Volume holographic microscopy shows cell depths in real-time

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Fluorescence images of human cell samples are a powerful tool for studying cancer migration.

Conventional optical imaging systems, such as optical coherence tomography (OCT)\textsuperscript{1} and confocal microscopy,\textsuperscript{2} have shown great promise in monitoring and detecting cancer cell structures for biological and biomedical applications. Some OCT systems can scan very fast, but since they use a coherent imaging technique, they cannot provide fluorescence information. Confocal microscopy uses a small aperture to reject out-of-focus light, allowing imaging of thin sections within thick biological samples. It can thus provide micron or submicron resolution and image approximately a few hundred microns deep. It has the added benefit of being able to acquire biochemical information of fluorescently marked samples.

Ideally, confocal and OCT techniques would provide 3D images that enable characterization of a biological sample volume. However, they typically require scanning. Efforts to improve efficiency by optimizing the scanning algorithm for confocal\textsuperscript{3,4} microscopy are ongoing. However, these methods are complicated and do not eliminate moving parts. The complex designs also increase device cost and reduce robustness.

We are working with volume holographic microscopy (VHM) to obtain multiple depth-resolved images. The volume hologram can be thought of as a spatial-spectral filter that has a characteristic optical path difference (OPD) response that defines how well the filter uses its high spectral selectivity to extract lateral and depth information from an object. VHM is beneficial in biological and clinical applications because it can provide structural and biochemical imaging from different depths in real-time without scanning.

Figure 1 shows VHM imaging of a fluorescent object using a broadband source. The VHM set up consists of a 4\textsubscript{f} configuration of lenses with a hologram in the central (Fourier) plane. The hologram comprises two multiplexed holographic gratings, each of which 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 red, green, and blue colors illustrate the longer, medium, and shorter wavelengths of the object’s emission light, respectively. Each slice shows a spatial plane in

Figure 1. Schematic of our VHM optical system. This arrangement can be used for real-time, 3D imaging without scanning through the multiplexed volume holographic gratings that are used as spatial-spectral filters.
the object at a different depth (z). If the object’s light is at a narrow spectral bandwidth, the width of the spatial image in the x field of view (x-FOV) will be narrow. However, since the object’s light is broadband, the x-FOV is wider. In addition, the parts of the spatial image are portrayed with different bands of the object’s emission spectrum. For instance, in Figure 2 the fluorescently labeled cell nuclei seeded in a 3D microfluidic platform, shown on the left of depth 1, were imaged with longer wavelength light, and we imaged those on the right using shorter wavelengths. This imaging property results from the coupling between wavelength and spatial position in the Bragg matching relations for volume holograms.5–7

Figure 2(a) shows our experimental results from a 3D microfluidic device with human endothelial and breast cancer cells seeded in the channels and attached on the polydimethylsiloxane (PDMS) wall. We illuminated the 3D microfluidic sample using a collimated broadband blue LED source with a central wavelength of 480nm and spectral bandwidth of 40nm. The separation between the two layers is ~30μm. Figure 2(b) shows fluorescence images within the microfluidic cell sample using a 355nm pump laser. The sample was stained with a fluorescent dye, 4’,6-diamidino-2-phenylindole (DAPI). We used an emission notch filter with a central wavelength of 480nm and spectral bandwidth of 40nm during imaging. Nuclei of ~5μm at two different depths are well resolved. Thus, fluorescence information from different depths can be provided simultaneously, which should offer promising applications in biological and biomedical research.

Finally, each multiplexed grating in our VHM system images a 2D slice of a different depth. The images are adequately separated on the camera plane because the signal beams corresponding to the two multiplexed holograms are separated by a sufficient angular distance. This system can therefore image in real-time three spatial (X,Y,Z) dimensions on a CCD or several cameras without scanning through spatial-spectral selective gratings.

We are working towards improving signal-to-noise and penetration depth using wavelength-coded techniques [7]. In the future implementation, the device can be extended with more multiplexed gratings to provide additional depth information, allowing us to image in 3D. From that, we can gather information on cancer cell rolling and migration in real-time within a 3D microfluidic tissue sample.

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