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Low-cost fabrication of PPIX liquid phantoms for use in fluorescence measurements

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ABSTRACT

This article presents a liquid phantom technology that simulates the fluorescent properties of protoporphyrin IX (PPIX). A technology was developed for the isolation of PPIX from dark egg shells with a final concentration close to the real values in human tissues. A comparative analysis of the transmittance coefficient of the manufactured phantom as well as the fluorescence spectra measured through the combined use of a CCD spectrometer and a hyperspectral camera is presented as results.

Keywords: optical phantom, fluorescence imaging, fluorescence spectroscopy, protoporphyrin IX.

1. INTRODUCTION

Current methods of optical medical diagnosis, in particular fluorescence analysis methods, allow for a high degree of accuracy in differentiating between areas of tumor transformations. Fluorescence imaging demonstrates considerable sensitivity in detecting the presence of pathological changes, including malignant changes, in biological tissues. However, the calibration and verification of such imaging systems requires the development of optical phantoms of biotissues with known and quantified optical and mechanical properties.

Currently, to simulate the properties of biotissues, various groups of scientists have developed and proposed a variety of optical phantoms for both imaging methods[1-4] and spectroscopic methods[5, 6]. The manufactured phantoms can be different in structure, depending on the specific research tasks, where for fluorescence imaging systems solid elastic samples with stable mechanical and temperature properties are most preferable, as for example presented in our previous work[7]. However, to reproduce fluorescent properties, the simplest variant is liquid optical phantoms consisting, for example, of aqueous colloidal solutions, with the addition of absorbing dyes, as well as substances having pronounced intrinsic fluorescence when excited in the desired wavelength range[8, 9]. Such phantoms allow the use of buffer solutions with the required pH value as a matrix material to provide an environment compatible with the natural structure of organic molecules that simulate certain properties of a biotissue[10].

Thus, the aim of this work is to develop a liquid phantom that simulates the fluorescence of PPIX. The mechanisms of selective accumulation of protoporphyrins in tumors by binding to lipoproteins and collagen are known[11], which makes their fluorescent analysis as markers of malignant structures possible. It is already known to create liquid phantoms with the addition of dimethylformamide as a solvent and powder PPIX, which, although it meets the chemical requirements, is also expensive[12]. So, the actual task is to optimize the technology of liquid phantoms with the development of the technology of PPIX extraction from cheap available raw materials.

2. MATERIALS AND METHODS

To produce a liquid phantom with the addition of PPIX, a technology was developed for its isolation from dark egg shells. PPIX is known to be the main pigment responsible for the brown color of eggshells[13]. Thus, the first step to isolate PPIX was to use carefully washed, dried, and crushed eggshells that were mixed with 1M HCl. The reacting mixture was incubated until carbon dioxide was no longer released, after which it was passed through a paper filter. Ethyl acetate was added to the filtrate and shaken to transfer the protoporphyrin to the organic layer. The resulting emulsion was centrifuged, then the upper layer was selected, which was evaporated on a water bath in a flask until the

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solvent was completely removed. The resulting precipitate was dissolved in DMCO. As a result, a solution containing PPIX was obtained at a concentration of approximately 150 μmol/L, which corresponds to real values in human blood[14] and correlates with concentrations used by other scientific groups in the manufacture of similar phantoms (Figure 1)[15].

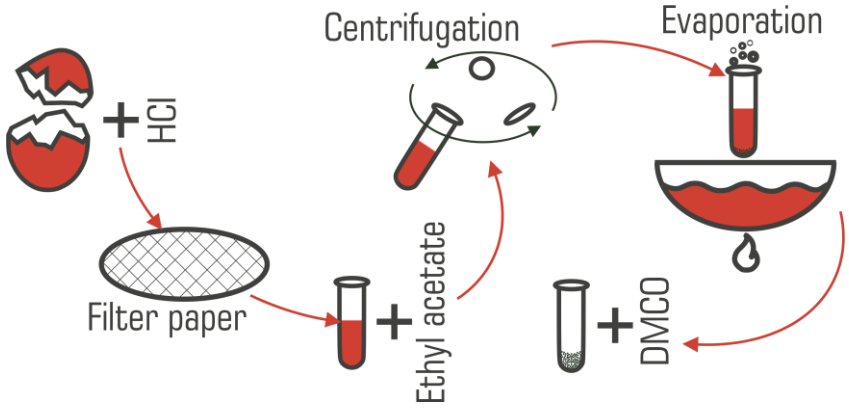

Figure 1. Schematic representation of the main phantom manufacturing steps, containing PPIX.

To confirm the optical characteristics of the fabricated solution containing PPIX, its diffuse transmittance was measured using a spectrophotometer with an integrating sphere (Shimadzu, Japan)[16] shown in Figure 2. Based on the results, optical characteristic of PPIX are clearly visible in the prepared solution, which also correlates with the absorption peaks (on 510, 530, 580 and 630 nm) shown for example in this papers[17, 18].

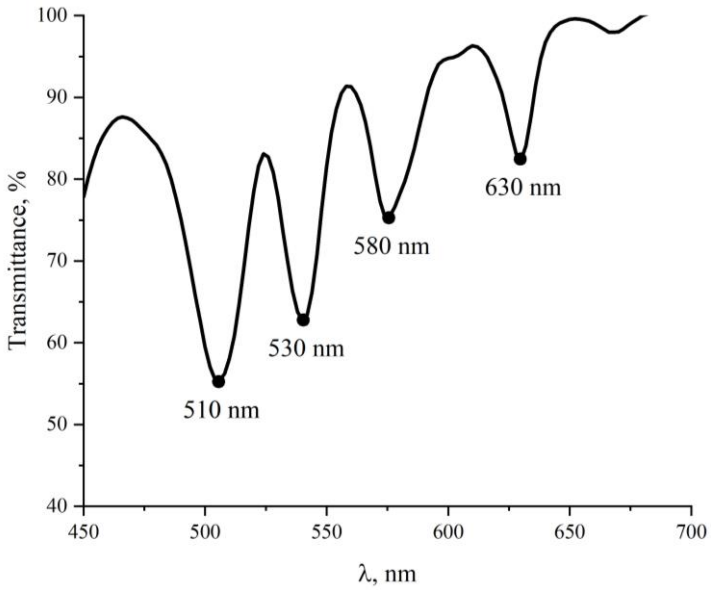

Figure 2. PPIX liquid phantom transmission spectra.

To confirm the fluorescence properties of the manufactured phantom, measurements were performed using an experimental setup that combined a hyperspectral camera and a CCD spectrometer, shown in Figure 3. For measurements, the prepared solution with PPIX was applied to a coverslip.

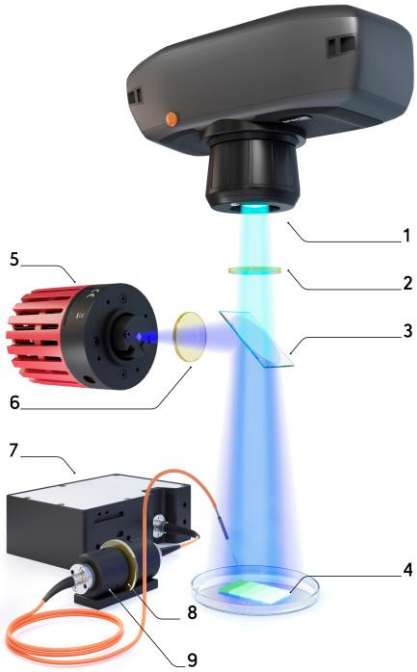

Figure 3. Experimental setup for hyperspectral imaging and fluorescence spectroscopy: 1 – hyperspectral camera; 2 – long-wave emission filter; 3 – dichroic filter; 4 – samples being characterized; 5 – LED source; 6 – bandpass filter; 7 – CCD spectrometer; 8 – long-wave emission filter; 9 – filter holder.

In this setup, emission from a 450 nm M450LP1 LED source (Thorlabs, USA) passes through the MF445-45 bandpass filter (Thorlabs, USA). The emitted band of radiation passes through a MD416 dichroic filter (Thorlabs, USA) and is directed to the liquid phantom, exciting the fluorescence of PPIX. Back-reflected radiation from the source is removed by a dichroic filter and a 500 nm filter FELH0500 (Thorlabs, USA). The remaining fluorescence emission of the sample is recorded by a Specim hyperspectral camera (Spectral Imaging Ltd., Finland) in the spectral range of 400-1000 nm. In the fluorescence spectroscopy channel, spectra are recorded using an optical fiber FLAME-T-VIS-NIR-ES CCD spectrometer (Ocean Insight, USA) in the spectral range of 350-820 nm.

3. RESULTS AND DISCUSSION

As a result, hyperspectral fluorescence images of the manufactured phantom containing PPIX were obtained, shown in Figure 4.

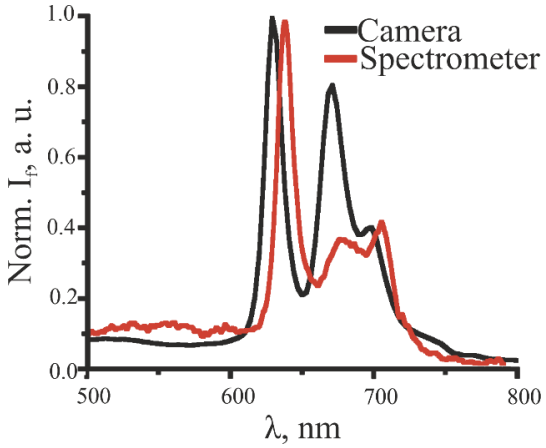

Figure 4. Normalized fluorescence spectra of the optical phantom obtained with a hyperspectral camera and a CCD spectrometer.

Images were recorded with a camera exposure time of 500 ms, and average irradiance of 0.5 mW/cm^{-2}. In the image analysis, the fluorescence intensity values were averaged within the area of interest (over the entire area of the phantom). The fluorescence spectra recorded by the CCD spectrometer were averaged over 3 spectra to compare with the data obtained by the hyperspectral camera.

The fluorescence spectra of the manufactured phantom were calibrated to the PPIX maximum fluorescence value, shown in Figure 4. The results clearly show characteristic fluorescence peaks of PPIX at wavelengths of 635, 675, and 715 nm, which also correlates with the protoporphyrin data we obtained in our past research[19]. The difference in the spectra form and the shift of the fluorescence peaks, between the measurement channels, can be explained by their different diagnostic volume, as well as by the influence of temperature effects. Due to the fact that the measurements of the two channels were separated in time, the thermal quenching processes characteristic of PPIX in the physiological temperature range may possibly[20]. As we suppose, the influence of light as processes such as photooxygenation and photodegradation on PPIX in this study should be minimal, since external illumination was excluded as much as possible at all stages of the presented work.

4. CONCLUSION

This article presents a technique for manufacturing an optical phantom that simulates the fluorescence of PPIX. On the basis of obtained results, the presented liquid phantom, with confirmed scattering and fluorescence properties, indeed allows reproducing the skin fluorescence spectra in the specified wavelength range with a sufficiently high accuracy. Application of the developed optical phantom will make it possible to test, standardize and calibrate systems for fluorescence imaging, as well as fluorescence spectroscopy instruments coupled with a fiber-optic probe. However, the presented liquid phantom is affected by temperature effects and does not meet the conditions of stationarity. Accordingly, further work will be directed to the development of a solid-based phantom containing PPIX isolated by the presented technology. As an improvement and further development of the presented in this work, we plan to create optical phantoms with varying concentrations of PPIX in the range characteristic of tumor changes in the tissue.

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