Segmentation Based on Determinate Chaos of Histological Specimen Images

Valery Orel, Andriy Gusynin, Hanna Selezneva, Serge Kolesnik, Hanna Stendyk, Helen Komisarova

National Technical University of Ukraine "Kyiv Polytechnic Institute", Department of Medical Cybernetics and Telemedicine, Kyiv, Ukraine, e-mail: selezneva_anna@ukr.net, gusynin@gmail.com

Neuroblastoma is one of the most abundant tumors in infancy and ranks the fourth place among all malignant tumors of children, after acute leukemia, tumors of central nervous system and malignant lymphomas. Histological examination of atypical cell structures is mandatory for diagnosing and forecasting of neoplasm growth .. At present, the image processing of histological specimens is made by a professional histologist manually and is based on visual perception of the histologist. Naturally, visual approach has some shortcomings. Therefore, for objective estimation of medical images, increasing diagnosis accuracy and information processing speed, the computer analysis of histological images is a current need. In this paper, the features of the digital images of histological specimens and methods for digital analysis of medical images are presented. An innovative information system for instant diagnosis of the stage of neuroblastoma based on digital images analysis of histological specimens is developed. In presented studies, a new algorithm, based on deterministic chaos of digital images of histological specimens which uses original formalized indicators (irregularity of contoured zone of diagnostic interest, its structures heterogeneity including cells, nuclei, mitochondrion etc.) have been implemented.

Key words and phrases: image analysis, segmentation, histological specimen, neuroblastoma.

Introduction

Cancer is the second most common cause of death in developed countries and the percentage is still increasing. Numerous medical researches show that approximately 33% of human population either already have a tumor, or with a certain degree of probability the tumor will develop [1].

Treatment effectiveness of many oncological diseases considerably depends on early detection and treatment mode selection. Any pathological process, regardless of the degree of functional disorders caused, begins on the subcellular level. Therefore, histological examination of atypical cell structures is mandatory for diagnosing and forecasting of neoplasm growth .. The the clinical diagnosis is based on the result of histological examination .. At present, the imaging analyzing of histological specimens is made by a professional histologist manually [2—3] and is based on visual perception of the histologist. Naturally, this visual approach has somedrawbacks. Firstly, the diagnostic possibilities of images analyzing are limited and according to psychological laws of Weber-Fechner and Stevens, changes in medical images are perceived in logarithmical progression. Secondly, diagnostic effectiveness in visual approach is restricted and essentially depends on experience and psychoemotional condition of a physician [4]. Therefore, for objective estimation of medical images, increasing of diagnosis accuracy and information processing speed, the computer analysis of histological images is up-to-date task.

The goal of this paper is to develop a segmentation method, based on determinate chaos of histological specimen images, for innovative information system for instant diagnosis of the stage of a neuroblastoma.

Material and methods

Neuroblastoma is the most abundant tumor in infancy and ranks the fourth place among all malignant tumors of children, after acute leukemia, tumors of central nervous system and malignant lymphomas. The possibility of neuroblastoma expansion throughout the world is practically equal. Near 90% of neuroblastoma have revealed till the age of 5. Only 2% of tumors have revealed at the age after 10 and in adult patients [1].

Neuroblastoma is the malignant tumor of sympathetic nervous system and is undifferentiated tumor consisting of small round cells with dark-spotted nuclei (Fig. 1).

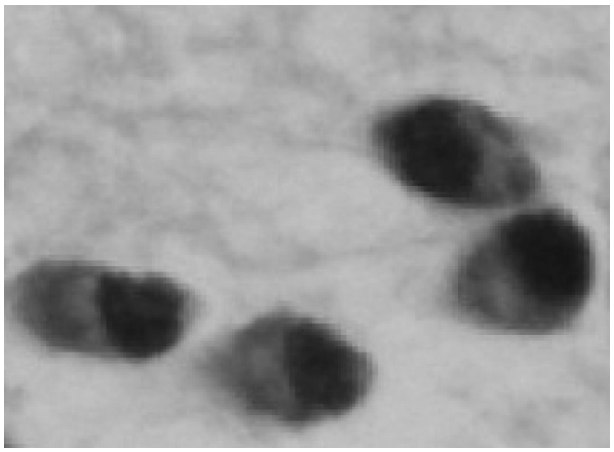

Fig. 1. Neuroblastoma image.

Histological images are the special type of medical images. Digital images of histological specimens are characterized by several features [5]. Firstly, such images are characterized by background irregularity. Secondly, through the low tinction quality, the boundary between cell cytoplasm and nucleus may be poorly distinguished. Thirdly, cells can be gathered closely to each other. Fourthly, digital images of histological specimens are characterized by high variability of geometric and optical indicators. Fifthly, the strong intensity drop may occur inside cell nucleus.

There are three main software methods for digital analysis of medical images [5]: method of edge detection of image objects (threshold segmentation, algorithm of active contours, high-frequency filtering etc.), method for contouring at the template of object shape, method for region definition (clustering, block segmentation, region growing, image marking etc.). Lack of specialized and poor effectiveness of existing methods of medical images analysis is an obstacle for automated microscopy systems development. So, the methods for edge detection of image objects does not allow to solve the problem of nuclei segmentation qualitative, if background is not homogeneous cells gathered closely to each other, strong variation of intensity inside nuclei exists. Using the method for contouring at the template of object shape, we receive many wrong decisions and results depend on accuracy of selected template. In method for region definition there is a problem of selecting accurate start point of the segmentation.

Proposed algorithm

For facilitating and increasing accuracy of histological specimen images segmentation for the further calculation of morphometric indicators the innovative algorithm, based on determinate chaos of digital images which uses of original formalized indicators (irregular of contoured zone of diagnostic interest, its structures heterogeneity including cells, nuclei, mitochondrions etc.) was developed [6—7]. Proposed algorithm relies primarily on thresholding. The algorithm block diagram is shown on Fig. 2.

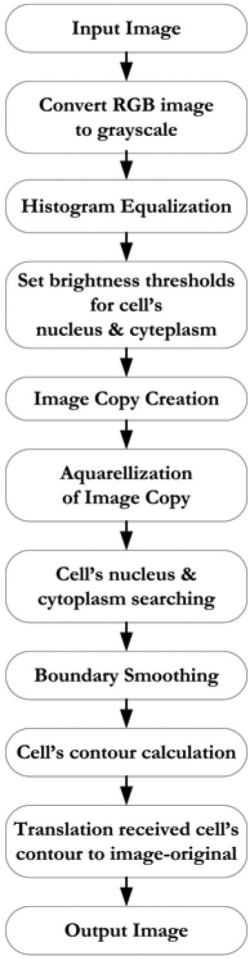

Fig. 2. Algorithm Block Diagram.

Histological specimen images are captured using binocular microscope mounted with a digital camera. Typical image capture devices such as CCD cameras produce row image data in the RGB color space. However, as medical researches proved, human eyes have different sensitivity to colour and brightness. The eye is more sensitive to change in brightness than changes in colour [8]. Moreover, application of processing methods to RGB images frequently causes unwanted results. Therefore, proposed algorithm deals with input images that have been transformed from the standard colour space RGB to the device independent colour representation HSV.

The algorithm for transformation RGB color space to HSV is as follows:

$$H = \begin{cases} \text{undefined}, & \text{if } MAX = MIN \\ 60 \times \dfrac{G - B}{MAX - MIN} + 0^\circ, & \text{if } MAX = R, \text{ and } G \geq B \\ 60 \times \dfrac{G - B}{MAX - MIN} + 360^\circ, & \text{if } MAX = R, \text{ and } G < B \\ 60 \times \dfrac{B - R}{MAX - MIN} + 120^\circ, & \text{if } MAX = G \\ 60 \times \dfrac{R - G}{MAN - MIN} + 240^\circ, & \text{if } MAX = B \end{cases},$$

$$S = \begin{cases} 0, & \text{if } MAX = 0; \\ 1 - \dfrac{MIN}{MAX}, & \text{otherwise} \end{cases},$$

$$V = MAX,$$

where MAX and MIN are maximum and minimum values from R, G and B, respectively.

In HSV space, in the sequel, we will use only one channel (black-and-white), since use of all three channels have some shortcomings. First of all, it demands more computing power. Secondly, cell images can be made in any illumination that affects the image color gamma and at last, overlaying of the image cells with different colors can lead to new color creation, which complicates the recognition process.

Enhancing the contrast and quality of the image

Images obtained from digital light microscope, as a rule, do not have enough sharp and contrast, and carry many noises. While it is possible to improve the image sharpness and contrast by using hardware methods (e.g. changing of video camera), then to improve the image quality, it is reasonable to use software methods.

Images can be characterized by their gray-level histogram, from which global qualities, such as brightness, contrast, entropy and signal-to-noise ratio, can be determined. Histograms are simple to calculate, and are the bases for a number of real-time images processing techniques.

Normally, the image histogram is dense in one side of the spectrum. This will cause the image to be very dark or very bright, different parts of the image with different grey intensity will not be well detected by eyes. Spreading out the spectrum of the histogram will enhance the contrast of the image [9-–10]. Herewith, new pixel values will be following:

$$G_{eq}(I(x,y)) = \frac{M}{N}\sum_{i=0}^{k} n_i ,$$

where: M is brightness maximum,
N is pixels amount,
I (x,y) is pixel intensity,
k is brightness level, determined by I(x,y),
n_i is pixels amount with brightness from 0 to k.

The manipulated image (Figure Fig. 3) is more clear than the input image in HSV space (Figure Fig. 4).

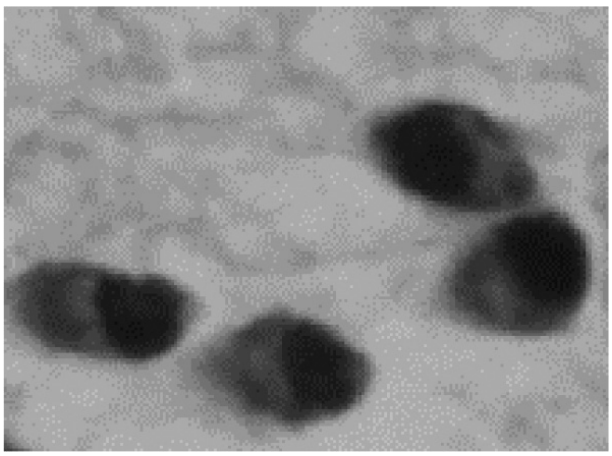

Fig. 3. Transformed image to grayscale.

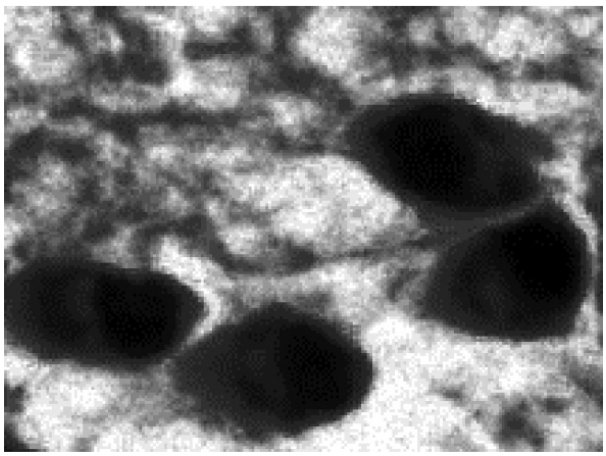

Fig. 4. Enhanced Image.

Human eye can now detect easily the full scale of the grey intensity. In addition, the real cell boundary appears more clear (if the image was made in too light room, the excess image brightness will disappear automatically, similarly, if the image was made in too dark room – the brightness will equalize), so the position of the nucleus and cytoplasm are detected more accurately.

Setting of brightness threshold for cell boundary

Two brightness threshold levels need to be determined by user: one for nucleus and one for cytoplasm. The first

threshold is selected to separate the cell nucleus from the background of the image. The second one is selected to find the cell cytoplasm around the nucleus. As practice shows, there are many types of cells, which can be obtained in different ways, so it is impossible to create a program, which recognizes all feasible cases. Therefore, at the beginning user can use the mean values of brightness for cell boundary (85 and 170, i.e. 1/3 * 255 and 2/3 * 255), since image histogram is extended to 0…255). The next step is to search by algorithm the cell nucleus and cytoplasm and boundary smoothing. After that, user evaluates the similarity of received cell contouring, and, if it is necessary, corrects the brightness boundary, and the process iterates.

Searching cell nucleus and envelope

Create an image copy and aquarellizate it. The formula for image aquarellization is as follow:

$$G_{eq}(I(x,y)) = \frac{M}{N}\sum_{i=0}^{k} n_i ,$$

where M is brightness maximum,
L is aquarellization degree (8),
I (x,y) is pixel intensity.

The next step is iterating along image pixels and choice from each 15 in a circle the average value. After this manipulation, image will decrease by four times and user can carry out searching of pixels lying abroad of cell nucleus (0…85 for middle boundary). We will save these pixels and their coordinates. Aquarellized image is shown on Fig. 5.

The binary mask for object, that will contoured is formed. For each pixel lying inside of cell nucleus, the image's coordinates before decreasing and aquarellizing are calculated and it is assumed that we have one pixel on cell.

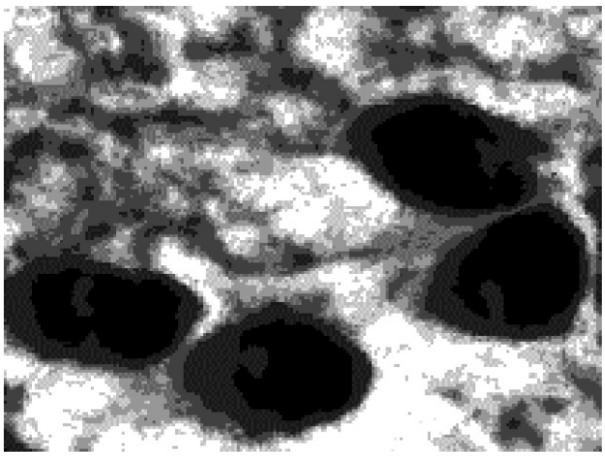

Fig. 5. Aquarellizated image.

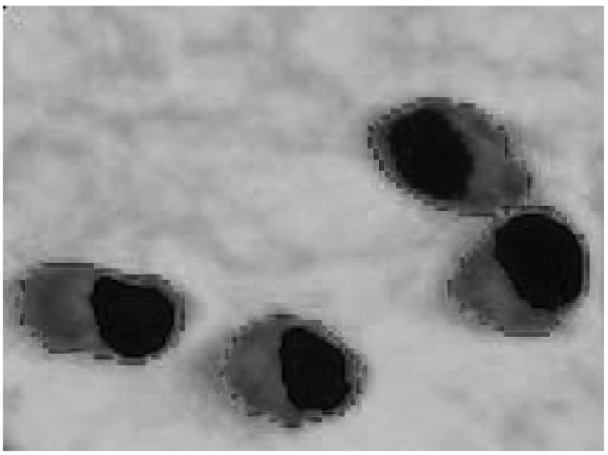

Fig. 6. Autorecognized cell contour.

Pixels amount is the amount of future analyzed cells. Launching the algorithm for pixels search along the image with given brightness boundary. It will be the cell nucleus. After that, similar algorithm works with cytoplasm boundary. As a result we will receive an image, wherein recognized cells are painted by certain colors.

Boundary smoothing

For boundary smoothing, operations of dilation and erosion are used: carrying out four times the contouring of received cell contour on the one pixel and restoring the previous boundary by means of quadruple elimination of contour. It gives the possibility to smooth the sharp aliasing in cell cytoplasm, which can appear in the time of shooting errors.

For better functionality user can make not quadruple, but N-tuple, where N is a number given by user. Value N depends on image quality and size. After smooth operation the cell contour will be received (Fig. 7).

As a result, we received the black-and-white aquarellizated image with equalized histogram, some regions (cell contours), painted by certain color, with contoured boundary. So, we should attach these contours on the image-original (not edited by effects) or save for further edition. Received cell contours will be used further for calculation of cell parameters. If it is necessary, histologist can select cells from the image region, and system will separate cells nucleus and cytoplasm in selected region and calculate necessary parameters.

Feature generation and classification

Numerous medical studies have shown that various on the histological accessory tumors are strictly specific as on tissular, cellular and structural levels. However, there are a number of morphological and cytological methods for evaluation of malignant tumorstage. Several features, in spite of the variety of tumors, are common

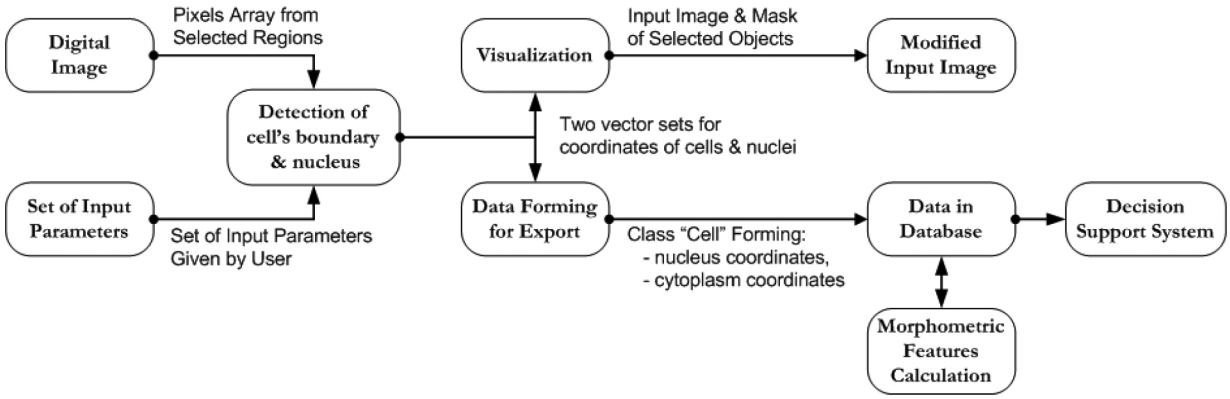

Fig. 7. The process chart of proposed system.

for all of them. These features are: cell sizes, nucleus sizes and nuclear-cytoplasmic ratio etc. Thus, image analysis of histological slices of any topology is reduced to a morphological evaluation of histopathologic feature.

The evaluation process of the nature neuroblastoma development, using the digital microscopic specimens consists of the sequential implementation of a number of stages: image acquisition, image enhancing, image segmentation, contouring, selection, extraction, classification, signs and issue conclusions.

After cell nucleus and cytoplasm contouring, the developed information system calculates morphometric features of tumor cells in the database (cell area, cytoplasm area, mathematics expectation and dispersion of cell nucleus and cytoplasm). These morphometric features are the set of features, which are commonly used by histologist for manual microscopic diagnosis. On the basis of calculated tumor cells morphometric features by information system, the evaluation of the stage of malignant neuroblastoma, based on neuroblastoma differentiation and on the presence or absence of Schwann stroma, is carried out.

Proposed system

Developed information system for evaluation of malignant neuroblastoma level should provide following functions:

Maintenance of the history of patients histological studies;

- Patient search;
- Sets of medical images storing and execution of comparative analysis;
- Semi-automatic segmentation of histological images (contouring the cell's cytoplasm and nucleus);
- Calculation of morphometric indicators of tumor cells in database (cell area, cytoplasm area, mathematics expectation and dispersion of cell nucleus and cytoplasm);
- Evaluation of the stage of malignant neuroblastoma;
- Obtained results storing in system database.

Nonfunctional requirements to system can be: intuitive interface, easiness of maintenance and the possibility of system mark expansion.

The process chart of proposed system is shown on the Fig. 7. Interface and image processing units are developed using Java Standard Edition Development Kit and also Swing components library. Cache Intersystem was used as database.

Conclusion

In the paper, we proposed the segmentation method, based on determinate chaos of histological specimen images. Proposed algorithm relies primarily on thresholding. The algorithm allows considerably facilitate and increase the contouring accuracy of neuroblastoma nucleus and cytoplasm for the further calculation of morphometric indicators ((irregular of contoured zone of diagnostic interest, its structures heterogeneity including cells, nuclei, mitochondrions etc.). In addition, proposed algorithm gives an opportunity to carry out segmentation of histological images of tumor cells nucleated as well as unnucleated. By system work results, expert evaluation about the malignant tumor stage is produced.

The information system for instant diagnosis of the stage of neuroblastoma at the condition of time limitation and with diagnostic decision support is developed. In addition, system can be used for diagnostics of non-oncological diseases in the others spheres of medicine and veterinary. System uses Cache database, which performs the work logic, processing and storing of image processing results.

References

[1] Anticancer society of Russian, http://www.pror.ru

[2] Canete, A., Navarro S., Bermudez J., Pellin A., Castel V., Liombart-Bosch A. et al. "Angiogenesis in neuroblastoma: relationship to survival and other prognostic factors in a cohort of neuroblastoma patients",. *Journal of Clin. Oncol*., No. 18(1),, 2000: pp. 27-–34, 2000.

[3] Carvalho, Ade A.C., Parra E.R., Zerbini M.C., Alves V.A., Capelozzi V.L., Antonangelo L. et al. "Morphometric evaluation of NB84, synaptophysin and AgNOR is useful for the histological diagnosis and prognosis in peripheral neuroblastic tumors (pNTs)",. *Clinics* (Sao Paulo), No. 62(6), 2007:pp. 731–-740, 2007.

[4] Orel, V., Kozarenko T., Galachin K., Romanov A., Morozoff A. et al. "Nonlinear dynamics"., *Journal of Psychol. Life Sci*., No. 11(3), 2007: pp. 309-–331, 2007.

[5] Orel, V., Gusynin A., Selezneva H., Kolesnik S., Stendyk H., Komisarova H. et al. "Methods of digital images analysis of histological specimens"., III International Conference "Biomedical engineering and technology"., March 15-–16, Ukraine, 2012,: p.40 ([in Russian]).

[6] Sokolova, N., Orel V., Gusynin A., Selezneva H. et al. "Database Design for Information System of Neuroblastoma Development Forecasting based on the Object-Oriented Approach"., *Bulletin Kherson National Technical University*, No. 1(44), 2012: pp. 86-–91, 2012 ([in Russian].).

[7] Orel, V., Gusynin A., Selezneva H., Kolesnik S., Stendyk H., Komisarova H. et al. "Algorithm for digital images analysis of histological specimens"., International Conference "Telemedicine-experience @perspective"., March 19-–20, Ukraine, 2012,: pp.25-–26 ([in Russian)].

[8] Deshpanae, G., and M. Borse M. "Image Retrieval with the use of different color spaces and the texture feature",. International Conference on Software and Computer Applications, IPCSIT, V. 9,. Singapore, 2011:, pp. 273-–278.

[9] Soni, J. "Advanced image analysis based system for automatic detection of malarial parasite in blood images using SUSAN approach",. *Int. jJ. of eEngineering sScience and tTechnology*, 2011, V.3, No(.6), 2011:pp. 5260-–5274.

[10] Arun, K.S., and Sarath K.S. Sarath. "Evaluation of SUSAN filter for the identification of micro calcification", ". *Int. jJ. of cComputer aApplications*, 2011, V.15(, No.3), 2011:pp. 41-–44.