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Authors

Azartash, Kaveh
Gratton, Enrico

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Measuring the Cell-induced Deformation of Collagen Matrix Detected with Digital Holographic Microscopy

Kaveh Azartash/University of California Irvine

Enrico Gratton /University of California Irvine

ABSTRACT

A modified Mach-Zender set-up in reflection is applied to record and reconstruct holographic amplitude and phase images. A charged couple device (CCD) is used to record a hologram and numerical reconstruction algorithms are then applied to rebuild the hologram for obtaining both phase and amplitude information. One could also focus on multiple focal planes from a single hologram, similar to the focusing control of a conventional microscope.

The morphology and behavior of mammalian cells is determined by an interaction between signals from the intracellular matrix and the cellular responses. It is important to note that the physical aspect of the extracellular matrix is as significant as the chemical nature of it. Specifically the stresses, mechanical forces, and the profile of the external environment have major effects on cell behavior.

The mechanical and physical characteristics of a tissue are greatly dependent on a hierarchical spatial arrangement of its extra-cellular matrix components. A key player in the ECM is collagen which exhibits significant tensile strength on the cellular scale. Digital holographic microscopy (DHM) is applied to study the deformation of collage matrix in response to cell migration.

INTRODUCTION

Advancements of coherence interferometric imaging techniques in terms of digital holography are applied to evaluate living

biological samples in-vitro providing physiological environment. It is very problematic for conventional bright-field microscope to differentiate different components of the cell from one another due to living cells not being able to exhibit amplitude contrast. To boost the scientific understanding of the behavior of intracellular organelles, various phase-contrast imaging techniques such as Zernike, Normarsky and dark-field microscopy have been developed. However these methods still lack the essential of phase-contrast imaging which is quantification. Quantitative phase-contrast imaging (QPI) is indispensable due to its ability for determination of the optical thickness variations from the measured optical path length with sub-wavelength accuracy. [1]

Digital holographic microscopy is a phase contrast imaging technique that offers sub-wavelength resolution with real time observation capabilities. These high resolution readings originate from the diffraction of a beam off microscopic objects which provide the amplitude and absolute phase that can be reconstructed into a spatial intensity profile of the interference pattern (hologram)[2]. Nano-meter or subnano-meter longitudinal resolution can be achieved in air or dielectric media, respectively. Digital holography microscopy allows the reconstruction of both phase and amplitude of an object wavefront from acquisition of only one digital hologram a CCD or other type of imaging detectors have the ability of capturing the interference between a

reference and an object beam. Different numerical formulations have been developed to reconstruct the recorded hologram such as the single Fourier transform formulation(SFTF) or simply the convolution method. Other methods such as the Huygen's method and the angular spectrum method (ASM) have also been applied to formulate the reconstruction of the wavefront.[3]

The principles of creating holograms, attaining and reconstructing the wavefront from digital holograms are applied to study the deformation of collagen fibers.

DISCUSSION

Measurements are planned on individual collagen fibers in artificial tissue by implanting gold nano-rods onto the collagen matrix to quantify the mechanical deformation of the fibers. Other mechanical parameters are also to be measured by applying the classical theories of rubber elasticity. This will be accomplished by measuring the persistence length of the collagen matrix which will be calculated by tracing individual fibers in the network.

Thus digital holography provides the framework to seize a better comprehension of the mechanics of collagen matrix that in turn will open up new avenues for enhanced approaches in tracking particles in 3-dimensions.

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