

PATTERN RECOGNITION BASED SEGMENTATION METHOD OF CELL NUCLEI IN TISSUE SECTION ANALYSIS

P. Spyridonos¹, D. Glotsos¹, D. Cavouras², P. Ravazoula³, V. Zolota³ and G. Nikiforidis¹

¹ Computer Laboratory, School of Medicine, University of Patras, Rio, Patras 265 00, Greece
gnikif@med.upatras.gr

² Department of Medical Instrumentation Technology, Technological Education Institution of Athens, Ag. Spyridonos Street, Aigaleo, 122 10, Athens, Greece
cavouras@hol.gr

³ Department of Pathology, University Hospital, Rio, Patras, 265 00, Greece

Abstract: A pattern recognition-based segmentation (PRS) system was developed for segmenting cell nuclei in tissue sections of urine bladder tumors and brain tumors. One hundred and thirty eight image samples from HE-stained tissue sections of urine carcinoma and brain astrocytomas were selected. Half of them were used for designing the PRS-system and the rest for evaluating its performance. PRS-system can be designed by employing one of three classifiers: minimum distance, Bayesian, and multi-layer perceptron (MLP) classifier. Classifier design was based upon two sets of training data, the nuclei set and the surrounding tissue set, which were derived from textural features. According to the pathologists' evaluation, 88% of the segmented nuclei were registered correctly and 12% incorrectly. The MLP classifier proved superior in sensitivity and discrimination of nuclei from background when compared with Bayesian and minimum distance classification performance. The PRS-system proved efficient when tested under different types of histopathological tissue samples providing an index for potential generalization of the technique.

1. INTRODUCTION

Tissue section analysis of urine bladder specimens provides an index of disease severity with tumor classification determining the choice and form(s) of treatment [1-2]. Tissue carcinomas present a wide spectrum of disease, with highly variable biological behavior and response to therapy [3-4]. Thus, prognosis varies widely. Tumor stage and grade are generally applied to predict the course of disease and select the most appropriate treatment. Once the physician has determined that the tumor exists, the next step is to clarify the tumor's status [5]. The assessment of specimen's malignancy is specified according to the perception of the pathologist validating biological information that is derived from cytological attributes, among which the most significant are emanated from the morphological and textural characteristics of cell nuclei [7-9]. Segmentation of cell nuclei comprises a critical procedure for the extraction and quantification of nuclei 'hidden' biological information [10-12]. Considering that the segmentation algorithm constitutes an essential part of computer-aided medical decision systems, the development of fast, stable and reproducible methods for automatic nuclei segmentation affects determinative grade classification assessment, thus, therapy and prognosis decision.

Previous studies have been mostly relied upon the traditional methods of cell segmentation, thresholding and edge detection with post processing [13-15]. Effective segmentation resulted employing Fleugen staining technique images [16-18], in contradiction to the most widely accepted method of Hematoxylin and Eosin (HE) staining that is adopted in clinical routine [19]. The variability in color distribution of HE stained

images renders ineffective global thresholding techniques for segmentation, which result in insufficient tissue components discrimination.

In this study, we propose a new approach for automatic cell nuclei segmentation in tissue section analysis applications. A PRS-system was developed, providing a flexible and adaptive method to extract nuclei 'hidden' biological information in the form of quantitative nuclei attributes, utilizing different classification algorithms according to the particular type of histopathological tissue samples.

2. MATERIALS AND METHODS

The histological material used in this study was collected from the Department of Pathology of the University Hospital of Patras, Greece. The database comprised 138 image samples from HE-stained tissue sections of urine bladder carcinomas (figure 1) and brain astrocytomas (figure 2). Registration of each color image was performed at a magnification of x400 using a light microscopy imaging system consisting of a Zeiss KF2 microscope and an Ikegami color video camera.

Half of the image samples, randomly selected, were used for designing the PRS-system (training set) and the rest (test set) for evaluating its performance. For the design of the classifier, adequate numbers of 5x5 nucleus and background image samples were isolated from each image of the training set. After the formation of the nucleus and background training classes, 3 textural nuclear features were extracted from the autocorrelation function. More specific, features that were calculated contained a) the sum of autocorrelation function for all possible displacements m, n inside the

window 5x5, b) the cross relation $S(1,1)$ and c) the second degree spread $S(2,2)$:

$$S(u, v) = \sum_{m=0}^T \sum_{n=-T}^T (m-n_m)^u (n-n_n)^v A_F(m, n) \quad (1)$$

where,

$$n_m = \sum_{m=0}^T \sum_{n=-T}^T m A_F(m, n) \quad (2)$$

$$n_n = \sum_{m=0}^T \sum_{n=-T}^T n A_F(m, n) \quad (3)$$

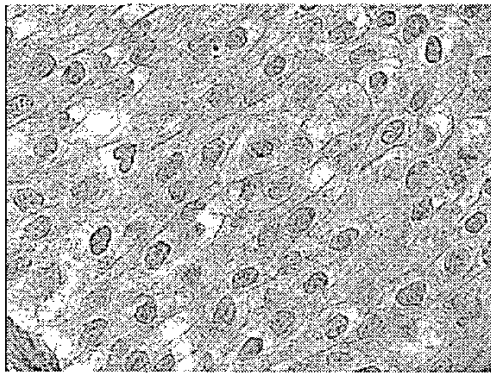


Figure1: Typical tissue sample of urine bladder carcinoma

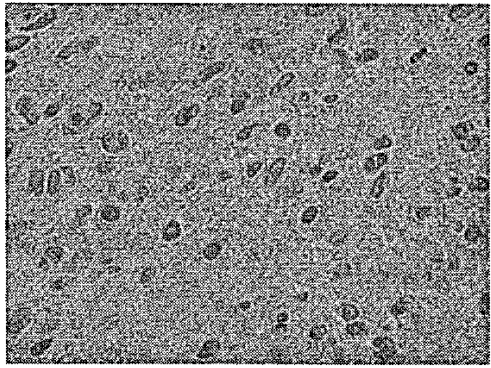


Figure2: Typical tissue sample of brain tumor astrocytomas

$$A_F(m, n) = \sum_j \sum_{n=-T}^T (m-n_m)^u (n-n_n)^v A_F(m, n) \quad (4)$$

The autocorrelation function has been suggested by many researchers as a texture measure [20-21]. From equation (4) it is apparent that a sub-region will exhibit higher correlation for a fixed shift (m, n) than a region of fine texture. The sum of autocorrelation function for all displacements over the window 5x5 could be

regarded as coarseness index that is the higher the value of the feature the coarser the region is.

Since we estimate the sum of autocorrelation function, for all displacements, it would be possible two different textural fields to exhibit the same value. To improve the texture discrimination, the texture features $S(1,1)$ and $S(2,2)$ were estimated additionally, which provide the extra information of two-dimensional spread measures of the autocorrelation function.

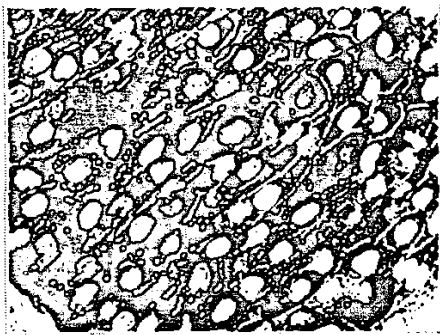
Segmentation was performed sweeping each image with a 5x5 mask. The autocorrelation based textural features were calculated at each position and the central pixel was classified as belonging to nucleus or surrounding tissue. Pixel classification was performed by either employing the minimum distance (MD) (figure 3a), the Bayesian (figure 3b) or the MLP classifier (figure 3c). The MLP was designed with two hidden layers containing 3 and 6 nodes in each layer respectively, and one output unit. In the resulted binary image (nucleus-surrounding tissue), pixels that were classified as emanating from surrounding tissue were turned black and those belonging to nucleus as white (figure 3). To reject noisy regions the binary image was further processed by morphological filters [22]: 1/fill holes and 2/close and open morphological operations (figure 4). Finally, nuclear delineation was achieved by superimposing the resulting image on the original image and performing logical AND operation (figure 5-6).

3. RESULTS AND DISCUSSION

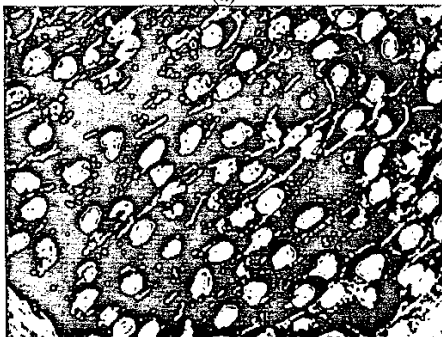
PRS-system classification was optimized using the MLP classifier (figure 3c). The correct rate in assigning pixels to nuclei was about 52.3%, 63% and 75% for MD, Bayes and MLP classifier respectively. The classifiers performed in a stable and reproducible way for all cases tested. PRS-system validation was performed by the pathologist comparing the segmented images of the test set against the original images and marking wrongly segmented nuclei. Under this perspective, 88% of all delineated nuclei on the tested images were registered correctly and 12% were registered incorrectly. Considering that segmented nuclei from each section field ranged between 18 and 80, the misclassification error of 12% may be regarded as of limited significance.

Since the proposed segmentation method was validated for two-types of histo-pathological images, this may lead to the suggestion that this technique can be of value for various-different types of tissue section analysis applications.

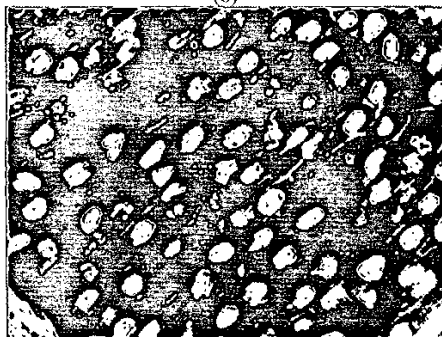
In conclusion, the selected textural features and the design of the MLP algorithm are efficient tools in detecting nuclei regions, however, for further improving the segmentation method more investigation would be required in refining the nuclei shape determination, since morphological filters are of limited accuracy.



(a)



(b)



(c)

Figure 3: comparative segmentation results employing (a) MD, (b) Bayes, and (c) MLP classifier.

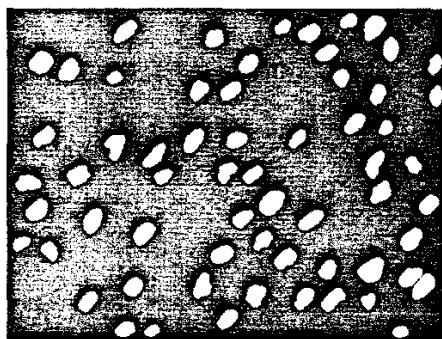


Figure 4: binary image resulted after morphological operations

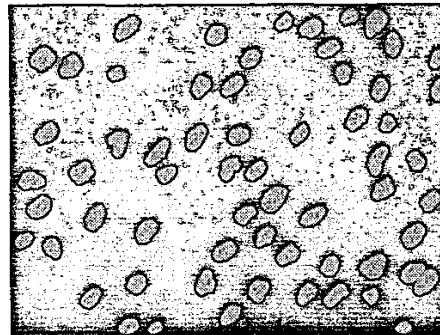


Figure 5: segmented nuclei from the urinary bladder tissue sample

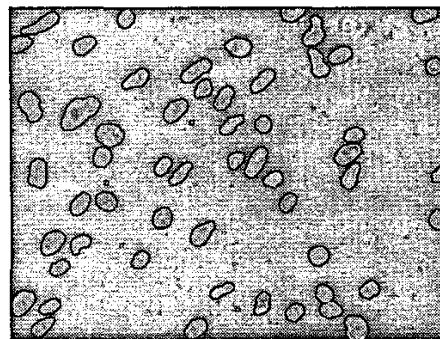


Figure 6: segmented nuclei from the brain tumor astrocytomas tissue sample

REFERENCES

- [1] D. G. Bostwick, D. Ramnani and L. Cheng, "Diagnosis and grading of bladder cancer and associated lesions", *The Urologic Clinics of North America*, Vol. 26, No.3, 1999, pp. 493-507
- [2] Patrick J. Kelly, M.D., "Astrocytomas", Department of Neurological Surgery, New York University Medical Center, (<http://mcns10.med.nyu.edu/tumors/astro.html>)
- [3] R. Oyasu, "World Health Organization and International Society of Urological Pathology classification and two-number grading system of bladder tumors", *Cancer*, Vol. 88, No. 7, 2000, pp. 1509-1512.
- [4] "World Health Organization international: Histological typing of tumours of the central nervous system, 2nd edition", Berlin, Springer-Verlag, 1993.
- [5] H. G. Van der Poel, "Quantitative light microscopy in Urologic Oncology", Urologic thesis, University of Nijmegen, 1994.
- [6] V. E. Reuter, "Bladder: Risk and prognostic factors-a pathologist's perspective", *The Urologic Clinics of North America*, Vol. 26, No. 3, 1999, pp. 481-492.

- [7] R. Nafe, S. Roth and P. Rathert, "Analysis of criteria for grading bladder cancer in urine cytological tumor diagnosis by means of an expert system", *European Urology*, Vol. 21, No. 2, 1992, pp. 103-109.
- [8] R. P. Pauwels, R. F. Schapers, A. W. Smeets, F. M. Debruyne and J. P. Geraedts, "Grading in superficial bladder cancer. (1). Morphological criteria", *British Journal of Urology*, Vol. 61, No. 2, 1988, pp. 129-134.
- [9] W. N. Street, W. H. Wolberg and O. L. Magasarian, "Nuclear feature extraction for breast tumour diagnosis", *International Symposium on Electronic Imaging: Science and Technology*, San Jose, California, 1995, 861-870.
- [10] Pascal Bamford and Brian Lovell, "Unsupervised cell nucleus segmentation with active contours", *Signal Processing*, Vol. 71, 1998, pp. 203-213.
- [11] Ge Cong and Bahram Parvin, "Model-based segmentation of nuclei", *Pattern recognition*, Vol. 33, 2000, pp. 1383-1393.
- [12] C. Sowter, G. Slavin and D. Rosen, "Morphometry of bladder carcinoma: 1. The automatic delineation of urothelial nuclei in tissue sections using an IBAS II image array processor", *Journal of Pathology*, Vol. 153, 1987, pp. 289-297.
- [13] U. De Meester, I. T. Young, J. Lindeman and H. C. van der Linden, "Towards a quantitative grading of bladder tumors", *Cytometry: the Journal of the Society for Analytical Cytology*, Vol. 12, No. 7, 1991, pp. 602-613.
- [14] J. Martin, McKeown and David A. Ramsey, "Classification of Astrocytomas and Malignant Astrocytomas by Principal component Analysis and a Neural Net", *Journal of Neuropathology and Experimental Neurology*, 1996; Vol. 55, No. 12, 1996, pp. 1238-1245.
- [15] H. Martin and D. Schmidt, "Malignancy grading of glial tumours: 1. Astrocytomas", *Zentralblatt Fur Allgemeine Pathologie Und Pathologische Anatomie*, Vol. 130, No. 6, 1985, pp. 451-462.
- [16] H. K. Choi, J. Vasko, E. Bengtsson, T. Jarkrans, P. U. Malmström, K. Wester and C. Busch, "Grading of transitional cell bladder carcinoma by texture analysis of histological sections", *Analytical Cellular Pathology: the Journal of the European Society for Analytical*, 1994, Vol. 6, No. 4, 1994, pp. 327-343.
- [17] R. F. Schapers, R. P. Pauwels, J. T. Wijnen, J. W. Arends, F. B. Thunnissen, J. W. Coebergh, A. W. Smeets, and F. T. Bosman, "A simplified grading method of transitional cell carcinoma of the urinary bladder: reproducibility, clinical significance and comparison with other prognostic parameters", *British Journal of Urology*, Vol. 73, No. 6, 1994, pp. 625-631.
- [18] T. Jarkrans, J. Vasko, E. Bengtsson, H. K. Choi, P. U. Malmström, K. Wester and C. Busch, "Grading of transitional cell bladder carcinoma by image analysis of histological sections", *Analytical Cellular Pathology: the Journal of the European Society for Analytical Cellular Pathology*, 1995, Vol. 8, No. 2, 1995, pp. 135-158.
- [19] Dr. Tom Hollinger, "Histological Stains", University of Florida, College of Medicine, (<http://www.medinfo.ufl.edu/dental/denhisto/stains.html>).
- [20] W. K. Pratt, "Digital Image Processing", John Wiley and Sons, 1991, pp. 578-579.
- [21] O. Faugeras and W. Pratt, "Decorrelation methods of texture feature extraction", *IEEE Transactions on Pattern Analysis and Machine Intelligence*, PAMI-2,14, pp. 323-332.
- [22] R. C. Gonzalez and R. E. Woods, "Digital Image Processing", Addison-Wesley, 1992, pp. 518-528.