REVIEW ARTICLE

Intralipid-Based Phantoms for the Development of New Optical Diagnostic Techniques

Maria Lepore[1] and Ines Delfino[2,*]

[1]*Department of Experimental Medicine, University of Campania - 'Luigi Vanvitelli', Napoli, Italy*
[2]*Department of Biological and Ecological Sciences, Tuscia University, Viterbo, Italy*

Abstract: Intralipid is a material widely employed for the preparation of phantoms for optical imaging and biophotonics applications in medical field. The development of new optical diagnostic equipment in these fields requires the use of well-designed phantoms with optical properties (including scattering and absorption) mimicking those of biological tissues in all the pre-clinical stages of investigations. For this reason, great research effort has been devoted to optically characterize Intralipid and at preparing optimal phantoms. In this short review, we summarize the principal physico-chemical characteristics of Intralipid and the main contributions in the assessment of its scattering and absorption properties. In addition, the most largely used Intralipid-based homogeneous and non-homogeneous phantoms are discussed. Even though other materials are available for the preparation of phantoms, the use of Intralipid still offers an inexpensive and easy-to-use method for preparing phantoms with finely tuned optical properties.

1. INTRODUCTION

In the last years, the popularity of optical techniques as powerful methods for studies in the biomedical field have been continuously increasing [1 - 9]. Among the other outcomes, biophotonics research has made new approaches available for the development of innovative diagnostic tools [9]. In particular, time-resolved Near-Infrared (NIR) spectroscopy has been demonstrated to be particularly appealing [10 - 13] for realizing imaging systems aimed at investigating human tissues and bodies, for diagnostic and therapeutic purposes. By using various geometries and experimental set-ups, the propagation of light in opaque media was used to design new approaches and devices enabling to image real opaque systems, such as human tissues and parts of the human body (see Supplementary Materials for a short description of the basic principles of light propagation-related techniques and theoretical descriptions) [14, 15]. The development and assessment of new optical diagnostic equipment require the use of well-designed tissue phantoms in all the pre-clinical stages of investigations [16]. For obtaining phantoms with optical properties mimicking those of biological tissues, Intralipid is largely employed. Intralipid indicates an aqueous suspension of lipid droplets that is sterile and suitable for intravenous feeding of patients and

drug delivery [17]. It is also used also as an antidote against anesthetic toxicity and various toxins [18] and as a cardio-protective agent [19].

In the last years, other materials have been adopted for preparing phantoms for reference and calibration tests of optical diagnostic tools and instruments [20, 21], but Intralipid is still an interesting choice because it is easy to handle and has a low price. For these reasons, we believe that it can be interesting to revise the main examples of Intralipid phantoms reported in the literature and the procedures to realize them also with the aim of stimulating new biotechnological research in the field.

In order to give a general but not exhaustive overview of the topic, in this review, we will focus on different phantoms prepared with Intralipid and other additional components usefully employed in preclinical testing of optical diagnostic instrumentation adopting time-resolved techniques developed for breast and brain imaging applications. In particular, we will report on homogeneous and inhomogeneous phantoms in the liquid and solid state. Homogeneous phantoms have been largely used for investigating the performances of the various optical techniques in assessing the optical properties of biological tissues. Inhomogeneous phantoms are strictly required for evaluating optical imaging systems performances. In this framework, there are two main classes of phantoms: phantoms in which small inclusions are present and layered

* Address correspondence to this author at Department of Biological and Ecological Sciences, Tuscia University, Viterbo, Italy; Tel: +39 0761357026; E-mail: delfino@unitus.it

phantoms. The former ones are mainly used for imaging small tumour lesions in different tissues and the latter for brain imaging. We will discuss the general physico-chemical properties of Intralipid, with particular attention to its optical properties, and we will give information about some other additional substances used in the preparation of Intralipid-based phantoms. After this, we will present just some particularly significant Intralipid phantoms which are indicative of the large amount of work available in the literature. Similar consideration has to be done for the cited references, the list is far from being exhaustive, but it is only demonstrative of the large amount of literature available in this field, witnessing the great importance of the topic in the present biomedical research. The results of a survey about recent patents regarding Intralipid phantoms will be also discussed, witnessing, on one hand, the economic importance of these phantoms as testing elements for new medical equipment and, on the other hand, the number of patents devoted at presenting new methods for realizing Intralipid based phantoms.

2. PHYSICO-CHEMICAL PROPERTIES OF INTRALIPID

Intralipid (mainly produced by Pharmacia (USA), Fresenius Kabi (Germany), Baxter (USA), Kabivitrum (USA)) is an aqueous suspension of lipid droplets that is sterile and suitable for intravenous feeding of patients. It is available as Intralipid-10%, Intralipid-20% and Intralipid-30% (the percentage indicating the relative amount of lipids; for instance, 10% indicates 10 g of lipid per 100 ml of suspension). The constituents of Intralipid-10% in a 500 mL (497.75 g) bottle according to the manufacturers are soybean oil (50 g),

lecithin (6 g), glycerin (11.25 g), water (430.5 g). Intralipid suspension is composed mainly by quasi-spherical colloidal particles with size falling in the range 25-625 nm, consisting of soybean oil encapsulated within a monolayer membrane of lecithin with a thickness of approximately 2.5-5 nm [22]. The glycerin is dissolved into individual molecules in the water. A description of the colloidal particles as concentric spheres can be reasonably adopted. In Intralipid-10%, approximately half of the amount of lecithin is used for encapsulating the soybean oil, and the excess of lecithin forms small bilayer vesicles Fig. (**1**).

Some parameters largely investigated in Intralipid solutions are the particle average diameter, size distribution and polydispersity because they influenced the delivering speed of the emulsion and also its efficiency in drug release. Electron microscopy permits determination of both the size and the shape of the scattering particles in Intralipid-10%. Van Staveren *et al*. [22] reported the results obtained by using a magnification of 18000x at 100 kV in a Philips EM 420 electron-microscopy. They showed the size differences in Intralipid-10% particles and their generally spherical shape. A shell structure was not apparent, thus proving that a sharp boundary (in the case of a bilayer membrane) between lecithin and soybean oil was absent. Hence, they concluded that the layer of lecithin was a monolayer. The measured particle sections were classified into 14 particle diameter classes (see Fig. (**7**) of Ref. [22]) in Intralipid-10%. The mean particle diameter of this distribution was 97 $\pm$ 3 nm. A form factor (Perimeter Eccentricity (PE)) was computed for the particle sections. This form factor for an area *A* with perimeter *s* was defined according to the following Eq. (1).
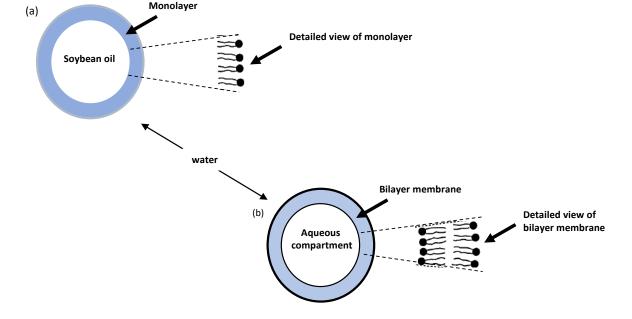

Fig. (1). (a) Schematic drawing of an Intralipid-10% particle after sonication. **(b)** Model of a lipid vesicle in the water environment.

$$PE = \frac{4\pi A}{s^2} \qquad (1)$$

The closer the form factor is to 1 (for any non-spherical shape PE < 1), the closer the particles are to a spherical shape. A plot of the form factor versus the particle diameter [22], allowed the authors to affirm that a spherical shape can be reasonably assumed.

As reported by Rotenberg *et al.* [23] Quasi-elastic Light Scattering (QLS) measurements can also be used for the evaluation of the particle sizes in various Intralipid samples. Measurements were carried out on a Malvern photon correlation spectrometer (Model 4700), equipped with an Argon laser operating at λ = 488 nm. All the samples were diluted 100-2000-fold in 2.5% glycerol prior to being measured. Dilution of the samples in either 2.5% glycerol, in 0.15 M NaCl or water did not affect the size of the particles in the suspension. Measurements were carried out at 25°C, both at a fixed angle of 90 degrees, or multiple angles (45-105 degrees). The results indicated that large emulsion particles with mean diameters ranging from 265 nm in 10% Intralipid to 400 nm in Intralipid-30% were present. These data are consistent with the previous reports on QLS size measurements of particles from 10% and 20% emulsions of various commercial sources [24, 25]. Electron microscopy viewing supported the QLS measurements and ruled out the presence of even smaller aggregates (dimension << 50 nm) which would not have been detected by light scattering analysis.

Intralipid particle size was also measured by using Photon Correlation Spectroscopy (PCS) [26]. This is a laser-light scattering technique that uses fluctuations in scattered light intensity to measure the velocity of Brownian diffusion of small particles that is influenced by the particle diameter. Using PCS, it is possible to determine particle average diameter, size distribution and polydispersity. This technique is also able to measure zeta-potential when combined with electrophoresis. For Intralipid -20% an average diameter ranging between 309.5 + 5.3 and 344.0 + 12.9 nm was measured in dependence of the batch. In the paper of Komatsu *et al.* [26] also data for zeta-potential are discussed. PCS technique was also employed by Li and Caldwell [27] for determining differences in particle size between Intralipid-10% and -20%. They found that the Intralipid-10% emulsion has an average particle size (average diameter: 265 nm) smaller than its 20% analogous (average diameter: 367 nm) although the manufacturer gives the same value for both of them (average diameter: 500 nm).

Ferezou *et al.* [28] investigated Intralipid-10% by using electron microscopy. They evidenced the coexistence of artificial chylomicrons (mean diameter: 260 nm) and liposomes (average diameter: 43 nm). The size (mean diameter: 43 nm) of the liposomes present in Intralipid-10% is smaller than that usually assumed.

3. OPTICAL PROPERTIES OF INTRALIPID

As said before, Intralipid is one of the most extensively studied reference materials in the biomedical optics community and for this reason, its optical properties have been investigated in a number of papers. Here, we will report the results discussed in some of the most cited ones [22, 29 - 31].

As well-known biological tissues can be considered as turbid media when light propagates inside them. The light propagation can be essentially described taking into account four optical parameters: the absorption coefficient, μ_a (the inverse of the mean free path before absorption event), the scattering coefficient, μ_s (the mean number of photon scatters per unit length), the anisotropy *g*-factor (the mean cosine of the scattering angle) and the refractive index *n* (the ratio between the speed of light in the vacuum and in the medium). Usually, μ_s and *g* are combined in the reduced scattering coefficient μ_s' which is defined as $\mu_s' = (1-g) \mu_s$. Another parameter that can be used for taking into account all these quantities is the diffusion coefficient, D, that can be expressed Eq. (2) as:

$$D = \frac{1}{3(\mu_a + \mu_s')} \qquad (2)$$

The numerical values of the above-introduced optical parameters for soft human tissue are reported in Table **1**.

Table 1. Typical values for the optical parameters of soft human tissue [20, 22, 29, 30].

Optical Parameter	Value for Soft Human Tissue
Absorption Coefficient	~ 0.05 cm^{-1} in the near infrared wavelength region
Scattering Coefficient	1-5 cm^{-1} for 600-1300 nm wavelength
Anisotropic g-Factor	0.95-0.98
Refractive Index	~ 1.4

For describing optical properties of Intralipid, Driver *et al.* [29] introduced an effective attenuation coefficient given by the following expression in Eq.(3):

$$\mu_{eff}^2 = 3\mu_a(\mu_a + \mu_s') \qquad (3)$$

In the paper by Driver *et al.* [29], aqueous suspensions of Intralipid-20% were examined. The mean particle size was measured and found to be 444 $\pm$ 84 nm. The effective attenuation coefficient of Intralipid solutions at concentrations ranging from 0.05 to 1.0% in volume was determined at a wavelength of 630 nm by measuring the space irradiance at different source-detector distances using an isotropic source and detector submerged in the solution. In Fig. (**2**), the main results are reported.

Van Staveren *et al.* [22] measured the absorption, scattering, and anisotropy coefficients of the fat emulsion Intralipid-10% at 457.9, 514.5, 632.8, and 1064 nm. The main results are reported in Table **2** along with results from other studies.
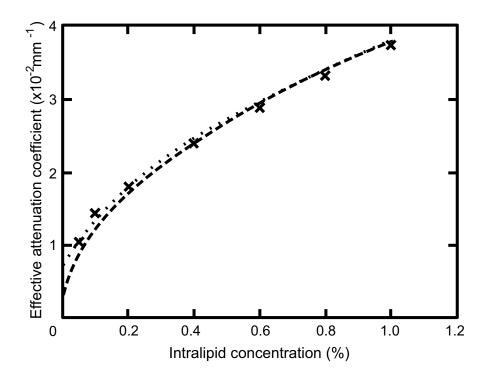
Fig. (2). Effective attenuation coefficients vs Intralipid concentration: experimental points (x), theoretical estimations with different approaches reported in [29]. Reprinted with permission from Driver *et al.* [29]. © Institute of Physics and Engineering in Medicine. Reproduced by permission of IOP Publishing. All rights reserved.

Table 2. Optical parameters of Intralipid-10% at 633 nm.

Absorption Coefficient (cm^{-1})	Scattering Coefficient (cm^{-1})	Anisotropic g-factor	Reduced Scattering Coefficient (cm^{-1})	References
0. 169	0.476	0.796	-	[22]
0.57 $\pm$ 0.15	-	0.71$\pm$0.03	-	[46]
0.18	0.34	0.825	-	[30]
0.16	-	-	150	70
0.0261$\pm$0.0008	-	-	185	[29]
-	-	-	266	[31]

Flock *et al.* [30] extended the measurements of the absorption and reduced scattering coefficients from 460 to 690 nm and the total attenuation coefficient from 500 to 890 nm. These measurements showed that, for stock Intralipid- 10%, the absorption coefficient varied from 0.015 to 0.001 cm^{-1} between 460 and 690 nm, the reduced scattering coefficient was in the 92 to 50 cm^{-1} range between 460 and 690 nm, the total attenuation coefficient changed from 575 to 150 cm^{-1} between 500 and 890 nm, and the average cosine of the scattering angle was in the 0.87 to 0.82 range between 460 and 690 nm.

In 2014, Spinelli *et al.* [31] reported a multi-center study aimed at accurately characterizing the optical properties of Intralipid at NIR wavelengths. Nine research laboratories adopting different measurement techniques, instrumental set-ups, and data analysis methods determined the optical pro-

perties and relative uncertainties of Intralipid suspensions. The authors reported intrinsic reduced scattering coefficient of Intralipid-20% with an uncertainty of about 1%.

An interesting feature of Intralipid was evidenced by Bodenschatz *et al.* [32] that considered the layering properties of this substance that is generally assumed to have homogeneous optical properties. The image of a sample obtained by using Intralipid and ink is shown in Fig. (**3**): the formation of a surface layer is evident. The surface layer is present also when no absorber is used, but in this case, is less evident to the eye. Using frequency domain reflectance and an analytical solution for a two-layer media, Bodenschatz *et al.* investigated the characteristics of this surface layer when suspensions with different Intralipid concentrations are used [32]. The obtained results indicated that a highly scattering layer is formed and, thus, has to be taken into account when using Intralipid phantoms. In order to minimize the surface layer effects, the authors also suggested to avoid long-standing times for Intralipid before measurements.

The results so far discussed are representative of a very large number of studies aimed at best characterizing the optical properties of Intralipid [33 - 41]. Due to the ready availability, low cost, long-term stability and batch-to-batch reproducibility of Intralipid, it can be considered a fundamental compound for preparing phantoms for the calibration and performance assessment of optical spectroscopy instrumentation for optical imaging, even if some attention has to be paid to the fact that Intralipid cannot be rigorously considered an optical standard [31, 38].

Fig. (3). Surface layer on a water-Intralipid-ink dilution ($\mu a \approx 0.3$ mm-1 and $\mu s' \approx 3$ mm-1) formed after 15 min of standstill and subsequent gentle stirring. This camera image is enhanced in contrast to improve the visibility of the flow marks. Reprinted with permission from Bodenschatz *et al.* [32]. © Institute of Physics and Engineering in Medicine. Reproduced by permission of IOP Publishing. All rights reserved.

4. INTRALIPID-BASED PHANTOMS

In this section, the characteristics of the more largely used homogeneous and non – homogeneous Intralipid based phantoms are reported. For the fabrication of these phantoms, Intralipid is generally used as scattering agents, some other materials are used as absorbing agents and some substances are sometimes employed as a hardener. In the Supplementary Materials, a short note about the principal properties of inks and hardeners is reported. It has to be underlined that Intralipid phantoms have high stability, resulting to be of some months for liquid phantom and up to one year for solid ones, when some simple precautions are taken in their storage [42 - 45].

4.1. Liquid Homogeneous Phantoms

Given the above mentioned characteristics of Intralipid suspensions, many different researchers adopt it for preparing liquid homogeneous tissue phantoms useful for the development of time-resolved imaging systems [20, 31, 46 - 50]. A description quite accurate about the preparation of this kind of phantoms is reported in Ref. 30. As said before, in that paper, Flock *et al.* preliminarily collected data on optical coefficients of Intralipid measured at 633 nm (Table **1** of [30] and after extended the measurements of the absorption and reduced scattering coefficients from 460 to 690 nm and the total attenuation coefficient from 500 to 890 nm. Using these measured data, they described the different steps of preparation of an optically tissue-equivalent phantom consisting of Intralipid and black India ink.

They used the similarity relations [51, 52], which can be formulated to show that two media (labelled with subscripts 1 and 2) will exhibit "similar" diffuse reflectance when:

$$\frac{\mu_{s,1}(1-g_1)}{\mu_{a,1}} = \frac{\mu_{s,2}(1-g_2)}{\mu_{a,2}} \qquad (4)$$

According to Flock *et al.*, this relation predicts that a medium with $g = 0$, $\mu_a = 1$ mm^{-1} and $\mu_s = 9.5$ mm^{-1} will show a reflectance similar to that of a medium with $g = 0.5$, $\mu_a = 0.5$ mm^{-1} and $\mu_s = 9.5$ mm^{-1}. Eq. (4) is valid if $\mu_s(1\text{-}g) \gg \mu_a$, for both the media. This condition is necessary because, in the derivation of Eq. (4), it is assumed that the radiance within the medium is linearly anisotropic. Flock *et al.* [30] used Eq. (4) to design a tissue-like optical phantom consisting of Intralipid and a purely absorbing medium whose characteristics are reported in Fig. (**4**). The authors assumed that the black India ink acts as a pure absorber even though a molecular dye would be a better choice.

4.2. Solid Homogeneous Phantoms

A recipe largely used for easily preparing solid homogeneous phantoms was proposed by Cubeddu *et al.* [53]. They suggested that it is possible to prepare this kind of solid phantom by using agar, Intralipid and black ink. The preparation procedure is fast and easily implemented with standard laboratory equipment. A highly purified agar powder is dissolved in distilled water at 1% concentration and heated up to the melting temperature (95 °C) using a microwave oven. The agar solution alone has negligible absorption and very low turbidity. Thus, the desired optical properties of the phantom are obtained by adding appropriate amounts of Intralipid as scattering medium, and India ink as absorbing medium, taking into account that agar induced a systematic decrease in the μ_s' value. In [49], all the useful indications about the further steps for obtaining solid phantoms are given. The samples were placed in small transparent plastic boxes (height = 5 cm, width = 5 cm, depth = 2 cm) for the initial optical characterization of the phantom medium that was performed by using time-resolved transmittance experimental set-up. The proposed phantom was characterized by good overall repeatability with a maximum variation of the optical properties < 3% and a standard deviation < 1% of the mean value. All the phantoms were prepared from the same bottles of Intralipid and ink. The authors showed also that it was possible to accurately tune the optical properties in a well-controlled way [53]. These phantoms were also stable for a two months period if stored at room temperature in closed containers.
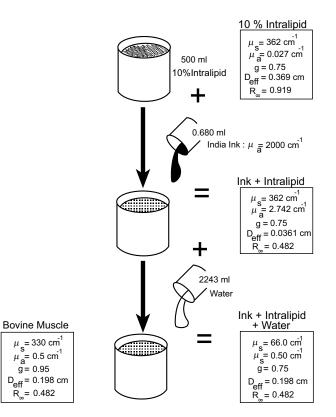
Fig. (4). A diagrammatic representation of the design of a phantom using Intralipid-10% and black India ink that will exhibit a diffuse reflectance, R, and effective penetration depth, Deff, like that of bovine muscle at 633 nm. Reproduced with permission from Flock *et al.* [30]. © John Wiley and Sons Publishers.

Many other authors [54 - 57] used similar phantoms, instead of agar, acrylamide was employed as a hardener by some researchers [58].

4.3. Inhomogeneous Phantoms

When inhomogeneous phantoms have to be prepared three different approaches are usually adopted: liquid-liquid, liquid-solid, and solid-solid configurations, each of them presenting peculiar advantages and disadvantages [59]. Liquid-liquid phantoms are usually constituted by placing a liquid sample (generally prepared with Intralipid and ink) in a small glass or plastic container suspended within another homogeneous medium. Using the liquid-liquid approach, it is possible to change the optical properties of the inclusion and hosting medium in a very easy way. Moving the inclusion inside the hosting medium and preparing a homogeneous reference sample is also easily done in these phantoms. The main problem regards the presence of light guiding effects in the walls of the small container.

For the liquid-solid phantoms agar or polyacrylamide are used for preparing a solid inclusion. This kind of samples can be handled more easily and the change of the inclusion characteristics is simple and light-guiding effects are not present. However, the tuning of optical properties is more difficult because different preparations are necessary for the liquid and solid phases and the refractive index mismatch between the solid inclusion and the liquid medium has to be taken into account.

Solid–solid phantoms can be an optimal choice, but they have fixed characteristics that cannot be changed. For this reason, they are useful only in well-defined situations.

The above described procedures for solid homogeneous phantoms can be adopted for preparing inhomogeneous ones in different ways [53]. For example, holes can be fabricated in a homogeneous agar matrix and it is possible to fill them with solutions having different optical properties in order to mimic a mass embedded in a healthy tissue for controlling imaging optical technique performances. Solid inclusion can be inserted in a liquid medium [60]. In this case, no artifacts due to the solid-liquid interface are present, since the agar matrix is mainly made of water and there is no refraction index mismatch at the solid-liquid interface.

Within the framework of the European nEUROPt Project, an inhomogeneous phantom based on the suspension of black Polyvinyl Chloride (PVC) cylinders within a water suspension of Intralipid and ink was proposed [59]. In Fig. (**5**), a photograph of this phantom is reported. A scattering cell made of black PVC is a suitable container to prepare liquid phantoms. Photons hitting the black walls are reflected or absorbed and guiding effects are not present. Boundary conditions can be precisely considered both in the Monte Carlo (MC) simulations and with analytical models. The container has small transparent windows that allow the light to enter into the diffusive medium and the diffused light to be collected. Different distances for collecting light are available by considering the different transparent windows. Small black PVC cylinders with different sizes (Fig. **5**) immersed in the
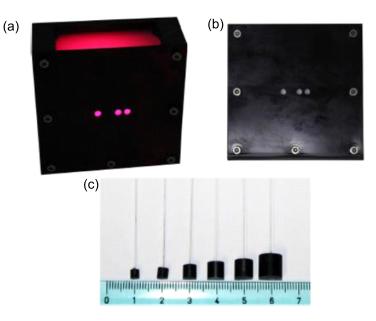
Fig. (5). (**a**) Scattering cell used for liquid phantom experiments; (**b**) Detailed view of the frontal wall; (**c**) polyvinyl chloride (PVC) cylinders of various size. Reprinted from Martelli *et al.* [59] under Open Access conditions.

liquid phantom were used for simulating absorbing objects. The PVC cylinders were held by a rigid metallic wire (0.5-mm music wire) painted white in order to reduce the perturbation. The characteristics of this phantom were carefully investigated by using MC simulations and analytical solutions of the diffusion equation and it was experimentally validated by comparing time-resolved measurements with the results of MC simulations.

4.4. Bilayer Phantoms

Among the inhomogeneous Intralipid-based phantoms, the preparation of multilayer structures useful for developing optical applications for the brain-skull-skin of the human head

for the measurement of blood oxygenation in the brain deserves special attention.

A first approach exploits solid phantoms made of agar or other hardener substances like gelatin, Intralipid, and ink for reproducing multilayer structures, by means of different subsequent depositions [60]. In this case, two-layered gelatin phantoms (230x230x55 to 70 mm) were fabricated with thicknesses of the upper layers equal to 10 or 15 mm. The employed base material was a gelatin solution, that can be considered as a transparent material. By adding Intralipid as the scattering agent and greenish-brown ink as absorber, optical coefficients of human layered tissue were simulated. Further details about the preparation protocol are available in [60].
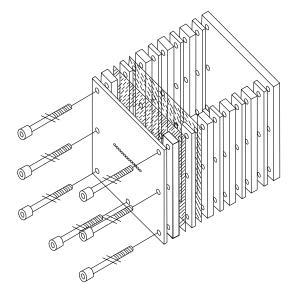

Fig. (6). Schematic representation of the proposed phantom. Reprinted from Del Bianco *et al.* [61] under Open Access conditions.

An alternative approach was proposed in 2004 by Del Bianco *et al.* [61]. It consists in preparing a phantom using Intralipid aqueous suspensions with known absorption and reduced scattering coefficients and thin membranes for separating layers with different optical properties. In Fig. (**6**), a schematic view of the proposed phantom is reported [61, 62]. The cell was fabricated by using black polyvinyl chloride (refractive index 1.54). A thin layer was placed between the spacers to separate aqueous suspensions (refractive index 1.33) with different optical properties. The spacers were sealed with a thin film of silicone and kept together by screws. Small transparent windows (the small circles in the front wall in Fig. (**6**) were used to send light to the diffusive medium and to collect the diffuse reflectance at different distances from the light source. The windows were made of polished Plexiglas layers. In addition to Intralipid used as scattering medium, India ink or Cresyl blue were employed as absorbers. The cell was 115 mm high, 100 mm wide, and 60 mm thick. The thickness and the material for the membrane were carefully chosen. In fact, the membrane should separate volumes with different optical properties without introducing appreciable perturbations on light propagation. In particular, a 23-μm thick film of Mylar was chosen. In [61, 62] all the details of a complete characterization are also reported.

5. SURVEY ON PATENTS

Given the importance of Intralipid in the preparation of phantoms for designing and testing the applications of optical methods in medical fields, we examined also the deposited patents concerning Intralipid phantom preparation using Google Patents and Scopus Patents. Using as descriptors the words "Intralipid phantoms" some thousands of papers were found (1994-2018). The largest part of them regards the development of new optical methods and systems, involving the bare use of Intralipid phantoms in the testing step before the use in clinics, thus confirming very large use of this compound in biophotonics research and the economic relevance of the use of Intralipid-based phantoms in this crucial preliminary step. By refining the survey adding the word "preparation" to the descriptors, one hundred patents are still present. After reading the title, summary or report, as inclusion criteria, and avoiding duplicate a small number of patents remains to be considered [63 - 66]. This confirms that there is still room for realizing new methods aimed at preparing Intralipid-based phantoms with optical and physical properties properly suited for testing equipment with increasing performances. The most representative and well-characterized patent product is the one due to Frangioni and De Grand [63] that mimics any desired tissue and can be imaged using one, two, or more (*e.g.,* four) different imaging modalities (*e.g.,* X-ray Computed Tomography (CT), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), and Near-Infrared (NIR) fluorescence) simultaneously.

CONCLUSION

In this short review, the main properties of Intralipid together with the characteristics of the more generally used Intralipid- based homogeneous and non-homogeneous phan-

toms have been reported. From this rapid and not exhaustive survey, it is clearly evident that Intralipid phantoms can still play a relevant role in the development, validation and translation of biophotonics technology in the medical field. In fact, other approaches [67 - 70] result to be more expensive and difficult to handle notwithstanding new methods recently developed to facilitate the fabrication of tissue phantoms for diagnostic and therapeutic purposes.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

REFERENCES

[1]   Baker MJ, Trevisan J, Bassan P, *et al.* Using Fourier transform IR spectroscopy to analyze biological materials. Nat Protoc 2014; 9(8): 1771-91.
[http://dx.doi.org/10.1038/nprot.2014.110] [PMID: 24992094]
[2]   Delfino I. Light scattering methods for tracking gold nanoparticles aggregation induced by biotin-neutravidin interaction. Biophys Chem 2013; 177-178: 7-13.
[http://dx.doi.org/10.1016/j.bpc.2013.03.001] [PMID: 23563185]
[3]   Sahl SJ, Hell SW, Jakobs S. Fluorescence nanoscopy in cell biology. Nat Rev Mol Cell Biol 2017; 18(11): 685-701.
[http://dx.doi.org/10.1038/nrm.2017.71] [PMID: 28875992]
[4]   Ryder AG. Cell culture media analysis using rapid spectroscopic methods. Curr Opin Chem Eng 2018; 22: 11-7.
[http://dx.doi.org/10.1016/j.coche.2018.08.008]
[5]   Stender AS, Marchuk K, Liu C, *et al.* Single cell optical imaging and spectroscopy. Chem Rev 2013; 113(4): 2469-527.
[http://dx.doi.org/10.1021/cr300336e] [PMID: 23410134]
[6]   La Gatta A, Ricci G, Stellavato A, *et al.* Hyaluronan hydrogels with a low degree of modification as scaffolds for cartilage engineering. Int J Biol Macromol 2017; 103: 978-89.
[http://dx.doi.org/10.1016/j.ijbiomac.2017.05.091] [PMID: 28549864]
[7]   Alizadeh M, Abbasi F, Khoshfetrat AB, Ghaleh H. Microstructure and characteristic properties of gelatin/chitosan scaffold prepared by a combined freeze-drying/leaching method. Mater Sci Eng C 2013; 33(7): 3958-67.
[http://dx.doi.org/10.1016/j.msec.2013.05.039] [PMID: 23910302]
[8]   d'Apuzzo F, Perillo L, Delfino I, Portaccio M, Lepore M, Camerlingo C. Monitoring early phases of orthodontic treatment by means of Raman spectroscopies. J Biomed Opt 2017; 22(11): 1-10.
[http://dx.doi.org/10.1117/1.JBO.22.11.115001] [PMID: 29110445]
[9]   Handbook of optical biomedical Diagnostics. Ed V Tuchin, SPIE Press. 2016.
[10]   Yankelevich DR, Ma D, Liu J, *et al.* Design and evaluation of a device for fast multispectral time-resolved fluorescence spectroscopy and imaging. Rev Sci Instrum 2014; 85(3)034303
[http://dx.doi.org/10.1063/1.4869037] [PMID: 24689603]
[11]   Pifferi A, Contini D, Mora AD, Farina A, Spinelli L, Torricelli A. New frontiers in time-domain diffuse optics, a review. J Biomed Opt 2016; 21(9): 091310.

[http://dx.doi.org/10.1117/1.JBO.21.9.091310] [PMID: 27311627]

[12]   Lepore M, Delfino I, Ramaglia A, Vigilante F, Indovina PL. An experimental comparison between time-resolved transmittance and reflectance techniques for optical characterization of scattering media. Proc SPIE 1999; 3597: 168-80.
[http://dx.doi.org/10.1117/12.356836]

[13]   Yamada Y, Suzuki H, Yamashita Y. Time-domain near-infrared spectroscopy and imaging: A review. Appl Sci (Basel) 2019; 9: 1127.
[http://dx.doi.org/10.3390/app9061127]

[14]   Patterson MS, Chance B, Wilson BC. Time resolved reflectance and transmittance for the non-invasive measurement of tissue optical properties. Appl Opt 1989; 28(12): 2331-6.
[http://dx.doi.org/10.1364/AO.28.002331] [PMID: 20555520]

[15]   Esposito R, De Nicola S, Lepore M, Delfino I, Indovina PL. A perturbative approach to characterize absorptive inclusions in diffusing media by time-resolved contrast measurements. J Pure & App Phys A 2004; 6: 736-41.

[16]   Hassaninia I, Bostanabad R, Chen W, Mohseni H. Characterization of the optical properties of turbid media by supervised learning of scattering patterns. Sci Rep 2017; 7(1): 15259.
[http://dx.doi.org/10.1038/s41598-017-15601-4] [PMID: 29127385]

[17]   Patel P, Bhatia J. Total parenteral nutrition for the very low birth weight infant. Semin Fetal Neonatal Med 2017; 22(1): 2-7.
[http://dx.doi.org/10.1016/j.siny.2016.08.002] [PMID: 27576106]

[18]   Ok SH, Hong JM, Lee SH, Sohn JT. Lipid emulsion for treating local anesthetic systemic toxicity. Int J Med Sci 2018; 15(7): 713-22.
[http://dx.doi.org/10.7150/ijms.22643] [PMID: 29910676]

[19]   Lucchinetti E, Lou PH, Hatami S, *et al.* Enhanced myocardial protection in cardiac donation after circulatory death using Intralipid® postconditioning in a porcine model. Can J Anaesth 2019; 66(6): 672-85.
[http://dx.doi.org/10.1007/s12630-019-01322-x] [PMID: 30790198]

[20]   Pogue BW, Patterson MS. Review of tissue simulating phantoms for optical spectroscopy, imaging and dosimetry. J Biomed Opt 2006; 11(4)041102
[http://dx.doi.org/10.1117/1.2335429] [PMID: 16965130]

[21]   Hwang J, Ramella-Roman JC, Nordstrom R. Introduction: feature issue on phantoms for the performance evaluation and validation of optical medical imaging devices. Biomed Opt Express 2012; 3(6): 1399-403.
[http://dx.doi.org/10.1364/BOE.3.001399] [PMID: 22741084]

[22]   van Staveren HJ, Moes CJ, van Marie J, Prahl SA, van Gemert MJC. Light scattering in Intralipid-10% in the wavelength range of 400-1100 nm. Appl Opt 1991; 30(31): 4507-14.
[http://dx.doi.org/10.1364/AO.30.004507] [PMID: 20717241]

[23]   Rotenberg M, Rubin M, Bor A, Meyuhas D, Talmon Y, Lichtenberg D. Physico-chemical characterization of Intralipid emulsions. Biochim Biophys Acta 1991; 1086(3): 265-72.
[http://dx.doi.org/10.1016/0005-2760(91)90169-I] [PMID: 1742317]

[24]   Hajri T, Férézou J, Lutton C. Effects of intravenous infusions of commercial fat emulsions (Intralipid 10 or 20%) on rat plasma lipoproteins: phospholipids in excess are the main precursors of lipoprotein-X-like particles. Biochim Biophys Acta 1990; 1047(2): 121-30.
[http://dx.doi.org/10.1016/0005-2760(90)90037-X] [PMID: 2248969]

[25]   Westesen K, Wehler T. Investigation of the particle size distribution of a model intravenous emulsion. J Pharm Sci 1993; 82(12): 1237-44.
[http://dx.doi.org/10.1002/jps.2600821211] [PMID: 8308703]

[26]   Komatsu H, Kitajima A, Okada S. Pharmaceutical characterization of commercially available intravenous fat emulsions: estimation of average particle size, size distribution and surface potential using photon correlation spectroscopy. Chem Pharm Bull (Tokyo) 1995; 43(8): 1412-5.
[http://dx.doi.org/10.1248/cpb.43.1412] [PMID: 7553988]

[27]   Li J, Caldwell KD. Structural studies of commercial fat emulsions used in parenteral nutrition. J Pharm Sci 1994; 83(11): 1586-92.
[http://dx.doi.org/10.1002/jps.2600831114] [PMID: 7891280]

[28]   Férézou J, Gulik A, Domingo N, *et al.* Intralipid 10%: Physicochemical characterization. Nutrition 2001; 17(11-12): 930-3.
[http://dx.doi.org/10.1016/S0899-9007(01)00667-0]   [PMID: 11744343]

[29]   Driver I, Feather JW, King PR, Dawson JB. The optical properties of aqueous suspensions of Intralipid, a fat emulsion. Phys Med Biol 1989; 34: 1927-30.
[http://dx.doi.org/10.1088/0031-9155/34/12/015]

[30]   Flock ST, Jacques SL, Wilson BC, Star WM, van Gemert MJC. Optical properties of Intralipid: A phantom medium for light

propagation studies. Lasers Surg Med 1992; 12(5): 510-9.
[http://dx.doi.org/10.1002/lsm.1900120510] [PMID: 1406004]

[31]   Spinelli L, Botwicz M, Zolek N, *et al.* Determination of reference values for optical properties of liquid phantoms based on Intralipid and India ink. Biomed Opt Express 2014; 5(7): 2037-53.
[http://dx.doi.org/10.1364/BOE.5.002037] [PMID: 25071947]

[32]   Bodenschatz N, Krauter P, Foschum F, *et al.* Surface layering properties of intralipid phantoms. Phys Med Biol 2015; 60(3): 1171-83.
[http://dx.doi.org/10.1088/0031-9155/60/3/1171] [PMID: 25590919]

[33]   Giusto A, Saija R, Iati MA, Denti P, Borghese F, Sindoni OI. Optical properties of high-density dispersions of particles: Application to intralipid solutions. Appl Opt 2003; 42(21): 4375-80.
[http://dx.doi.org/10.1364/AO.42.004375] [PMID: 12921288]

[34]   Michels R, Foschum F, Kienle A. Optical properties of fat emulsions. Opt Express 2008; 16(8): 5907-25.
[http://dx.doi.org/10.1364/OE.16.005907] [PMID: 18542702]

[35]   Wen X, Tuchin VV, Luo Q, Zhu D. Controling the scattering of intralipid by using optical clearing agents. Phys Med Biol 2009; 54(22): 6917-30.
[http://dx.doi.org/10.1088/0031-9155/54/22/011] [PMID: 19887711]

[36]   Cletus B, Künnemeyer R, Martinsen P, McGlone VA. Temperature-dependent optical properties of Intralipid measured with frequency-domain photon-migration spectroscopy. J Biomed Opt 2010; 15(1)017003
[http://dx.doi.org/10.1117/1.3290820] [PMID: 20210477]

[37]   Di Ninni P, Martelli F, Zaccanti G. Effect of dependent scattering on the optical properties of Intralipid tissue phantoms. Biomed Opt Express 2011; 2(8): 2265-78.
[http://dx.doi.org/10.1364/BOE.2.002265] [PMID: 21833363]

[38]   Ninni PD, Martelli F, Zaccanti G. Intralipid: Towards a diffusive reference standard for optical tissue phantoms. Phys Med Biol 2011; 56(2): N21-8.
[http://dx.doi.org/10.1088/0031-9155/56/2/N01] [PMID: 21160111]

[39]   Kanick SC, Krishnaswamy V, Gamm UA, *et al.* Scattering phase function spectrum makes reflectance spectrum measured from Intralipid phantoms and tissue sensitive to the device detection geometry. Biomed Opt Express 2012; 3(5): 1086-100.
[http://dx.doi.org/10.1364/BOE.3.001086] [PMID: 22567598]

[40]   Aernouts B, Zamora-Rojas E, Van Beers R, *et al.* Supercontinuum laser based optical characterization of Intralipid® phantoms in the 500-2250 nm range. Opt Express 2013; 21(26): 32450-67.
[http://dx.doi.org/10.1364/OE.21.032450] [PMID: 24514839]

[41]   Aernouts B, Van Beers R, Watté R, Lammertyn J, Saeys W. Dependent scattering in Intralipid® phantoms in the 600-1850 nm range. Opt Express 2014; 22(5): 6086-98.
[http://dx.doi.org/10.1364/OE.22.006086] [PMID: 24663943]

[42]   Marois M, Bravo J, Davis SC, Kanick SC. Characterization and standardization of tissue-simulating protoporphyrin IX optical phantoms. J Biomed Opt 2016; 21(3): 35003.
[http://dx.doi.org/10.1117/1.JBO.21.3.035003] [PMID: 26968385]

[43]   Lai P, Xu X, Wang LV. Dependence of optical scattering from Intralipid in gelatin-gel based tissue-mimicking phantoms on mixing temperature and time. J Biomed Opt 2014; 19(3): 35002.
[http://dx.doi.org/10.1117/1.JBO.19.3.035002] [PMID: 24604534]

[44]   Wróbel MS, Popov AP, Bykov AV, Kinnunen M, Jędrzejewska-Szczerska M, Tuchin VV. Multi-layered tissue head phantoms for non-invasive optical diagnostics. J Innov Opt Health Sci 2015; 8(3)1541005
[http://dx.doi.org/10.1142/S1793545815410059]

[45]   Ohmae E, Yoshizawa N, Yoshimoto K, *et al.* Stable tissue-simulating phantoms with various water and lipid contents for diffuse optical spectroscopy. Biomed Opt Express 2018; 9(11): 5792-808.
[http://dx.doi.org/10.1364/BOE.9.005792] [PMID: 30460162]

[46]   Moes CJM, van Gemert MJ, Star WM, Marijnissen JPA, Prahl SA. Measurements and calculations of the energy fluence rate in a scattering and absorbing phantom at 633 nm. Appl Opt 1989; 28(12): 2292-6.
[http://dx.doi.org/10.1364/AO.28.002292] [PMID: 20555514]

[47]   Choukeife JE, L'Huillier JP. Measurements of scattering effects within tissue-like media at two wavelengths of 632.8 nm and 680 nm. Lasers Med Sci 1999; 14: 286-96.
[http://dx.doi.org/10.1007/s101030050096]

[48]   Delfino I, Esposito R, Piccirillo B, Lepore M. Static and dynamic light scattering properties of Intralipid aqueous suspensions for tissue phantoms preparation and calibration. Proc SPIE 2008; 687068700P
[http://dx.doi.org/10.1117/12.769364]

[49]   Rehman A, Ahmad I, Rehman K, Anwar S, Firdous S, Nawaz M. Optical properties measurement of highly diffusive tissue phantoms for biomedical applications. Laser Phys 2015; 25025605
[http://dx.doi.org/10.1088/1054-660X/25/2/025605]

[50]   Fujisaka SI, Ozaki T, Suzuki T, *et al.* A clinical tissue oximeter using NIR time-resolved spectroscopy. Adv Exp Med Biol 2016; 876: 427-33.
[http://dx.doi.org/10.1007/978-1-4939-3023-4_54] [PMID: 26782242]

[51]   Wyman DR, Patterson MS, Wilson BC. Similarity relations for the interaction parameters in radiation transport. Appl Opt 1989; 28(24): 5243-9.
[http://dx.doi.org/10.1364/AO.28.005243] [PMID: 20556035]

[52]   Yoon G, Prahl SA, Welch AJ. Accuracies of the diffusion approximation and its similarity relations for laser irradiated biological media. Appl Opt 1989; 28(12): 2250-5.
[http://dx.doi.org/10.1364/AO.28.002250] [PMID: 20555507]

[53]   Cubeddu R, Pifferi A, Taroni P, Torricelli A, Valentini G. A solid tissue phantom for photon migration studies. Phys Med Biol 1997; 42(10): 1971-9.
[http://dx.doi.org/10.1088/0031-9155/42/10/011] [PMID: 9364593]

[54]   Hernández-Quintanar L, Fabila-Bustos DA, Hernández-Chávez M, Stolik-Isakina S, de la Rosa-Vázquez JM. Characterization of intralipid-10% in the range of 400-700 nm using Light Emitting Diodes. International Conference on Electronics, Communications and Computers.
[http://dx.doi.org/10.1109/CONIELECOMP.2018.8327182]

[55]   Martelli F, Binzoni T. Heuristic model for ballistic photon detection in collimated transmittance measurements. Opt Express 2018; 26(2): 744-61.
[http://dx.doi.org/10.1364/OE.26.000744] [PMID: 29401955]

[56]   Isler H, Kleiser S, Ostojic D, Scholkmann F, Karen T, Wolf M. Liquid blood phantoms to validate NIRS oximeters: Yeast *versus* nitrogen for deoxygenation. Adv Exp Med Biol 2018; 1072: 381-5.
[http://dx.doi.org/10.1007/978-3-319-91287-5_61] [PMID: 30178375]

[57]   Shahin A, Bachir W. Broadband spectroscopy for characterization of tissue-like phantom optical properties. Polish J Med Phys Eng 2017; 23(4): 121-6.
[http://dx.doi.org/10.1515/pjmpe-2017-0020]

[58]   Wagnières G, Cheng S, Zellweger M, *et al.* An optical phantom with tissue-like properties in the visible for use in PDT and fluorescence spectroscopy. Phys Med Biol 1997; 42(7): 1415-26.
[http://dx.doi.org/10.1088/0031-9155/42/7/014] [PMID: 9253049]

[59]   Martelli F, Di Ninni P, Zaccanti G, *et al.* Phantoms for diffuse optical imaging based on totally absorbing objects, part 2: Experimental

implementation. J Biomed Opt 2014; 19(7)076011
[http://dx.doi.org/10.1117/1.JBO.19.7.076011] [PMID: 25023415]

[60]   Sato C, Shimada M, Yamada Y, Hoshi Y. Extraction of depth-dependent signals from time-resolved reflectance in layered turbid media. J Biomed Opt 2005; 10(6)064008
[http://dx.doi.org/10.1117/1.2136312] [PMID: 16409073]

[61]   Del Bianco S, Martelli F, Cignini F, *et al.* Liquid phantom for investigating light propagation through layered diffusive media. Opt Express 2004; 12(10): 2102-11.
[http://dx.doi.org/10.1364/OPEX.12.002102] [PMID: 19475045]

[62]   Martelli F, Del Bianco S, Spinelli L, *et al.* Optimal estimation reconstruction of the optical properties of a two-layered tissue phantom from time-resolved single-distance measurements. J Biomed Opt 2015; 20(11)115001
[http://dx.doi.org/10.1117/1.JBO.20.11.115001] [PMID: 26524677]

[63]   Frangioni JV. De Grand AM inventors; Tissue-like phantoms. United States patents US 2006/0056580 A1 2006.

[64]   Sujatha NU, Vijayaragavan V. Balanivetha K inventors; Multilayer skin tissue models and methods of preparation. WO 2019167059 A1 2019.

[65]   Hielscher HA, Mourant JR. Bigio IJ inventors; Characterization of highly scattering media by measurement of diffusely backscattered polarized light. United States patents US 20150209449 A1 2015.

[66]   Pan Y, Du C, Ren H. Volkow N inventors; Intralipid as a contrast agent to enhance subsurface blood flow imaging. United States patents US 20170266323 A1 2017.

[67]   Cerussi AE, Warren R, Hill B, *et al.* Tissue phantoms in multicenter clinical trials for diffuse optical technologies. Biomed Opt Express 2012; 3(5): 966-71.
[http://dx.doi.org/10.1364/BOE.3.000966] [PMID: 22567589]

[68]   Diep P, Pannem S, Sweer J, *et al.* Three-dimensional printed optical phantoms with customized absorption and scattering properties. Biomed Opt Express 2015; 6(11): 4212-20.
[http://dx.doi.org/10.1364/BOE.6.004212] [PMID: 26600987]

[69]   Sato C, Shimada M, Tanikawa Y, Hoshi Y. Estimating the absorption coefficient of the bottom layer in four-layered turbid mediums based on the time-domain depth sensitivity of near-infrared light reflectance. J Biomed Opt 2013; 18(9)097005
[http://dx.doi.org/10.1117/1.JBO.18.9.097005] [PMID: 24057194]

[70]   Wilson BC, Patterson MS, Burns DM. Effect of photosensitizer concentration in tissue on the penetration depth of photoactivating light. Lasers Med Sci 1986; 1: 235-44.
[http://dx.doi.org/10.1007/BF02032418]