

DIGITAL HOLOGRAPHIC MICROSCOPY FOR THE STUDY OF MORPHOLOGICAL CHANGES IN CELLS UNDER SIMULATED MICROGRAVITY CONDITION

Pache C.^(1,2), Westphal K.⁽²⁾, Parent J.⁽¹⁾, Franco-Obregon A.⁽³⁾, Depeursinge C.⁽¹⁾, Egli M.⁽²⁾

⁽¹⁾Ecole Polytechnique Fédérale de Lausanne, Advanced Photonics Laboratory, 1015 Lausanne, Switzerland
Email: christophe.pache@epfl.ch

⁽²⁾Eidgenössische Technische Hochschule Zurich, Space Biology Group, 8005 Zürich, Switzerland

⁽³⁾Eidgenössische Technische Hochschule Zurich, Institute of Biomedical Engineering, 8005 Zürich, Switzerland

ABSTRACT

Previous investigations on mammalian cells have shown that real microgravity in space and/or simulated microgravity causes severe cellular modifications. Aim of the study was to use Digital Holographic Microscopy (DHM) to investigate morphological changes of cells under simulated microgravity generated by the Random Positioning Machine (RPM) in real-time. Preliminary studies were carried out on mouse myoblasts (C2C12). Besides showing cellular modification under simulated microgravity similar to the results obtained by the using conventional methods, an increase of the phase height of the cell was measured which have not been reported yet.

Our technique provides a new approach in the study of cellular adaptations to microgravity, providing real-time images of living cells.

1. INTRODUCTION

Several studies have shown morphological modifications of cells when subjected to microgravity condition including reorganization of the cytoskeleton [1]. However, these studies were mostly performed on fixed cells which were previously exposed to microgravity, either simulated by a device like RPM or during actual space missions. To get more insights of the dynamic processes responsible for the microgravity induced morphological changes, real-time measurements on living cells is needed. Phase contrast images of biological samples fulfill these requirements, making DHM the method of choice.

The aim of the study was to adapt DHM for the use on the RPM and to validate the system.

2. MATERIALS AND METHODS

2.1 Digital Holographic Microscopy

DHM is an innovative interferometric imaging method able to measure optical path length (OPL) in transmission or reflection. The OPL is defined by:

$$OPL = \int n(x)dx \quad (1)$$

Because n represents the refractive index and x the light propagation distance, DHM can be used to evaluate topology of three-dimensional objects or detect structures of different refractive indexes. The axial

resolution is in the nanometer range. The lateral resolution is diffraction-limited, depending on the numerical aperture (NA) of the microscope objective. DHM measures the phase of the transmitted or reflected light and the phase changes can be related to the height of the object and/or a refractive index change. The captured phase contrast images are convertible into a quantitative optical path difference (OPD) measurement by using the relation, where λ is the wavelength of light and φ , the phase delay [0°; 360°].

$$\frac{OPD}{\lambda} = \frac{\varphi}{360} \quad (2)$$

To obtain a hologram in transmission, the object is illuminated by a collimated light beam O and the transmitted light interferes with a reference wavefront R on a CCD camera, as shown in Fig. 1. In contrast to the reference beam, the wavefront O experiences a sample induced disturbance.

The hologram is then virtually illuminated by a plane wave and propagated over the reconstruction distance, which can be set in order to get the image in focus [3].

Cells are typical phase objects, meaning that phase delays will be induced on a light wave passing through cells but the intensity of the beam will not be significantly affected. Phase changes are created by refractive index differences between the cells and their environment or structures within cells.

The main advantages of DHM for cell imaging are:

- quantitative 3D images
- real-time measurement
- no invasive, no phototoxicity
- digital focusing (extended depth of focus)
- robust regarding environmental disturbances (e.g. vibrations)
- numerical correction of the aberrations [2]

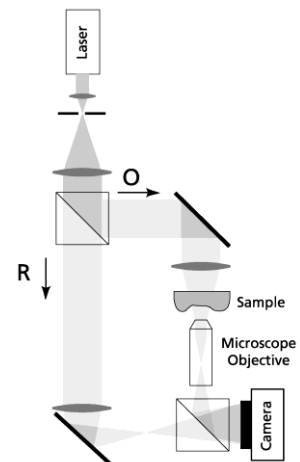


Fig. 1: DHM in transmission

2.2 Cells

C2C12 cells were cultivated in flasks and continuously observed by DHM (magnification: 40x) under normal and under simulated microgravity. Ground controls were recorded as reference. The frame grabbing period was set to one second.

2.3 Setup

DHM was mechanically adapted to allow an installation on the RPM (Fig. 2). An embedded computer (PC) acquired and stored the recorded holograms. At the end of the experiment, all the gathered data were transmitted to a second computer (PC 2). The final hologram reconstructions as well as image processing were performed on the second computer afterwards.

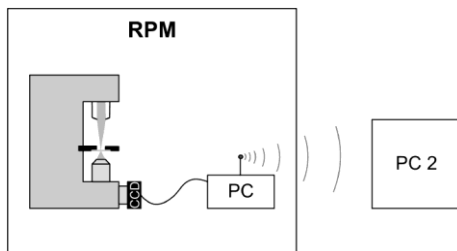


Fig. 2: Scheme of the whole setup

3. RESULTS

DHM technique allows a detailed analysis of morphological aspects of living cells: their topography can be revealed as well as internal cell structures (due to differences of the refractive index) (Fig. 3).

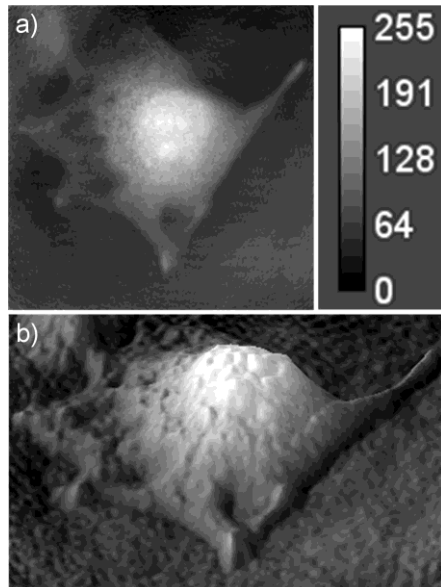


Fig. 3: DHM images of a living cell under simulated microgravity condition on the RPM. a) Phase image (vertical scale in degrees [°]); b) Perspective image of a). FOV: $70 \times 70 \mu\text{m}^2$

Morphological analysis of the dynamic processes of cells under simulated microgravity indicated an increase of the phase height (Fig. 4).

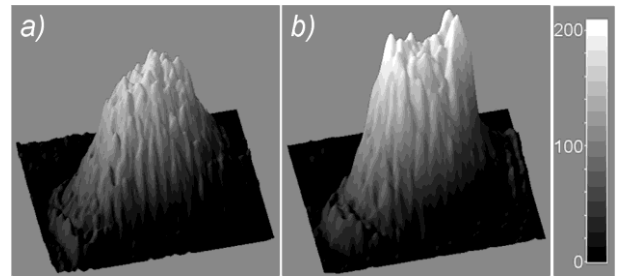


Fig. 4: Phase images, vertical scale in degrees [°] demonstrating the observed behaviour: a) a cell in normal gravity and b), the same specimen after 1h30 under simulated microgravity conditions.

The comparison of the two images in Fig. 4 (a and b) clearly demonstrate the difference in the phase height. First signs of this morphological alteration can be seen after only two minutes simulated microgravity exposure. In contrast, 1 g control cells did not change their phase height during the whole observation period.

4. DISCUSSION

Our novel setup allows a three-dimensional study of living cells exposed to simulated microgravity. Real-time changes of single cells can be observed during a long time period (several hours). Moreover, the method is non-invasive and no fixation of cells, which may produce undesired effects.

Our first observation on living cells showed an increase in the phase height within the initial phase of simulated microgravity exposure. This increase can be due to two modifications: an increase of the refractive index or the actual cell height. We hypothesize that in our experiments, an altered refractive index is responsible for the effect observed. This might be due to a redistribution of the protein concentration, especially around the nucleus.

5. REFERENCES

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