0.04	0.03	0.03	0.04
P/mW	2.29	2.54	2.76	2.87	3.06	3.29	3.28	3.45	3.68	3.83	3.88	3.67

L/cm	0.05	0.06	0.06	0.07	0.08	0.09	0.1	0.11
P/mW	3.45	3.23	3.22	3.03	2.85	2.66	2.50	2.28

μ_t_ph = 6.40 cm^{-1} [C]: agar_mix_01.m

b) **P_0 = 4.75 .. 4.94 mW**

L/cm	0.11	0.1	0.09	0.09	0.08	0.07	0.06	0.06	0.05	0.03	0.03	0.05
P/mW	1.32	1.64	1.93	1.85	2.12	2.36	2.73	2.81	3.05	3.53	3.81	3.08

L/cm	0.06	0.06	0.07	0.08	0.09	0.09	0.1	0.11
P/mW	2.71	2.70	2.36	2.16	1.96	1.92	1.67	1.34

μ_t_ph = 12.31 cm^{-1} [C]: agar_mix_02.m

c) **P_0 = 4.765 mW**

L/cm	0.11	0.1	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.03
P/mW	0.82	1.01	1.05	1.27	1.43	1.76	1.98	2.28	2.59	3.11	3.11	3.01

L/cm	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	0.1	0.11
P/mW	3.08	2.65	2.35	2.00	1.78	1.43	1.26	1.08	1.12	0.81

μ_t_ph = 15.71 cm^{-1} [C]: agar_mix_03.m

d) **P_0 = 4.705 mW**

L/cm	0.11	0.1	0.09	0.08	0.08	0.07	0.06	0.06	0.05	0.04	0.03	0.03
P/mW	1.42	1.71	1.93	2.07	2.04	2.34	2.55	2.57	2.88	3.15	3.52	3.54

L/cm	0.04	0.05	0.06	0.06	0.07	0.08	0.08	0.09	0.1	0.11
P/mW	3.15	2.93	2.68	2.67	2.45	2.12	2.15	1.96	1.80	1.47

μ_t_ph = 10.61 cm^{-1} [C]: agar_mix_04.m

e) **P_0 = 4.755 mW**

L/cm	0.11	0.1	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04
P/mW	1.46	1.68	1.73	1.92	2.10	2.35	2.42	2.86	3.28	3.46	3.55	3.34

L/cm	0.05	0.06	0.07	0.08	0.09	0.1	0.1	0.11
P/mW	2.82	2.50	2.32	2.09	1.91	1.72	1.71	1.49

μ_t_ph = 10.59 cm^{-1} [C]: agar_mix_05.m

f) **P_0 = 4.58 mW**

L/cm	0.11	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04	0.05
P/mW	0.91	1.10	1.30	1.51	1.80	2.00	2.27	2.65	3.11	3.18	2.83	2.42

L/cm	0.06	0.07	0.08	0.09	0.1	0.11
P/mW	1.97	1.85	1.50	1.35	1.18	0.91

μ_t_ph = 15.01 cm^{-1} [C]: agar_mix_06.m

g) **P_0 = 4.615 mW**

L/cm	0.11	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04	0.05
P/mW	1.08	1.33	1.49	1.63	1.98	2.20	2.52	2.80	3.10	3.20	2.83	2.50

L/cm	0.06	0.07	0.08	0.09	0.1	0.11
P/mW	2.18	1.98	1.66	1.45	1.32	1.06

μ_t_ph = 13.23 cm^{-1} [C]: agar_mix_07.m

h) **P_0 = 4.845 mW**

L/cm	0.11	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04	0.05
P/mW	1.69	1.86	2.06	2.20	2.59	2.71	3.12	3.44	3.74	3.75	3.47	3.18

L/cm	0.06	0.07	0.08	0.09	0.1	0.11
P/mW	2.77	2.61	2.26	2.01	1.87	1.68

μ_t_ph = 10.21 cm^{-1} [C]: agar_mix_08.m

i) **P_0 = 4.845 mW**

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04	0.05
P/mW	0.77	0.94	1.10	1.42	1.62	2.00	2.35	2.88	2.89	2.38	2.02

L/cm	0.06	0.07	0.08	0.09	0.1
P/mW	1.64	1.44	1.11	0.94	1.76

μ_t_ph = 18.85 cm^{-1} [C]: agar_mix_09.m

k) **P_0 = 4.775 mW**

L/cm	0.11	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04	0.05
P/mW	0.66	0.82	0.99	1.20	1.42	1.68	2.07	2.44	2.90	2.84	2.45	2.08

L/cm	0.06	0.07	0.08	0.09	0.1	0.11
P/mW	1.70	1.42	1.19	1.00	0.83	0.66

μ_t_ph = 18.26 cm^{-1} [C]: agar_mix_10.m

l) **P_0 = 4.74 mW**

L/cm	0.11	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.03	0.04	0.05
P/mW	2.76	2.84	3.05	3.19	3.37	3.48	3.66	4.00	4.09	4.18	3.94	3.72

L/cm	0.06	0.07	0.1	0.11
P/mW	3.52	3.39	2.88	2.70

μ_t_ph = 5.21 cm^{-1} [C]: agar_mix_11.m

conclusion: μ_s_agarvaso = 3750 cm^{-1}

σ = 18.9 %

[C]: agar_mix_vasovgl_a.m

μ_a_ink = 2650 cm^{-1}

σ = 45 %

[C]: agar_mix_inksolvgl.m

7. Measurement of the absorption coefficient μ_a of the Agar-phantom with added ink at different concentrations for two samples each (a, b).

Date: a), b) 30.11.2004 c) 24.11.2004

Phantom compo: 97 ml water, 2g Agar,

a) 300 µl blue ink

b) 500 µl blue ink

c) 100 µl blue ink

Dish Volume: 4000 µl deionised water

Laser Wavelength: λ = 633 nm

Background light: 0 W

Detector settings: Filter: OUT

Power Range: AUTO

Average Over: 3s

Wavelength: λ = 633 nm

a1) **P_0 = 4.98 mW**

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02
P/mW	3.33	3.53	3.60	3.80	3.85	3.95	4.15	4.50	4.69

μ_t_ph = 4.04 cm^{-1}

[C]: agar_ink_2a.m

a2) **P_0 = 4.83 mW**

L/cm	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02
P/mW	3.28	3.60	3.64	4.00	4.14	4.44	4.52	4.98

μ_t_ph = 5.59 cm^{-1}

[C]: agar_ink_2b.m

b1) **P_0 = 4.85 mW**

L/cm	0.07	0.06	0.05	0.04	0.03	0.02
P/mW	3.36	3.84	3.94	4.36	4.50	4.89

μ_t_ph = 7.01 cm^{-1}

[C]: agar_ink_3a.m

b2) **P_0 = 4,85 mW**

L/cm	0.1	0.09	0.08	0.07	0.05	0.04	0.03
P/mW	2.35	2.57	2.79	2.98	3.69	3.95	4.14

μ_t_ph = 8.40 cm^{-1}

[C]: agar_ink_3b.m

c) **P_0 = 4.95...5.10 mW**

L/cm	0.09	0.08	0.06	0.05	0.04	0.03
P/mW	3.97	4.20	4.38	4.58	4.79	5.01

μ_t = 3.67 cm^{-1}

[C]: agar_ink_1_mod.m

8. Measurement of the absorption coefficient μ_a of the Agar-phantom with added Metylrosanilin.

Date: a), b) 10.12.2004 c) 20.12.2004

Phantom compo: 97 ml water, 2g Agar, 3 drops Metylrosanilin

a) measured directly after cutting

b) measured after 2.5 hours kept in deionised water

c) measured after more than one week

Dish Volume: 5000 µl deionised water

Laser Wavelength: $\lambda = 633$ nm

Background light: 0 W

Detector settings:

Filter:	OUT
Power Range:	AUTO
Average Over:	3s
Wavelength:	$\lambda = 633$ nm

a)

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02
P/mW	3.08	3.29	3.53	3.65	3.78	3.96	4.11	4.30	4.55

μ_t_ph = 4.58 cm^{-1}

[C]: agar_metros_1.m

b)

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02
P/mW	3.28	3.32	3.58	3.72	3.92	4.05	4.16	4.28	4.42

μ_t_ph = 3.90 cm^{-1}

[C]: agar_metros_2.m

c)

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04
P/mW	3.60	3.61	3.73	4.11	4.27	4.30	4.44

μ_t_ph = 3.98 cm^{-1}

[C]: agar_metros_3.m

9. Measurement of the scattering coefficient μ_s of the Agar-phantom with added Vasolipid at different concentrations for two samples each.

Date: 30.11.2004

Phantom compo: 97 ml water, 2 g Agar,

a) 1000 µl Vasolipid

b) 500 µl Vasolipid

Dish Volume: 5000 µl deionised water

Laser Wavelength: λ = 633 nm

Background light: 0 W

Detector settings: Filter: OUT

Power Range: AUTO

Average Over: 3s

Wavelength: λ = 633 nm

a1) **P_0 = 5.35 mW**

L/cm	0.1	0.07	0.06	0.05	0.04	0.03	0.02
P/mW	0.66	1.36	1.62	1.83	2.27	2.58	2.97

μ_s_ph = 18.55 cm^{-1}

[C]: agar_vaso_2a.m

a2) **P_0 = 5.35 mW**

L/cm	0.08	0.07	0.06	0.05	0.04	0.03	0.02
P/mW	1.10	1.24	1.48	1.73	2.14	2.75	3.11

μ_s_ph = 18.14 cm^{-1}

[C]: agar_vaso_2b.m

comparison of a1) and a2): mean (μ_s_ph) = 18.345 cm^{-1} σ = 1.6 %

b1) **P_0 = 5.28 mW**

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03
P/mW	1.91	2.10	2.27	2.59	2.80	3.00	3.26	3.50

μ_s_ph = 8.75 cm^{-1}

[C]: agar_vaso_3a.m

b2) **P_0 = 5.28 mW**

L/cm	0.1	0.09	0.08	0.07	0.06	0.05	0.04
P/mW	1.90	2.14	2.29	2.60	2.84	2.98	3.13

μ_s_ph = 8.48 cm^{-1}

[C]: agar_vaso_3b.m

comparison of b1) and b2): mean (μ_s_ph) = 8.615 cm^{-1} σ = 2.2 %

10. Measurement of the homogeneity of the Agar-phantom with added Vasolipid, ink or both substances.

Date: a) 03.12.2004 b), c) 13.01.2005

Phantom compo: a) 97 ml deionised water, 2 g Agar, 500 µl Vasolipid

b) 48.5 ml deionised water, 0.5 g Agar, undef. amount of ink

c) 37 ml deionised water, 0.5 g Agar, 100 µl Vasolipid, undefined amount of ink

Dish Volume: 5000 µl deionised water

Sample thickness: a) L = 0.7 cm b), c) L = 0.8 cm

Laser Wavelength: λ = 633 nm

Background light: 0 W

Detector settings: Filter: OUT

Power Range: AUTO

Average Over: 3s

Wavelength: λ = 633 nm

a)

P_0/mW	5.87	5.87	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.89
P/mW	2.59	2.59	2.66	2.64	2.59	2.66	2.66	2.60	2.60	2.62

μ_s_ph = 11.54 cm^{-1} σ = 1.42 % [C]: agar_vaso_homo.m

b)

P_0/mW	4.90	4.90	4.90	4.90	4.90	4.90	4.90	4.90	4.90
P/mW	4.31	4.29	4.32	4.29	4.29	4.28	4.31	4.28	4.33

μ_s_ph = 1.63 cm^{-1} σ = 3.21 % [C]: agar_inksol_homo.m

c)

P_0/mW	4.83	4.83	4.83	4.83	4.83	4.83	4.83
P/mW	1.98	1.99	1.89	2.03	1.85	1.95	1.86

μ_s_ph = 11.44 cm^{-1} σ = 3.94 % [C]: agar_mix_homo.m

Used substances:

- Ink: Parker Quink, black, Parker Pen Products, Newhaven, England
  Parker Quink, blue, Parker Pen Products, Newhaven, England
  Artline stamp pad ink, black, Shachihata Inc., Malaysia
  Artline xylene free marking ink, black, Shachihata Inc., Malaysia
- Märkbläck, metylrosanilin 2%, Apoteket, Umeå, Sweden
- Agar: Bacto™ Agar, Becton Dickinson Microbiology Systems, Sparks, USA {1}
  Difco™ Agar, granulated; Becton, Dickinson and Company, Sparks, USA
- Vasolipid 200mg/ml, B. Braun Medical AB, Bromma, Sweden
- Dye: Sudan III, Alcohol 99,5%, Aceton
- Jodopax Hud & Sår 1%, Cederoth International AB, Upplands Väsby, Sweden
- Aceton, Gripen, SC Johnson Scandinavia, Kista, Sweden
- Absolut Finsprit 99,5% (Ethanolum), Kemetyl AB, Haninge, Sweden
- Jojoba Oil
- Graphite
- Oil paint

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