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Holographic microscopy for the three-dimensional exploration of light scattering from gold nanomarkers in biological media

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The 3D structure of light scattering from dark-field illuminated live 3T3 cells marked with 40 nm gold nanomarkers is explored. For this purpose, we use a high resolution holographic microscope combining the off-axis heterodyne geometry and the phase-shifting acquisition of the digital holograms. Images are obtained using a novel 3D reconstruction method providing longitudinally undistorted 3D images. A comparative study of the 3D reconstructions of the scattered fields allows us to locate the gold markers which yield, contrarily to the cellular structures, well defined bright scattering patterns that are not angularly tilted and clearly located along the optical axis. This characterization is an unambiguous signature of the presence of the gold biological nanomarkers, and validates the capability of digital holographic microscopy to discriminate them from background signals in live cells.

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Keywords: Digital Holography, Holographic Microscopy, Light scattering, Gold Nanoparticles

1. Introduction

Digital Holographic Microscopy is gaining increasing interest in biological research [1, 2] because it enables the 3D reconstruction of both the amplitude and the quantitative phase images from a single recorded hologram and without any mechanical scanning. Since Gold nanoparticles (NPs) are non cytotoxic and benefits from interesting optical properties, their use as biological markers is advantageous [3, 4]. In this communication, we used a new holographic microscope that combines

the off-axis geometry [5] and phase-shifting acquisition of holograms [6] based on an original 3D reconstruction-without-distortion method to image live 3T3 mouse fibroblasts marked with 40 nm gold NPs. The proposed reconstruction method is a two step method providing longitudinally undistorted 3D reconstructed images and corrected for the off-axis tilt and lens aberrations. We showed that important information can be derived not only from the intensity of the bright spots caused by the gold NPs, but also from the 3D shape of the light scattering pattern, which is easily accessed using holography. We showed, in particular, that the speckle signal keeps memory of the illumination direction, while the particle signal does not.

2. Methodology

A. Sample preparation

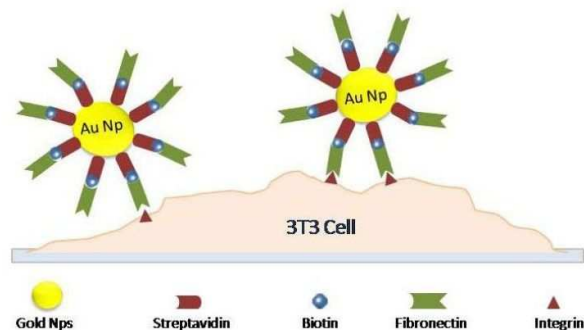


Fig. 1. Biological specimen preparation.

We use cultures of NIH 3T3 mouse fibroblasts incubated with 40 nm gold bioconjugates. Gold NPs were previously functionalized with Fibronectin proteins allowing their bonding with the Integrin surface cellular receptors of the cells (See Fig. 1). The bio-conjugation and functionalization protocols that we used to prepare the biological samples are the same protocols described in details in Ref. [7]. *Novel Biophotonic Techniques and Applications*, edited by Henricus J. C. M. Sterenborg, I. Alex Vitkin, Proc. of

B. Optical setup

The optical setup is a modified Mach-Zender interferometer (Fig. 2). Coherent light is produced by a laser source which is divided by a polarizing beam splitter (PBS). One of the two splitted beams is used to illuminate the sample in Total Internal Reflection (TIR). A microscope objective (MO) collects the scattered light and forms the object wave which interferes with a reference beam to produce the hologram that is recorded by a digital CCD camera.

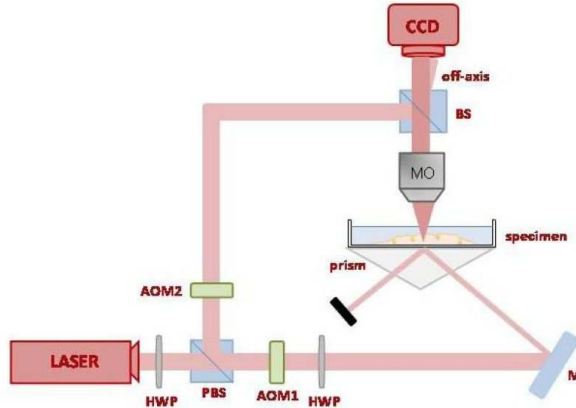


Fig. 2. Optical setup, M: Mirror, HWP: Half Wave Plate.

C. Numerical reconstruction

Holograms are recorded in an off-axis geometry.⁵ Phase-shifting interferometry is performed using two synchronized Acousto-optic modulators (AOM), one in each arm of the interferometer, allowing independent frequency modulation [8–10]. The combination of off-axis and phase-shifting is an effective configuration to reduce parasitic noise and eliminate images aliases.⁹ The hologram, carrying both amplitude and phase information, is then numerically treated and reconstructed using our novel reconstruction-without-distortion method that uses two Fourier Transforms (FFT). A first FFT is performed to obtain a clear bright disk which is the image of the MO exit pupil plane in the reciprocal space. Since the light scattered by the sample is collected by the MO, all interesting information is included inside this disk. A circular spatial mask is thus applied to eliminate all parasitic signal and keep only this interesting signal. The off-axis tilt is then compensated by translating the circle to the center of the reciprocal space. Using two FFT [11], the object field at the object plane is then reconstructed from the image, through the MO, of the recorded hologram at the CCD plane. 3D images are obtained by performing this 2FFT reconstruction at varying reconstruction distances. Because the propagation is done in free-space, the pixel size is kept constant and the method do not suffer from longitudinal distortions of the 3D images.

3. Experimental results

We imaged a 3T3 cell marked with 40 nm gold bioconjugates. The cell can be clearly seen of Fig. 3(a), which is a direct brightfield image. Fig. 3(b) is the reconstructed holographic image. The brightest point (arrow 1 on Fig. 3(b)) is a gold marker and the bright point marked by arrow 2 is a speckle hot spot. To better characterize the particles signal with respect to hot spots, we have analyzed the 3D images of the wave-field obtained by reconstruction for 512 different reconstruction planes and we performed cuts, at the position of the gold particle (point 1) and at the position of the hot spot (point2), along the x plane that is parallel to the yz incidence plane of the sample illumination beam. We can see on the scattering patterns images of Fig.4 that, contrarily to the

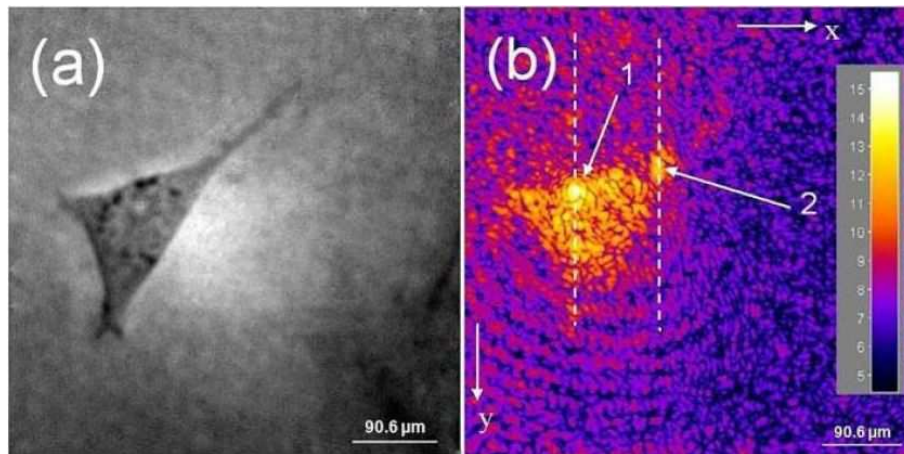


Fig. 3. Images of 3T3 mouse fibroblast marked with 40 nm gold particle : (a) direct image under white light illumination, (b) reconstructed holographic intensity image in logarithmic scale.

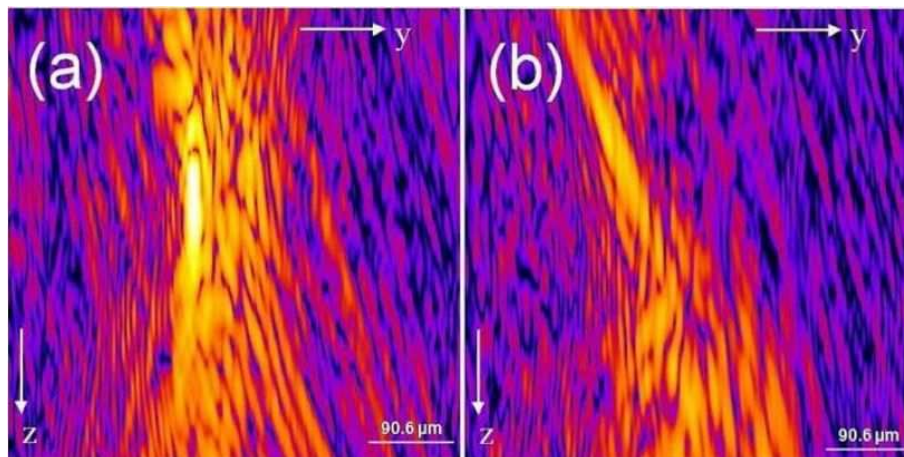


Fig. 4. Cuts along the x plane : (a) the cut corresponds to the white dashed line 1 of Fig.2(a), (b) the cut corresponds to the white dashed line 2 of Fig. 2(a).

particle, the hot spot signal extension along the vertical axis is quite large. Moreover, the hot spot image is angularly tilted in the yz plane while the particle signal is a well defined straight bright pattern clearly located along the optical axis z . This effect is a direct consequence of the non-similarity of the angular distribution of the light scattered from biological tissues and from gold nanoparticles, which depends on the anisotropy factor g (with $g \simeq 1$ for cells) giving a forward scattering regime while gold nanoparticles scatters light isotropically since their anisotropy factor is null [12].

4. Conclusion

Off-axis holographic microscopy is well adapted to the detection of weakly scattering objects. Image reconstructions are done using a novel 3D reconstruction-without-distortion method potentially effective for thick volume objects imagery. The sensitivity, SNR and selectivity of the technique allow the localization of gold nanoparticles of a few tens of nanometers. Biological environments, however, are difficult to address since cell features generate strong parasitic speckle. Here, we have reported the detection of 40 nm particles attached to the surface of live 3T3 cells. We show that, in addition to a stronger scattering signal, gold particles induce a relatively isotropic scattering, whereas biological features are characterized by mostly forward scattering. This dissimilarity in the scattering patterns, explained by the inconsistency of the refractive indexes and anisotropy parameters g , is easily characterized by digital holography, making it an excellent tool for the 3D detection of gold markers in biological environments.

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