НАБЛЮДАВАНЕ НА КЛЕТКИ ALGAE *CHLORELLA VULGARIS* С ЦИФРОВ ХОЛОГРАФСКИ МИКРОСКОП
CELL OBSERVATION OF *CHLORELLA VULGARIS* ALGAE BY DIGITAL HOLOGRAPHIC MICROSCOPY

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Abstract

Imaging of microscopic objects is an essential art, especially in life sciences. Digital holographic microscopy is a new imaging technique, which is very advanced when compared to optical microscopy. Digital holographic microscopy does not require any preliminary preparation of the samples and can be used to study live objects. This new type of microscopy is very advanced because it yields a 3D volume image from a single hologram. By combining several images, reconstructed from the same digital hologram, but at different focal planes, an increased depth of field can be obtained, which is vastly superior to the depth of a field achieved with traditional light microscopy.

It is shown that digital holographic microscopy is capable of visualising live cells with dimensions of 5–10 μm without any preliminary preparation. A big advantage of the new technique is that it can be used for dynamic visualisation of live cell deformations to study their interactions with other particles as well as the surrounding environment. The new technique was successfully employed to observe the growth and proliferation of *Chlorella vulgaris* algae. It was illustrated that digital holographic microscopy can be used for label-free morphology analysis of cells and label-free studies of cell division and migration.

Key words: digital holographic microscopy, *Chlorella vulgaris*, prolification.

INTRODUCTION

Holography is a technique by which a wavefront can be recorded and subsequently reconstructed in the absence of the original wavefront i.e. a 3D image is observed just as if the object was still present and being illuminated in the same way as when the holographic recording was made (Jones et al., 1989). In conventional holography, invented by Gabor (Gabor, 1948), the holograms are photographically recorded and optically reconstructed.

The digital holography does not require wet chemical processing of a photographic plate. Once the amplitude and phase of the light wave are recorded numerically, these data can be subjected to a variety of manipulations, and so digital holography offers capabilities not available in conventional holography. The remarkable aspect of the digital reconstruction – its possibility to refocus at different depths inside a transparent object, depending on the reconstruction distance, makes this technique very suitable for biological cells studies and could have many applications in life sciences.

Digital holographic microscopy (DHM) can provide quantitative marker-free imaging that is suitable for high resolving investigations of transparent and reflective surfaces as well as for fast analysis of living cells under usual laboratory conditions. One of many interesting applications of DHM is to study cells without staining or labeling them and without affecting them in any way.

MATERIALS AND METHODS

Digital in-line holographic microscope (DIHM) was constructed and developed at the Agricultural University of Plovdiv (Peruhov et al., 2012).

The light source is a diode laser (*Lasiris*) with wavelength of 673.2 nm and output of 6.98 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 non-diffracted wave interfere and are recorded as a hologram on a CCD sensor. The intensity and the phase are reconstructed numerically (Skotheim et al., 2001; Seifi et al., 2012).

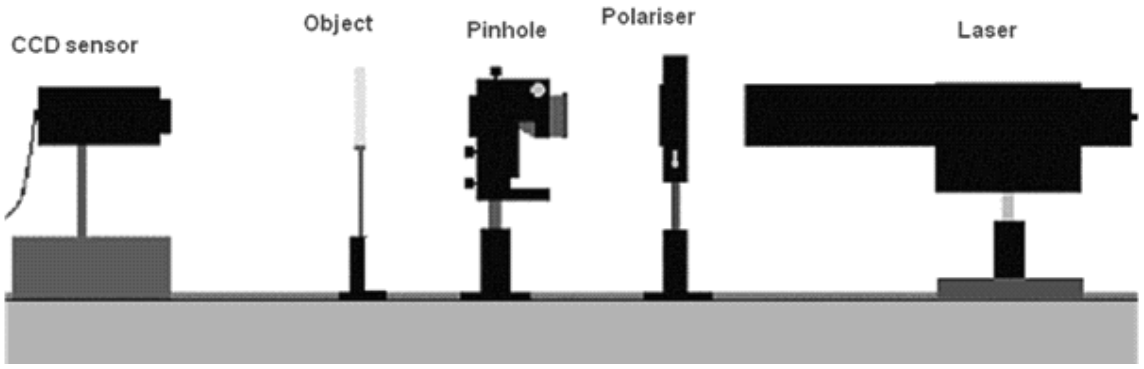
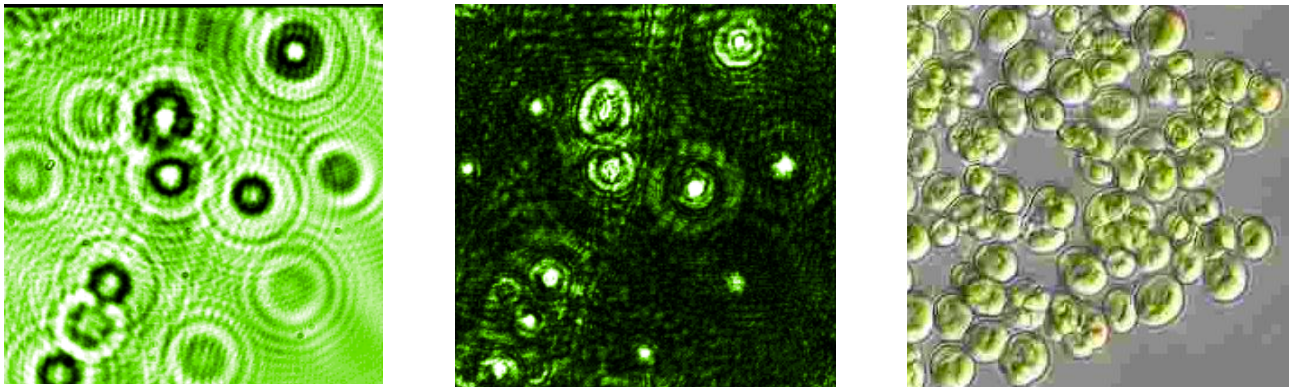


Fig. 1. *Optical set-up of the digital in-line holographic microscope*



a **b** **c**

Fig. 2. *Images of algae Chlorella vulgaris, approximately 5-8 μm in diameter: a) digital hologram; b) the numerically reconstructed wavefront intensity of* ***a****; c) image from a light microscope*

RESULTS AND DISCUSSION

DIHM was applied to visualise live algae cells of *Chlorella vulgaris*, without any preliminary preparation. Digital reconstruction of the recorded interference patterns is performed using appropriate software (Skotheim et al., 2001; Seifi et al., 2012).

Figure 2 shows a digital hologram and the reconstructed intensity to represent the object. The reconstructed intensity illustrates the possibility of observation of the structure and morphology of live algae cells obtained from a digital hologram. These experiments illustrate the capability of DHM for non-invasively visualizing and quantifying biological cells and tissues. That's why DHM can be successfully used for: cell counting, observation of cell growth and proliferation. etc.

Figures 3-5 present digital holograms of algae cells *Chlorella vulgaris* taken at different stages of their life cycle and the reconstructed intensities of these holograms. The cells morphology is visible on the images showing the reconstructed intensities.

These experiments illustrate the capability of DHM for:

- label free morphology analysis of cells
- label free studies of cell division and migration
- label-free analysis of subcellular motion in living tissues etc.

CONCLUSIONS

It is shown that digital holographic microscopy is capable of visualising live cells with dimensions 5–10 μm without any preliminary preparation. A big advantage of the new technique is that it can be used for dynamic quantitative visualisation of live cell deformations to study their interactions with other particles as well as the surrounding environment. This makes the DIHM a valuable technique for many life science applications. It is demonstrated that digital holographic microscopy can provide quantitative marker-free imaging of transparent micro-objects as well as for fast analysis of living cells under usual laboratory conditions. The new technique was successfully employed to observe growth and proliferation algae *Chlorella vulgaris*.

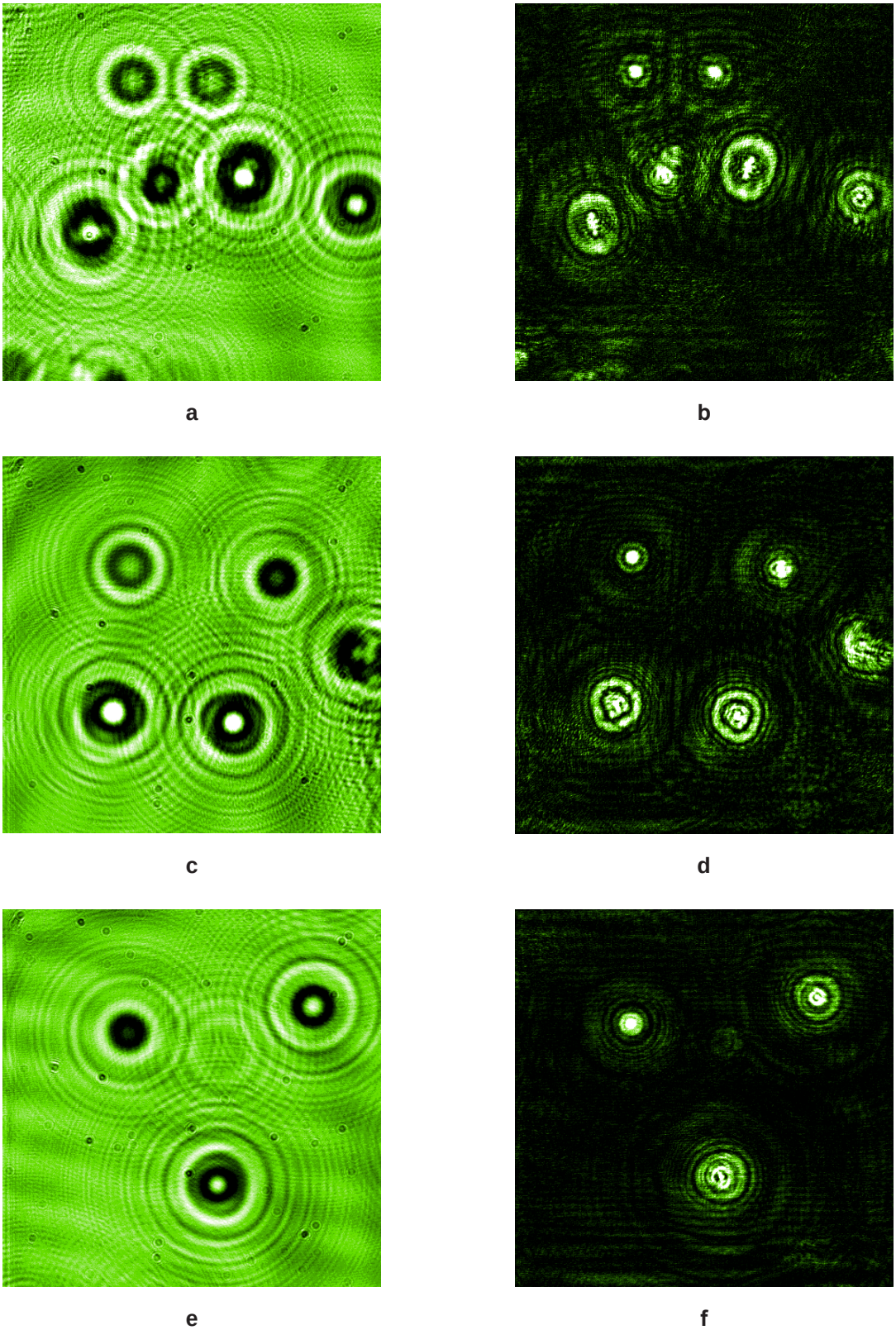
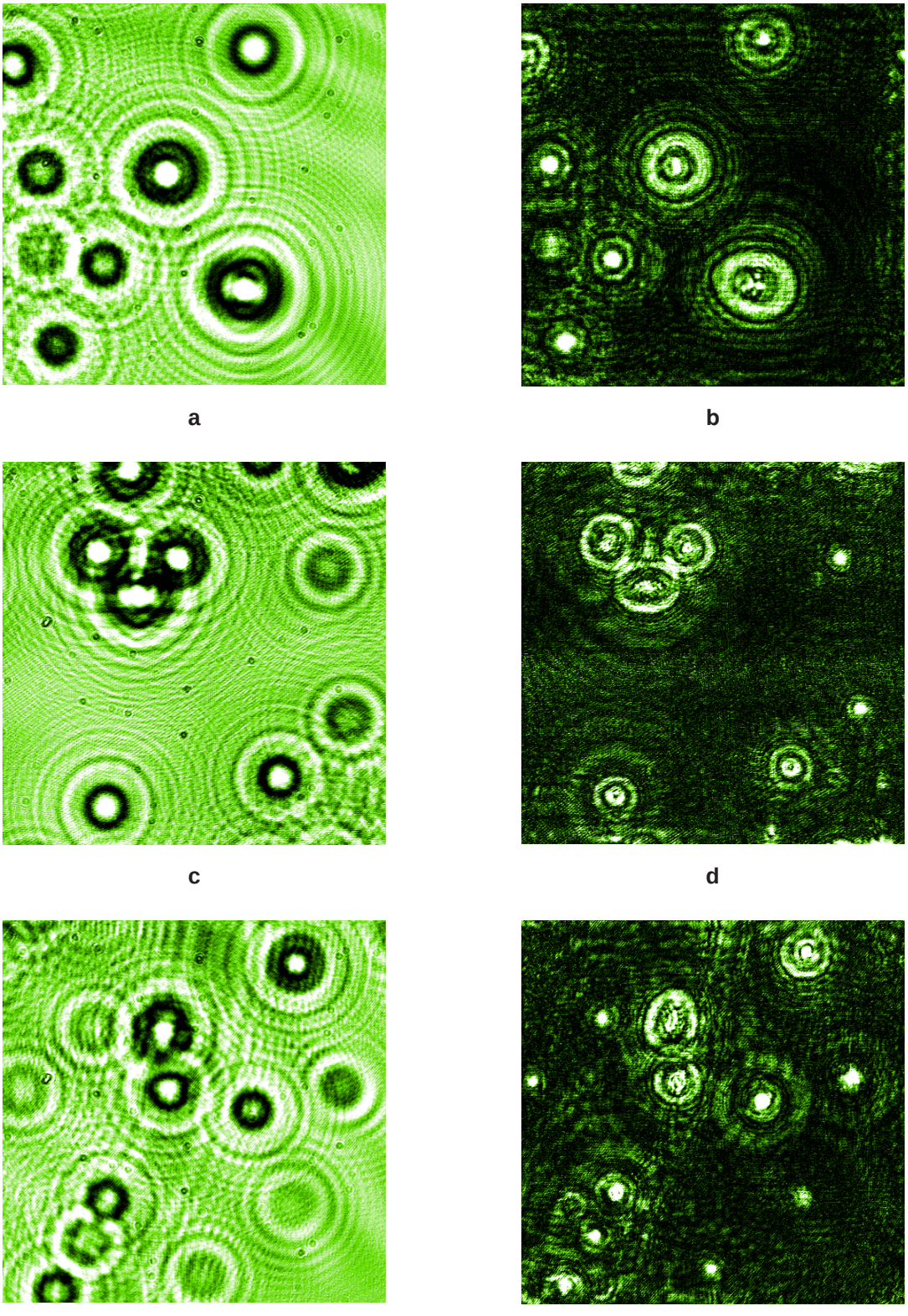


Fig. 3. *Images of algae Chlorella vulgaris, approximately 5-8 μm in diameter: a) digital hologram of one day old cells; b) the wavefront intensity of* ***a*** *c) digital hologram of one day old cells; d) the wavefront intensity of* ***c*** *e) digital hologram of one day old cells; f) the wavefront intensity of* ***e***



a **b**

c **d**

e **f**

Fig. 4. *Images of algae Chlorella vulgaris, approximately 5-8 µm in diameter: a) digital hologram of 5 day old cells; b) the wavefront intensity of* ***a*** *c) digital hologram of 5 day old cells; d) the wavefront intensity of* ***c*** *e) digital hologram of 5 day old cells; f) the wavefront intensity of* ***e***

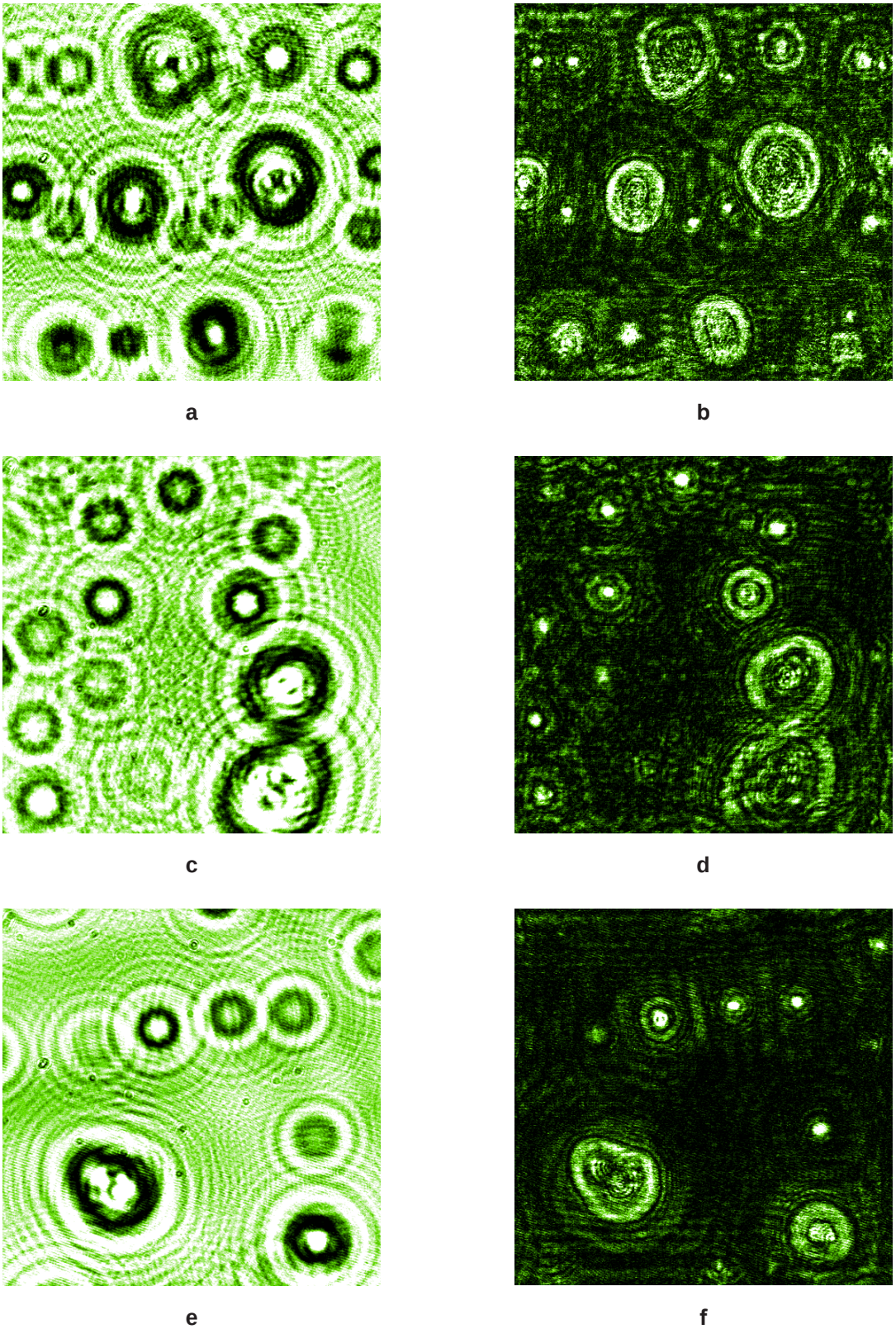


Fig. 5. *Images of algae Chlorella vulgaris, approximately 5-8 µm in diameter:*
a) digital hologram of 8 day old cells; b) the wavefront intensity of ***a***
c) digital hologram of 8 day old cells; d) the wavefront intensity of ***c***
e) digital hologram of 8 day old cells; f) the wavefront intensity of ***e***

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