The Fractal Analysis of Image Structures for Microbiologic Application

Mária Veselá, Oldřich Zmeškal, Michal Veselý, Martin Nežádal
Brno University of Technology, Faculty of Chemistry
Purkyňova 118, 612 00 Brno
vesela@fch.vutbr.cz

The methods for an image analysis are used in biology and medicine more often. This is due to development of image recording equipment (digital cameras, scanners), more efficient personal computers and their peripheries (A/D converters, TV cards, Video CD, DVD) and special software for image data processing.

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]. These software are developed for special purposes so that are not fully suitable for many applications.

At the Faculty of chemistry was developed software HarFA [4], which attempts to solve the problems of image analysis more complex, although the main area of its application is focused on fractal analysis. Just this method was used for analysis of microbiological specimens for the determination of number of yeast cells in digital image (*Figure 1*).

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 (10 μm per 48 pixels).

The number of cells was determined by following expectations:

1. the cells are of round shape,
2. the cells are similar in size,
3. the cells differs form background by colour.

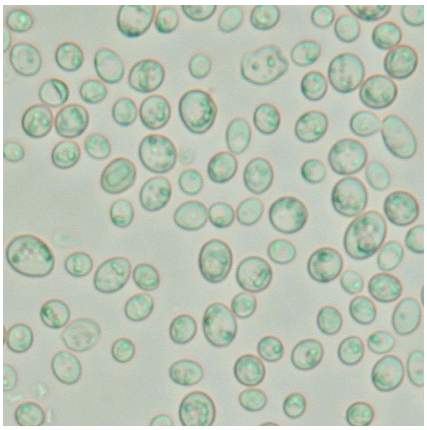

Figure 1: An image of analysed microbiologic specimen

The procedure of cell size determination and its number determination was as follows:

1. By means of proper colour separation (RGB channel, intensity, brightness, contrast) the masking procedure is made for colour adjustment – white (W) for cell and black (B) for background.
2. By means of fractal analysis the fractal dimension and fractal measure is determined for such masked image including interface (K_{WBW}, D_{WBW}) and cell interface (K_{BW}, D_{BW}). According to following equations

$$N_{BW}(\varepsilon)=K_{BW}\varepsilon^{-D_{BW}}, \quad N_{WBW}(\varepsilon)=N_W(\varepsilon)+N_{BW}(\varepsilon)=K_{WBW}\varepsilon^{-D_{WBW}}, \tag{1}$$

the quantity is connected to number of white ($N_W(\varepsilon)$) and partially white ($N_{BW}(\varepsilon)$) squares of network of size $\varepsilon\times\varepsilon$ pixels. From such determined constants is possible by using of the following equations

$$N_{BW}=x\frac{\pi(2r+\varepsilon)}{\varepsilon}\approx x\frac{2\pi r}{\varepsilon}, \quad N_{WBW}=x\frac{\pi r^2}{\varepsilon^2}, \tag{2}$$

to determine number of cells x and their round shape radius r.

From equation (1) and (2) we get

$$x=\frac{K_{BW}^2\varepsilon_m^{-2D_{BW}}}{4\pi K_{WBW}\varepsilon_m^{-D_{WBW}}}=\frac{K_{BW}^2}{4\pi K_{WBW}}\varepsilon_m^{D_{WBW}-2D_{BW}}, \tag{3}$$

where ε_m is network size, with maximum of fractal dimension. For smaller values of ε $(\varepsilon < \varepsilon_m)$ will be a border line formed by pixels of image discrete (a fractal dimension of interface will decrease), for $(\varepsilon > \varepsilon_m)$ will be a border line broad (it causes the decreasing of fractal dimension again).

In both cases it is displayed by decreasing the number of investigated cells *x*, or by increasing their radius (*Figure 3*), respectively. From this figure is possible to determine the number of cells stated by fractal analysis ($x = 100$) and their mean radius ($r = 29$ pixels).

Comparing these results with values, which are easy to estimate from *figure 2* ($x \approx 85$) we can see, that the error of determination of number of cells in this case is smaller than 15 %. The higher accuracy is achievable by optimal choice of cell size in image (it can be easy modified by change of optical magnification) and by optimal cell count in image (can be changed by dilution of cell culture).

The most proper parameters (cell size, number of cells in image) can be established by their evaluation in two ideal cases:

1. for different number *N* of round shaped cells with the same radius *r*,
2. for fixed number *N* of round shaped cells with the various radius *r*.

It was found, that the optimal size of cells (the mean radius *r*) for the image analysis is 35 pixels, the optimal cell number is approx. 50. From the analysed structure can be seen that parameters of analysed image structure are at the edge of suggested conditions. The further information will be presented on the poster.

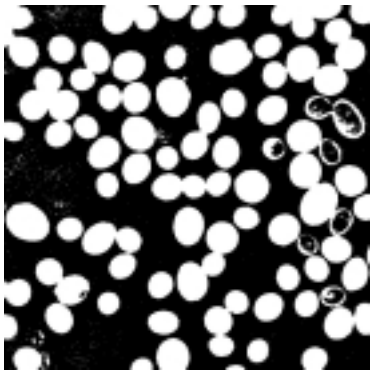

Figure 2: The masked image of microbiologic specimen

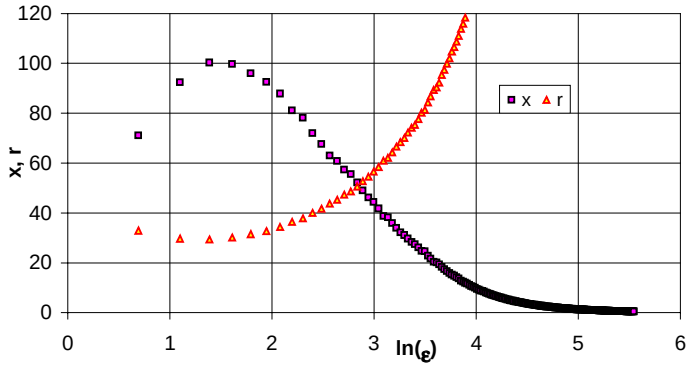

Figure 3: The determination of number of cells N and their radius r (x = 100, r = 29 pixels)

Conclusion

It is evident that the cell count determined by the fractal analysis differs from true value to about 15 %. This difference is caused by unequal cell size and shape, by quality of specimen (it should be removed the thermal noise of CCD element, should be performed the correction of illumination non-homogeneity, gamma correction) and by colour differences of individual cells. From these results we can claim that the mean size of analysed cells is 12 μm.

Literature

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)