Diffuse light suppression of back-directional gating imaging in high anisotropic media

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Abstract. We experimentally demonstrate that back-directional gating in an imaging setup can potentially remove unwanted diffuse light to improve the contrast of an object embedded in a high anisotropic surrounding medium. In such back-directional gating, the high anisotropic property of the surrounding medium can serve as a waveguide to deliver the incident light to the embedded object and to isolate the ballistic or snake-like light backscattered from the object in a moderate depth. We further discuss the effects of back-directional gating in the image formation in terms of the image resolution and the depth of field. Although backscattering detections of biological tissue have recently received considerable attention, we, for the first time to our knowledge, show its potential advantage for the contrast improvement in high anisotropic media. *© 2009 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.3156801]

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Light elastically scattered from scattering media including biological tissue can be classified into three major components: ballistic light, snake-like light, and diffuse light. The ballistic or snake-like components are usually masked by the multiple scattered diffuse component, which generates complicated scattering paths and deteriorates contrast in images. Recent advances in deep tissue imaging are based on the separation of the ballistic or snake-like light from the diffuse light to improve the image contrast. Among various approaches, the simplest method to select the ballistic or snake-like light is directional gating (or spatial filtering), because it propagates along the direction of the incident light, whereas the diffuse light exits the medium at oblique angles with respect to the optical axis.[1] Such directionality of the ballistic or snake-like light implies that the anisotropy factor of the scattering medium may play an important role in the image formation of an embedded object. Given that one of the unique optical properties of biological tissue is the tendency that light is scattered in the same direction with respect to the incident direction,[2] image formation by back-directional gating could potentially remove complicated scattering paths that would otherwise deteriorate the contrast in images. Although imaging of light backscattered from biological tissue has recently received considerable attention to probe structural alternations at subcellular levels,[3,4] its potential advantage for image contrast improvement in high anisotropic media such as biological tissue has not been fully investigated yet.

In tissue optics, the overall directional tendency of the scattered light is represented by the anisotropy factor g. g is defined as the average cosine of the scattering angle: $g = \langle \cos \Theta \rangle = \int_{4\pi} p(\Theta) \cos \Theta d\Omega$, where $p(\Theta)$ is the phase function that describes the angular distribution from a single scattering event, and Θ is the scattering angle with respect to the forward direction. Because most biological tissue including major organs is highly anisotropic with $g > 0.8-0.9$, the light scattered from biological tissue will slightly deviate from its original incident direction during relatively low-order scattering events for moderate depths. The effects of the high anisotropic property resulting from subcellular scatterers or aligned microstructures are often considered to hamper accurate imaging or tomography.[5] Indeed, the potential advantages of the high anisotropic property in directional gating in the backward direction have not been investigated. In this letter, we demonstrate that simple back-directional gating imaging can be an effective method to significantly remove unwanted diffuse light and thus to improve the contrast of an object embedded in a high anisotropic scattering medium. To take advantage of the high anisotropic property, we implement an optical imaging setup that allows imaging of a relatively large area in several solid angles of backscattering. We further investigated the effects of back-directional gating on the image formation in terms of the resolution and the depth of field (DOF).

To exploit the high anisotropic property for the suppression of unwanted diffuse light in back-directional gating image formation, we built a relatively large area imaging setup, as shown in Fig. 1. In brief, a beam of broadband continuous wave light from a 75-W xenon arc lamp was highly collimated by a 4-f lens system in the delivery arm (divergence $=0.1$ deg) and delivered onto the sample with the illumination diameter of 15 mm. To avoid the specular reflection from the sample surface, the incident beam was orientated at an angle of 15 deg normal to the sample surface. An interference filter was placed in the delivery part to select the central wavelength of 600 nm with the spectral width of 70 nm. The light backscattered from the sample was collected by a sequence of a beamsplitter and another 4-f lens imaging system and a CCD camera. The pixel size of the CCD camera was $13\ \mu\text{m} \times 13\ \mu\text{m}$, and the imaging area was approximately $10\ \text{mm} \times 10\ \text{mm}$. Importantly, by changing the aperture size (A in Fig. 1) in the 4-f imaging system in the detection arm, images formed by different solid angles θ from 2 deg to 6 deg in the backward direction were recorded onto the CCD camera without any magnification.

In order to vary the anisotropy factor of scattering media, we used aqueous suspensions of different sizes of polystyrene microspheres.[6,7] Our samples consisted of a United States Air Force (USAF) contrast target (Edmund Optics, Inc.) embedded in the aqueous suspension; the USAF contrast target was placed in different optical thicknesses below the sample surface, as shown in Fig. 1. We calculated the optical properties of the scattering media using Mie theory.[6,8] For the aqueous

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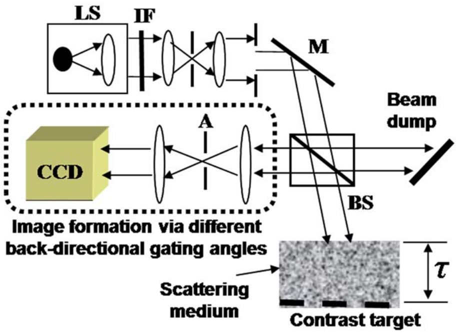

Fig. 1 Schematic of the back-directional gating imaging system. LS: light source; IF: interference filter; M: mirror; BS: beamsplitter; and A: aperture. By varying the aperture size in the 4-f imaging system, images are formed by different backscattering angles. Our sample consists of the USAF contrast target embedded in the aqueous suspension of the microspheres.

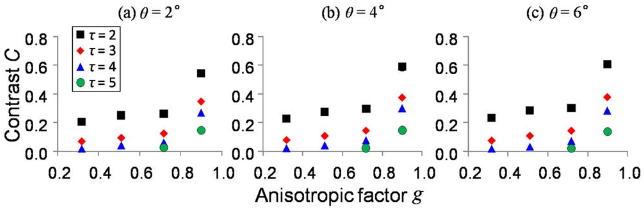

Fig. 3 The contrast of the target embedded in the scattering media of the different anisotropy factors and the optical thicknesses. The error bars represent the standard deviation.

suspensions, we used four different sizes of microspheres of the nominal diameter of 0.19 μm (Bangs Laboratories, Inc.), 0.24 μm (Bangs Laboratories, Inc.), 0.33 μm (Thermo Fisher Scientific, Inc.), and 0.76 μm (Bangs Laboratories, Inc.), which corresponded to four different anisotropy factors of $g=0.32$, 0.51, 0.72, and 0.90 at $\lambda=600$ nm, respectively. In terms of the anisotropy factor, the scattering medium of $g=0.90$ can be considered to be close to most biological tissue. The target embedded in the scattering medium was placed on the conjugate imaging plane of the CCD camera. We changed the optical thickness τ between the medium surface and the contrast target, as shown in Fig. 1. We varied $\tau=2$ to 5 by changing the scattering coefficient of light in the medium μ_s with the fixed physical thickness of $T\sim 5$ mm ($\tau=\mu_s T$). We also imaged the contrast target through the different scattering media using three backscattering angles ($\theta=2$ deg, 4 deg, and 6 deg).

Figure 2 shows representative images of the contrast target embedded in two different anisotropic scattering media of $g=0.90$ (a) and $g=0.72$ (b). In Fig. 2, the images were formed by the solid angle of $\theta=2$ deg in the backward direction, and τ between the medium surface and the contrast target was 4. Interestingly, the contrast target was significantly more visible through the scattering medium of $g=0.90$ than that of $g=0.72$. This strongly indicates that a subtle change in the anisotropic property of the surrounding medium can significantly affect the image contrast of an embedded object. Given that biological tissue is highly anisotropic, we hypothesized that the image formed through the back-directional gating in high anisotropic scattering media can isolate the ballistic and snake-like light by significantly removing the diffuse light. Our hypothesis was based on the idea that a high anisotropic scattering medium can enhance back-directional gating, because it can deliver the incident light to an embedded object along the direction of the incident light, and because it can guide the ballistic and snake-like light reflected from the object to scatter along the opposite direction of the incident light in a moderate depth.

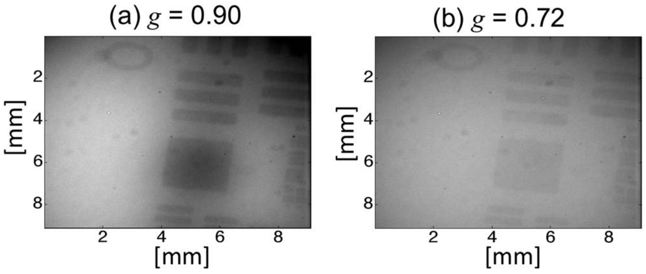

Fig. 2 Representative images of the contrast target embedded in the scattering media of two different anisotropic factors with the optical thickness kept constant as $\tau=4$. The images were formed by the solid angle of $\theta=2$ deg in the backward direction. (a) The surrounding medium has the anisotropy factor $g=0.90$. (b) The surrounding medium has the anisotropy factor $g=0.72$. The slight increase in the anisotropy factor of the surrounding media provides more visibility of the contrast target due to back-directional gating.

We quantified the image contrast of the target embedded in scattering media of several g through a few different τ. We defined the image contrast as $(I_{\text{bright}}-I_{\text{dark}})/[(I_{\text{bright}}+I_{\text{dark}})/2]$, where I_{bright} and I_{dark} were the average intensities of the bright area and the dark area, respectively, and the denominator represented the overall background intensity. Figure 3 clearly shows that the image contrast is improved as g increases. In particular, when $g\leqslant 0.72$, the image contrast remained relatively unchanged, although there was a small gradual increase in the contrast over g. More interestingly, when g reached 0.90, the image contrast significantly increased, and the three different backscattering collections showed the similar improvement of the image contrast. This result demonstrates that the image formation by the back-directional gating ($\theta\leqslant\sim 6$ deg) can effectively improve the contrast of an embedded object when the surrounding scattering medium is highly anisotropic. Because in low anisotropic media, light is uniformly scattered to all directions, the back-directional gating does not isolate the ballistic or snake-like light. On the other hand, high anisotropic surrounding media in directional gating can serve as a waveguide to deliver the incident light to the embedded target and to isolate the ballistic or snake-like light scattered from the target. As a result, the directional gating in the backward direction can be a simple and effective method to remove the diffuse light that otherwise deteriorates the image contrast. In addition, Fig. 3 shows that when τ is small, the contrast is high because of the insignificant diffuse light through the scattering medium, as expected.

We further investigated the contrast improvement derived from the high anisotropic property of the surrounding medium over several τ. In Fig. 4, we plot the ratio of the contrast of $g=0.90$ to that of $g=0.72$ over τ of the surrounding scattering medium to study the rate of the contrast improvement of

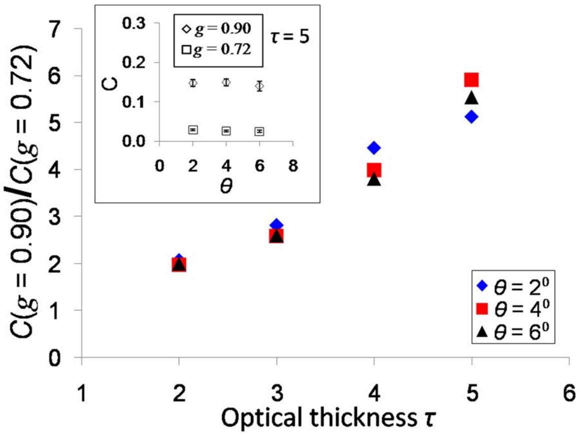

Fig. 4 The ratio of the image contrast through the surrounding medium of $g=0.90$ to that through the surrounding medium of $g=0.72$ over several τ. The relative image contrast improvement significantly increases with τ. The inset shows that the image contrasts by different backscattering angles ($\theta \leq \sim 6$ deg) are not significantly affected when the surrounding media have relatively high $g=0.72$ to 0.90. As τ increases, our back-directional gating is more effective to separate the ballistic or snake-like light from the diffuse light that would deteriorate the contrast, potentially offering an ideal imaging platform for relatively large tissue areas.

back-directional gating imaging ($\theta=2$ deg to 6 deg). Surprisingly, Fig. 4 shows that the relative contrast improvement increased as τ of the surrounding scattering medium increased. When $\tau=2$, there was a twofold increase in the ratio. However, when $\tau=5$, the relative contrast improvement was approximately 5.5. To test whether different back-directional angles in the range of $\theta=2$ deg to 6 deg significantly affect the contrast in such high anisotropic media, we used an analysis of variance (ANOVA) for the three different angles when $\tau=5$. There were no statistically significant differences with p-value$=0.71$ and p-value$=0.25$ for $g=0.90$ and $g=0.72$, respectively. Overall, the result demonstrates that our back-directional gating imaging ($\theta \leq \sim 6$ deg) in a relatively thick surrounding medium with a high anisotropy factor is a simple, yet effective, tool to significantly suppress the diffuse light.

Because the backscattering angle in our imaging system, which was determined by the aperture size in the 4-f imaging system, defined the numerical aperture (NA) of the detection arm, we also investigated the level of detail in the contrast image of back-directional gating imaging. To estimate the image resolution of the contrast target embedded in different anisotropic scattering media, we obtained a line spread function (LSF) using a modified knife-edge method.[9] Using this method, we estimated the image resolution from the LSF calculated from a skewed edge on the contrast target and we used the full width at half maximum (FWHM) of the LSF as our image resolution of the contrast target. From the images that we analyzed for the contrast improvement, the image resolutions of the target embedded in the surrounding scattering medium of $\tau=2$ to 3 were 6 to 8 pixels (pixel size$=13$ μm), and our back-directional gating angles ($\theta \leq \sim 6$ deg) played a minimal role on the image resolution of the embedded target. The image resolution of the target also did not depend strongly on the anisotropy factor of the surrounding medium in the relatively moderate depths. Back-directional gating imaging mainly reduced the overall background intensity, increasing the image contrast as discussed. Thus, moderately thick anisotropic media minimally affect the image resolution of the embedded object in our back-directional gating of relatively large area imaging. In addition, in our imaging setup, back-directional gating imaging provided not only the contrast improvement in high anisotropic media, but also a large DOF. We used a DOF target (Edmund Optics, Inc.) to estimate the DOF of different backscattering angles. The DOFs of $\theta=2$ deg, 4 deg, and 6 deg were approximately 7, 4, and 3 mm, respectively. Therefore, such small backscattering angles yield large DOFs and subsequently maintain the image quality over a large field of view.

In conclusion, we experimentally demonstrate that in back-directional gating imaging, a high anisotropic surrounding medium can serve as a waveguide to deliver the incident light to an embedded object and to isolate the ballistic or snake-like light backscattered from the object in a moderate depth. Such back-directional gating can be used to remove the diffuse light that otherwise deteriorates the image contrast of the embedded object. Since most biological tissue has a high anisotropic property, back-directional gating imaging of biological tissue has the potential to visualize an object such as an organ or a tumor in small animals through a moderate thickness of tissue in a relatively large area. Moreover, our findings can be easily implemented into other imaging platforms such as wide-field imaging or tomography.

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