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Optimized stereo matching algorithm for integral imaging microscopy and its potential use in precise 3-D optical manipulation

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ABSTRACT

In this paper, we present an integral imaging microscopy, which can achieve continuous depth extraction. The multiple stereo matching algorithm is optimized with data point interpolation and calculation range correction, which can improve the computing efficiency and accuracy. A water suspension of silica-embedded magnetic particles in light-trap environment is used as a specimen. The elementary image array is obtained through sequentially imaging the specimen by the objective lens and microlens array, which contains continuous depth information that can give the optical tweezers a feedback for precise 3-D manipulation. Experimental and calculated results show the feasibility of our method.

1. Introduction

The research of living cells has been at the forefront of life science for years. It is very important to analyze the position–function relationships of organizations, cells and organelles on the cellular scale. Optical tweezers are a typical tool for trapping and manipulating small particles [1]. The holographic optical tweezers show great potential in multiple 3-D manipulations on the cellular scale [2,3]. However, due to the lack of stereoscopic vision in traditional optical tweezers, operators can only receive 2-D scenery, which will reduce the operational accuracy inevitably [4,5].

The function of stereoscopic vision can be realized by the stereo microscopy or the scanning super-resolution microscopes. However, the low magnification and small numerical aperture of the stereo microscope still need further improvements [6]. On account of the time-sequential scanning process and the structural complexity, the scanning super-resolution microscopes cannot provide 3-D information in real time [7–12].

The practical light field microscopy (LFM) was firstly proposed by Levoy in 2006 [13]. In his method, the microlens array (MLA) plane is considered as an imaging plane, and the angular information is recorded on its rear focal plane. The light field inside the camera is parameterized by two planes. The *uv* plane is the principal plane of the lens, and the *st* plane is the sensor plane. Any rays in the light field can be represented by a four dimensional function-$LF(u, v, s, t)$ [14]. In the LFM, 4-D light field can be recorded and 3-D objects can be reconstructed [15–17]. Particularly to the application of optical tweezers, the discontinuity of the depth reconstructing calculation of LFM limits its accuracy in extracting the depth information of little particles. In integral imaging method [18–20], the elementary image array (EIA) is generated by using MLA and the depth information can be extracted from the parallax. Usually, only the target particles in close proximity to the focal point can be captured by the optical tweezers. The trapping force will be too weak to function when the distance is too far. Therefore, in traditional optical tweezers, the laser needs to be adjusted in the vertical direction several times due to the particle's depth position is unclear. However, in integral imaging method, the depth information can be extracted from the EIA and can provide us an accurate guidance for operation. What is more, if we want to drag the target particle along the optical axis, the moving distance can be calculated out from the EIA directly without other auxiliary measuring methods. Thus, the operational accuracy can be greatly improved by using the feedback information extracted from the EIA. Integral imaging is an appropriate method for application in optical tweezers.

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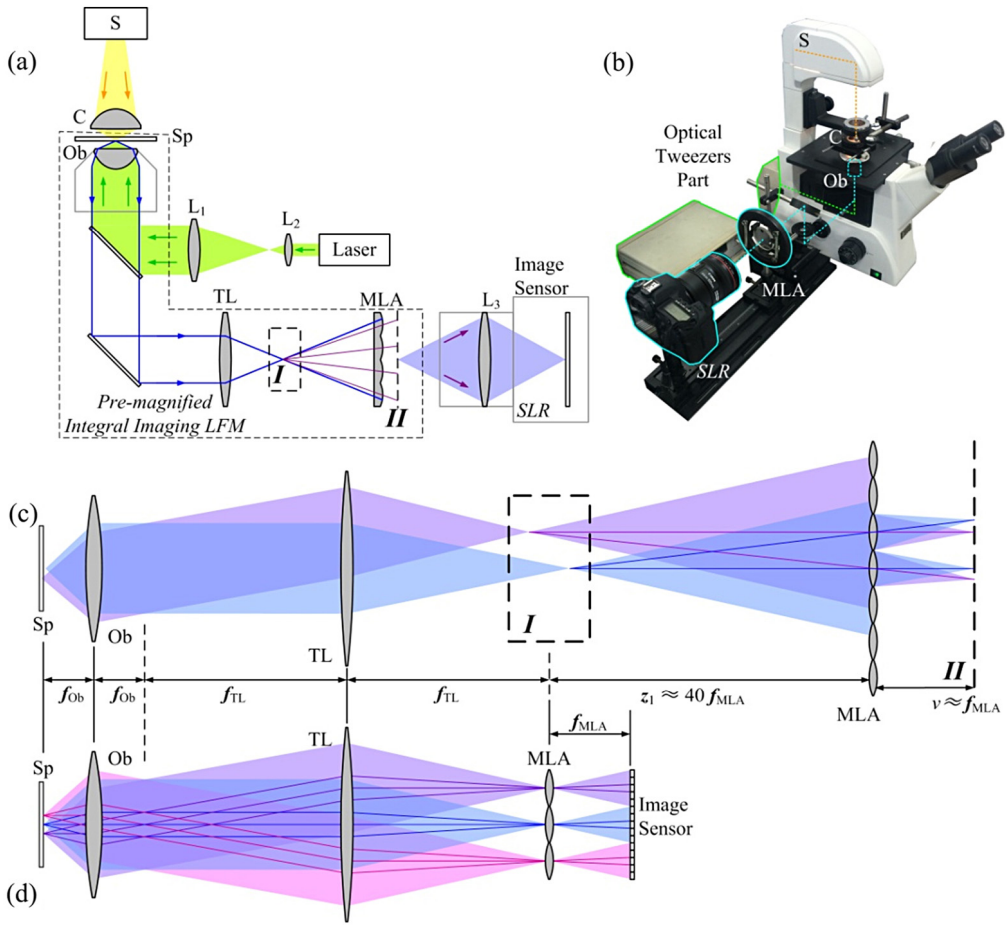

Fig. 1. (a) Schematic diagram of optical tweezers with integral imaging microscopy. (b) Photograph of our prototype system (Yellow: Illumination subsystem; Green: Optical tweezer subsystem; Cyan: integral imaging microscopy subsystem). (c) Diagram of the optical structure of integral imaging microscopy subsystem. (d) Diagram of the optical structure of conventional LFM. S: halogen light source; C: condenser; Sp: specimen; Ob: objective lens; L1, L2: collimating lens; TL: tube lens; L3: SLR lens; I: first imaging region; II: second imaging plane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In this paper, we propose a new integral imaging microscopy based on optimized multiple stereo matching algorithm and apply this method to optical tweezers. We consider MLA as the key device of integral imaging system and obtain EIA on its imaging plane. To analyze the parallax and extract the depth information, an interpolation-optimized integral stereo matching algorithm is used. We study the distribution of water suspension of magnetic particles embedded in silica, which are captured by light trap. The depth map of the magnetic particles is calculated to verify the feasibility of our method.

2. Optical setup of optical tweezers with integral imaging microscopy

The setup of the optical tweezers and integral imaging microscopy are based on the framework of an inverted microscope, including optical tweezers and an integral imaging microscopy, as shown in Fig. 1(a) and 1(b). The white light from a halogen light source (S) illuminates the specimens (Sp) after converged by a condenser (C) with a numerical aperture of 0.95. The optical tweezers and the integral imaging microscopy share a dry objective (Ob) of $NA = 0.9$. In the subsystem of optical tweezers, the 532 nm laser beam from a second harmonic generation with Nd: YAG laser is collimated by L1, L2 and strongly focused by the objective, which generates a light field with intensity gradients (“light trap”) in the specimen cell. Small objects can be trapped and manipulated on the focal point by the optical gradient forces in the intensity gradient field.

In the integral imaging microscopy subsystem, the objective (Ob) and a tube lens (TL) generate a magnified real image of the specimens (Sp) in region I, which is then multiply imaged by a MLA of 9 mm focal length and 192 μm interval (distance between the center of lenses). On plane II, an EIA of the real image with full parallax is generated. We re-image the EIA using a SLR camera with 1:1 macro lens (L3). The SLR camera is Canon EOS 5D Mark II. The sensor area is 36 mm × 24 mm and the effective pixel number is 5616 × 3744. The pixel size is approximately 6.4 μm × 6.4 μm. The camera lens is a macro lens with a focal length of 100 mm.

Fig. 1(c) and 1(d) show the different setups of the integral imaging microscopy in our method and a conventional LFM. In a conventional LFM, the MLA is placed directly on the real image plane of the specimen, whose purpose is to record 2-D spatial information of the 4-D light field. The detection plane is set on the rear focal plane of the MLA, whose purpose is to record the 2-D angular information of the 4-D light field. In this way, researchers pay more attention to the reconstruction process of the light field more than numerically estimate the depth position of any image point. Especially when applied to optical tweezers, the lack of ability of continuous depth of LFM is not sufficient to meet the requirements. In the integral imaging microscopy, the lens unit of MLA is regarded as both an imaging lens and an objective lens. The depth “signal” is magnified in the first imaging process, then extracted from the parallax of EIA in the second imaging process, which makes it easier to estimate depth value continuously.

The microlens array is the key device of integral imaging microscopy. The elementary interval p of the microlens array influences the count