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Dual-mode digital holographic and fluorescence microscopy for the study of morphological changes in cells under simulated microgravity

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ABSTRACT

A dual mode microscope is developed to study morphological evolution of mouse myoblast cells under simulated microgravity in real time. Microscope operates in Digital Holographic Microscopy (DHM) and widefield epi-fluorescence microscopy modes in a time sequential basis. DHM provides information on real time cellular morphology. EGFP transfected actin filaments in mouse myoblast cells function as the reporter for the fluorescence microscopy mode. Experimental setup is fixed in the RPM to observe microgravity induced dynamic changes in live cells. Initial results revealed two different modifications. Disorganized structures become visible in the formed lamellipodias, and proteins accumulate in the perinuclear region.

Keywords: microscopy, holography, digital holographic microscopy, fluorescence microscopy, microgravity, cell morphology, cytoskeleton

1. INTRODUCTION

Exposure of living organism to weightlessness causes various physiological alterations such as orthostatic intolerance, cardiac susceptibility to ventricular arrhythmias, and muscle atrophy. At cellular level, weightlessness in space flight like conditions was shown to cause rearrangement of cytoskeleton[1,2]. Cytoskeletal dynamics are explained by tensegrity model in which tensional integrity of the entire structure is preserved by the balancing of tension and compression on the microfilaments and microtubules[3]. Parabolic flight based living cell experiments indicated to formation of lamellipodias and redistribution of the actin network[2]. In case of space flight related muscle atrophy, loss of the force and peak power in the muscle tissue has strong ties with the cytoskeletal structures in a bottom up hierarchy[4,5,6]. In spite of the existence of this relationship, previous research works express the lack of the real time observation of myoblast cytoskeletal dynamics under microgravity. In the frame of this work, a dual mode microscope that sequentially operates in digital holographic microscopy (DHM) and fluorescence microscopy is built to observe cytoskeletal modifications in C2C12 mouse myoblast cells under simulated microgravity. Simulated microgravity environment is maintained using a random positioning machine (RPM)[7]. RPM simulates the microgravity condition for biological samples positioned at its 2D rotation center[8]. DHM provides real time phase images containing the optical thickness information of the transmission type sample with nanometer scale axial accuracy[9]. Robustness, noninvasive nature and accuracy of this technique opened the path for applications in 3D cell microscopy[10] and microgravity studies[11]. Fluorescence microscopy mode of the microscope is targeted on the enhanced green fluorescent protein (EGFP) transfected actin filaments of C2C12 cells to observe the dynamics of the actin network. These two microscopy methods in combination have the potential to provide deep understanding on 3D cell morphology and cytoskeleton dynamics in simulated microgravity.

2. METHOD AND EXPERIMENTAL SETUP

DHM is based on the basics of classical holography with a physical recording in off-axis geometry and reconstruction in numerical environment[12]. In classical holography, interference pattern of a known wave and modified version of it by an object, namely reference and object waves, is recorded on a photosensitive plate. Reconstruction of the 3D image of the

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object is achieved by the reference wave illumination of the photosensitive plate. A charge coupled device (CCD) camera replaces the photosensitive plate, and reconstruction takes place in numerical environment for DHM. Depending on the existence of an angle between the propagation axes of reference and object waves, recording scheme is named as in-line or off-axis. Off-axis geometry enables the separation of object wave spatial frequency information from others. Hence it can be filtered as a part of the hologram numerical treatment[13]. This numerical reconstruction methodology also brings the advantage of numerical corrections for various aberrations[14]. Numerically reconstructed complex object wave field is separated into amplitude and phase images that give object absorption and optical path length (OPL) respectively[12]. OPL is equivalent to object topology for reflection type DHM and optical thickness for transmission type DHM.

Experimental setup is fixed on the RPM and encloses both DHM and fluorescence microscope in the same housing. Laser beam at the wavelength of 653nm emerging from the laser diode is collimated by a lens and separated into two beams by a beam splitter. Width of the reference wave is increased using a beam expander, and it reaches to the Andor Luca electron multiplying CCD camera after being manipulated by the dichroic beam splitter in the filter cube. This dichroic beam splitter has its cut-off wavelength at 500nm and 85/15 transmission-reflection ratio at the laser wavelength. Meanwhile, other beam as the object wave is condensed by a lens and passes through the cell sample. A microscope objective with 60X magnification and 0.85 NA images the cell sample on the camera through the dichroic beam splitter. The interference pattern of object and reference waves is recorded for DHM mode. Then laser diode is turned off and LED light source with the center wavelength of 470nm and 30nm spectral width is turned on for the epi-fluorescence microscopy mode. Fluorescence excitation satisfies the Koehler illumination condition. For this purpose, LED light is shaped using a lens and a diaphragm. This excitation light reaches to the cell sample through the microscope objective after passing through the excitation filter and being reflected by the dichroic beam splitter in the filter cube. Emission from the EGFP transfected actin filaments of C2C12 cells is collected and imaged on the camera following the path of microscope objective, dichroic beam splitter and emission filter. Following image illustrates the optical schematic of this dual mode microscope.

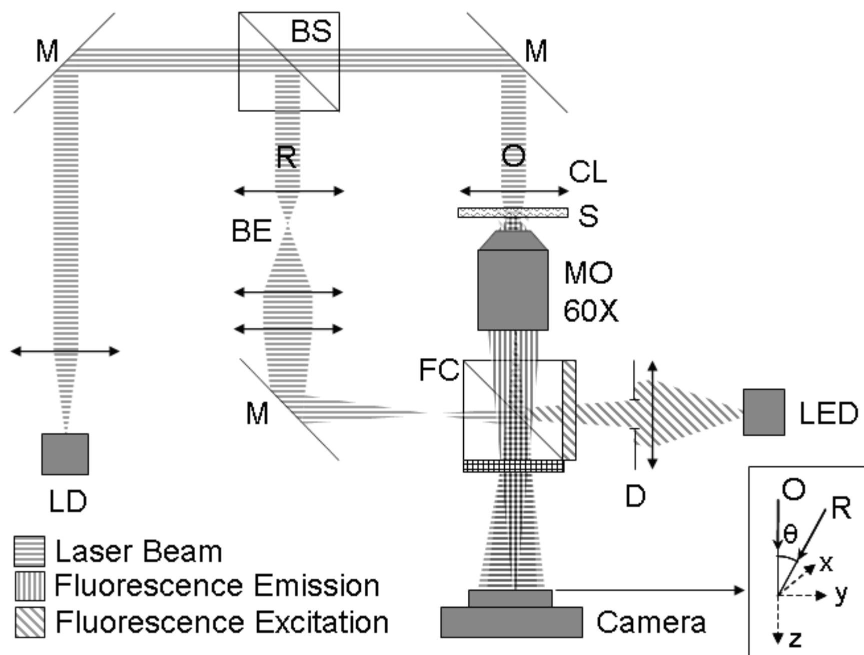

Figure 1 Simplified schematic of the microscope with LD: laser diode, M: mirror, BS: beam splitter, BE: beam expander, CL: condenser lens, S: sample, MO: microscope objective, FC: filter cube, D: diaphragm, O: object wave, and R: reference wave

On behalf of the microscope, RPM accommodates a built in computer and control box with power and signal line connectors. The built in computer is linked to a remote server over Wi-Fi link and transfers lossless compressed images from DHM and fluorescence microscopy modes to server and directs exposure time and gain settings to the camera from server. Control box provides power to laser diode and LED and switches them on and off sequentially based on the TTL

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level signal from the built in computer. Phase images are reconstructed on the server in real time from the transferred holograms and post processed for out-of focus and lateral shift when required.

3. RESULTS

Initial experiments are carried out for DHM and fluorescence modes separately. For fluorescence mode, fixed C2C12 cells with EGFP transfected actin filaments are imaged and post processed to evaluate the performance of this mode. Figure 2 a shows a sample fluorescence mode image. As it can be observed from this image, fluorescence mode provides sufficient level of detail with high contrast. DHM mode initial experiments are based on living C2C12 cells. These initial experiments in simulated microgravity showed two different kind of alterations. Some of the cells under this simulated microgravity conditions formed lamellipodias in time. Phase image series in time of an exemplary cell region forming lamellipodia is shown in figure 2 b. In the beginning of microgravity loading this region has a low optical thickness in the order of 50° or less. After one hour of continuous exposure to microgravity, thickness increases to level of 75° and reaches to steady state after 2 hours of exposure with final thickness of 100°. Protein accumulation at the perinuclear region is the other type of modification observed in these experiments. Another series of phase images are shown in figure 2 c to illustrate this accumulation by the optical thickness increase around nucleus. These observations are also visible on the fluorescence images of the actin network with well agreement (data not shown).

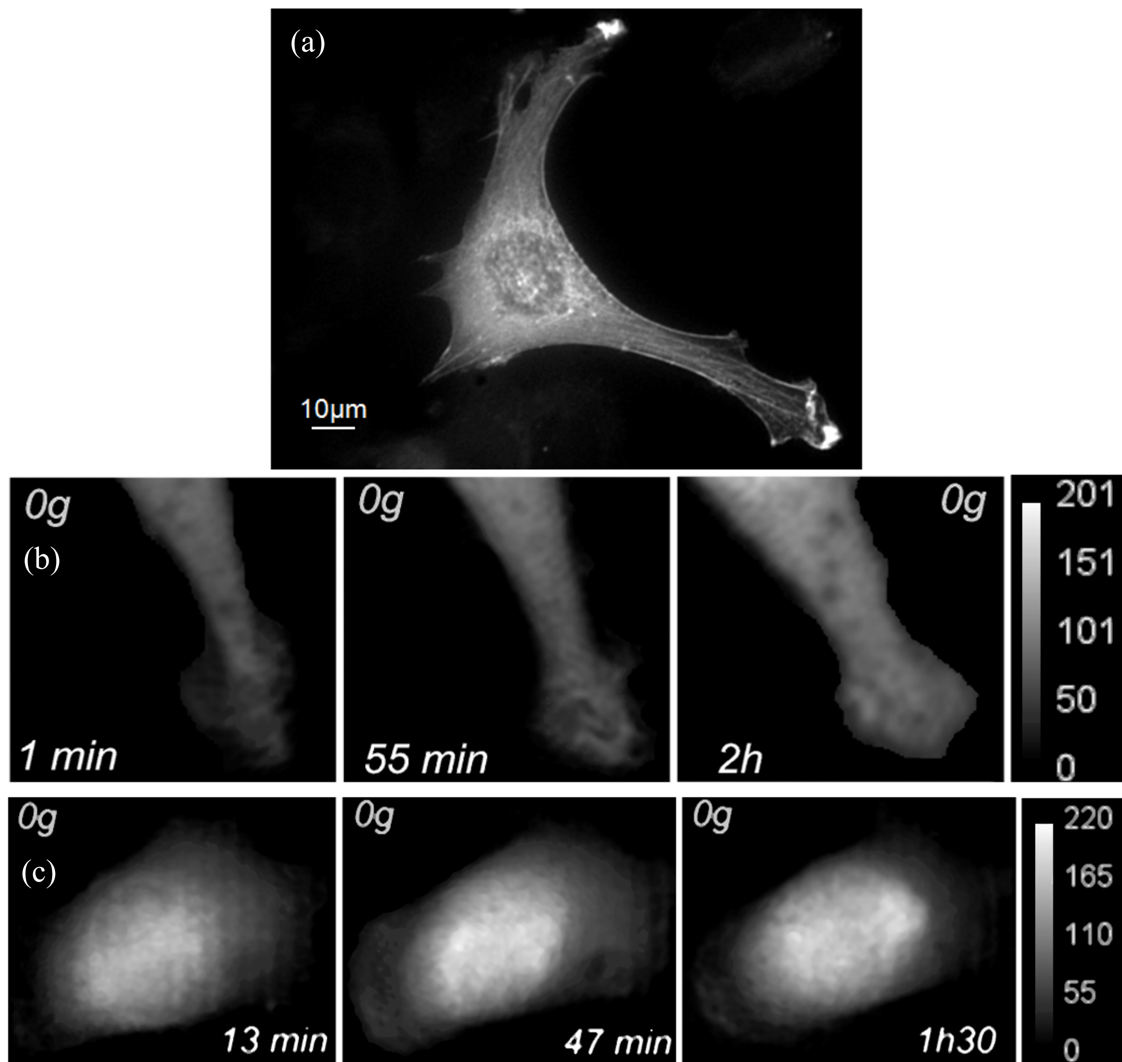

Figure 2. Fluorescence microscopy mode image of EGFP transfected actin filaments in a fixed C2C12 cell (a), phase images at different time instances showing the lamellipodia formation by a living cell under simulated microgravity (b), and protein accumulation around nucleus (c) with grayscale bars in degree

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4. CONCLUSION

In this work, a dual mode DHM and fluorescence microscope operating in time sequential mode is developed to study cytomorphological modifications in C2C12 mouse myoblast cells. Microscope is fixed on the microgravity simulation platform, RPM, with required peripheral instruments. DHM mode provides real time phase images of cells while epi-fluorescence microscopy mode unveils actin filament network distribution in real time taking advantage of EGFP transfection. Initial experiments with fluorescence mode show sufficient microscope performance. Results obtained from DHM mode indicated to lamellipodia formation and protein accumulation around the nucleus. Future work involves experimentation on living C2C12 cells using both microscopy modes sequentially on RPM, improvement of the close loop operation, and microscope miniaturization.

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