Review of tissue simulating phantoms for optical spectroscopy, imaging and dosimetry

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Abstract. Optical spectroscopy, imaging, and therapy tissue phantoms must have the scattering and absorption properties that are characteristic of human tissues, and over the past few decades, many useful models have been created. In this work, an overview of their composition and properties is outlined, by separating matrix, scattering, and absorbing materials, and discussing the benefits and weaknesses in each category. Matrix materials typically are water, gelatin, agar, polyester or epoxy and polyurethane resin, room-temperature vulcanizing (RTV) silicone, or polyvinyl alcohol gels. The water and hydrogel materials provide a soft medium that is biologically and biochemically compatible with addition of organic molecules, and are optimal for scientific laboratory studies. Polyester, polyurethane, and silicone phantoms are essentially permanent matrix compositions that are suitable for routine calibration and testing of established systems. The most common three choices for scatters have been: (1.) lipid based emulsions, (2.) titanium or aluminum oxide powders, and (3.) polymer microspheres. The choice of absorbers varies widely from hemoglobin and cells for biological simulation, to molecular dyes and ink as less biological but more stable absorbers. This review is an attempt to indicate which sets of phantoms are optimal for specific applications, and provide links to studies that characterize main phantom material properties and recipes. [DOI: 10.1117/1.2335429]

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1 Introduction

1.1 *Medical Tissue-Simulating Phantoms*

The development of all diagnostic imaging systems and most physical therapeutic interventions has required the use of tissue-simulating objects to mimic the properties of human or animal tissues. These so-called "phantoms" are used for a number of purposes,[1–3] including:

1. initially testing system designs
2. optimizing signal to noise in existing systems
3. performing routine quality control
4. comparing performance between systems

When systems are established and in routine clinical use with regulatory approval, there are generally requirements or recommendations for quality control phantoms that need to be imaged for validation of system performance and use. Regulatory bodies such as the American College of Radiology (ACR), and medical physics associations such as the American Association of Physicists in Medicine (AAPM) and/or Canadian Organization of Medical Physicists (COMP) make recommendations on requirements for phantoms to be used for minimum performance criteria for new systems and for routine monitoring of existing systems. The benefit of this procedure is that system performance can then be made more uniform between institutions and over time.

Access to these phantoms is made possible through commercial distributors who can manufacture them economically. Unfortunately, in the development phase of imaging systems, the status of tissue phantoms can be inconsistent and change over time, making comparison of research systems more difficult. In addition, considerable wasted effort occurs in redeveloping tissue phantoms that have already been well designed by previous groups. In the case of optical or near-infrared imaging and spectroscopy, the field has developed considerably over the past several decades, yet routine widespread clinical use has not been established for many systems. In addition, the spectral range and geometrical range of optics applications are so diverse that development of systems and tissue phantoms has not been a straightforward linear progression. In this study, an overview of the various types of tissue simulating phantoms and their applications is outlined. An attempt is made to discuss the strengths and weaknesses of each phantom type, and issues such as system purpose, geometry, and tissue type are included. The tradeoffs between structure and biological or chemical function are also in-

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cluded, in an effort to provide the most comprehensive listing possible at this stage of development.

The history of tissue simulating phantoms for optical or near-infrared spectroscopy and imaging of tissue began in the early 1980s with the surge of clinical interest in near-infrared transillumination for breast cancer imaging, also termed diaphanography.[4–7] Later interest also arose from applications in photodynamic therapy treatment planning and pulsed laser treatment planning,[8–14] where knowledge of the optical fluence distribution in tissue was critical to achieving treatment efficacy. In the early 1990s, the introduction of spatially resolved, time-resolved, and frequency-domain light signals spurred a larger number of researchers to investigate spectroscopy and imaging of tissue, leading to the generation of many different types of tissue phantoms.[15–23] In recent years, the applications of light in medicine have increased dramatically, with cosmetic laser surgery being a major commercial driving force, and fluorescence and reflectance diagnostics emerging as serious contenders for commercial success. Research into near-infrared tomography,[24–29] photodynamic therapy dosimetry,[8,12,30] luminescence imaging,[31–33] fluorescence molecular imaging,[34–36] and optical coherence tomography[37] among other applications, keeps the area of tissue phantoms progressing and important. Experimental progress toward molecular imaging applications requires tissue phantoms that have some of the specific molecular features of human tissue. At the same time, companies are developing tissue imaging and spectroscopy devices that will require well-calibrated tissue phantoms for routine system comparison, evaluation, and quality control. For all of these reasons, the improvement and standardization of tissue optical phantoms is essential and likely inevitable, even though this work has low priority in most research labs.

1.2 *Tissue Optical Properties*

The key to matching tissue properties in phantoms is a comprehensive understanding of the key physical and biochemical characteristics of tissue that influence its interaction with light.[13] For small scale (<1 mm) applications, it is likely important to match the absorption coefficient $\mu_a(\lambda)$, the scattering coefficient $\mu_s'(\lambda)$, and the anisotropy coefficient $g(\lambda)$, which is defined as the average cosine of the scattering angle. Over larger distances (more than 3 to 5 scattering lengths, a scattering length being defined as the reciprocal of the scattering coefficient $1/\mu_s$) matching the reduced scattering coefficient μ_s' [also called the transport scattering coefficient, defined as $\mu_s'=(1\text{-}g)\mu_s$] is all that is required.[21] This "reduced" approximation follows observations in neutral particle scattering that over multiple scattering event lengths, an anisotropic scattering process appears identical to an isotropic scattering process with a reduced value for the effective scattering coefficient.[12,38–40] In many cases of thick tissue transmission, it is possible to get away with mimicking the effective attenuation coefficient of a tissue, defined in the wavelength regime where diffusion theory is accurate as $\mu_{\text{eff}}=(3\mu_a\mu_s')^{-1/2}$. This is possible because steady-state attenuation in homogeneous media is affected in the same way by the same relative change in absorption or scattering. Over long distances, diffusive processes appear to be attenuated exponentially with this single coefficient, and only when boundaries or temporal signals are introduced is there a discernable separation of the effects of μ_a and μ_s'. If the goal is to mimic the tissue transmission, then matching μ_{eff} can often be sufficient, but in most tissue spectroscopy applications where the goal is to separate $\mu_a(\lambda)$ and $\mu_s'(\lambda)$ to allow spectral fitting, the tissue must have representative values for both these parameters. An excellent compendium of tissue optical properties was compiled in the late 1980's by Cheong, Prahl, and Welch,[41] and updated in 1995.[42] Since that time, many more spectra have been produced for dozens of different tissue types, including breast,[43–47] brain,[48] skin,[49,50] esophagus, and cervix.[51–54]

1.3 *Molecular/Flow/Structural Complexities of Optical Phantoms*

Most of the early studies in tissue phantoms were focused on creating regular-shaped objects that mimicked tissue reduced scattering μ_s' and absorption μ_a at specific wavelengths. In the past decade, focus has shifted to providing phantoms that reproduce tissue properties over broader wavelength ranges, matching the full spectrum of tissue $\mu_a(\lambda)$ and $\mu_s'(\lambda)$ values. There is also significant interest in developing biochemical and biologically compatible tissue phantoms, which can utilize biologically important molecules such as hemoglobin, melanin, or endogenous fluorophores such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD)[55] or exogenous fluorphores such as porphyrins or cyanine dyes.[56] Extending the biochemical capacity to measuring transient biochemical species such as radicals or singlet oxygen has also been demonstrated, and provides actinometry capabilities for therapy planning and optimization.[57,58]

Generation of hybrid phantoms with specific characteristics for multimodality imaging, such as elastic properties,[59] biochemical properties, water/lipid concentrations,[60] electrical properties,[61] magnetic resonance properties, and thermal properties, together with optical properties, is becoming increasingly useful.[62]

Along the lines of dynamically changeable phantoms, there is also a need in some developments to study motion or mass displacement with optical signals. Several methods have been developed to image motion in tissue, which ultimately provides a good measure of mass flow, either by Doppler shift measurements[63,64] or correlation analysis of speckle.[65–68]

In recent years, with advances in tissue engineering, a new emphasis has been placed on engineered tissue structures as tissue-simulating phantoms for studies that investigate biological chemistry or complex biochemical signatures.[69] This approach and the use of *ex-vivo* tissue[70,71] have become established areas of investigation, although their use is distinctly different from the standard concept of a tissue phantom. The ability to better test systems in realistic situations with thin tissue layers, anisotropic properties, and extracellular scaffolding is essential in some applications. Each of these subjects is addressed in detail in this work.

1.4 *Optical Tissue Phantom Composition Choices*

In choosing the most useful phantom materials and design, the region of the spectrum to be used is important, as are the geometrical design parameters of thickness, heterogeneities, container, and possible machining constraints. The biological compatibility in terms of biochemical action or inclusion of

Table 1 Scattering constituents of optical phantoms.

Scatterer material	Permanent	Biologically compatible	Organic chemical	Particle size [nm]	Index of refraction	Particle distribution function	Recommended Use	References
Lipids	N	Y	Y	10 to 500 nm	1.45	Exponentially weighted to smaller sizes, impossible to get a single size distribution	Intralipid, milk, mixture Theory/experimental tests and multiple phantom contrast studies	7,30,60,80,81,148,149
Polymer microspheres	Y	Y	Y	50 nm to 100 μm	1.59	Single size function as ordered, with possible 1 to 2% variance.	Most accurate theoretical prediction of properties Used with all aqueous, resin, and RTV phantoms	1. Bangs Laboratories (Fishers, IN) 2. Polysciences Inc., Warrington, PA, and Eppelheim, Germany) 3. Duke Scientific Inc. (Palo Alto, CA). 55,109,150,151
TiO_2 Al_2O_3 powders	Y	Y	Y	20 to 70 nm	2.4 to 2.9	Exponentially weighted or single size can be ordered	Used with gelatin, RTV, and resin phantoms	Sigma-Aldrich Inc. commonly cited Many possible manufacturers and distributors Many different forms
Quartz glass microspheres	Y	Y	Y	250 nm	N/A	Single size function, with 10% variance	Used with resin phantoms	Darmstadt Inc., Germany[152]

biologically relevant chromorphores and fluorophores is critical as well. Since one of the important features of optical and near-infrared (NIR) spectroscopy is the spectral sensitivity to molecular features of tissue, it has become increasingly important to develop reliable phantoms that accurately mimic the chemistry of tissue. This requires a shift away from solid nonorganic polymers and silicone phantoms toward biologically compatible structures such as agar, gelatin, or collagen matrixes that allow easy inclusion of cellular constituents such as blood or fat and fluorescent molecules such as NADH, FAD, porphyrins,[72,73] and other exogenous organic luminescent molecules.[74–76].

In this survey, the strengths of each approach are put alongside the ease of use, and in Tables 1–8 a summary of these is included, along with recommendations for use for each type of phantom. Because of the wide variety of phantoms and their constituents, it is not possible to have a single comprehensive table of constituents without having significant redundancy and overwhelmingly long tables. In an effort to streamline the presentations, the important parameters for tissue optical phantoms are separated into scattering particles and matrix material. In the sections that follow, more detailed discussion of each is provided to include all the pertinent details, and to reference the key studies that provide more complete directions of how to make and use these phantoms.

1.5 *Purposes of Phantoms and the Criteria for Determining Their Value*

In general, the purposes of tissue optical phantoms can be roughly divided into the following categories:

1. validation of physical models and simulations
2. instrument performance testing and optimization
3. instrument calibration and testing of stability and reproducibility
4. interlaboratory comparison and standardization.

The properties of the ideal phantom depend on its intended use. For example, validation phantoms need to be precisely characterized, but stability and reproducibility might not be as important as in phantoms intended for interlaboratory comparisons. Thus, as the different phantoms are discussed, these four uses are kept in mind.

An "ideal" phantom that could be used for any application would have the properties listed as follows. As stated before, in real applications only some of these properties are important and the others can be neglected or given a lower priority.

1. Absorption and scattering properties can be varied as in different tissues.
2. Wavelength dependence of these properties is similar to tissue.
3. Molecules of specific interest can be incorporated (e.g., NADH, FAH, collagen, tetrapyrroles, fluorophores, and actinometers).
4. Properties are stable over time and environmental conditions (e.g., temperature, humidity, and photobleaching).
5. Index of refraction close to that of tissue (e.g., index of tissue $\approx$1.4).
6. Ability to incorporate regions with different optical properties (e.g., inclusions mimicking tumors or layers mimicking skin).

7. Mechanical and surface properties are similar to tissue (e.g., Young's modulus near 4 to 20 kPa).[77]
8. Ability to incorporate Brownian motion or flow in the phantom.
9. Ability to include thermal properties similar to tissue.
10. Ease of manufacturing.
11. Inexpensive to produce.
12. Easily transported between different sites.

Again, as the different compositions are analyzed, these features are raised and discussed to compare each phantom material with alternatives.

2 Scattering Particles in Optical Phantoms

In most tissue phantoms, the choice of a scattering agent is separate from the choice of matrix composition, as the volume fraction of the scattering material is typically less than 5% of the total, and often less than 1%. There have been three main choices: lipid microparticles, polymer microparticles, and white metal oxide powders and a brief list is shown in Table 1. The benefit of lipid microparticles is that they are biologically similar to what is thought to cause scattering in tissue, namely the bilipid membrane of cells and organelles. The next most common choice has been the polymer microsphere, with polystyrene being the most popular. This is an excellent choice from a scientific perspective, because it is produced in regular sizes with good quality control over the size and index of refraction. Thus, the repeatibilty and theoretical prediction of the spectra are excellent. The third choice is common titanium dioxide or aluminum oxide powder. These are often the main pigment in white paint and white plastics, due to their high scattering coefficients, and they can be obtained in well-controlled spherical formulations, although the use of these is less well established. Finally, in recent years scattering gold nanoparticles have been developed, and their use in tissue diagnostics and therapy has considerable promise due to their high scattering cross section and potential biocompatibility.[78,79] While their use in phantoms is not well established, their significant Mie scatter cross section makes them a good potential scatterer. Each of these is discussed in more detail later, and summarized in Table 1.

2.1 *Commercially Available Lipid-Based Scatterers*

The most widely used phantoms for optical imaging and spectroscopy have been the liquid type, made from milk[149] or emulsified oil suspensions initially, and later being largely replaced with the well calibrated, commercially available lipid emulsion with the trade name Intralipid.[148,152] These are listed in Table 2.

Commerical supplies of calibrated lipid solutions are possible due to their production for intravenous feeding.[82] There is a number of commercial manufacturers, and the trade name of the product varies between manufacturers. Intralipid™ (Kabi-Pharmacia, Erlangen Germany; Pharmacia and Upjohn, Clayton, New Jersey; and Kabi-Vitrum Incorporated, Stockholm, Sweden) is the most commonly cited word, with other versions called Nutralipid™ (Pharmacia, Quebec, Canada) and Liposyn II™ (Abbott Labs Incorporated, Montreal). This solution is readily available in all hospital pharmacy departments, and the uniformity between batches is thought to be excellent, although there is some contention about the consistency in the optical properties between batches. Clearly this is an emulsion suspension, and thorough mixing is required for homogeneity. The homogeneity lasts for a period of hours, while reuse of the solution has been reported over many days. When used for ultraviolet studies, the lipid content in this medium is likely to fluoresce which may interfere with transmission or remission studies, and so care must be taken to use nonorganic scattering materials such as are described in the next two sections.

Table 2 Summary of lipid emulsion-based phantoms.

Scatterer	Function	Limitations	Stability	References
Milk	Readily available	Not highly reproducible between samples	Hours	6,149,153
Oil/fat/lipid	Used to custom-make scattering and lipid/water phantoms	Must be emulsified and blended reproducibly	Hours	7,60,106
Intralipid/ Nultralipid	Reproducible source of lipid solution	Stability better than 10%	Days	80,81,148

2.2 *Scattering Coefficient Spectrum of Intralipid*

An excellent survey of the properties of Intralipid can be found at the website http://omlc.ogi.edu/spectra/intralipid/. The most cited study by van Staveren et al.[81] used measurements of optical transmission as well as electron microscopy and Mie theory calculations to estimate the scattering spectrum. They also proposed a simple power law for the wavelength dependence of the reduced scattering coefficient, which has been utilized by many researchers. For a standard 10% stock solution, the formulas for scattering and anisotropy coefficients are:

$$\mu_s(\lambda) = 16\lambda^{-2.4},$$

units of mm^{-1}, when λ is in microns, and

$$g(\lambda) = 1.1 - 0.58\lambda,$$

resulting in the equation for reduced scattering coefficient of:

$$\mu_s'(\lambda) = 9.3\lambda^{-1.4} - 1.6\lambda^{-2.4} \quad (\text{units of } mm^{-1}).$$

For a more complete spectrum across the visible range, inclusion of Rayleigh scattering is likely needed, requiring a third term having the standard λ^{-4} power function, but requiring fitting for the coefficient. Many analyses retain only the first term of this latter formula, and fit $\mu_s'(\lambda)$ to the functional form $\mu_s'(\lambda) = a\lambda^{-b}$, with a and b as free parameters. When fitting is restricted to the near infrared, this can be a reasonable assumption over a limited wavelength range.

2.3 *Polymer Microspheres*

From a scientific viewpoint, polystyrene microspheres provide the best standard phantom, as they are well controlled in

Table 3 Phantom matrix options to hold the scatterers, absorbers, and fluorophores.

Phantom matrix material	Permanent	Solid/ liquid/ flexible	Biologically compatible	Organic chemical compatible	Inclusions possible?	Adjustable absorption	Adjustable scattering	Index of refraction	Recommended use	References
Aqueous suspension	N	L	Y	Y	Y	Y	Y	1.34	Initial use and multiple phantom contrast studies	7
Gelatin/agar matrix	N	F	Y	Y	Y	Y	Y	1.35	Detailed heterogeneity phantom studies bioabsorbers and fluorophores	55
Polyacrylamide gel	N	F	Y	Y	Y	Y	Y	1.35	Thermal therapy studies	154
Polyester or epoxy resin	Y	S	N	N	Y	Y	Y	1.54	Calibration and routine validation Intersystem comparisons	109,111,155,152
Polyurethane resin	Y	S	N	N	Y	Y	Y	1.50	Calibration and routine validation Intersystem comparisons Inclusion of dyes	114
RTV silicone	Y	F	N	N	Y	Y	Y	1.4	Complex geometries with permanent flexible phantoms	117

size and index of refraction.[15] Their use has been included in fundamental Mie scattering theory modeling studies, and their scattering coefficient properties validated in dilute and bulk samples. The ability to have a phantom that matches the predicted scattering coefficient of Mie theory provides a level of validation that does not exist in any other system. Thus, this phantom is perhaps the best for validation of absolute optical property calculations. Microspheres of different composition can be obtained from commercial suppliers: 1. Bangs Laboratories, Fishers, Indiana); Polysciences Incorporated, Warrington, Pennsylvania and Eppelheim, Germany; 3. Duke Scientific Incorporated, Palo Alto, California.

Prediction of the scattering coefficient based on the Mie theory has been repeatedly shown to be a valid way to predict the bulk scattering properties of polystyrene microspheres in solution. Following the derivation provided by Bohren and Huffman,[83] the following equation for the reduced scattering coefficient can be obtained:

$$\mu_s'(\lambda) = N\sum_{i=1}^{p} f(a_i)C_{\text{scat}}(m,a_i,\lambda)[1-g(m,a_i,\lambda)],$$

where C_{scat} is calculated by a series expansion of Ricatti-Bessel functions, N is the particle number density in the solution, and $f(a)$ is the normalized particle distribution function that is summed over all p values of particle size. Here, g is calculated by:

$$g = \langle \cos(\theta) \rangle = \int_{4\pi} P(\theta)\cos(\theta)d\Omega,$$

$$\text{where } P(\theta) = \frac{1}{C_{\text{scat}}}\frac{dC_{\text{scat}}}{d\Omega}.$$

These formulas have been used to show that with polystyrene in water or glycerin, the measured scattering matches the predicted value quite accurately. Computational solvers for these Mie expressions are available at several locations, with comprehensive resources at the following websites http://www.t-matrix.de/ or http://diogenes.iwt.uni-bremen.de/vt/laser/wriedt/New/new.php3. These all refer to the original programs developed by Bohren and Huffman in 1983,[83] but several of the newer versions have more efficient solvers for the Bessel function expansion, and provide solutions in newer programming languages, such as MATLAB and Mathematica.

Addition of absorbers of all kinds is possible with these phantoms; however, molecular absorbers are probably preferable, as the phantoms can then last for years and be reused as needed,[84,85] similar to the period that the polystyrene spheres would last. However, in the case of specific biochemical or biological studies, organic molecules and biological cells can readily be added to these suspensions to create accurate and realistic phantoms.[86]

Table 4 Absorbers and fluorophores that can be added to aqueous phantoms.

Absorber or fluorophore	Function	Limitations	Stability	References
Whole blood	Provide realistic tissue spectra and oxygenation function		Hours to days	67,86,156,157
Ink	Provide nearly flat absorption spectra	Not stable nor repeatable unless taken from a calibrated sample	Days (if remixed)	92,97,110,152
Molecular dyes	Provide spectra with wavelength peaks		Days to weeks	74,152
Fluorophores	Compatible with aqueous dissolving compounds	May need to avoid aggregation effects with addition of additional agents	Days to weeks	158
Heterogeneities (scattering/absorption /fluorescent)	Test tomography and imaging capabilities Used to fill inclusions in solid phantoms	Clear enclosures need to be avoided due to light channeling Index of refraction changes may be significant for solid inclusions	Days	159

2.4 *Titanium Dioxide and Aluminum Oxide*

Titanium dioxide (TiO_2) powder is perhaps the most common choice for scatterering in science and engineering, and this stems from its wide availability as the main pigment in common white paint. Aluminum oxide or barium oxide powders are also excellent scatterers, and are commonly used for coating the interior of integrating spheres where exceptionally high scatter and low absorption are required. TiO_2 powder comes in several forms and purities, including preformed microspheres, available from Dupont Chemical (http://www.specialchem4polymers.com/tc/Titanium-Dioxide/).

The main disadvantage of TiO_2 powder is that it resides in suspension in most media, and so settles when not stirred. This is not a problem in resin or agar phantoms once they are set, but is an issue for aqueous phantoms. Continuous stirring of the aqueous suspension produces a homogeneous phantom. For resin- or agar-based phantoms, mixing for extended periods is also important to ensure that the particles are uniformly distributed. Automated stirring for more than 30 min has been a reliable approach for manufacture of reproducible resin phantoms. Liquid-based stock supplies of TiO_2 are now available from Sigma, and these may be a more reliable additive, as the scattering properties are better controlled than a powder mixed in suspension.

3 Bulk Matrix Materials for Optical Phantoms

The choice of the bulk material for the phantom has perhaps the largest impact on how the phantom can be used. Different matrix materials are optimal for different applications, and the major types are summarized in Table 3. The use of these phantom matrix compositions is discussed throughout the remainder of the work.

4 Aqueous Suspension Phantoms

Water-based phantoms can employ any of the three main scatterers mentioned before lipid, microspheres, or TiO2 power in suspension. The absorption of such phantoms is due mainly to water throughout most of the visible and near-infrared wavelengths. This absorption coefficient is sufficiently low below 700 nm that it can be ignored ($\mu_a < 0.002$ mm^{-1}), and absorbers can be added to tailor the absorption coefficient and spectrum to that of tissue. The water absorption spectrum can be reliably assumed to match the measurements of Hale and Querry,[87] and an excellent overview of the water spectra available and their conversion between different units is found at the website http://omlc.ogi.edu/.[56] A brief summary of absorbers and fluorophores used is listed in Table 4.

4.1 *Exogenous Absorbers in Aqueous Phantoms*

Addition of absorbers and fluorophores to aqueous phantoms has been demonstrated in hundreds of studies, and this phantom design has proven to be extremely valuable in the initial validation of an imaging/spectroscopy system. Typically the goal has been to mimic tissue, so the addition of erythrocytes,[58,156–164] whole blood, or hemoglobin have all been reported. When attempting to preserve the oxygen bind-

ing function of hemoglobin, the use of saline rather than distilled water is important, otherwise the blood cells will lyse and the heme will dissociate from the hemologlobin molecule. However, for simplicity, many researchers have chosen to use ink or molecular absorbing dyes to simulate the absorption of blood or melanin in tissue.

Addition of fluorophores[158] has been reported in many studies, with hydrophilic molecules used most successfully. Aggregation of certain hydrophobic dyes such as protoporphyrin 9 is possible, but addition of 5% Tween-29 (Fisher Scientific, USA) as an emulsifying agent has been found to correct this and result in a monomerized form of the fluorophore. The absorption and fluorescence spectra are similar to those observed when the dye is dissolved in a dilute organic solvent.

4.2 *Inclusions and Heterogeneous Lipid Solution Phantoms*

An important complication in the use of lipid solution phantoms is the choice of container and the possibility of light channeling through the container walls, rather than through the solution. This is especially problematic over longer distances and in cases when inclusions or heterogeneities are to be incorporated in phantoms. Early studies in tomography used containers with thin mylar walls to hold liquid inclusions,[88,89] but it is apparent that the mylar itself does perturb the light field. Correction for this effect can be performed by filling the inclusion with the same solution as the background medium and using this as the "homogeneous" reference phantom. However, for smaller inclusions and lower contrast inclusions, this approach not accurate, and solid phantoms are preferable.

Light channeling along the top surface of Intralipid over long distances has also been noted, yet little discussed in publications. It is important when using this or any optical phantom to shield the surfaces of the phantom properly so that signals can enter and exit the phantom only at desired locations. This can be achieved with black masking of the phantom surfaces, using any opaque acrylic or plastic material.

Lipid-based solutions have been used with great success in conjunction with solid phantoms, where holes or channels have been left in the solid phantom to allow dynamic variation of the heterogeneity optical properties.[90–92] This approach has been used in many studies to assess detectability of objects of differing contrast. This approach allows the use of contrast-detail analysis as well, to determine the minimum contrast detectable for each size of inclusion in the phantom.[90] There is clearly concern that the transition from solid to liquid matrix involves a change of refractive index, yet experiments appear to indicate that this is a manageable, if not insignificant, artifact.

5 Hydrogel-Based Phantoms

Most substances that encapsulate water as a main component and form a stiff matrix that has limited water mobility are in the category of hydrogels. Gelatin and agarose are two of the most common examples, and in biological laboratories there are hundreds of varieties of these. In this section, agar and gelatin are discussed separately because of their long history as phantom matrix materials. Agar-based phantoms have been used in magnetic-resonance imaging (MRI) and ultrasound imaging for decades,[93–95] and they were adopted in optical tissue phantoms in many laboratories in the mid 1990's.[58,96–99] Agar and gelatin allow inclusion of organic molecules and cellular-based constituents, while providing a semisolid object that can have a variety of shapes. Gelatin and agar phantoms have had an equally rich period of development in ultrasound imaging, and a large number of papers describe the diversity of phantoms developed here.[62,100,101] More recently, the whole area of hydrogels has been studied for biocompatibility and drug delivery applications, and this encompasses most biological scaffolds that alter the behavior of water.

Polyacrylamide hydrogels have been used as scaffolds for collagen and other matrices,[102,103] and polyvinyl alcohol hydrogels are reviewed in Sec. 8.1, as they have intrinsic scattering properties as well as being a matrix medium. Polyacrylamide hydrogel use has undergone enormous development in biological laboratories for use in electrophoresis and molecular separation techniques, yet there are only a few reports of testing of these matrices as phantom materials.[102,103]

5.1 *Scattering Composition*

Since gelatin phantoms are usually used for periods of a day to a week or more and then discarded, they are commonly made with less expensive scattering particles. Construction with polystyrene microspheres is possible but is quite expensive. Use of titanium dioxide (TiO_2) power or aluminum oxide (Al_2O_3) powder is the norm, as they are inexpensive and provide a reasonably reliable means to mix a scatterer into the liquid gelatin or agar solution while it is cooling. The major complicating factor in production of these phantoms is the need for careful attention to detail and procedure. For example, the TiO_2 scatterers in the phantom readily precipitate out, and when ordered in bulk comes in clumpy power form, requiring continuous stirring for approximately 20–30 min to ensure homogeneous dispersion in the phantom. Liquid-based TiO_2 is also available and is a reliable method to add controlled amounts to a solution without the concerns of being able to mix and declump the suspension. In addition, TiO_2 does settle over time, so the final scattering coefficient of phantoms can vary significantly from one to another. Despite careful procedures with TiO_2 suspensions, repeated studies in our laboratory show that up to 50% variation can occur. Making multiple phantoms from a large batch of agar can reduce this intersample variation. In addition, the bottom of each phantom typically has a large precipitation of TiO_2, indicative of a scattering gradient along the vertical direction. The precipitated TiO_2 is thought to be from larger particles of the powder, which have higher gravitational force acting on them. Increased mixing time reduces the number of these particles. However, in the end, it is imperative to be able to independently measure the scattering coefficient prior to use in these types of phantoms, due to inability to exactly predict the scattering coefficient from a set recipe.

5.2 *Additives to Gelatin Phantoms to Improve Function*

Table 5 summarizes some main additives used to improve

Table 5 Additives that can be used in gelatin/agar phantoms.

Additives	Function	Limitations	Stability	References
EDTA Penicillin	To avoid bacterial growth (0.5 g/L) Sigma Chemical Co., St. Louis, MO		Days to weeks	61,92,98,104,105
Yeast Sodium azide	Remove molecular oxygen		Hours	98,157
Formaldehyde	Increase melting temperature above room temp. Requires 0.2%		Years	101,104
Whole blood	Provide realistic tissue spectra	Oxygen saturation is not easily changed	Days	98,160
Ink	Provide flat absorption spectra	Not stable nor repeatable unless highly calibrated and repeatably mixed	Days to years	
Organic molecules (i.e., glucose)	Matrix holds most organic compounds	Stability of each molecule must be assessed	Days	55,96
Fluorophores	Compatible with aqueous dissolving compounds Gelatin provides additional capabilities to deaggregate	May need to avoid aggregation effects with addition of additional agents	Days to weeks	55,58,98
Heterogeneities	Test tomography and imaging capabilities Inclusions can be liquid or solid	Clear enclosures need to be avoided due to light channeling Index of refraction changes significantly for solid inclusions	Days to weeks	160
Gadolinium Copper Sulphate	Provide varying levels of magnetic resonance contrast Approx. 1 mg/ml		Years	62,104,105,160
Actinometry agents	Provide measure of photochemical dose deposition	Unstable over long periods of time	Hours	57,58

the function of gelatin based phantoms. Inclusion of 0.2% formaldehyde in gelatin phantoms increases the melting temperature of the gelatin matrix by increasing the crosslinking of the fibers while preserving the lower Young's modulus.[101,104] This allows the phantom to be used at room temperature without need for refrigeration. This can also be achieved with agar-based phantoms, but these can become fragile and crumble under applied stress. Gelatin can be ordered from different biological origins, and with different bloom levels—increasing the level of bloom results in a stiffer gelatin phantom. A pig-skin-based gelatin with a bloom of 175 provides a good stiffness for reliable phantoms.[104]

Table 6 Additives that can be used in resin phantoms.

Additive	Function	Limitations	Stability	References
TiO_2	Provide stable and calibrated scatter spectra	Not exactly representative of tissue scatter spectra	Years	[23]
Microspheres	Provide precise scattering value	Cost	Years	[109]
Ink (India ink) (900NP ink)	Provide flat absorption spectra	Not stable nor repeatable unless highly calibrated	Years	900NP[109] India[90,91,161]
Heterogeneities	Holes can be embedded in these with machining, or using preformed inclusions	Clear enclosures need to be avoided due to light channeling Index of refraction changes may be significant between resin and aqueous inclusions	Years	Vessels[162,163] Cylinder[90,91] Spheres[113]

Inclusion of biochemically toxic species such as wood preservative (0.01 g/L),[61] mild acid ethylenediamine tetraacetic acid (EDTA) at 0.02 g/L,[92,104,105] or sodium azide[98] provides a stable phantom that lasts for many days and weeks without bacterial growth. The EDTA additive is probably most common, because its lower toxicity simplifies handling procedures. Inclusion of penicillin has also been reported for the same reason.[98] While these additives will maintain good biological stability for many days and weeks, they will not keep the gelatin from drying out, and the phantoms must be kept sealed in airtight enclosures such as plastic bags or containers. Keeping the phantoms in vegetable oil has also been reported as an excellent way to preserve the water content.[104] This process can provide an intact matrix for years of use of a single gelatin phantom, although the other biochemical molecules included may not last as long as the gelatin matrix itself.

Blood has been added to gelatin phantoms[55,98] and provides an excellent model of tissue spectra in the near infrared, where the dominant absorbers are hemoglobin and water. Inclusion of fat has been reported, but without extensive study of this capability.[106]

For therapeutic study use, these phantoms are ideal, as they can have the same elastic properties as human tissue and similar thermal properties.[107] Inclusion of actinometry agents has been demonstrated and used to compare photodynamic dose deposition from cw and pulsed laser sources.[57,58] Similarly, measurement of oxygen in phantoms and tissues can be achieved with fluorescent reporters.[108] The potential for biochemical similarity to tissue, together with the potential for therapy studies, makes these tissue phantoms the best for complex tissue geometries and biophysical study.

6 Polyester and Polyurethane Resin Phantoms

Polyester resin phantoms were introduced by Firbank, Delpy, and Oda using both TiO_2[23] and polystyrene particle scatterers.[152,155] The construction of these phantoms requires mixing a resin and hardener to create a transparent solid resin, which typically sets within a few days at room temperature or within a few hours at elevated temperature. A detailed outline of this procedure can be found at the University College London website http://www.medphys.ucl.ac.uk/research/borl/research/NIR_topics/phantom_recipe.htm. An alternative recipe can be found at http://esperia.iesl.forth.gr/~jripoll/resin.html. This material can be obtained from a number of manufacturers and in different compositions including: 1. Araldite epoxy (MY753) and hardener (XD716), Aeropia Chemical Supplies, (Crawley, United Kingdom), or 2. Araldite resin (GY502) and hardener (HY832), D. H. Litter Incorporated, (Elmsford, New York). Thorough mixing of the resin and hardener is critical to obtain a homogeneous volume that cures in a timely manner. There is significant heat and gas generated during this process. Degassing of the phantom during the initial curing process is critical to avoid large numbers of air bubbles embedded in the phantom. Initial degassing during the curing process will cause a massive expansion of the resin due to the large amount of gas present; however, delaying the onset of the degassing, or repeated rapid degassing and repressurizing cycles, can break the bubbles present in the phantom and gradually reduce the phantom volume to be predominantly resin with little gas.

A recipe used in our laboratory for a reliable phantom is as follows,[90,92] mixing 100 parts Araldite GY502 mixed with 30 parts of the hardener HY 837. Prior to mixing in the hardener, the scatterer and absorber can be mixed into the Araldite thoroughly and degassed to allow a homogeneous mixture of optical properties prior to initiation of the hardening process. Then when the hardener is added, it can be slowly mixed to minimize inclusion of air bubbles during the mixing process. It has been found that 3.5 g of TiO_2 powder per liter of resin provides a scattering coefficient near 1.0 mm^{-1} at 800 nm, which is proportional to this concentration. Mixing for an

extended period of time with a magnetic stir bar or an electric mixer is strongly recommended.

In previous studies, the bulk absorption coefficient of the medium was set by adding 25×10^{-6} liters of ink per liter of resin, which was found to increase the absorption coefficient to a range between 0.006 to 0.009 mm^{-1}, but different ink bottles and solutions will vary significantly, so well calibrated and mixed samples of ink stock solution must be used. Particulate ink absorbers, such as India ink, produce a relatively flat absorption spectrum across most of the visible and near infrared, as they are composed of carbon particles suspended in an emulsion. It is important to note that particulate inks also scatter light,[110] so quantification of the absorption coefficient of ink in standard spectrophotometers is not possible. Instead, it must be measured in a standard "added absorber" experiment. Use of molecular absorber inks such as 900NP has been firmly established.[111–113] Many types of these nonorganic dyes have been successfully added to this matrix and provide wavelength-dependent absorption across the near infrared, and have no significant scattering coefficient as they are smaller molecules. With consistent procedures, it is possible to obtain a process where phantoms produced successively have absorption and scattering properties within 10% of their target value. A summary of additives typically used is in Table 6.

Polyurethane phantoms were more recently described by Vernon et al.[114] and suggested as a superior alternative to polyester resin, due to their better compatibility with infrared dyes to better match the absorbing and fluorescent molecules of tissue. It is stated that these resins provide less bleaching of the dyes, but extensive testing has not been reported. The transparency and index change are similar to polyester, making these phantoms otherwise quite similar.

7 Room-Temperature-Vulcanizing Silicone Phantoms

Room-temperature-volcanizing (RTV) silicone-based soft phantoms were introduced by Bays et al.[150] and Beck et al.[116] The merits of these phantoms are that they are quickly produced, have a soft rubber texture similar to stiff tissue, and can include nonorganic scatterers and absorbers. The RTV-based compounds can be obtained from a number of manufacturers (RTV Elastosil 604, Wacker, Munich Germany[116,117]) (Rhodorsil RTV 141, Rhone-Poulenc, France[115]), (RTV-141, Medford Silicone, Medford, New Jersey[59]). Preparation of the material is similar to the resin-based phantoms described in the previous section. Mixing the RTV with its hardener initiates a chemical process that solidifies the compound, and heat and gas generation require pumping under vacuum. This degassing removes the bubbles that are generated when it is curing.

A summary of some additives used with RTV phantoms is used in Table 7. Beck et al.[116] examined ways to embed absorbers and scatterers into the medium, with the conclusion that certain stable dyes could be added, but organic molecules such as porphyrins were not stable in this polymer. Jiang et al. examined controlling stiffness by lowering the hardener concentration. They showed that the elastic modulus of the phantom could be lowered by a factor of 3, (from 230 to 80 kPa), making it closer to the stiffness of soft human tissues. Shaping this material into biologically relevant configurations with the stiffness of human tissue has been the main argument for its use. This was demonstrated by Bays et al. for esophageal phantoms[115] intended for dosimetry for photodynamic treatment planning. Jiang et al. used this material for breast phantoms[92] to help in calibration of an optical tomography system. Lualdi et al. have used these phantoms to study imaging of skin lesions using melanin and absorbers that mimic skin lesions.[118] The only major drawbacks of this matrix material are cost and hardening time, but these are not prohibitive, and a pliable tissue phantom can be quite useful for applications where the mechanical contact to tissue is important.

Table 7 Additives in RTV silicone phantoms.

Additive	Function	Limitations	Stability	References
TiO_2 Al_2O_3	Scattering	Mixing consistency is critical Degassing to remove air bubbles is critical	Years	115,116,92,117,164
Molecular absorbers	Possible		Years	150
Heterogeneities	Test tomography and imaging capabilities		Years	118

8 Novel Materials for Optical Phantoms with Intrinsic Scattering

In addition to the materials discussed in the previous two sections, there are a number of materials that have been used for phantoms that have intrinsic matrix and scattering properties that are interlinked. These are less clearly organized than the previous group, but have properties that could make them useful options for certain studies. These range from polyvinyl alcohol gels, dough, and teflon, to "engineered" or excised tissues. Each of these are briefly mentioned in Table 8, and summarized in the following subsections.

8.1 *Polyvinyl Alcohol Phantoms*

Perhaps the most promising and widely used of these options are the polyvinyl alcohol gels,[119,120] sometimes referred to as cryogels, due to the fact that their scattering coefficient and stiffness increase with repeated freeze/thaw cycles, allowing them to be tailored for specific applications. These were originally used in ultrasound and MRI[121–123] research, and have recently been adopted for photoacoustic tomography, where the combination of elastic and optical scattering properties makes them ideal for this hybrid imaging approach.[124] Kharine et al. report reduced scattering coefficients near 0.8 mm^{-1} after seven freeze/thaw cycles. They also demonstrated the ability to create pliable phantoms this way, without increased scattering, by including dimethyl sufoxide (DMSO), thereby reducing the apparent "whiteness" produced by water

Table 8 Phantom materials and tissues with intrinsic scattering within the matrix material.

Phantom material	Permanent	Solid/ liquid/ flexible	Biologically compatible	Organic chemical compatible	Inclusions possible?	Adjustable absorption	Adjustable scattering	Index of refraction	Recommended use	References
Polyvinyl alcohol gel	Y	F	N	N	Y	Y	Y	1.36	Heterogeneity studies with deformation Thermal studies Acoustic studies (higher cost OK)	119,120,124
Dough	N	F	Y	Y	Y	Y	N	1.35	Heterogeneity phantom studies (fixed μ_s')	N/A
Engineered tissue models	N	F	Y	Y	Y	Y	N	1.35	Scientific study of biochemistry and biology	127
Ex vivo tissue	N	F	Y	Y	Y	N	N	1.35	Reality check scientific studies	134–136, 134,137–141

freezing in the cycles. This phantom can then be considered a clear matrix, in which microspheres or TiO_2 could be embedded to create well-controlled optical scattering phantoms while the elastic properties are set independently. These phantoms appear highly promising for use in these hybrid applications where optical and stiffness properties need to be separately controlled.

These gels can be obtained with average molecular weight of 85 to 140 kDa from Sigma-Aldrich (USA) (catalog number 36 314-6), and are dissolved at a concentration of 20% by weight in distilled water while being heated to 90 °C for 2 h with continuous stirring. After cooling for a few hours to allow air bubbles to migrate to the surface, it is then poured into a mold and frozen at −20 °C for 12 h. This gel is then thawed at room temperature and refrozen to produce a stiffer gelatin matrix, and this can be repeated several times to produce stiffer and stiffer phantoms. Without the addition of DMSO, the scattering coefficient will increase with each cycle as well.[124] This matrix is sensitive to humidity and will likely require storage and preparation under humidity controlled conditions.

8.2 *Dough-Based Phantoms*

While the concept of using dough or Play-Doh™ may appear unscientific, these phantoms have considerable promise because of their ease of construction, ease of use, and long storage time. Composition of these phantoms is based on a recipe for the children's toy, playdough. The standard mixture can be obtained from hundreds of websites, but one such recipe is 250-ml flour, 125-ml salt, 15-ml vegetable oil, 30-ml cream of tartar, and 250-ml water. After mixing flour, salt, and oil, slowly add the water. Heat slowly and stir until dough becomes stiff. When a homogeneous dough ball forms, the mixture is then cooled and left to set. Various absorbers can be easily mixed into the dough as well, with India ink being used successfully. This composition leads to a pliable phantom with $\mu_s' = 1.6\ \mathrm{mm}^{-1}$ at 800-nm wavelength. Repeated mixtures had similar scattering coefficients, and were successfully used in tomography phantom studies (unpublished data). The absorption coefficient appears to track linearly with the addition of higher and higher absorber concentration, as would be expected.

8.3 *Engineered Tissues as Phantoms*

Tissue engineering tools have evolved in the past decade to the point where structures can be created or grown in culture that mimic the structural properties of tissues.[108,125–132] These tissues are most important in situations where the subtle complexities of the biochemistry or thin-layered structure of the tissue are simply not well understood, and therefore cannot be fully reproduced by inert tissue phantoms. This issue is especially important in optics for anisotropic scattering due to structures such as collagen matrix[133] or muscle fibers, or where the layered sequence of tissues affects the light transport into or out of the tissue.

Study of epithelial squamous tissues has been a primary area for this approach,[126] mainly due to the possibility of growing epithelial cells on a thin collagen matrix, with medium flowing above and below the culture. This is called a "raft" culture system, because the cells float on a raft of collagen. The layered structures of squamous epithelium can be spontaneously developed, allowing *in-vitro* study of cellular growth, differentiation, and expression of proteins, so this system is "organotypic" in structure and function. Spectroscopy of these layered structures reveals a biochemical spectrum in which the influence of the layered features of the tissue is unique and not well modeled by a simple phantom.[127,128]

While this field is arguably still in its infancy, the potential is reasonably good for these models to become main stream tools in molecular imaging studies. As engineered tissues become more reproducible between laboratories, this becomes a viable option. Another rationale for the use of these structures is as a replacement for animal studies. Alternatives to animal models are usually welcome in laboratories as long as the model is a true representation.[127,133]

8.4 *Ex-Vivo Tissue*

While *ex-vivo* tissue is not technically a phantom, its widespread use in tissue spectroscopy and imaging merits some mention. Because of the biological complexity of the absorption and fluorescence spectrum of tissue, as well as the complexity of mimicking layered and scattering structures accurately,[55] it is often useful to avoid phantoms and simply use excised tissue.[71] This has been common in light transport studies, especially where the goal has been to study transport and the anisotropy that can occur in structured tissues.[134–136] In diffuse imaging applications, there has been widespread use of chicken or bovine muscle as an *ex-vivo* tissue to test transmission measurements[134,137–141] as a reality check on how the modeling or measurement system performs in real tissue. While phantoms are useful, there is always the concern that the phantom does not really mimic the tissue properties well, and an *ex-vivo* sample can serve as a useful intermediate prior to initiating human or animal studies. Chicken breast tissues are often used, as they are extremely low in blood concentration and have low scattering coefficient values, providing a tissue with exceptionally good light penetration.[141] Bovine muscle or liver offer increasingly darker pigmentation to test penetration, and can be quite homogeneous as well.[41,42]

It is likely true that the bulk scattering coefficient may not be altered significantly when the tissue is excised, but it is certainly true that the absorption due to blood will decrease as the blood volume decreases after removal. Also, energetic changes associated with NADH, FAD, and hemoglobin oxygenation will also change as oxygen is consumed and all tissue will become ischemic within several seconds of removal. Preservation of the tissue oxygen and energy level state can only be achieved with cryogenic freezing of the tissue before, during, or immediately after the removal process.[142–144] For optical therapy studies, excised tissue may preserve the thermal properties of the tissue and offer a good model of nonperfused organs such as the cornea 1.[145] However, the heat convection due to blood flow is lost *ex vivo*, and *ex-vivo* tissue is not a good model for long term heat distribution studies in perfused tissues.

9 Conclusions

This summary of phantoms and phantom materials is an attempt to identify common themes in a field that has a large diversity of applications and methods distributed throughout hundreds of research laboratories. Major problems exist in tissue phantom work due to the lack of uniformity and the lack of a "gold standard" for comparison. However, the strengths and weaknesses of phantom technology are best discussed in terms of the application, as mentioned at the beginning of this work.

For application of phantoms in validating theoretical or experimental systems, optimal choices are based on well calibrated and known quantities, and so microspheres or Intralipid are excellent choices, and allow the use of aqueous emulsions or gelatin-based solid phantoms. This approach has become the *de facto* standard, although there is really no universally accepted method to measure phantom optical properties. Integrating sphere setups for reflectance and transmittance measurement are widely considered the best way to assess phantom properties, but this approach is still prone to discrepancies in the absolute values obtained. Intersystem comparison measurements have been completed by several laboratories, and routinely show at best a 10 to 15% agreement between groups,[146,147] with some measurements having close to 50% disagreement. Clearly the status of repeatability in absorption and scattering properties is far from ideal at the current moment, mainly due to a lack of a gold standard measurement system, a standardized model phantom to compare to, and systematic variation in the preparation of phantoms.

Standardized phantoms to establish the accuracy and repeatibilty of newly developed instruments are an area that should take on a new level of priority in the scientific community, as optical imaging and spectroscopy systems achieve regulatory approval for marketing and clinical use. It is generally agreed that polyethylene or polyurethane phantoms are needed with well-controlled and repeatable optical properties to calibrate and test the performance of such systems. Unfortunately at this time, commercial production and distribution of these phantoms is not commonly available, although several researchers have taken the responsibility of distributing recipes in an attempt to provide uniformity to the field.[146,147] This process needs to continue, and ultimately, as in all clinical radiology systems, a company should produce phantoms with well controlled and known optical properties in different geometries. This is similar to what is available for optical reflectance standards, but would have independent validation as is currently available for CT, mammography, or MRI phantoms approved by the American College of Radiology.

This survey provides the first steps in summarizing progress in the field. The uses of optics are so diverse that it is not likely an exhaustive review, but the references in near-infrared spectroscopy and imaging are comprehensive and should prove useful. Multimodality phantoms are an emerging field, and a significant number of developments are likely in this area as optical imaging and spectroscopy become utilized alongside and within standard clinical imaging systems. This summary should logically be followed by a push toward development of standardized or recommended phantoms based on specific applications, and eventually by commercial products.

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