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AN APPROACH TO QUALITY EVALUATION OF EMBRYOS ON THEIRS GEOMETRICAL PARAMETERS

Kutsenko A. S., DSc Tech, Professor, E-mail: kuzenko@kpi.kharkov.ua,
Megel Y.Ye, DSc Tech, Professor, E-mail: megelye@gmail.com
Kovavenko S. V., PhD, Assoc. Professor, E-mail: adbc@ukr.net,
Kovavenko S. N., PhD, Assoc. Professor, E-mail: svetkovalenko@gmail.com

The process of automation of quality evaluation of embryos is directly related to determining the degree of their development and the possibility of further use of viable embryos in modern biotechnologies. The first step of determining the viability of the embryos, during the morphological quality evaluation, is the evaluation of the various stages of their development (oocyte, morula, blastocyst), i.e. determination of the cells number within the pellucid zone [1, 2]. Thus, automatic image processing at an early stage involves recognizing the object by the number of blastomer cells. Further, the quality of embryos can be performed by comparison with the reference image and classification as a certain stage of development.

The measured quantitative parameters of the embryo (such as the thickness of the pellucid zone, sphericity of the embryo itself, the density of the blastomer, the ratio of the area of the blastomer to the area of the perivitelline space, etc. [1, 3]) can serve as the criteria to make a decision on viability according to geometric characteristics. But the problem of quality evaluation of embryos is not only to make a decision about its viability, but also to obtain meaningful information and to distinguish the most informative features on the image. The block diagram of the process of solving the problem is shown in Fig 1.

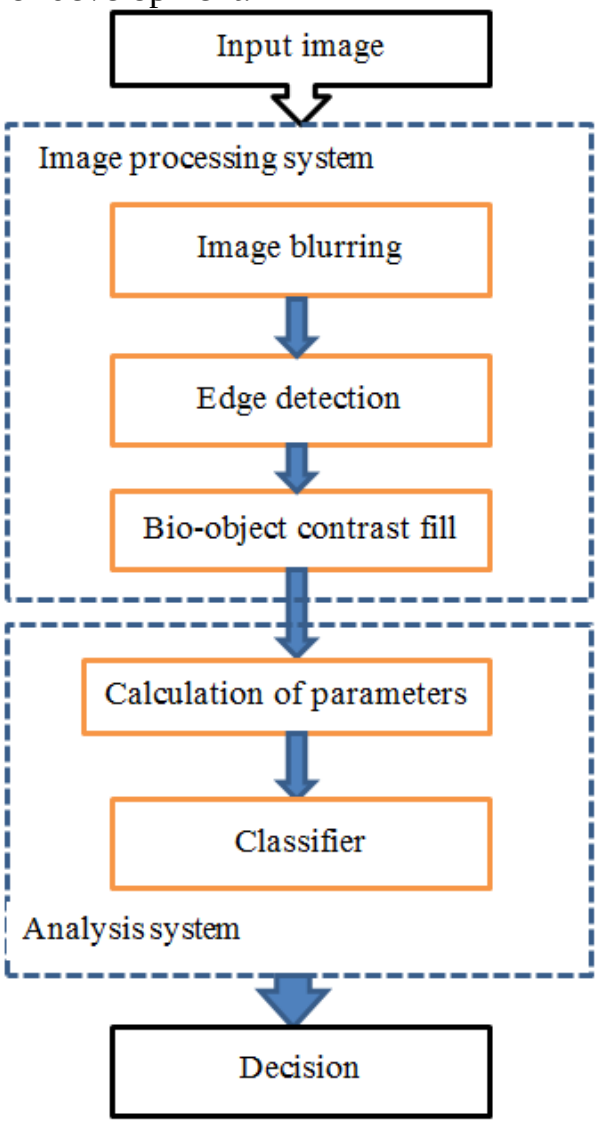

Fig. 1. Block diagram of the solution of the problem

In order to evaluate the parameters of the object, use the methods of computer graphics, where the main directions is image processing, with subsequent recognition [3].

To calculate the measured parameters such as a diameter d_p, a square S_p and a perimeter P_p of a bio-object

and its isoperimetric ratio $K_f = P_p^2 / S_p$, choose 2 images of microbiological objects of different shapes (Fig. 2). The dimensions of each of these images are 455×455 pixels.

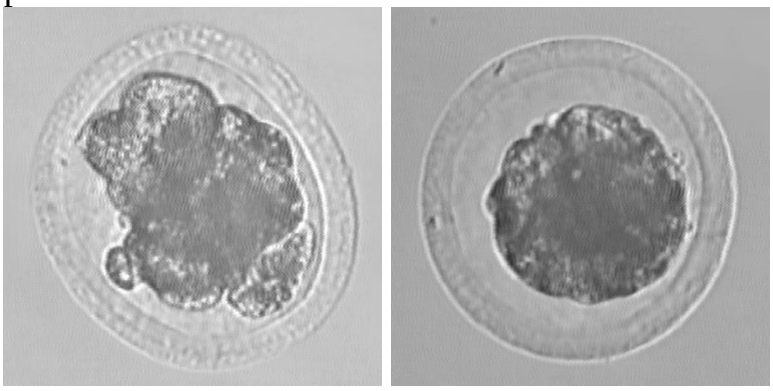

Fig. 2. Initial pictures for processing

For solving said problem, it is necessary to perform edge detection of objects in the images. Data filtering is divided into linear filtering (with the use of convolution matrix) and non-linear one [4].

The idea of linear filtering resulted from the features associated with two-dimensionality of the information. For images filtering, two-dimensional filters are used, which represent apertures (masks) of various configurations on the plane. The specified number, which further will be called as a weighting factor $H(i, j)$ corresponds to each element of the aperture.

Usually [4, 5], filtering is performed by moving the filter aperture by one pixel from left to right and from top to bottom. At each position of the aperture, same type operations are performed, namely, multiplication of weighting factors $H(i, j)$ by the corresponding values of color components of an original image $A(i, j)$ and summing the obtained results. In other words, convolution is performed. The resulting value is divided by a predetermined number - the normalizing factor, and then the resulting value is assigned to the central element of the aperture. This is called a filter output. Schematically, the principle of linear filtering is presented in Fig. 3.

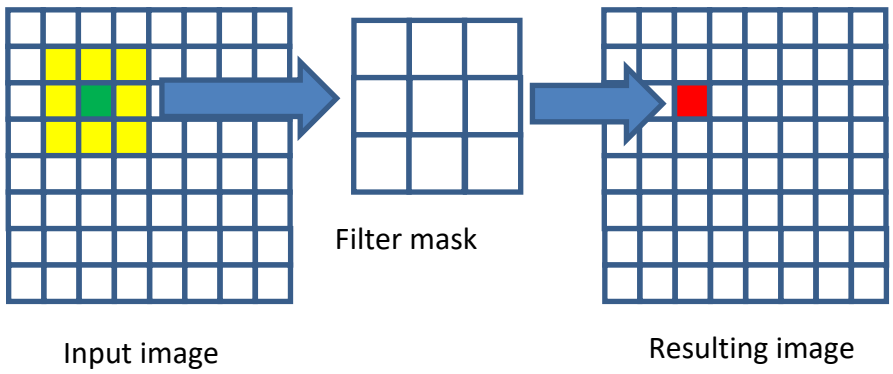

Fig. 3. The principle of linear filters

The size of the aperture is taken in such a way to identify the central element unambiguously. The most popular mask sizes are 3×3 and 5×5. The term local filtering indicates that the size of the aperture is smaller than the size of the original (filtered) image. Otherwise, filtering is called global one. Thus, the above-mentioned transformation can be represented as

$$B(x, y) = \sum_{i \in S_{x,y}} \sum_{j \in S_{x,y}} H(i, j) A(i, j)$$

where $A(i, j)$ are colour components of input image, $B(x, y)$ is a resulting image, $H(i, j)$ is a mask (convolution matrix) of weighting coefficients, $S_{x,y}$ is a raster aperture.

To eliminate random noise, it is appropriate to apply an averaging filter. One of the most frequently used is a Gaussian filter, averaging pixels around a point according to Gaussian function.

$$G_\sigma = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}}$$

The advantage of this filter is the absence of artifacts after its application. The Gauss filter matrix of size 5×5 at $\sigma = 1$ has the form:

$$H_G = \begin{bmatrix} 0,003 & 0,013 & 0,022 & 0,013 & 0,003 \\ 0.013 & 0.059 & 0.097 & 0.059 & 0.013 \\ 0.022 & 0.097 & 0.159 & 0.097 & 0.022 \\ 0.013 & 0.059 & 0.097 & 0.059 & 0.013 \\ 0.003 & 0.013 & 0.022 & 0.013 & 0.003 \end{bmatrix}$$

For discrete images, the calculation of partial derivatives is as calculating the difference in brightness of neighboring pixels in various ways. Thus, we return to linear spatial filtering using convolution in two directions.

$$G_x = \sum_{i \in S_{x,y}} \sum_{j \in S_{x,y}} H_x(i,j) A(i,j),\ G_y = \sum_{i \in S_{x,y}} \sum_{j \in S_{x,y}} H_y(i,j) A(i,j),$$

where H_x is a filter mask applied along the horizontal axis, H_y is a filter mask applied along the vertical axis.

The resulting image can be obtained using the expression:

$$B(x,y) = \sqrt{G_x^2 + G_y^2}\ .$$

Consider convolution matrices for the Sobel filter. There are four matrices for delineating the boundaries of the vertical, horizontal and $45°$ axes.

$$H_S(x) = \begin{pmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{pmatrix},\ H_S(y) = \begin{pmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{pmatrix},$$

$$H_S(xy) = \begin{pmatrix} -2 & -1 & 0 \\ -1 & 0 & -1 \\ 0 & -1 & 2 \end{pmatrix},\ H_S(yx) = \begin{pmatrix} 0 & 1 & 2 \\ -1 & 0 & 1 \\ -2 & -1 & 0 \end{pmatrix}.$$

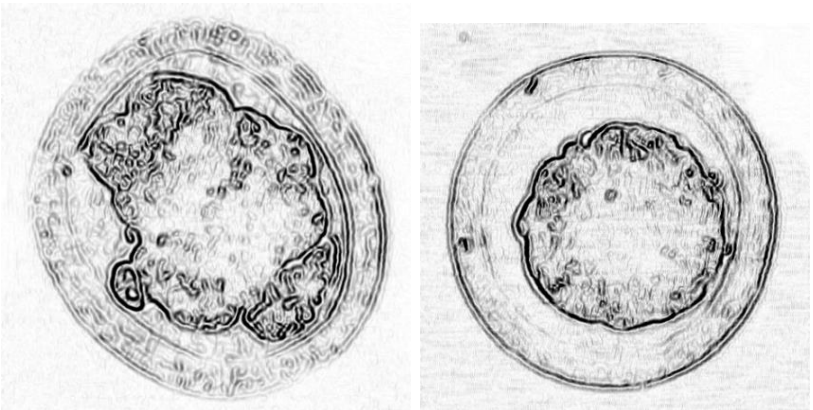

Fig. 4. Result of blurring and edge detection

The result of blurring and edge detection is shown in the Fig. 4. For convenience, it is possible to get images in invert color.

The same colors inside and outside the object should be separated for the next step. Filling the area around the embryo is not

difficult – use the fill up to the specified color. For this purpose, the flood fill algorithm can be used [4]. The essence of it is that the fill color successively spread from the initial coordinate up to some boundaries (see Fig. 5).

In our case, the starting point is located in the upper left corner of the image, i.e. in all the examples it purposely is outside the embryo. The flood fill algorithm can be implemented with or without recursion.

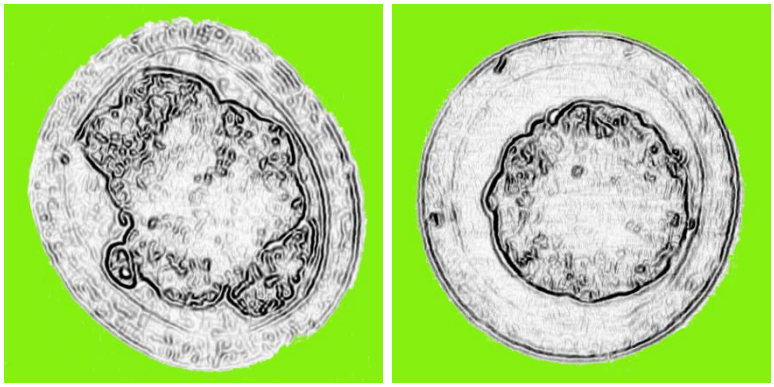

Fig. 5. The result of the colour fill around the bioobject

Based on the small size of the image, a recursive version was chosen. It must be taken into account that four-directional filling should be implemented (see Fig. 6) since the thickness of the contour of the embryo itself may be one pixel in some places.

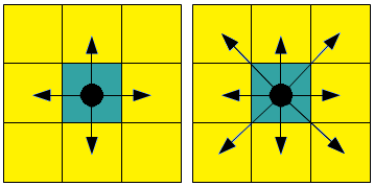

Fig. 6. Ways to fill adjacent pixels (four and eight directions)

Filling the area inside the embryo may be different, i.e. to speed up the process, in our view it is preferable to fill the entire image line at once. This method uses less computer resources and allows performing an internal fill in fewer steps (see Fig. 7).

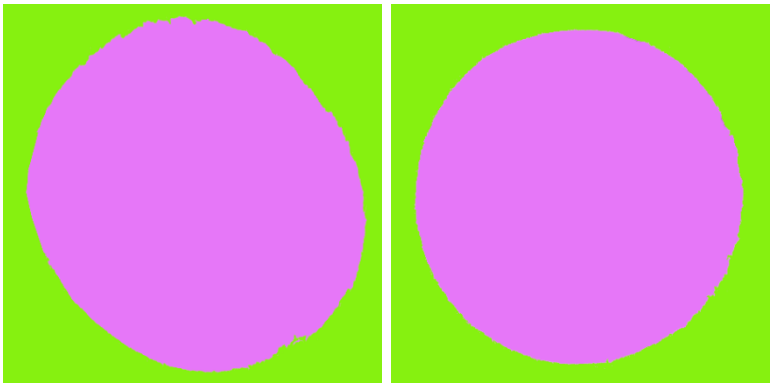

Fig. 7. The result of fill inside and outside the bio-objects

As a result of the performed operations, the procedure of segmentation of objects was implemented, which allows to operate with images composed of only two colors (denote the color inside the embryo as $Color_1$, and outside the embryo as $Color_2$).

By calculating the number of pixels of these colours, we can get the measured parameters of the studied images of biological objects, including the isoperimetric ratio of the biological object $K_f = P_p^2 / S_p$. To obtain the area S_p, it is necessary to count the number of pixels with $Color_1$. For the first image, this value is 131472 pixels. With a total number of pixels of 207025 (which corresponds to a size of 455×455 pixels) and a resolution of 289 pixels per inch for this image, we have an area value $S_p = 16\ \text{cm}^2$ (taking into account the optical magnification of the microscope). Obtaining the perimeter value of P_p involves calculation the number of pixels located on the border of two colors. The calculation was made using a 3×3 matrix, which slides over the entire image and captures the differences of the neighboring colors $Color_1$ and $Color_2$. Due to the fact that there are possible transitions from $Color_1$ to $Color_2$ and from $Color_2$ to $Color_1$,

then the total number of such transitions will be 2 times more than their actual number. Thus, the value $P_p = 1330$, and, therefore, $K_{f_1} = 13.45$ for the first image, $K_{f_2} = 12.61$ for the second one. For an ideal circle, the ratio is $K_f = 4\pi$ [6]. Therefore, we can conclude that the shapes of the studied bio-objects are close enough to circle.

Thus, to determine the geometrical characteristics of a micro-biological object, a block diagram of an automated system with uniformly distributed hardware and software for a multi-gradient image representation is proposed, which allows increasing the information capabilities of the system. The proposed method of two successive differential operators (masks) makes it possible to provide higher measurement accuracy and increasing the objectivity of the decision on the viability of the microbiological object and, thus, complements the morphological evaluation.

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