Spectrum resolved fluorescence imaging in multi-focal volume holographic microscopy

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Spectrum resolved fluorescence imaging in multi-focal volume holographic microscopy

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ABSTRACT

A real-time three dimensional (3D) fluorescence imaging system incorporating wavelength-coded and multiplexed holographic gratings is presented. Holographic gratings formed in thick Phenanthrenquinone- (PQ-) Doped Poly (methyl methacrylate) (PMMA) have narrowband spectral-spatial transmittance filtering properties to generate wavelength-spectrum selective multi-focal planes within a biological object. We demonstrate the imaging modality to obtain laser-induced fluorescent tissue structures from different depths at the excitation wavelength of 355nm.

Keywords: Volume hologram, Grating, Multiplexing.

1. INTRODUCTION

Conventional optical imaging systems such as optical coherence tomography (OCT) [1] and confocal microscopy [2] have been applied for a variety of biological and biomedical applications, and these systems have shown great promise for monitoring and detecting cancer cell structures. Although some OCT can scan at a very fast rate, OCT is a coherent imaging technique and cannot provide fluorescent information. Confocal microscopy has been developed for a variety of bio-imaging applications. Confocal microscopy uses a small aperture to reject out-of-focus light, allowing imaging of thin sections within thick biological samples. Confocal microscopy can provide micron or submicron levels resolution and image approximately a few hundred microns deep, and it has the additional benefit of being able to acquire biochemical information of fluorescently marked samples.

Ideally, confocal and OCT would provide three-dimensional images, enabling characterization of a volume of a biological sample, but they typically require scanning. Efforts to improve scanning efficiency by optimizing the scanning algorithm for confocal [3,4] are ongoing. However, these methods are complicated and do not eliminate the need for moving parts, and the complex designs also increase device cost and reduce robustness.

2. CONVENTIONAL VOLUME HOLOGRAPHIC MICROSCOPY

Volume holographic microscopy (VHM, Figure 1) [5-8] incorporating a volume hologram to obtain multiple depth-resolved images is a unique imaging system. The volume hologram can be thought of as a “spatial-spectral filter”, which has a characteristic optical path difference (OPD) response function that defines how well the filter uses its high spectral selectivity to extracts lateral and depth information from an object. The VHM is particularly beneficial to biological and clinical applications in that it can provide both structural and biochemical imaging from different depths in real-time without scanning.
Figure 1. Schematic drawing of the optical system. This arrangement can be used for real-time 3D imaging with no need of scanning through MVH gratings as spatial-spectral filters. Figure 1 shows that the conventional VHM is imaging a fluorescent object. The VHM consists of a 4-f configuration of lenses with a hologram placed in the central (Fourier) plane. The hologram is composed of two multiplexed holographic gratings, and each grating behaves as a highly selective spatial-spectral filter in the imaging system. Each depth is then probed by its corresponding grating and projected to a different lateral location on the camera. The colors “red,” “green,” “blue” are meant to illustrate the longer, median and shorter wavelengths of the object’s emission light, respectively. Slice 1 and 2 show spatial planes in the object at a different depth z.

Figure 2. Experimental result obtained from the same 3D microfluidic sample consisting of fluorescent nuclei (DAPI). The fluorescently labeled nuclei were uniformly illuminated by a 355nm pump laser beam. The two slices shown were 30μm apart in the z direction.

In Figure 2, the fluorescently labeled nuclei of cells seeded in a 3D microfluidic platform shown on the left side of depth 1, are imaged with longer wavelength light and those on the right side form with shorter wavelengths. This imaging property results from the coupling between wavelength and spatial position in the Bragg matching relations for volume
holograms. However, the conventional VHM is limited to obtaining only a single fluorescent color from multiple depths within an object.

3. SPECTRUM RESOLVED VOLUME HOLOGRAPHIC MICROSCOPY

In this paper, we present a spectrum resolved VHM based on the wavelength coded technique for color fluorescence imaging. The spectrum-resolved VHM incorporates a wavelength-depth selective multiplexed volume hologram (MVH) to simultaneously obtain different fluorescent colors from multiple depths in real-time. Each multiplexed grating within the hologram is sensitive to a specific depth. Each depth within a tissue sample is probed by its corresponding fluorescent color and projected to a distinct lateral location on a digital camera. This is particularly beneficial for 3D fluorescence imaging to take advantage of its deeper penetration, and it gives the advanced spectrum-resolved VHM improved contrast and penetration depth.

Figure 3 shows the operation of the spectrum resolved VHM that is imaging a multi-spectral fluorescent object illuminated using laser excitation. Different spectrum illustrates the longer and shorter fluorescent wavelengths of the object’s emission light from different depths within a biological sample. The system consists of a 4-f configuration of lenses with a wavelength-depth selective MVH in the central (Fourier) plane. Each multiplexed grating within the wavelength-depth selective MVH selects a specific depth by its corresponding wavelength and displays on a digital camera at a different lateral location. In our approach, a longer wavelength is designated into a deeper depth, allowing greater imaging penetration within a biological tissue sample.

![Figure 3. Schematic setup of the spectrum-resolved VHM to simultaneously obtain multiple planes emitting different fluorescent information.](image)

4. IMAGE ACQUISITION

Figure 4 shows two depth-resolved fluorescence images from a 3D volumetric tissue sample with human breast cancer cells [9] using the DS-VHI system. The cell sample was stained with two diluted fluorescent green and red quantum-dots solutions (Evident Technologies ED-C11-TOL-0520 and ED-C11-TOL-0620). The stained tissue sample was excited using a 355nm UV pump laser and simultaneously emitted in green and red color, with respective central wavelengths of ~520nm and ~620nm and same spectral bandwidth of 40nm.
5. CONCLUSION AND DISCUSSION

Laser induced fluorescence (LIF) has been developed for a variety of clinical applications. However, many of the existing biomedical imaging systems typically require scanning in two lateral dimensions as well as depth focusing. Efforts to improve scanning efficiency by optimizing the scanning algorithm or increasing the number of focal points are ongoing. However, these methods can increase system complexity and do not eliminate the need for moving parts. We use wavelength-depth selective multiplexed holographic gratings to simultaneously obtain information from multiple depths and specific fluorescent spectrum corresponding to each depth within biological samples with no scanning and moving parts. Compared with conventional biomedical imaging systems, in our approach specific fluorescent wavelengths can be designated to beneficial depth locations within a biological object. We used thick Phenanthrenquinone- (PQ-) Doped Poly (methyl methacrylate) (PMMA) [10-14] to form wavelength-depth selective multiplexed holographic gratings to provide spectral-spatial filtering properties in the imaging system. We demonstrate the imaging modality to obtain laser-induced fluorescent tissue structures with red and green emitting wavelengths at the excitation wavelength of 355nm. In the future work, the spectrum-resolved VHM can be operated simultaneously at fluorescent as well as bright-field mode to provide complementary information of 3D tissue structure and biochemistry, enabling characterization of a volumetric tissue in real-time.

6. ACKNOWLEDGEMENT

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