Fluorescence multicolor hologram recorded by using a macrolens array

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We present a new efficient method for obtaining multicolor digital Fresnel holograms of three-dimensional (3-D) objects emitting incoherent fluorescent light. For each emitted fluorescent light wavelength, a single monochromatic digital modified Fresnel hologram of the 3-D scene is generated. Using a macrolens array, only nine projections of the 3-D scene are captured in a single camera shot and are then digitally processed to yield the hologram. The 3-D reconstructed images from all the monochromatic holograms are composed into a single multicolor image of the 3-D scene. The proposed holographic technique is demonstrated by experiments. © 2008 Optical Society of America

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Fluorescence radiation is useful for various applications in the fields of medical imaging, microscopy, biology, and chemistry. High sensitivity, low background noise, and thus good detectability and localization [1] are only some of the advantages of fluorescence imaging that have encouraged the foundation of fluorescence three-dimensional (3-D) imaging methods.

Holography is considered a reliable 3-D imaging technique, capable of creating an authentic illusion of 3-D objects without special observation devices. Moreover, digital holograms can store 3-D information in a very dense and encrypted way. However, classic holographic methods demand wave interference, requiring meticulous stability of the entire optical system, as well as a high intensity and long coherence length of the laser used.

New techniques for generating holograms of 3-D objects emitting fluorescent (incoherent) light have the advantages of fluorescence over regular illumination and yet eliminate most of the disadvantages of conventional holography. Fluorescence scanning holography [2] is an example of such a technique. However, this method is relatively slow and complicated because of the mechanical scanning required. Multicolor Fresnel incoherent correlation holography [3] is a different technique that does not use mechanical scanning. However, three different holograms for each emitted wavelength should be recorded for generating the final hologram by multicolor Fresnel incoherent correlation holography, in contrast to the method proposed herein.

By using multiple viewpoint projection (MVP) holography [4,5], it is possible to generate digital and optical holograms of 3-D, real-existing objects illuminated by incoherent light. These holograms are generated by first acquiring MVPs of the 3-D scene from various perspectives and then digitally processing the MVPs to yield the hologram of the scene.

For acquiring these MVPs, the digital camera can be shifted mechanically [4,5]. However, this process is slow and inadequate when the objects move faster than the scanning camera. Instead, it is possible to utilize a microlens array for acquiring the entire set of MVPs in a single camera shot [6]. However, for a high-resolution hologram, an array of many microlenses is needed. Since the total size of the microlens array is limited, the diameter of each microlens is small, and thus each projection is imaged with a relatively poor resolution.

To obtain MVP holograms of moving objects with a satisfactory resolution, here we propose to use a macrolens array. The image plane of the macrolens array contains a small number of projections, each of which has an acceptable resolution. The camera captures the entire macrolens image plane in a single snapshot. Afterward, the view synthesis algorithm [7] is used to predict the middle projections by performing 3-D interpolations on the acquired projections. Finally, the entire projection set is used to compute the two-dimensional (2-D) digital incoherent modified Fresnel hologram (DIMFH) [8] of the 3-D scene. The DIMFH is generated by processing the MVPs directly and without limitation to small MVP acquisition angles, in contrast to the previous MVP Fresnel hologram (e.g., [5]).

In this Letter, we also show how to obtain multicolor holographic reconstruction of the fluorescent and nonfluorescent objects in the 3-D scene. This is done by recording a monochromatic DIMFH for each emitted fluorescent wavelength and an additional DIMFH for the nonflorescent white light reflected from the scene. All hologram reconstructions are fused together, yielding the composite fluorescent and nonfluorescent 3-D image.

Figure 1 illustrates the optical system used for capturing the 3-D scene MVPs. An excitation light from filter F_1 stimulates the fluorescent light to be emitted from the labeled objects. Then, the 3×3 array of negative lenses creates the projections simultaneously. We use negative lenses in this array to form virtual images of the scene and thus to avoid aberrations that might occur in the outer lenses in the case where using positive lenses would create real images

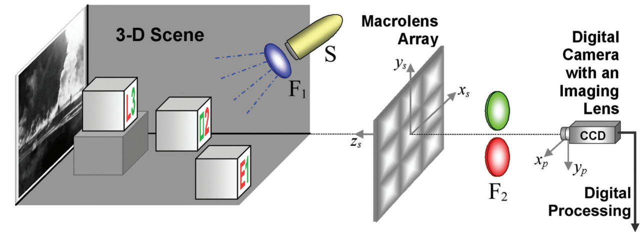

Fig. 1. Optical system for acquiring 3×3 perspective projections simultaneously by using a macrolens array.

of the scene. To take advantage of the entire array size, the lenses are cut into squares and stuck together.

For each fluorescent wavelength, the suitable bandpass filter F_2 is positioned in front of the monochrome digital camera. Each time the camera captures, through its imaging lens and in a single snapshot, another 3×3 projection set. An additional 3×3 projection set of the nonfluorescent light reflected from the scene is also captured with both filters F_1 and F_2 removed.

Next, the view-synthesis algorithm is used to digitally predict the middle projections between the 3×3 captured projections. Given two projections, this algorithm first calculates the correspondence map containing the displacements of each pair of corresponding pixels in the two given projections. Then, the algorithm predicts how the scene would look from a new middle viewpoint by interpolating the locations and intensities of the corresponding pixels [7].

Let us number the final MVPs with m and n, such that the middle projection is denoted by $(m,n)=(0,0)$, the upper-right projections by positive indices, and the lower-left projections by negative indices. The 2-D DIMFH is generated as follows:

$$H(m,n)=\iint P_{m,n}(x_p,y_p)\exp[i2\pi b({x_p}^2+{y_p}^2)]\mathrm{d}x_p\mathrm{d}y_p, \tag{1}$$

where $P_{m,n}(x_p,y_p)$ is the (m,n)th projection and b is an adjustable parameter. The mathematical relations between an arbitrary point (x_s,y_s,z_s) in the 3-D scene (where the coordinate origin is defined on the center of the middle macrolens) and its projected point (x_p,y_p) on the (m,n)th projection plane imaged on the camera are given as follows:

$$x_p=M_c|f|(x_s-m\alpha)/z_s, \qquad y_p=M_c|f|(y_s-n\alpha)/z_s, \tag{2}$$

where M_c is the magnification of the camera imaging lens, f is the focal length of each macrolens, and α is the camera gap between every two adjacent projections. Using Eqs. (1) and (2) a hologram of a single point object (x_s,y_s,z_s), having an infinitesimal size of $(\Delta x_s,\Delta y_s,\Delta z_s)$ and a value of $h(x_s,y_s,z_s)$ is given by

$$\begin{aligned}\hat{H}(m,n;x_s,y_s,z_s)&=\iint[h(x_s,y_s,z_s)\Delta x_s\Delta y_s\Delta z_s\delta(x_p'-x_p,y_p'-y_p)]\exp[i2\pi b({x_p'}^2+{y_p'}^2)]\mathrm{d}x_p'\mathrm{d}y_p'\\&=h(x_s,y_s,z_s)\exp\left[i2\pi bf^2M_c^2\frac{(x_s-m\alpha)^2+(y_s-n\alpha)^2}{z_s^2}\right]\Delta x_s\Delta y_s\Delta z_s.\end{aligned} \tag{3}$$

The volume integral over all single-point holograms, resulting from all 3-D scene points, yields the final hologram of the 3-D scene as follows:

$$\begin{aligned}H(m,n)&=\iint\int h(x_s,y_s,z_s)\exp\left[i2\pi bf^2M_c^2\frac{(x_s-m\alpha)^2+(y_s-n\alpha)^2}{z_s^2}\right]\mathrm{d}x_s\mathrm{d}y_s\mathrm{d}z_s\\&=\iint\int h(x_s,y_s,z_s)\exp\left[i2\pi b\left(\frac{M\alpha}{\Delta p}\right)^2\left[\left(\frac{\Delta p x_s}{\alpha}-m\Delta p\right)^2+\left(\frac{\Delta p y_s}{\alpha}-n\Delta p\right)^2\right]\right]\mathrm{d}x_s\mathrm{d}y_s\mathrm{d}z_s,\end{aligned} \tag{4}$$

where $M=M_c|f|/z_s$ is the magnification through the entire optical system and Δp is the pixel size of the digital camera. The first part of Eq. (4) is similar to a 2-D Fresnel hologram of the 3-D scene [5]. However, from the exponent of the second integral in Eq. (4), the hologram is in fact a sampling pattern of the scene by a 2-D sampling function, which creates unique transverse magnifications of $M_x=M_y=\Delta p/\alpha$. Thus, in contrast to a conventional imaging system, the DIMFH transverse magnifications are independent of the axial positions of the objects in the 3-D scene. This effect can be eliminated by scaling the reconstructed planes by M/M_x [8].

The DIMFH is reconstructed digitally by convolving it with quadratic phase functions scaled according to the reconstruction distances (digital Fresnel propagation). Finally, the reconstructed images from the different holograms are superimposed to form a single multicolor 3-D image.

To demonstrate the method experimentally, we implemented the optical system shown in Fig. 1. Three white cubes, each $2\text{ cm}\times2\text{ cm}\times2\text{ cm}$ in size, were positioned in front of a background picture. This is the first time that we have demonstrated that MVP holograms can be obtained even in the presence of a background. The letters "EOL" and the digits "123" were printed on the cubes and painted with red and green fluorescent dyes, red "E" and green "1" on the closest cube, located 42 cm from the macrolens array; green "O" and red "2" on the middle cube, located 50 cm from the macrolens array; and red "L" and green "3" on the farthest cube, located 58 cm from the macrolens array. The background picture was located 68 cm from the macrolens array.

To produce the 3×3 macrolens array, we used nine standard negative lenses, each of which had an original diameter of 5 cm and a focal length of −25 cm. Each lens was cut into a square of 3.53 cm $\times$3.53 cm, and a 3×3 macrolens array of 10.6 cm $\times$10.6 cm was formed by sticking the cut lenses together with an optical adhesive.

An optical bandpass filter F_1 [central wavelength (CWL) 456 nm, full width at half-maximum bandwidth (FWHM-BW) 38 nm, hereby the blue filter] was positioned in front of a halogen lamp S for excitation of the fluorescent dyes in the 3-D scene. Another bandpass filter F_2 was positioned in front of the imaging lens of the camera, where two different filters were used as F_2, each for creating a different monochromatic hologram. The first one (CWL 606 nm, FWHM-BW 74 nm, hereby the red filter) was used to pass the wavelengths around the red color. The second one (CWL 524 nm, FWHM-BW 89 nm, hereby the green filter) was used to pass the wavelengths around the green color.

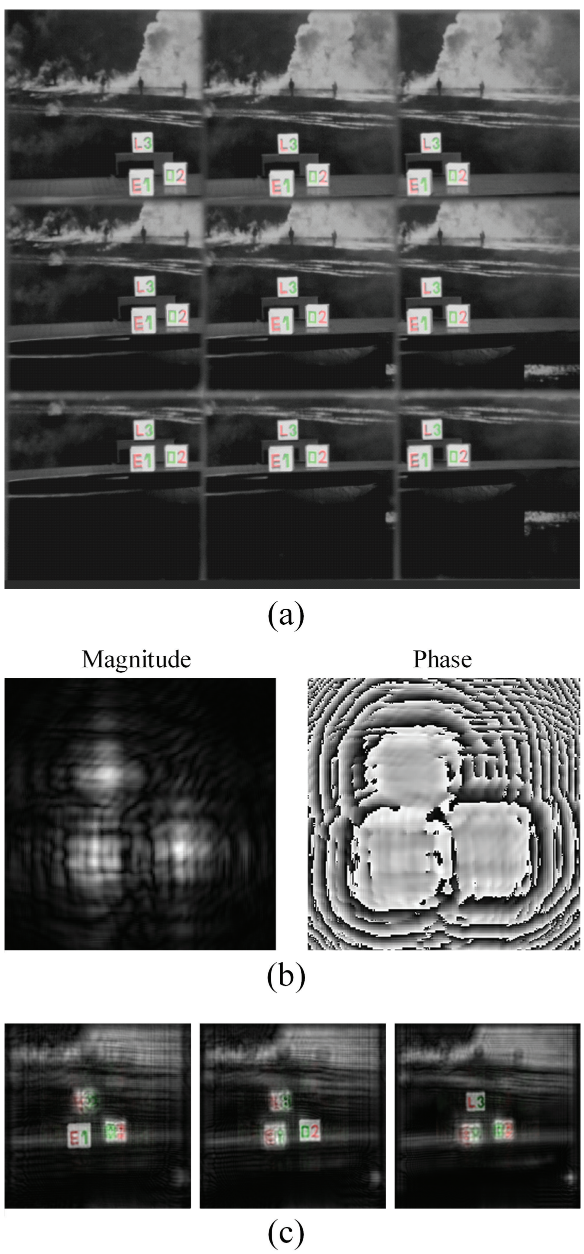

Fig. 2. (a) Composite image plane of the macrolens array. (b) Magnitude and phase of the nonflorescence DIMFH. (c) Multicolor best-in-focus reconstructed planes.

Figure 2(a) shows the composite macrolens-array image plane acquired by composing three image planes of three cases: when no filters were used, when F_1 and the red F_2 were used, and when F_1 and the green F_2 were used.

The view synthesis algorithm was applied to predict the middle projections in each of the three cases of the 3×3 projection sets, so that in each case 256 $\times$256 MVPs were obtained. For the first nonfluorescence image plane, we separated the background during the object segmentation in the view synthesis algorithm and created the hologram without the background. Then we used the background of the middle projection as the pseudo-located-at-infinity background of the reconstructed image.

Figures 2(b) show the magnitude and the phase of the nonfluorescence DIMFH generated by using Eq. (1). Similar DIMFHs are generated for the red and the green fluorescent light emitted from the labeled objects. Figures 2(c) show the unified three best-in-focus reconstructed planes obtained after digitally reconstructing each DIMFH, digitally pseudocoloring the reconstructed images from the red and green holograms in red and green, respectively, and finally fusing together all three reconstructions. As shown in Figs. 2(c), in each reconstructed plane, a different cube face is in focus, whereas the other two cube faces are out of focus. This validates the volumetric information encoded into the multicolor Fresnel hologram.

In conclusion, we have introduced and experimentally demonstrated a new efficient method for recording multicolor holograms of 3-D fluorescently labeled objects, using a macrolens array. The proposed method might be useful for various types of 3-D fluorescence imaging, including 3-D imaging in biology and medicine.

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