APPLICATION OF IMAGE ANALYSIS METHODS IN YEAST CELL STUDY

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Introduction

Recently, a great development has taken place in the field of light microscopy, which enables many applications in the microbiology research. New fluorescent dyes, better cameras and new microscopy techniques have been developed. The combinations of recent imaging cameras and computer image processing permit, for example, identification and calculation of objects in the visual field, measurement of their size or categorization into selected classes according to predefined conditions, or observation of continuous processes such as reproduction of microorganisms.

A significant role in the image analysis is played by the integral transformation, namely periodic (e.g. Fourier transformation) and wavelet (e.g. Haar transformation). The Haar transformation can effectively be used for the fractal analysis by the box counting method. The described method is a part of the HarFA software used for the analysis[1].

With the assistance of the box counting method, it is possible to investigate black and white fractal structures, which can be obtained from color pictures by a process called *thresholding*. The box counting method is based on laying the graticule on a black and white picture and finding the number of black N_B, white N_W and black and white N_{BW} squares. Based on the dependence of the number of black N_B, white N_W and black and white N_{BW} squares on their size n, it is possible to determine fractal dimension of white and black areas and their interface (D_{BBW}, D_{WBW}, D_{BW}).

The wavelet transformation (or the Haar one) makes the calculation of squares of different sizes of a laid mesh more effective with the box counting method provided that a square area is being analyzed. This transformation comes out from the system of orthogonal Haar functions which acquire values +1, 0, -1 multiplied by the power of number $2^{i/2}$, where $i = 0,1,2, \ldots$ The first two Haar rectangular functions are identical with the Walsh ones, the higher Haar functions are obtained from the lower (i.e. previous) ones by changing the measure and shift. Based on the coefficients of the Haar

transformation it is easy to determinate for black and white pictures the numbers of black N_B, partially black N_{BW} and white N_W squares for different mesh sizes n (1 × 1, 2 × 2, 3 × 3, ... pixels). From their power dependence on the measure size it is again possible to determine the basic structure parameters, a so-called fractal dimension D and fractal measure K of black and white areas and their interface. These parameters can be used for picture ordering evaluation, but also e.g. for specifying the number of defined objects without having to count them[2].

Experimental part

The fractal analysis was used when studying different species of yeast (*Saccharomyces cerevisiae*, *Saccharomyces fragilis*, *Candida vini*, *Kloeckera apiculata*, *Geotrichum candidum*, *Dipodascus magnesii*). Growth and reproduction of yeast in single cultivation under aerobic conditions was observed. Two culture media were used, namely wash enriched with vitamins and glucose-peptone medium containing the yeast extract. To record these processes, the combination of the light microscope and digital camera, or video camera, was used. A digital camera Nikon Coolpix 990 with resolution of 2048 × 1536 and CCD camera PixeLink with resolution of 1280 × 1024 were used, the Lucia Net 1.16.5 software being used in the process of using the video camera for records of pictures. The monitoring was employed in different ways of reproduction, primarily concerning multipolar and bipolar budding.

Figure 1 shows a record of budding of yeast *Candida vini* in wash using microscope Nikon Eclipse E200 with the phase contrast. Yeast was cultivated on an inclined agar by the room temperature for 17 - 24 hours and, then, was inoculated into a liquid culture medium where it was cultivated for a minimum of one hour prior to making the measurements so that it could adapt to the new culture environment. The thresholding of pictures was made by intensity ranging 0 - 88. A first bud appeared after two hours and 30 minutes after the inoculation into the liquid culture medium. The picture shows that the bud expanding into space also influences the value of the fractal dimension.

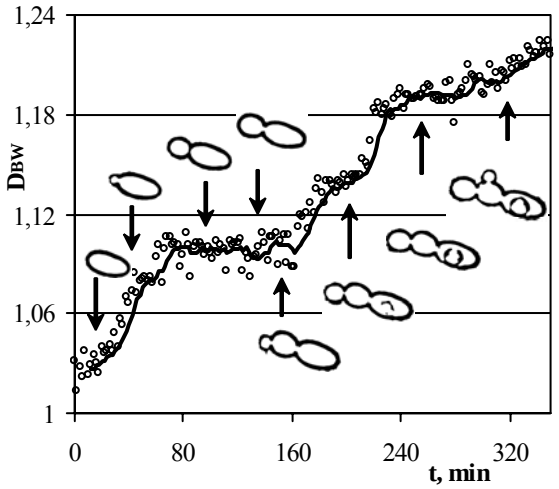

Fig. 1. *D_{BW} fractal dimension dependence on changing the structure of yeast Candida vini. Total magnification of 2400×.*

Furthermore, the fractal analysis was used for specifying the number of live and dead cells of yeast *Saccharomyces cerevisiae* in the picture. Usually, the direct microscopic counting that can be performed in counting chambers (by Thoma and Bürker) is used to detect the number of cells in a specimen. Different fluorescent dyes, e.g. acridine orange, can be used to distinguish the living and dead cells. The monomeric form of the dye is yellowgreen in living cells and the aggregated dye form is red in dead cells. With the assistance of fluorescent labeling it is possible to threshold either dead or living cells on a black color in one picture.

In Figure 2, the total number of cells is thresholded by intensity ranging 50 - 255, the living and dead cells being thresholded by coloured components of the RGB space (living cells: R = 0 - 254, G = 90 - 255; dead cells: R = 130 - 255, R = 0 - 255). To detect the convenient thresholding, it is possible to use the fractal spectrum, i.e. fractal dimension dependence on intensity or selected RGB component, which is accessible in the HarFA software as a tool referred to as *Fractal Analysis - Range.*

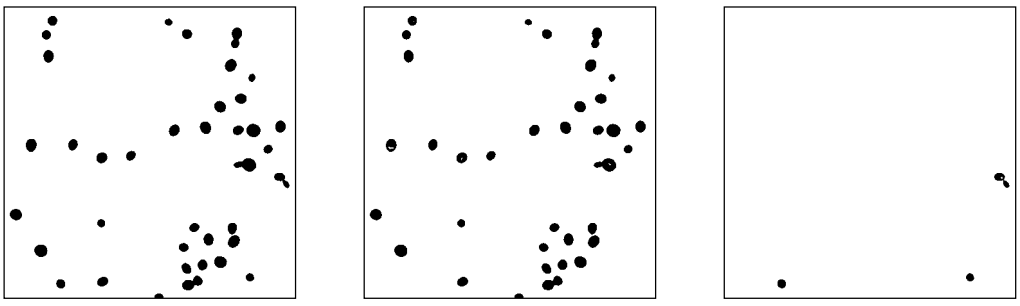

Fig. 2. *Thresholded pictures of yeast cells; total number (left), living cells (in the middle), dead cells (right).*

The number of cells was determined provided that cells are of spherical shape, similar in size and distinguishable on the background. For the calculation of cell numbers the following equation was used:

$$x = \frac{N_{BW}^2}{4\pi\left(N_B + N_{BW}\right)}$$

where N_B is the number of black boxes, N_{BW} is the number of black and white boxes. The resulting cell number x is derived from value x that is the maximum from the calculated values for different sizes of the mesh. The maximum value is select because the fractal structure is bordered most conveniently for the given mesh size. With a smaller mesh size, the picture border composed of pixels would not be continuous and, with a greater mesh size, the border would not be rather smooth[3].

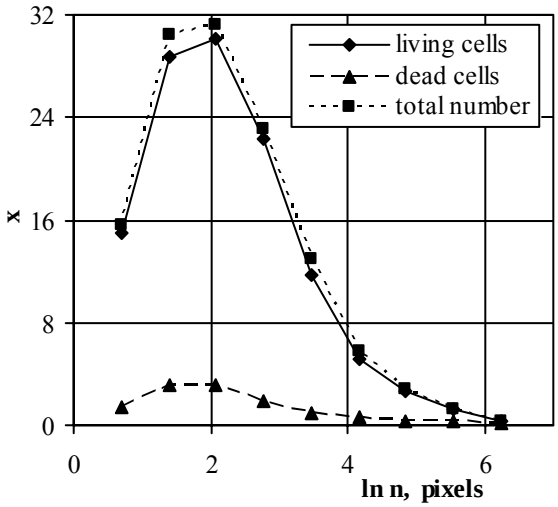

Fig. 3. *Determining number of cells x.*

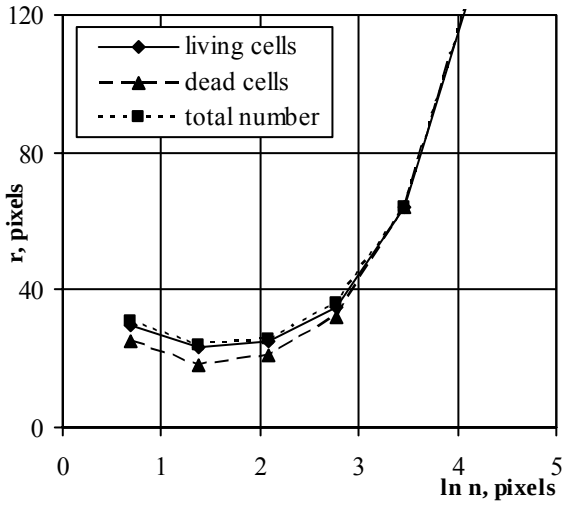

Fig. 4. *Determining average cell radius r.*

Results and Discussion

By appropriate setting of optical system parameters (optical filters, brightness, contrast), it is possible to facilitate the actual image analysis. When monitoring the growth and reproduction, it was discovered that after the appearance of a bud, the fractal dimension increases either continuously or in "fits and starts". A relaxation stage when that fractal dimension does not change was found between the individual leaps (Figure 1). During the relaxation stage, a cell complements its energy and substance reserves for the continuation of the division. The steepness of the fractal dimension increase is also determined by the number of buds developing simultaneously on the mother cell. The separation of a bud from the mother cell and subsequent division of the daughter cells was also recorded.

When calculating the living and dead cells, it was found out that the error of determining the number of cells depends on the size and number of cells. Error 18 % corresponds to the picture with 38 cells (total number), error 14 % corresponds to the picture with 35 living cells, and error 2 % corresponds to the picture with 3 dead cells. An error can also be caused by unequal size of cells and shapes, differences in coloring of the individual cells, quality of the recorded picture, etc. The picture resolution plays a significant role. If the number of cells is known, it is also possible to determine the average cell radius, which was 23 pixels again with an error of approximately 17%.

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REFERENCES

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