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# A Recipe for Near Infrared Spectroscopy and Diffuse Correlation Spectroscopy Phantoms with Tunable Optical and Dynamic Properties

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**Abstract:** We present a recipe of combined liquid phantoms for diffuse correlation spectroscopy and near-infrared spectroscopy with well-defined and easily tunable optical and dynamic properties. 
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**1. Introduction:** The combination of near-infrared diffuse optical techniques such as diffuse correlation spectroscopy (DCS) and near-infrared spectroscopy (NIRS) is an area of promising developments since they provide important and complementary information about the composition, structure and hemodynamics of the tissue under investigation [1]. NIRS techniques such as time-resolved spectroscopy (TRS), continuous wave or frequency domain optical spectroscopy allow the estimation of the concentration of chromophores like oxy- and deoxy-haemoglobin, water, lipids, collagen, and give information on cell structure [2]. Diffuse correlation spectroscopy (DCS) measures the microvascular blood flow in the tissue [1]. Combining the information retrieved by NIRS and DCS, it is possible to find important indicators in the diagnosis of several diseases, like for example malignant tumors, which are characterized by an increased metabolism and by the formation of new vessels, resulting in variations of blood flow and components' concentrations [3].

The development of reference standard phantoms with calibrated properties is necessary in order to implement and standardize NIRS and DCS measurements for clinical applications, to check for example the performance of a device and to calibrate it in order to perform absolute measurements [4]. The design of phantoms for NIRS must allow their fabrication with well-controllable optical properties. In literature we can find examples of solid phantoms of epoxy resin or silicon rubber and $TiO_2$ particles [5], and liquid phantoms made of suspension of small fat droplets in water (Lipofundin20% - B. Braun Melsungen AG, Germany -, Intralipid20% - Fresenius Kaby, Germany -) and ink [4]. The requirements for DCS phantoms have some additional constraints. DCS measures indeed the temporal auto-correlation of laser speckles in the turbid medium, which depends on the motion of the scatterers. Phantoms used up to now rely on the Brownian motion of the scatterers as the contrast. In this respect liquid phantoms made of water and Lipofundin20% or Intralipid20% are very common. Previous attempts in changing the particle motion varied from changing the temperature or the viscosity of the medium, or inducing motion by pumps. However, all these strategies to change scatterers motion present problems: viscosity is often changed by adding glycerol or methyl-cellulose, which changes the optical properties of the bulk solution, temperature also changes the viscosity of the bulk medium and can alter the composition of the fat scattering droplets, and, forced motion induced by pumps can lead to unpredictable dynamics.

In this contribution we propose a recipe to fabricate a liquid phantom for near-infrared diffuse optical spectroscopy and diffuse correlation spectroscopy, with well-defined optical and dynamic properties. The phantoms proposed are solutions of water and glycerol, with Lipofundin20% (B. Braun Melsungen AG, DE) as scattering element. The dynamic and scattering properties of the phantoms can be tuned considering that: i) the motion of the scatterers depends on the relative concentration of glycerol in water, which alters the viscosity of the solution [6,7], and ii) the reduced scattering coefficient of the system depends both on the concentration of scatterers and glycerol in the solution [7].

**2. Experiments and discussion:** Glycerol and water mixture allows the alteration of the viscosity of the medium in a controlled manner, but changes the bulk index of refraction [7]. The Brownian diffusion coefficient $D_b$ of a particle in a fluid depends indeed on the viscosity η of the solution [6]; considering two fluids with different viscosities $\eta_1$ and $\eta_2$ , we indeed have $D_{b1}/D_{b2} = \eta_2/\eta_1$. In this way, it is possible to tune the dynamic properties of the solution, simulating tissues with different hemodynamics. However, mixing glycerol alters the reduced scattering coefficient

$\mu_s'$ of the liquid phantom (according to the percentage of the glycerol that is being used) [7] which, in the independent scattering regime, depends linearly on the concentration of the scatterers.

For these reasons we performed TRS and DCS experiments in order to titrate the optical and dynamic properties of glycerol and water with Lipofundin as scattering element. We prepared different concentrations of glycerol in water ranging from 0% to 30%. For each bulk solution we have gradually increased the concentration of Lipofundin (scatterers) from 0.1% to approximately 4%. By multi-wavelength TRS experiments and DCS experiments, we measured the reduced scattering coefficient, the absorption coefficient and the Brownian diffusion coefficient of different solutions, developing a simple recipe in order to obtain the requested optical and dynamic properties mixing water, glycerol and Lipofundin.

Figure 1a shows the value of the reduced scattering coefficient retrieved, at a fixed wavelength of 785 nm, by varying the concentration of Lipofundin and glycerol. These results show that, for a given glycerol concentration, the increase of the reduced scattering coefficient is linear with the concentration of Lipofundin in the solution. Furthermore, increasing the concentration of glycerol the slope of the lines decreases almost linearly. This decrease of $\mu_s'$ per unit of Lipofundin concentration depends on an increase of the refractive index of the water/glycerol solution when the glycerol concentration increases. This leads to a decrease of the refractive index contrast between the scatterers and the solution in which they are suspended, reducing the overall scattering strength. In addition, the data acquired for different wavelengths show a decrease of the reduced scattering coefficient with increasing wavelength, as predicted by the 'empirical' Mie relation ($\mu_s' = A\lambda^{-B}$). The TRS measurements performed allow us to retrieve the absorption coefficient $\mu_a$ : we demonstrated that absorption is independent both from glycerol and Lipofundin concentrations.

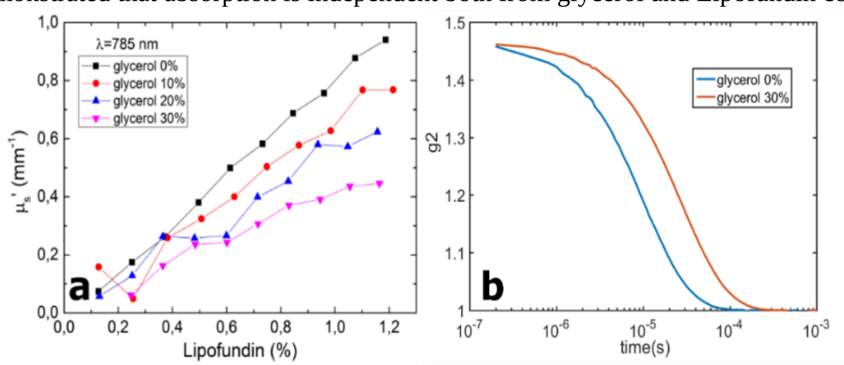


**Figure 1** a) Reduced scattering coefficient as a function of Lipofundin concentration at different glycerol concentrations; b) Intensity temporal autocorrelation curves ($g_2$) for different glycerol concntrations and same absorption and reduced scattering coefficient

We have furthermore performed DCS experiments, measuring the Brownian diffusion coefficient $D_b$ for solutions with glycerol concentration in water of 0%, 20% and 30% and varying Lipofundin concentration.Figure 1b reports an example of the intensity temporal autocorrelation curves acquired for solutions with different glycerol concentration but same optical properties, showing that increasing the glycerol concentration reduces the dynamics of the scatterers. The measurements done demonstrate that $D_b$ depends only on the glycerol concentration in the solution, without any dependence on Lipofundin concentration, and the ratio of measured$D_b$ of different solutions are equal to the inverse of the ratio of the viscosities of the solutions, as the theory of Einstein predicts ($D_{b1}/D_{b2} = \eta_2/\eta_1$).

In conclusion the results of the titration study performed allow the definition of a simple recipe to fabricate liquid phantoms with well-determined optical properties (absorption and scattering coefficients) and dynamic properties (Brownian diffusion coefficient) that can be used at the same time for near-infrared spectroscopy and diffuse correlation spectroscopy.

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