Non-invasive dry mass determination and monitoring at the single cell level with digital holographic microscopy.

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ABSTRACT

Digital holography microscopy (DHM) is an optical microscopy technique which allows recording non-invasively the phase shift induced by living cells with nanometric sensitivity. Here, we exploit the phase signal as an indicator of dry mass (related to the protein concentration). This parameter allows monitoring the protein production rate and its evolution during the cell cycle.

Keywords: digital holography, quantitative phase microscopy, protein production, dry mass, cell cycle, cell growth, fission yeast, Schizosaccharomyces pombe

1. INTRODUCTION

Cells reproduce by duplicating their content and then dividing by two. This cell-division cycle is the fundamental means by which all living things are propagated. Cell cycle genes are highly conserved between species and yeast cells, due to their ease of use and fast growing rate, have been extensively used to study the cell cycle machinery (Hartwell et al. 1974). Many mutant genes affecting various stages of the cell cycle have been discovered (Nurse et al. 1984) and used thoroughly to study the cell cycle.

The cell cycle is composed of 4 stages. After the previous cell division, the new cell starts with a gap stage (G1), where the protein machinery required for cytokinesis (division of cytoplasm by formation of a septum in the center of the cell) is put together. DNA synthesis occurs during the S stage and is followed by the second gap stage (G2), the longest stage in S. pombe. Nucleus division occurs during the final stage, mitosis (M) [see (Forsburg et al. 1991) for a review]. In rapidly growing S. pombe cells, S phase begins before cytokinesis is completed, and there is only a very short G1 phase. During the whole cell cycle, cell size and protein concentration are a highly controlled parameter (Nurse 1975; Fantes et al. 1977).

Monitoring the protein production can provide insights into the cell cycle completion. The most common ways to quantify proteins concentration involves colorimetric measurement of protein labellized with Coomassie (Bradford 1976) or Folin-phenol (Lowry et al. 1951). When studying yeast growth, and DNA or protein production the general approach involves cell lysis and absorption measurement of the lysate (Bostock 1970). These methods are invasive and do not allow monitoring changes in real time. Barer proposed in 1952 (Barer 1952) a way to relate the phase shift of light to the dry mass (an indicator of biomass related to the protein concentration). This relationship has already been used by (Zicha et al. 1995) to study the growth of chicken fibroblast with a classical interferometer microscope. However, this approach requires time-consuming post-processing of the data to compensate for the instability of the setup.

In this prodeeding we report how digital holographic microscopy (DHM) non-invasively monitors the phase shift of dividing cells and relates it the dry mass parameter used as an indicator of the cell cycle stage.

2. MATERIALS AND METHOD

2.1 Setup

The transmission DHM used for the present study has been described in details in Refs. (1) and (2). Results presented here have been obtained with a 60x 0.9 NA microscope objective (MO). The camera is a standard 1392 x 1040 pixels, 8 bits, black and white CCD, with a pixel size of 6.45 µm x 6.45 µm, and a maximum frame rate of 25Hz. For a 1024*1024 pixels hologram, the field of view is around 110 µm x 110 µm. With an INTEL Core 2 Duo 6600 2.4GHz, the phase image reconstruction described extensively in refs. (3) and (4), reaches a 15 frames/second rate. The light source is a laser diode with a wavelength of 663nm.

2.2 Cell culture and experimental conditions

Schizosaccharomyces pombe (fission yeast) were cultured in a classical Y5 rich medium before imaging. Experiments with the wild type strain 972 h- were conducted at 36°C. To obtain this temperature the microscope was placed in a closed Plexiglas box heated by hot air at 30°C ("the Cube" system, Life Imaging Services, Switzerland). The cell chamber was further heated at 36°C with a home-made PID controlled thermoresistance to ensure a perfect stability of the temperature along the experiment. One image was recorded each minute. All graphics are presented as running average of 3 values.

3. RESULTS AND DISCUSSION

Cell need to double their content during each cell cycle to preserve a constant size. A good indicator of the biomass is the dry mass, defined as the weight of the cell when water is evaporated. This parameter is mainly dependent on protein concentration (5). Monitoring the dry mass production is a good indicator of the evolution of the cell cycle. Barer already showed in 1952 (6) that the phase shift induced by a cell is related to its dry mass (DM) by the following equation:

$$DM = \frac{10\varphi\lambda S}{2\pi\alpha} \tag{1}$$

Where φ is the sum of the phase shift induced by each pixel of the cell, λ is the wavelength of the light source of the setup (663 nm), S is the surface of the cell (determined by a classical edge detection algorithm) and α is a constant known as the specific refraction increment (in kg/m^3 or g/dl) related to the protein concentration. α can be approximated by 0.002, when considering a mixture of all the component of a cell (6, 7).

In this series of experiments, we follow the growth of S. pombe. By recording the phase signal and the cell surface, the dry mass production of the cell is calculated from Eq. (1). The results are representative of 6 wild type cells. Figure 1 illustrates the evolution of the dry mass (in pg), the cell surface and the dry mass surface density (in $pg/\mu m^2$) during the recording period. In addition, 4 selected phase images of the monitored cell illustrate the main stages of the cell cycle. The two sister cells after cell division are considered as a single cell to better follow the continuity of the measured parameters.

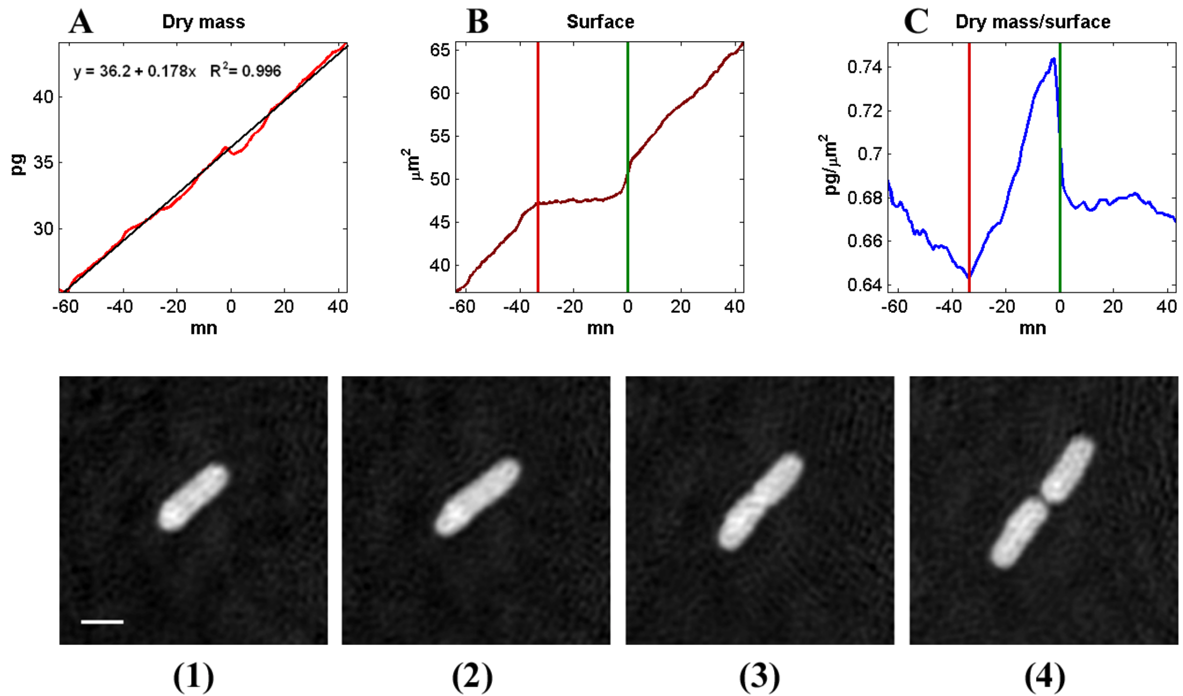

Figure 1: TOP: (A) dry mass (fit: linear regression), (B) cell surface and (C) dry mass concentration. Vertical lines: red, end of surface growth; green, cytokinesis. BOTTOM: representative phase images recorded at the beginning (1), at the end of cell growth (2), at cytokinesis (3) and at the end of the recording period (4). Scale bar 5 µm.

We can observe in Figure 1(A) a constant rate of dry mass production of 10.7 pg/h (as indicated by linear regression) which remains constant during the whole monitoring period. Such linear growth within a generation time was already reported for S. pombe by (8). Using this linear regression we can predict that the cell doubles its content in about 2.3 hours, a normal growth rate for this temperature. The constant growth in biomass is linked to protein and sugar synthesis for membranes construction during the cell cycle. The cell surface grows linearly during the first stage of the cell cycle and is followed by non-growing period of 35 mn before cytokinesis (indicated by the green vertical line). This probably occurs upon entry into mitosis, when cell growth at the tips stops and septum formation takes place.

Considering the dry mass concentration (obtained by dividing the dry mass by the cell surface), we observe a clear correlation with the stage of the cell cycle. During the 30 first minutes of the recording period the dry mass concentration slowly declines indicating a higher surface growth than dry mass production. When the surface growth pauses, the dry mass production remains constant thus producing this peak in dry mass concentration during the 35 mn preceding cytokinesis. This peak is probably linked to the recruitment of proteins required for the mitosis machinery, for the formation of the septum required for cytokinesis and for the S phase (DNA replication, histone synthesis). After cytokinesis the septum is degraded which leads to the huge drop in dry mass concentration observed at this moment (Figure 1(C), green line).

4. CONCLUSION

Living cells produce a phase shift of the light which is proportional to their dry mass or protein concentration. This parameter allows non-invasively monitoring cells growth and dry mass production. In this proceeding we have shown that the phase signal, which is non-invasively recorded in real-time by digital holography, can be related to the dry mass and readily used to monitor both cell growth and the stage of cell cycle.

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