The Use of Fractal Analysis for the Determination of Yeast Cell Diameter

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The advances in digital image recording techniques and especially the price reductions have made these technologies suitable and available for various techniques of image analysis. These digital techniques are now applicable in those segments of scientific research, where manual processes of image evaluation prevailed until nowadays.

There exist many ways of biological specimen image analysis and they are readily available as complete commercial software products. Anyway, the image analysis is not only applicable to biological and microbiological research. Software application HarFA [4] has been developed at our faculty. Originally, it has been designed for the image analysis of print patterns. This software enables the user to make various correction of the captured image, to apply filters, colour separations and both harmonic and fractal analysis. At the same time, it can be used for the image analysis of biological specimens as well.

The harmonic and fractal analysis of the image or its colour separations belongs to the basic methods for the image analysis. This analysis can be performed by various software equipment, which is commonly available [1], [2], [3]. However, this software is developed for special purposes, and therefore it is not applicable generally. Just this method was used for analysis of microbiological specimens for the determination of diameter distribution of yeast cells in digital image (*figure 1*).

It has been shown that under certain circumstances, HarFA can be used to analyse the image of a microscopic specimen. The circumstances are:

- cells have to be spherical or ellipsoidal,
- cells have to be similar in size,
- cells have to be coloured different from the background.

Under these conditions it is possible to determine the number of cells in the image, their size and the size distribution.

The image was taken by recording equipment, which consists of optical microscope SM-6, digital camera SONY and PC. The microscope magnification, resolution of digital camera gives the connection between image size and studied sample size (100 μm per 514 pixels).

According to the experience from previous work we tried to widen the field of application of this method. During the previous work, it was possible to determine the number and size of cells fairly correctly. The samples contained suitable estimated number of cells (100) of appropriate size (35 pixels). When compared with the standard manual method of counting by *Bürker box,* the error was less than 10 %.

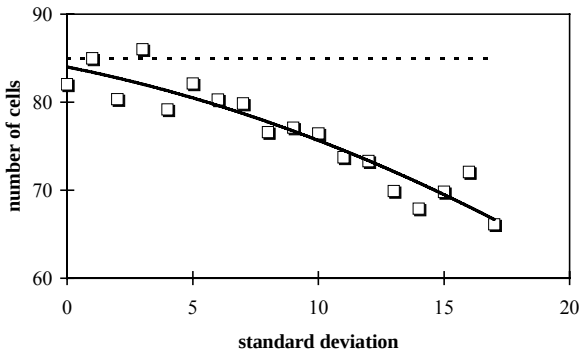

Figure 1: Calibration curve of the cell number

During the analysis of captured image, it is necessary to respect this optimal number and the deviation from correct values determined manually. Our proposed analytical process consists of the following steps:

1. It is necessary to calculate the calibration curve of real number of cells (85 cells were used) versus the number determined by fractal analysis for different distribution. The deviations are caused by just by the size

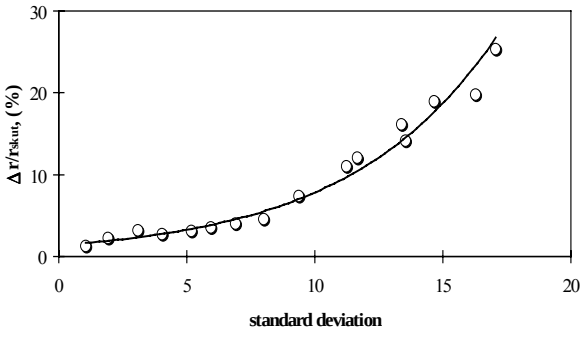

Figure 2: Calibration curve of radius deviation

distribution. This is true also for the deviations between the real radius and the radius determined by fractal analysis (*figure 1* and *2*).

The image analysis is based on:

2. The capturing of the digital image of the specimen (*figure 3*). The image must not contain larger number of cells the number for which the calibration curve was calculated (85). Naturally, the cells have to be easily recognisable.
3. The captured image is cropped so that it contains approximately the number of cells for which the calibration curve was calculated (85 cells, *figure 1*).
4. The image is Gaussian-blurred in order to remove noise, which deteriorates the results of masking.
5. The determination of the optimal colour level for masking. After masking, the image consists only of two colours, so the cells are well separated from the background (*figure 3*).
6. The fractal analysis of the masked image, by which the approximate average radius of the cell is determined.
7. The image is then resampled, so that the approximate cell radius is equal to the radius for which the calibration curve was calculated (35 pixels, *figure 2*).
8. The fractal analysis, by which the number of cells and the average radius are determined (*figure 4*). These values are distorted because of the cell size distribution.
9. The determination of cell size variation from the calibration curve of real number of cells versus the number determined by fractal analysis (*figure 1*).
10. The determination of real average cell radius from the calibration curve of percentual deviation of radius for the variation determined in the previous step (resampled image).
11. The determination of real average radius from the factor of resampling. The results of this analysis are the standard deviation and the average cell radius for the selected cropped image area.

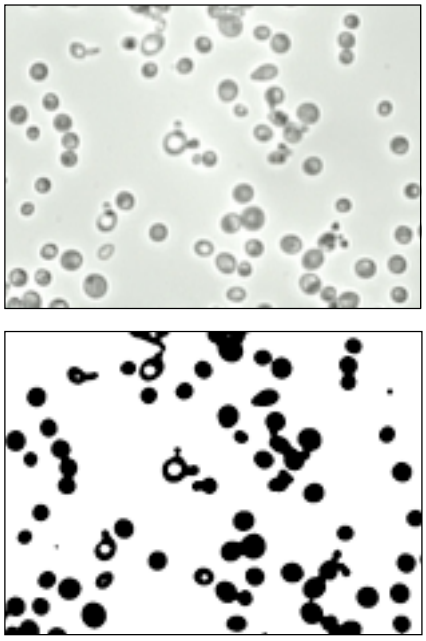

Figure 3: An image of specimen blurred by microscope (top), the same image after processing

The analysed specimen contained 81 cells whose average radius was 3.1 μm and the standard deviation 0.8 μm as determined by standard practice [2]. The following values were determined by the algorithm described above: average radius was 3.3 μm and the standard deviation 0.8 μm. By the evaluation of series of specimen it was determined, that the error of average radius and of standard deviation when compared with standard practice does not exceed 10%, even when the cells are not ideally spherical.

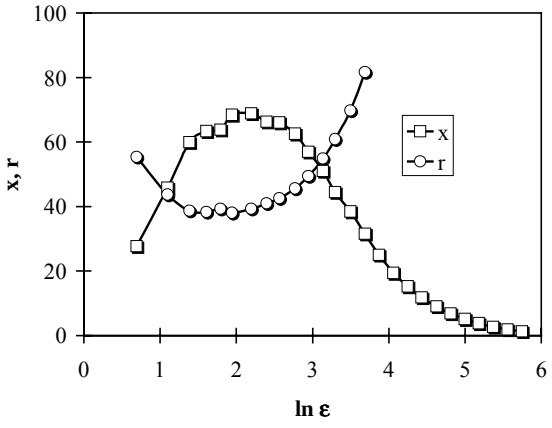

Figure 4: The determination of number and radius of cells

References

1. *Benoit*, Fractal Analysis System, True Soft International (1999)
2. *Lucia*, Image Archiv Plus, Users' guide, Laboratory Imaging, s.r.o., (1999)
3. Fooley J. D., van Dam A., Feiner S. K., Hughes J. F., Phillips J. R.: *Introduction to Computer Graphics*, Addison-Wesley Publishing Company, Reading USA (1997)
4. *HarFA*, Harmonic and Fractal Image Analyser, software for determination of fractal dimension, www.fch.vutbr.cz/lectures/imagesci. (2001)