# The Use of Fractal Analysis for the Determination of Cell Diameter – Model Calculation

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The methods of image analysis are being used in biology and medicine more often. This is due to the 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 color separations belongs to the basic methods for the image analysis. This procedure can be performed by various software tools, which are commonly available [1], [2], [3]. However, this software is developed for special purposes, and therefore it is not applicable generally.

For the past few years, the application HarFA (Harmonic and Fractal Image Analyser) [4] has been developed at the Faculty of Chemistry of BUT. This software enables the user to make various correction of the captured image, to apply filters, color separations and both harmonic and fractal analysis. It has been showed, that this software tool can be used for the characterization of the images of microscopic specimen. If we apply the fundamentals of fractal mathematics on the image of cellular structure complying with certain criteria (spherical shape of the cells, similarity of sizes and contrast background – *figure 1*), we can determine both their number and size.

If we cover the analyzed image by a virtual sampling mesh with the size of one box $\varepsilon \times \varepsilon$ pixels, we can formulate the following relations

$$N_{\mathrm{BW}}(\varepsilon)=K_{\mathrm{BW}}\varepsilon^{-D_{\mathrm{BW}}}, \quad N_{\mathrm{BBW}}(\varepsilon)=N_{\mathrm{B}}(\varepsilon)+N_{\mathrm{BW}}(\varepsilon)=K_{\mathrm{BBW}}\varepsilon^{-D_{\mathrm{BBW}}},$$

where $N_{\mathrm{B}}(\varepsilon)$ stands for the number of totally black and $N_{\mathrm{BW}}(\varepsilon)$ stands for the number of partially black boxes of that sampling mesh. $D_{\mathrm{BW}}$ ($D_{\mathrm{BBW}}$) is so called fractal dimension and $K_{\mathrm{BW}}$ ($K_{\mathrm{BBW}}$) so called fractal measure. Using these constants, it is possible to determine the number of cells $x$ and their radius $r$

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

From these equations we get

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

where $\varepsilon_m$ is the mesh size corresponding to the maximum of fractal dimension. For smaller values of $\varepsilon$ ($\varepsilon < \varepsilon_m$) will be a borderline 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). From the extremes of the curves at *figure 2*, it is possible to determine the number of cells and their radius.

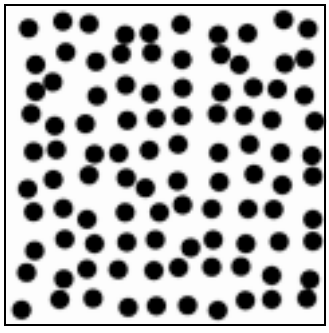

*Figure 1: Model cellular structure; 100 cells, radius 38 pixels*

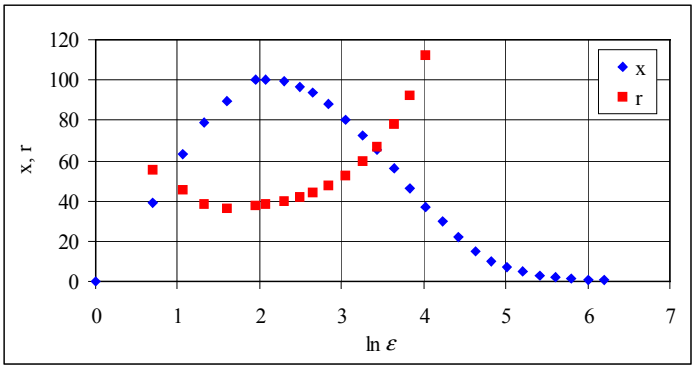


*Figure 2: The determination of number of cells x and their radius r (x = 100, r = 38 pixels)*

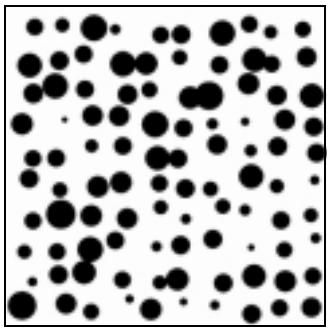

*Figure 3: Model cellular structure with Gaussian size distrubution; 100 cells, mean radius 38 pixels, standard deviation 4 pixels*

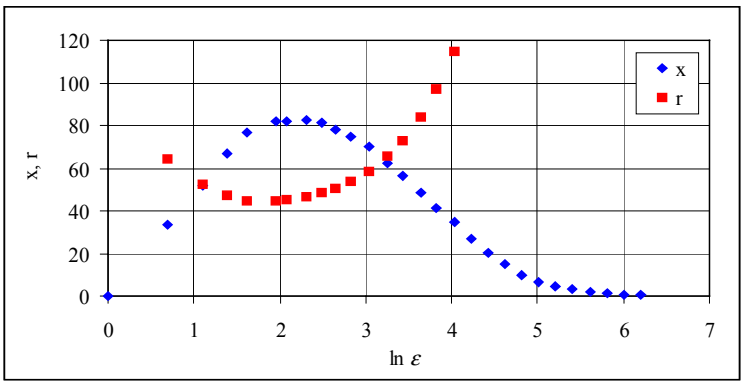


*Figure 4: The determination of number of cells x and their radius r (x = 82, r = 43 pixels)*

The determination of cell number and size by this method is valid only for structures consisting of cells of equal size. It was found that when the structures showing certain distribution of cell sizes (*figure 3*) were analyzed, the calculated numbers of cells were always smaller then the real numbers and on the other hand, the cell sizes were always bigger then the real sizes (*figure 4*). So, the relation between the real and calculated number of cells with respect to the distribution was studied.

For this purpose, model images were used. These images contained defined (Gaussian) distribution of cell sizes with varying standard deviation. The processing sequence described above was applied at these images and the cell number and size was calculated. Then, the dependences between the calculated number of cell and their radii *versus* selected standard deviation were evaluated. These dependences can be used as calibration curves of real cell number versus calculated cell umbers. (*figure 5, 6*).

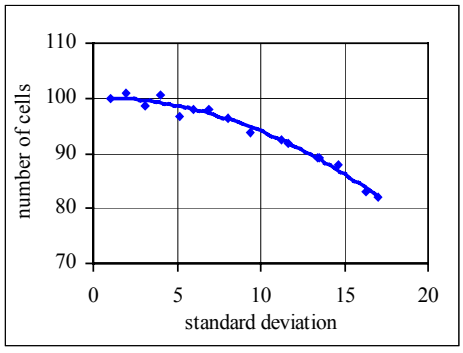


*Figure 5: Calibration curve for tthe cell number for x = 100, r = 38 pixels*

If we know the number of cells of certain structure, we can calculate their average radius and variation in the following way:

1. Perform the fractal analysis, by which we get a fictive number of cells and average radius (these values are distorted because of the cell size variation).
2. From the calibration curve of real cell size *versus* calculated size we get the cell radius variation (the standard deviation) (*figure 5*).
3. From the calibration curve of radius percentual deviation for the variation found it the previous step we get the real average radius (*figure 6*).

In this paper, a new method for the determination of size distribution of cellular structures is proposed. This method is not based on the area calculation, but on the determination of fractal parameters of the studied structure. The error of determination of average radius and of the standard deviation is less then 5 % when this method is applied to model data. The error does not exceed 15 % when real data is used.

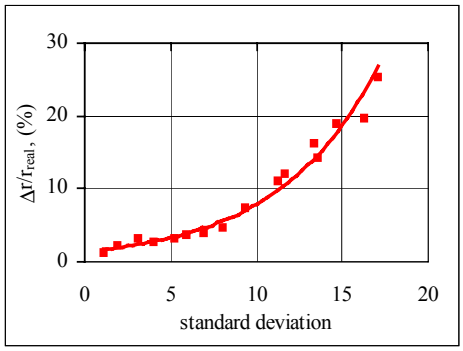


*Figure 6: Calibration curve for percentual deviation of cell radius for x = 100, r = 38 pixels*

**References**

1. *Benoit*, Fractal Analysis System, True Soft International (1999)
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