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Object reconstruction in scattering medium using multiple elliptical polarized speckle contrast projections and optical clearing agents

Tomer Moshe [a], Michael A. Firer [b], David Abookasis [a,*]

[a] Department of Electrical and Electronics Engineering, Ariel University, Ariel 40700, Israel
[b] Department of Chemical Engineering, Ariel University, Ariel 40700, Israel

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ABSTRACT

In this paper, we present a hybrid method for improving the imaging quality of objects obscured within a scattering environment by combining multiple elliptical polarized speckle contrast projections with the use of optical clearing agents (OCAs). Elliptically polarized light enables the probing of subsurface volumes, where OCAs decrease light scattering while increasing photons' penetration depth through the medium. Experiments were conducted on object sample and prostate cancer cells embedded within ex vivo biological samples (chicken breasts) in reflection configuration. After immersion with OCAs, the medium was irradiated with an elliptically polarized laser beam and multiple polarized speckled images obtained from a lens array were first converted to speckled contrast images and then processed using a self-deconvolution shift-and-add algorithm. The conversion to contrast images and multiple perspectives acquisition was found to emphasize contrast. Analysis of image quality indicated improvement in object visualization by the combination of elliptical polarization and OCAs. This enhanced imaging strategy may advance the development of improved methods in biomedicine field, specifically biomedical tomography.

1. Introduction

A variety of noninvasive and noncontact optical imaging methods have been developed and implemented for imaging turbid biological materials in the visible and near-infrared (NIR) regions over the past three decades, each of which has its own unique set of advantages and disadvantages [1,2]. These methods face the common obstacle of image degradation and blurring which limit the ability to identify objects with high resolution and contrast [3]. Currently, biomedical optical tomography (sectioning) is focus of development towards the goal of improving image quality and resolution of embedded targets, despite the repeated light scattering during both light propagation and due to the variable medium thickness [4,5]. Generally speaking, light scattering limits the imaging depth of most optical imaging techniques by decreasing the number of photons reaching a particular depth before significant scattering. To date, different approaches both with and without exogenous agents and with complex optical and/or computational schemes have been implemented that attempt to overcome the effects of photon scattering and to improve penetration depth [6–12], but there remains much room for improvement. In this work, we present a strategy that synergistically optimizes the capabilities of both light polarization and optical clearing methods.

Recently we suggested a relatively straightforward approach for enhancing the imaging of biological objects hidden in a diffuse medium (chicken breasts) in a transmission-mode using speckle contrast projections together with optical clearing agents (OCAs) [13]. The results showed improvement in image resolution with a more than two-fold increase in contrast-to-noise ratio in comparison to images obtained without OCAs and speckle contrast projections. While the technique is promising for imaging within scattering media, we believe that further contrast improvement and depth information can be achieved. Thus, in this study we present an improvement utilizing elliptically polarized light to simultaneously increase image quality and to perform imaging at different depths (optical sectioning). In addition, to be comparable with most of optical imaging and tomography modalities our previous mode of operation is now based on reflectance geometry rather than transmission, improving its functionality for biomedical applications.

It is well established that when NIR light is propagated in dense random media such as biological tissue; photons will mainly undergo multiple scattering events which arise from the non-uniform temporal and spatial distribution of the medium's refractive index [14]. When resolving an image, this effect limits performance leading to low spatial resolution and contrast. Attempts to image deeper layers of tissue therefore result in images of low quality and limited clinical utility because of a poor signal-to-noise ratio (SNR). Therefore, the suppression of photon scattering is required in order to improve image quality. Several techniques have been studied to this end, one of which

* Corresponding author.
E-mail address: davida@ariel.ac.il (D. Abookasis).

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temporarily decreases light scattering and improves light penetration. This optical clearing method produces an equalization (matching) of refractive indices between tissue components, alongside tissue dehydration and morphology change of collagen fibers [15,16]. Optical clearing is usually obtained by administration of non-reactive, biocompatible agents such as glucose, glycerol, polyethylene glycol (PEG), trazograph or similar pharmaceutical preparations. Indeed, OCAs enable deeper penetration through biological tissues and improve resolution during optical imaging by reducing multiple scattering but there remain four main issues required for their optimization: (i) the optimal OCA type and concentration to be used, (ii) immersion time, (iii) method of OCA application, and (iv) effects of OCA on tissue function. Proper optimization of all parameters is beyond the scope of this work but has been thoroughly investigated elsewhere [16–19]. In this study, we chose to use a relatively new optical clearing method based on a recently introduced mixture of liquid paraffin and glycerol [20]. This preparation significantly enhances images of rat skin in vitro and human skin in vivo when employed in optical coherence tomography (OCT). Although the use of OCAs is somewhat controversial, in recent years they have been widely applied in various optical imaging platforms to enhance imaging depth and the contrast of hidden objects [21–24].

While OCAs improve imaging depth and contrast, imaging depth quality can be further improved by use of polarized light. Although tissue multiple scattering randomizes incident polarization states, the degree of polarization can be observed under several circumstances. Subtraction between polarization states is usually conducted to separate the imaging signal according to tissue depth, known as polarization gating imaging [25–27]. Several groups have shown that optical polarization methods are beneficial to image reconstruction and improve image resolution at the surface and at different depths [28–33]. In addition, it was reported that linear and circular polarization states propagate differentially in biological tissues [34]. Circularly polarized light enables deeper layers to be probed whereas linearly polarized light is more sensitive to surface layers, since polarization of circularly polarized light is maintained through a larger number of scattering events than that of linearly polarized light.

Elliptically polarized light exhibits properties lying between those resulting from circular or linear polarization. Recently, Da Silva et al. reported the use of polarization gating imaging with elliptically polarized light [35,36]. They demonstrated the possibility of imaging differential tissue depths as a function of the polarization ellipticity. The novelty of elliptical light polarization lies in its ability to produce images free of surface and deep-volume scattering contaminations. In the current work, based on Da Silva's setup and signal processing approach, we further improved the setup by introducing a multiple imaging channel (lens array) in front of the camera and by including signal processing during object reconstruction based on lowpass spatial filtering obtained with speckle contrast algorithm [37,38] and self-deconvolution shift-and-add algorithm [39]. In addition, a laser light source was used in place of the incoherent light illumination (white halogen lamp) utilized in the Da Silva's setup. The present combination of elliptical laser light polarization, OCAs, multiple object viewpoints and reconstruction imaging algorithm distinguishes this study from the work of Da Silva et al. and other groups.

The next section describes the experimental configuration and material. Section 3 presents the results accompanied by discussion, demonstrating the efficacy of the combined technique. Conclusions follow in Section 4.

2. Material and methods

2.1. Sample and medium

As part of an unrelated project, a tumor mass was produced by the subcutaneous injection of human PC-3 prostate cancer cells into an immunocompromised mouse of strain nu/nu. After 21 days, the tumor was excised and a mass of dimension $8 \times 5 \times 4$ mm^3 was embedded between two layers of fresh chicken breast (an opaque, highly scattering layer) with overall thickness of ~8 mm; the top layer of the breast was ~2 mm and the bottom layer was ~6 mm. Fig. 1 shows a photograph of the sample before (Fig. 1(a)) and after it was covered with the upper breast tissue layer (Fig. 1(b)). Dimensions are given in the figure. The thickness of each tissue was measured using a digital micrometer (Mitutoyo); several measurements were taken at random locations across the tissue and average was used to determine the sample thickness. The sample volume V (in centimeters) was estimated as $V=(4\pi/3)\times[(X+Y+Z)/6]^3$ where X, Y, and Z are the three respective orthogonal dimensions (Fig. 1(a)). The optical properties, namely reduced scattering and absorption coefficients, of the chicken breast at 630 nm are approximately 0.25 mm^{-1} and 0.015 mm^{-1}, respectively [40]. In biological tissues, within the range of 600–1000 nm, the reduced scattering coefficient is greater than the absorption coefficient which is true in this study ($0.25/0.015 \approx 17$). It is true that it is easier to work with tissue-like phantom with known optical properties [35,36]. However, we felt

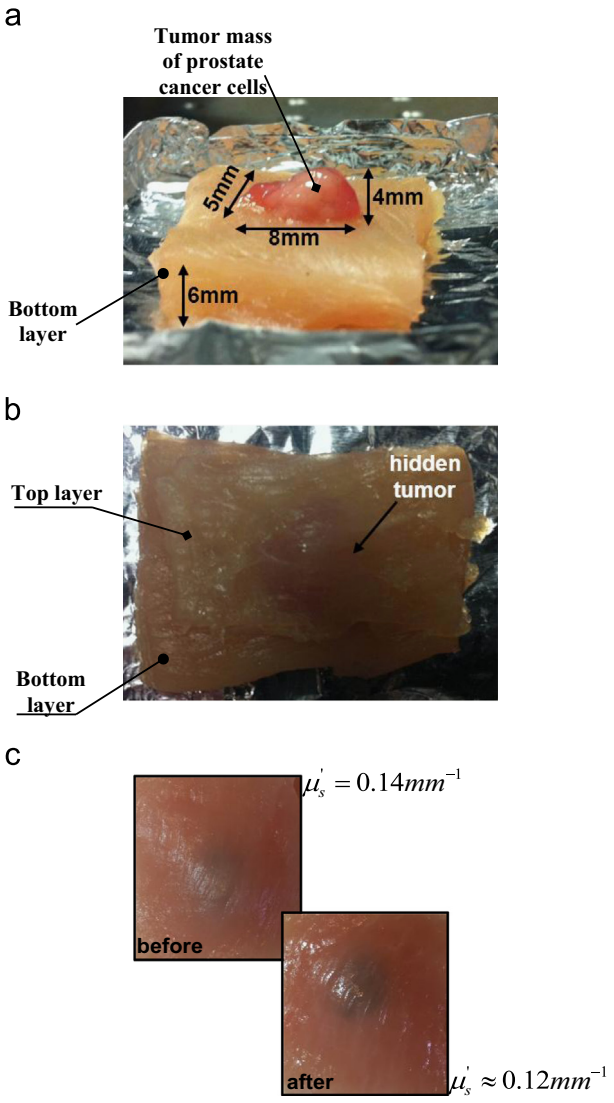

Fig. 1. Photograph of the prostate cancer cell tumor mass before (a) and after it was embedded inside the chicken breast (b). Dimensions are given in the figure. (c) Representative photographs of the tissue before and 50 min after application of OCA. Reduced scattering coefficients (μ_s') were taken from Ref. [13].