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Digital Holographic Microscopy for Characterization of *Fabiana Imbricata Ruiz & Pav.* Cell Suspension Cultures

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An application of digital holographic microscopy (DHM) for measuring the size of cell clusters in cell suspension cultures *in vitro* is reported for the first time. Undifferentiated plant cell cultures are widely used for fundamental and applied research, for the elucidation of biosynthetic pathways, production of secondary metabolites and as screening tools for biotechnology in pharmacy, food technology, and agriculture. Digital in-line holographic microscopy has been applied to visualize three different cell suspensions of *Fabiana imbricata Ruiz & Pav.*, named A, D, and MSD. Digital reconstruction of the recorded interference patterns was performed using an appropriate software. The reconstructed intensities represent the cell clusters in the suspensions under observation. Cell suspension cultures of *Fabiana imbricata Ruiz & Pav.* consists of cell aggregates dispersed and growing in shaking liquid media. Small cell aggregates with dimensions between 120 and 180 μm have been observed in all suspensions. The measurement of growth parameters in different cultures introduces diverse problems that must be addressed by using a specific methodology for each type of callus and cell suspension cultures. The attractive features of digital holographic microscopy are noncontact, nondestructive, marker-free *in vivo* imaging and quantifying biological cells and tissues. It is an advantageous technique for application in biological research and the agricultural sciences. It is shown that DIHM is a new microscopic technique easy to apply to study the size and the structure of cell aggregates in cell suspension cultures *in vitro*.

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1. Introduction

Imaging of microscopic objects is an essential need in technical and life sciences. The rapid progress in electronic detection and control, digital imaging, image processing, and numerical computation has been crucial in advancing modern microscopy. Digital holographic microscopy is a new imaging technology in the field of optical microscopy.

In the conventional in-line holography, invented by Gabor [1], the holograms are photographically recorded and optically reconstructed. Much more conveniently in digital holography, the holograms are recorded digitally, and the reconstruction is performed numerically. The digital holographic microscopy (DHM) has one more clear advantage over conventional holography as it yields a 3D volume image from a single image capture. DHM is capable of visualizing live cells with dimensions from 1 μm to 200 μm without any preliminary preparation. It can be applied to dynamic quantitative visualization of live cell deformations, to study their interactions with other particles and the surrounding environment as well. DHM is a valuable technique widely applicable in life sciences.

Fabiana imbricata Ruiz & Pav. is a plant used in Chilean folk medicine to treat diseases of the kidneys and urinary tract. It is a potent diuretic which also promotes digestion when prepared as a tea. *F. imbricata* has a long history of use in the treatment of general diseases as well. A mother tincture is occasionally applied in homeopathy for the treatment of the liver and urinary system, as well as for a general tonic, and water/alcohol extract is beneficial as an antiseptic [2]. The chemical composition of the plant *Fabiana imbricata Ruiz & Pav.* is less studied in detail. A particular interest is paid on the investigation of the metabolite profiles as a base for further isolation and characterization of valuable secondary metabolites. It has been reported that the principal components of the secondary metabolite mixture in the crude drug are the rutin, the coumarin scopoletin, oleanic acid and several sesquiterpenoids as well [3]. *Fabiana* plant has high vegetative propagation potential *in vivo* and *in vitro*, whereas the multiplication *in vitro* is faster, enabling rapid multiplication of valuable genotypes. Shoot regeneration is possible from callus culture [4].

The microscopic investigations of *Fabiana* cell suspension cultures are of great interest, giving information about the formation and dimensions of cell aggregates, the cell divisions and growth, and other dynamic studies. The digital holographic microscopy is promising microscopic technique, due to its application in live objects investigations.

2. Experimental procedure

2.1. Optical setup of the holographic microscope

Digital in-line holographic microscope (DIHM) was developed at the Agricultural University of Plovdiv [5].

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The light source is a diode laser (Lasiris) with a wavelength of 673.2 nm and output of 7 mW. The laser radiation is focused onto a pinhole after which the intensity is controlled by a polarizer (Fig. 1). After the pinhole, the spherical wave passes through the object: the diffracted by the object and the nondiffracted wave interfere as a hologram, which is recorded on a CCD sensor. The intensity and the phase are reconstructed numerically [6].

USAF Test Target 1951 was used to calibrate the holographic microscope. The optimum distance between the laser source and the object is 30 cm to record holograms of *Fabiana imbricata Ruiz & Pav.* cell suspension cultures. The distance from the object to the CCD camera in the experiments is 5 cm.

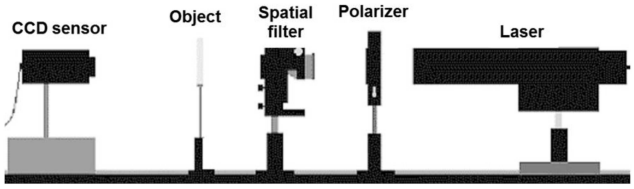

Fig. 1. Optical set-up of the digital in-line holographic microscope.

2.2. Fabiana imbricata Ruiz & Pav. cell suspension cultures

Plant cell suspensions were obtained following induction of callus on solid MSD medium and subsequently transferred to different variants of liquid culture media (Table I). The basal medium was MS [7], supplemented with sucrose (30 g/l) and pH = 5.7. Suspension cultures were developed and maintained on a rotary shaker at 110 rpm in darkness at 25 °C with a subculturing period of 10 days.

TABLE I

Variants of culture media for *Fabiana* cell suspensions.

Variants	Growth regulators	Concentration [mg/l]
MSD	2.4–D	0.2
D	2.4–D	4.0
A	2.4–D	0.25
	NAA	0.25
	kinetin	0.25
	+ vitamins MS	X 2

2.3. Preparation of samples

Cell suspension cultures of *Fabiana imbricata Ruiz & Pav.* consists of cell aggregates dispersed and growing in shaking liquid media. The samples for the investigation with the digital holographic microscope were prepared by the following method: 5 ml of plant cell suspension was transferred into a 10 ml glass tube; 5 min were given for the larger clusters to precipitate; the solution under investigation was taken with a pipette from the upper 1 cm layer; 0.5 ml of the solution containing plant cells were deposited on a microscopic glass slide and covered with a coverslip.

3. Results and discussion

DIHM was applied to visualize three different cell suspensions of *Fabiana imbricata Ruiz & Pav.* named A, D, and MSD. Digital reconstruction of the recorded interference patterns is performed using the "HoloVision 2.2" software [6]. Figures 2, 3 and 4 present the holograms and the reconstructed intensities to represent the objects.

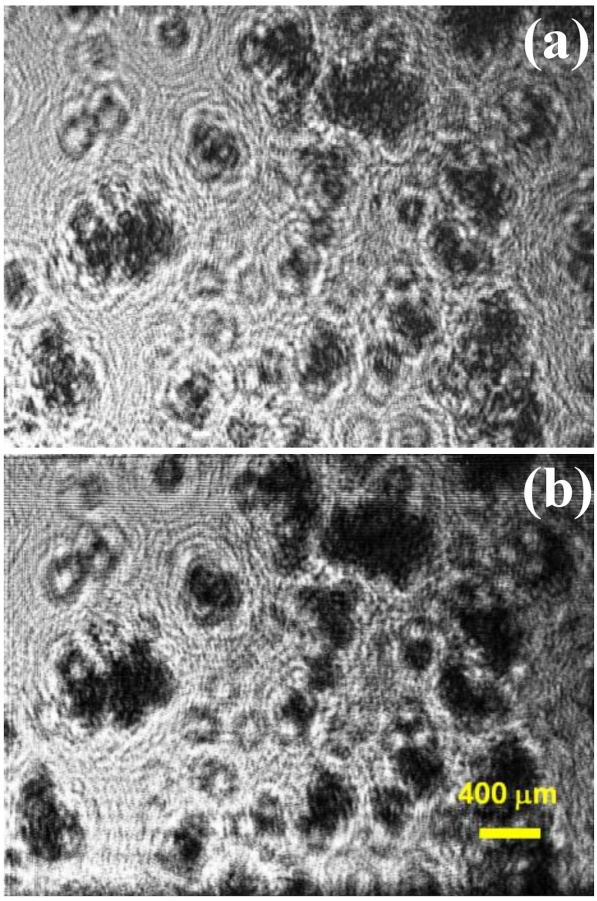

Fig. 2. Images of cell suspension culture A: (a) digital hologram, (b) the numerically reconstructed wavefront intensity of (a).

Small cell aggregates with dimensions between 120 and 180 μm have been observed in all suspensions. The large cell aggregates (140−180 μm) are characteristic for suspension A (Fig. 2). The cell aggregates in suspension D have dimensions between 120 μm and 150 μm (Fig. 3). Because the plant cell walls have a natural tendency to adhere, the obtaining of homogeneous cell lines that consist only of single cells is impossible. Some progress has been made in selecting cell suspensions with increased cell segregation in the cultures with medium

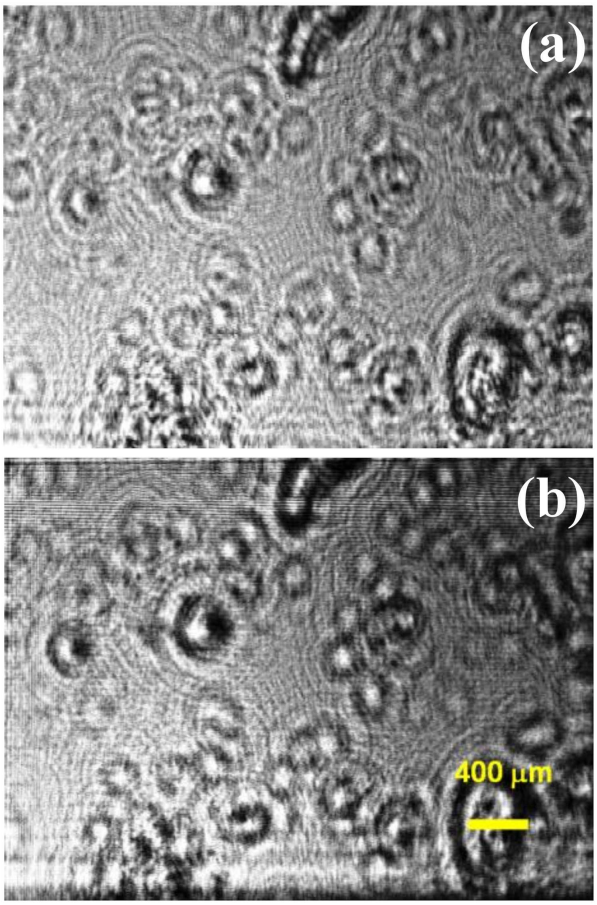

Fig. 3. Images of cell suspension culture D: (a) digital hologram, (b) the numerically reconstructed wavefront intensity of (a).

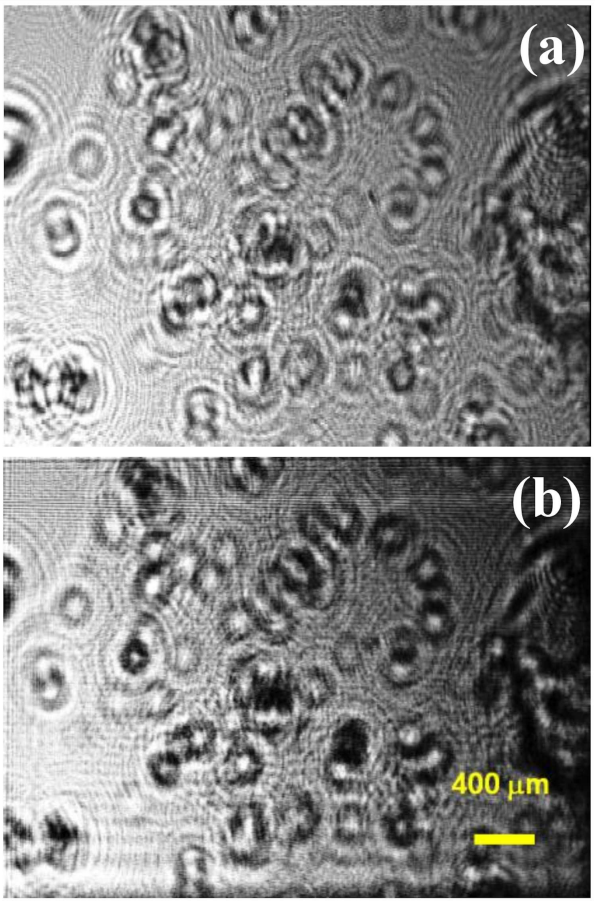

Fig. 4. Images of cell suspension culture MSD: (a) digital hologram, (b) the numerically reconstructed wavefront intensity of (a).

MSD (Fig. 4).The proportion and the size of the cell aggregates are genotype dependent and influenced by both the growth regulators and some nutrient medium constituents (such as calcium ions) in the culture. The measurement of growth parameters in different cultures introduces diverse problems that must be addressed by using a specific methodology for each type of callus and cell suspension cultures.

The reconstructed intensities illustrated the possibility of direct observation of live cell suspension cultures. These experiments demonstrate the capability of DHM for non-invasively visualizing and quantifying biological cells and cell clusters. Moreover, DHM can be successfully used for: cell counting, measuring the size of cells and cell clusters, label-free viability analysis of adherent cell cultures, etc.

4. Conclusion

We report for the first time an application of digital holographic microscopy (DHM) for measuring the size of cell clusters in cell suspension cultures *in vitro*. A method for sample preparation was developed. DIHM was applied to visualize three different cell suspensions of *Fabiana imbricata Ruiz & Pav.* and for assessing of the cell aggregates. In all suspensions, the cell aggregates with dimensions between 120 and 180 μm have been observed. The histological studies of suspension cultures are a slow and complicated process, while the application of DHM allows the evaluation of their development in dynamics. Present investigation demonstrated that DHM could provide an efficient label-free morphology analysis of cells and label-free studies of cell division and migration. Data for the dimensions of cell aggregates of *Fabiana imbricata Ruiz & Pav.* are reported for the first time.

The results obtained are promising for the future use of DIHM for accurate, fast, and reliable determination of cell division and growth, which is of critical importance in plant cell and tissue culture. DIHM is an attractive novel technique for application in life sciences, biological research and medical applications, and the agricultural science.

Acknowledgments

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