Super-depth and high-resolution optical imaging deep inside scattering media

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Abstract: Optical microscopy suffers from losing resolving power in imaging targets embedded in thick scattering media due to the dominance of strong multiple-scattered waves over the waves scattered only a single time by the targets. Here we present an approach that maintains full optical resolution in imaging deep within scattering media. We used both a time-gated detection and a spatial input-output correlation to identify those reflected waves that conserve in-plane momentum, which is the property of the single-scattered waves. By implementing a superradiance-like collective accumulation of the single-scattered waves, we enhanced single scattering signal to multiple scattering background ratio by more than three orders of magnitude. Imaging depth of 11.5 times the scattering mean free path was achieved with near-diffraction-limit resolution of 1.5 μm. Our method of distinguishing single from multiple-scattered waves will open new venues to deep-tissue imaging and studying the physics of light interaction with complex media.

1. Introduction

In life science and biomedicine, optical imaging is extensively used for examining specimens as it allows for the noninvasive investigation of living specimens and provides a spatial resolution high enough for intracellular organelles to be identified. Within the electromagnetic spectrum, visible light covers the highest energy band (or, in other words, the shortest wavelength) that does not damage biological molecules; as a result, optical imaging offers better resolving power than other noninvasive techniques, such as microwave and ultrasound imaging. However, its imaging depth has been extremely shallow due to multiple elastic light scattering, which irregularly changes the propagation direction of light waves carrying object information. The scattering mean free path (MFP) l_s defined by the average distance between successive scattering events is only about 100 μm in typical biological tissues, and this sets the depth limit of conventional bright-field imaging. Over the past years, my group explored various ways overcome the effects of multiple light scattering and also interesting approaches to make use of the disordered media [1-5]. In this presentation, I will introduce one of these past studies that dealt with the limitation of imaging depth.

2. Principle

In order to obtain a high-resolution image for a target object embedded within the scattering medium, it is necessary to extract single-scattered waves, which are the waves scattered only a single time by the target object and therefore retain object information, hidden in the multiple-scattered waves. As the target object is located deeper inside the scattering layers, however, this becomes an extremely difficult task. For an object located at a depth of 11 l_s, for example, single-scattered wave intensity is attenuated by a factor of $e^{-22} \cong 10^{-10}$. As a consequence, most of the light waves are reflected back without interacting with the target object, and those that do interact with the object tend to lose object information on their way out. In this Letter, we present a method to enhance single-scattered waves embedded within a background of random multiple-scattered waves. Using what we refer to as collective accumulation of single scattering (CASS) microscopy, we identified targets embedded at a depth of about 11.5 l_s with almost no loss of spatial resolution. In this method, we combined both a time-gated detection and a spatial input-output wave correlation to preferentially accumulate single-scattered waves over multiple-scattered waves.

3. Experimental setup

We constructed a reflection-mode interferometric microscope (Fig. 1) in which a superluminescent diode laser (center wavelength: 800 nm, bandwidth: 25 nm) was used as a low coherence light source to provide temporal gating. A spatial light modulator (SLM) was installed to control the angle of illumination to the sample. We chose N_{tot} = 2,500 phase ramps to be written on the SLM so that the incident wave vectors covered all the orthogonal input modes for the view field of 70×70 μm^2. The incident wave was delivered to the sample plane via an objective lens (20x, 0.4NA). The same lens captured the reflected wave from the sample and subsequently delivered it to the camera. The laser power at the sample plane was about 0.1 mW. The laser output reflected off the beam splitter BS1 served as a reference wave and interfered with the reflected wave at the camera. By controlling the optical path length of the reference wave using a scanning mirror, we selectively recorded reflected waves with the same time-of-flight as that of the reference wave with a temporal resolution of 76 fs, the coherence time of the light source. In the absence of multiple scattering, this temporal gating corresponds to a depth resolution of about 11.4 μm. Both the incident and the reference waves were linearly polarized, and reflected waves with the same polarization as that of reference wave was selectively recorded due to the interference. We matched the focal plane of the objective lens and the target depth set by the temporal gating. A

diffraction grating was used to set the propagation direction of the reference wave oblique relative to the optical axis and obtained a complex field map for the reflected wave using a Hilbert transform. As a test sample, we made a slab of scattering media by dispersing polystyrene beads of 1 μm diameter in the Polydimethylsiloxane (PDMS) and pasted the layer onto the United States Air Force (USAF) resolution target. Typical samples used in the experiment had l_s = 102 μm and a transport mean free path of 1.18 mm, values which approximate typical biological tissues.

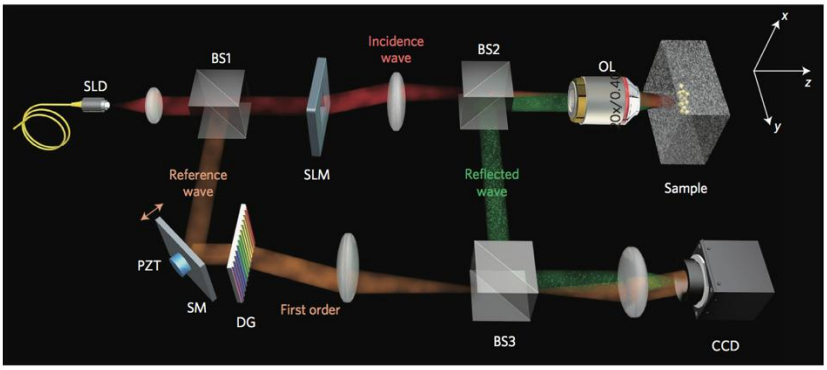

Figure 1. Experimental schematic diagram of CASS microscope. SLD: superluminal diode laser, OL: objective lens, BS1, BS2 and BS3: beamsplitters, SLM: spatial light modulator (working in the reflection mode, but indicated here as a transmission mode for simplicity), DG: diffraction grating. An aperture was used to select the first-order diffracted wave, SM: path length scanning mirror, and CCD: camera. We used red, green and dark goldenrod for indicating incident, reflected and reference waves, respectively, for clarity although their wavelengths are the same.

4. Results

We show the experimental results for various thicknesses of scattering layers placed on top of the USAF target. The scattering layer was made by dispersing polystyrene beads of 1 μm diameter in the Polydimethylsiloxane (PDMS). We separately measured the MFP of this scattering layer and it was about 102μm which approximate typical biological tissue. For a comparison, we present a single time-gated images for normal illumination, and angular compounding images, obtained by the summation of the intensity of time-gated images for each thicknesses (Fig. 2a, b). Individual time-gated images cannot identify the fine details of the target even for $1.60l_s$ case. With an increase of thickness, the image contrast of angular compounding image decreases, and at $9.7l_s$, it fails to identify the 9th group of USAF target whose smallest line pair separation was 1.56 μm. On the contrary, CASS microscopy show a good image contrast for 9th group of the target for up to $11.5l_s$ thick case (Fig. 2c).

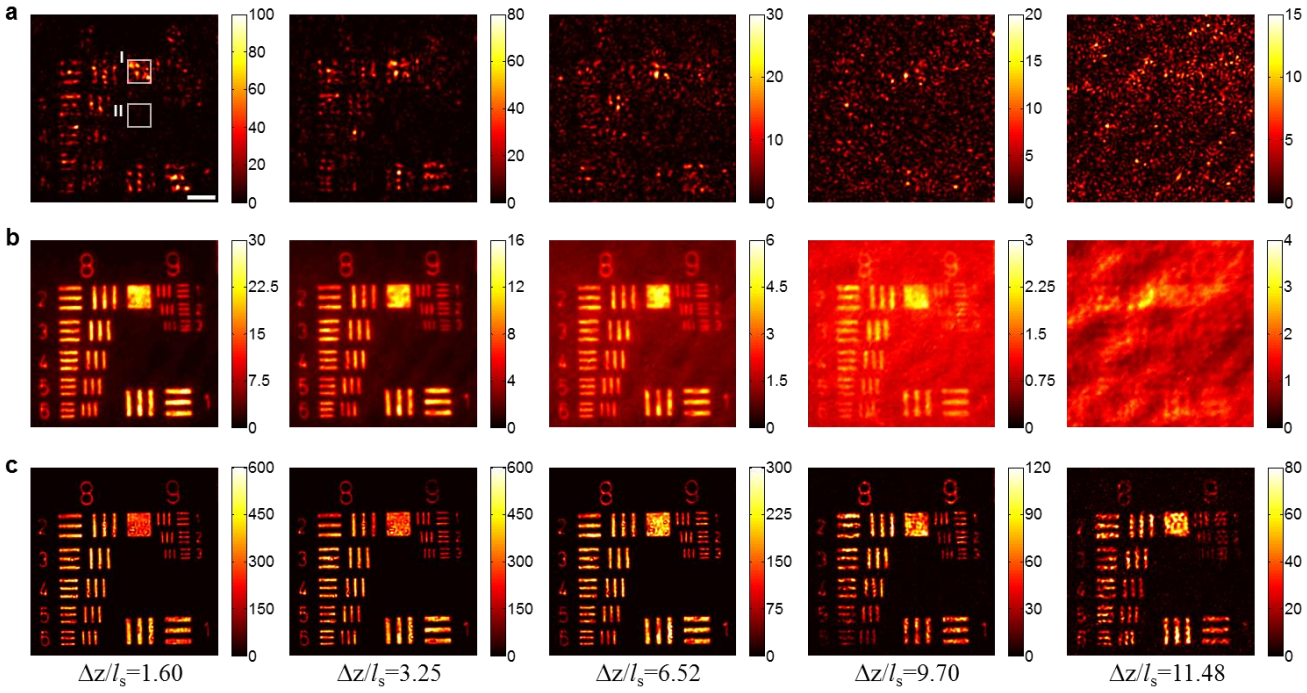

Figure 2. Demonstration of near diffraction-limit imaging in a thick scattering medium. a, Single-shot images under normal illumination. From the left, the thickness of the scattering layer is $1.60l_s$, $3.25l_s$, $6.52l_s$, $9.70l_s$, and $11.48l_s$, respectively. **b,** Angular compounding images for the same thicknesses. **c,** Reconstructed images using CASS for the

same thicknesses. All images were normalized by the mean values in the region indicated by square box II where there was no chrome coating. Because mostly multiple-scattered waves were present in this region, the color bars correspond to the ratio between the intensities of single and multiple-scattered waves. Scale bar, 10 μm.

5. Discussion

We have presented a method to collectively enhance single-scattered waves from a target object embedded in thick scattering media and experimentally demonstrated near diffraction-limited optical microscopic imaging up to a depth of 11.5 times the scattering mean free path. This imaging depth was mostly limited by the detector dynamic range in the present experiment and can be improved further by using a detector with a higher dynamic range than the camera used in the experiment. For *in vivo* applications, the increase of data acquisition speed would be the next step in order to deal with the decorrelation of scattered waves caused by the dynamics of a scattering medium. The acquisition of 2,500 angle-dependent wide-field images takes about 250 seconds mainly due to the slow refresh rate of the wave front shaping device used in the present experiment. This technical issue can be resolved by the use of a high-speed wave front shaping device along with a fast camera. Scanning the target depth set by the temporal and focus gating, which is necessary for volumetric imaging, will take the benefit of high-speed data acquisition. Since our method works for coherent light scattering process, it itself is not applicable to fluorescence imaging, but may be combined with two-photon or three-photon imaging techniques to exploit various contrast mechanisms of specimens. Our method of high-resolution imaging at an unprecedented target depth will lead to advances in life science and biomedicine in which the shallow imaging depth of conventional optical microscopy is a major obstacle. In addition, the ability to distinguish single and multiple-scattered waves will open new venues in the study of the physics of the interaction between light with complex med

6. References

[1] Imaging deep within a scattering medium using collective accumulation of single-scattered waves, Sungsam Kang, Seungwon Jeong, Wonjun Choi, Hakseok Ko, Taeseok D Yang, Jang Ho Joo, Jae-Seung Lee, Yong-Sik Lim, Q-Han Park, and Wonshik Choi, *Nature Photonics* **9,** 253 (2015)

[2] Scanner-free and wide-field endoscopic imaging by using a single multimode optical fiber, Youngwoon Choi, Changhyeong Yoon, Moonseok Kim, Taeseok Daniel Yang, Christopher Fang-Yen, Ramachandra R. Dasari, Kyoung Jin Lee, and Wonshik Choi, *Physical Review Letters* **109,** 203901 (2012)

[3] Measurement of the time-resolved reflection matrix for enhancing light energy delivery into a scattering medium, Youngwoon Choi, Timothy R. Hillman, Wonjun Choi, Niyom Lue, Ramachandra R. Dasari, Peter T. C. So, Wonshik Choi, and Zahid Yaqoob, *Physical Review Letters* **111,** 243901 (2013)

[4] Maximal energy transport through disordered media with the implementation of transmission eigenchannels, Moonseok Kim, Youngwoon Choi, Changhyeong Yoon, Wonjun Choi, Jaisoon Kim, Q-Han Park and Wonshik Choi, *Nature Photonics* **6,** 581 (2012)

[5] Overcoming the diffraction limit using multiple light scattering in a highly disordered medium, Youngwoon Choi, Taeseok Daniel Yang, Christopher Fang-Yen, Pilsung Kang, Kyoung Jin Lee, Ramachandra R. Dasari, Michael S. Feld, and Wonshik Choi, *Physical Review Letters* **107,** 023902 (2011)