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Improving depth sensitive fluorescence spectroscopy with wavefront shaping

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ABSTRACT

Depth sensitive optical spectroscopy detects optical spectra from different layers in layered samples, revealing crucial information about the samples, for example, and the progress of epithelial cancer. In depth sensitive fluorescence measurements, multiple light scattering in tissues significantly degrades the depth sensitivity to a subsurface target layer. To address this issue, feedback based wavefront shaping led by guide stars can be used to refocus light to increase the depth sensitivity to a target layer. However, the low target to background ratio caused by multiple scattering in tissue leads to weak fluorescence measurement from the target layer inside. In this study, we demonstrate that by using feedback based wavefront shaping, we can increase the signal contribution from the target and suppress that from the background region in tissue-like scattering phantoms. After wavefront optimization, the signal from the target can increase by quite a few times. Feedback based wavefront shaping can be very useful in depth sensitive fluorescence spectroscopy for the characterization of layered structures such as epithelial tissues.

Keywords: spectroscopy, wavefront shaping, spectral filter, scattering medium

1. INTRODUCTION

Depth dependent distribution of endogenous fluorophores and other intrinsic biomolecules reveals important diagnostic information about the progress of diseases such as cancer in epithelial tissues thus is critical to early diagnosis [1]. Depth-sensitive optical spectroscopy has been proposed to detect optical spectra from different layers in such layered tissues. Early optical probes based on fiber optics for depth-sensitive optical spectroscopy [2, 3] are rugged and convenient for in vivo hand-held measurements but suffer from low spatial resolution due to the divergence nature of light coming out of optical fibers. Similar techniques such as spatial offset Raman spectroscopy [4] have been applied to Raman spectroscopy. Later, optical probes based on an objective lens [5] were proposed to implement non-contact measurements to reduce the uncertainty in contact pressure and spectral acquisition, which also increase the spatial resolution since the light can be focused by the objective lens. Moreover, it requires the movement of the objective lens multiple times to collect optical spectra from a range of depths. To speed up depth sensitive measurements, our group proposed to use an axicon lens [6] to create a focal line covering a range of depths and detect optical spectra from all the depths in one measurement. While the above techniques for depth sensitive optical spectroscopy have made a significant improvement in reducing uncertainty and speeding up measurements, the depth sensitivity that can be achieved by these techniques will degrade dramatically with depth due to multiple light scattering in tissues. Various methods have been proposed to overcome the issue of multiple light scattering in the imaging of turbid media such as tissues. Optical coherence tomography (OCT) and related techniques [7] take advantage of coherence gating and can image up to 2 mm, but it does not work for fluorescence or Raman spectroscopy. Recently, wavefront shaping [8] and related techniques including transmission matrix measurements, etc. have emerged as a promising technique to provide an alternative for imaging through a turbid medium and focusing light in turbid media using linear or nonlinear optical techniques. Also, feedback based wavefront shaping is known for its versatility. Our results show that the depth sensitivity to fluorophores in the bottom layer of a two-layered tissue phantom in a reflection setup can be enhanced significantly with proper wavefront optimization compared to the situation without such optimization.

2. SIGNAL ENHANCEMENT

2.1 Backscattering Signal Enhancement

To demonstrate the ability of light refocusing, we use TiO_2 as a tissue-like phantom to create highly scattering medium. Multiple scattering will cause weak signal from the focal point to the detector. Fortunately, by using SLM to modulate light properly, we refocus the light into the target layer. As the result, it shows that wavefront shaping implemented by modulating the light with SLM could refocus in scattering medium to increase the backscattering signal inside the medium up to 6 times (Figure 1). Next, to address the problem of a thick sample with a different structure like the different layer of epithelial tissue, we test the technique by making two-layer phantom.

Figure 1. Enhancement reflectance signal by light modulation with SLM

2.2 Two-layered phantom

To facilitate the observation of fluorescence from each layer, a two-layered phantom sample is used in this study as shown in Fig. 2. Due to multiple scattering, even the bottom layer is placed on the focal plane of the objective, the detector can still capture strong signal from the top layer (Rhodamine 6G, i.e. R6G), which can be out-of-focus plane, due to the multiple scattering. The strong signal from the top layer would overwhelm the signal from the bottom layer (fluorescent microparticles) which we are more interested in. To overcome the issue, we use fluorescent emission from sample as the feedback signal to change the SLM pattern to optimize the incident light to refocus into the target layer. After optimization, we can observe that the signal from the target layer is increased without affecting the signal from top layer. The increase of signal can help us to distinguish the target signal from background noise. The sample is made by mixing TiO_2 in PDMS, the reduced scattering coefficient (μ_s') is 3 cm^{-1} .

Figure 2. Two-layered tissue-like phantom.

2.3 Spectral Filter

The effect of spectral filtering on the performance of wavefront optimization is evaluated in the cases without bandpass filters and with bandpass filters of different central wavelengths as shown in Fig. 3. Since the fluorescence peaks of the two-layer sample is around 550 nm (R6G in the top layer) and 610 nm (purple-color fluorescent particles in the bottom layer) respectively, spectral filtering with one of the two bandpass filters with different central wavelengths (618 ± 25 nm and 650 ± 30 nm) will increase the fluorescence contribution from the bottom layer as shown in Fig. 3(A). Fig. 3(C) shows a clear monotonic relation between the enhancement factor and emission ratio when using the bandpass filter 618 ± 25 nm, which demonstrates the positive effect of spectral filtering on the performance of wavefront optimization. In contrast, such a trend is unclear in the cases without bandpass filter and with the bandpass filter, 650 ± 30 nm as shown in Fig. 3(B) and 3(D). It is not surprising to see an unclear increasing trend in enhancement factor in Fig. 3(B) because fluorescence for guiding wavefront optimization can come from both layers without any bandpass filter. It is also worth noting in Fig. 3(D) that, when using 650 ± 30 nm bandpass filter, the enhancement factor seems more random than the other two cases. This can be most likely attributed to the relatively low signal-to-noise ratio in this case because the passband of the filter covers only the tail end of the fluorescence peak in the bottom layer. Consequently, the enhancement factor is influenced as discussed in the discussion section.

Figure 3. Effect of spectral filtering using bandpass filters on the performance of wavefront optimization.

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