

## AUTOMATIC GRADING OF ASTROCYTOMAS USING PROBABILISTIC NEURAL NETWORK CLUSTERING AND QUANTITATIVE NUCLEAR FEATURES

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**Abstract.** *A method for the automatic segmentation and classification of Hematoxylin and Eosin (H&E) stained nuclei images of astrocytomas is presented. 100 H&E microscopic sections of astrocytomas were selected of low, high or intermediate grade. From each section images were digitized and segmented by applying a pixel-based classification-clustering algorithm. Following segmentation, each case was represented by a feature vector comprising 33 morphological and textural features that were extracted from segmented nuclei to encode tumour malignancy. The most important features were selected using Principal Component Analysis. A novel Probabilistic Neural Network clustering algorithm was designed to distinguish low, intermediate, and high-grade tumours. The latter were identified with 90.2%, 88.3%, and 91.5% accuracies respectively. The proposed computer assisted microscopy system might be utilized in daily clinical practice without the need of performing specialized staining processes, that increase the time and cost of diagnosis, and without burdening histopathologists with the need to familiarize with new grading protocols.*

### 1 INTRODUCTION

Microscopic examination of brain tumour astrocytomas is the most significant step in the diagnostic chain because it affects treatment planning and patient management <sup>[1]</sup>. The most fundamental task is to accurately identify low from high-grade tumours, because these are treated differently. However, grading by visual inspection has been questioned mainly because the quality of diagnosis greatly depends on the experience and skills of the physicians (inter observer reproducibility can be as low as 51% <sup>[1]</sup>). Additionally microscopic images are characterized by large regional heterogeneity that makes it difficult to establish clear and understandable histological criteria, which will be used by experts to grade astrocytomas <sup>[2]</sup>. Computer-aided diagnosis has been reported to improve diagnostic accuracy and to enhance consensus among pathologists (inter-observer reproducibility) <sup>[2-5]</sup>.

The majority of previous studies <sup>[2-5]</sup> have been focused to the separation of low from high-grade tumours. In this study, a Probabilistic Neural Network clustering algorithm has been specifically designed to identify low from high grade tumours, and additionally those cases of intermediate grade that may need re-examination.

### 2 MATERIAL AND METHODS

One hundred (100) H&E stained biopsies of astrocytomas were collected from the Department of Pathology of the University Hospital of Patras, Greece. Tumour malignancy was defined as low or high according to the World Health Organization (WHO) grading system <sup>[7]</sup> by a pathologist (P.R.). For each biopsy, the pathologist specified the most representative region. From this region, images (Fig. 1) were digitized (576x256x8 bit) using a light microscopy imaging system consisting of a Zeiss Axiostar-Plus microscope (ZEISS; Germany) and a Leica DC 300 F color video camera (LEICA; Germany).

## 2.1 Image Segmentation

Images were segmented to isolate nuclei from surrounding tissue. Tumour malignancy was encoded by means of a set of features derived from the segmented nuclei. The segmentation process comprised four stages: **Stage A**: Initially, each image was scanned by a 5x5 pixel window frame, with 1-pixel step. At each frame position two features were calculated, namely the spread  $a(1)$  and cross relation  $a(2)$  of the autocorrelation function<sup>[8]</sup>:

$$a_i = [a(1), a(2)] \quad (1)$$

$$a(1) = \sum_{m=0}^S \sum_{n=-S}^S (m - n_m)(n - n_n) A_F(m, n) \quad (2)$$

$$a(2) = \sum_{m=0}^S \sum_{n=-S}^S (m - n_m)^2 (n - n_n)^2 A_F(m, n) \quad (3)$$

where  $A_F$  is the autocorrelation function inside an  $S \times S$  window,  $m, n$  represent all possible displacements inside the  $S \times S$  window and

$$n_m = \sum_{m=0}^S \sum_{n=-S}^S m A_F(m, n) \quad \text{and} \quad n_n = \sum_{m=0}^S \sum_{n=-S}^S n A_F(m, n) \quad (4)$$

In this way, the differences in texture between nuclei, surrounding tissue and boundaries were encoded, since coarser regions (i.e. regions of surrounding tissue, boundaries) resulted in higher values for both spread and cross relation, whereas smoother textured regions (i.e. interior of nuclei) resulted in lower values.

**Stage B**: The feature vectors (spread, cross relation) generated at each frame position, were fed to a Probabilistic Neural Network (PNN) clustering algorithm, presented by our group elsewhere<sup>[9]</sup>. The output of the clustering procedure decided on nuclei or surrounding tissue on the basis of each input feature vector and assigned the central pixel of each 5x5 frame as belonging to nuclei or surrounding tissue cluster. **Stage C**: A binary image was created by illuminating white only those pixels that corresponded to nuclei regions, whereas the remaining pixels were considered as background and lighted black. The resulted binary images were further processed for the elimination of nuclei intersecting image borders, small noisy regions with area less than 200 pixels, by means of size filtering, fill-holes operations and morphological filtering<sup>[8]</sup>. **Stage D**: Finally, nuclei segmentation was accomplished by superimposing the binary processed image to the original image.

## 2.2 Image Classification

After segmentation of all 100 images, a similar procedure was followed in order to classify low, high and of intermediate grade images. For each patient one image was used. Two kinds of features were generated from each segmented nuclei: 18 morphological features related to the size and shape of cell nuclei and 15 textural features (first-order, co-occurrence, run-length based) that encoded chromatin distribution and nuclear DNA content<sup>[2, 8]</sup>. After feature generation, each image was represented by a 33-dimensional feature vector, where each vector element was the mean feature value of all examined nuclei. Principal component analysis was performed in order to determine the best feature vector combination for highest tumour grading accuracy. Following, the PNN-based clustering algorithm was activated to search for two distinct clusters corresponding to the low and high grade groups or to outliers (unclassified cases of intermediate grade).

## 3 RESULTS

Figure 1a depicts a digitized H&E stained image of astrocytomas. The performance of the PNN-based clustering algorithm in locating nuclei regions is illustrated in figure 1b. In figure 1c nuclei regions are corrected by morphological-size filtering processing and the final segmentation is obtained by superimposing the original image to the morphological processed image (figure 1d). Concerning image classification, high-grade tumours were classified with 91.5% accuracy, low-grade tumours with 90.2% and ‘suspicious’ cases of intermediate grade with 88.3%. Overall accuracy was 90.0% (table 1).

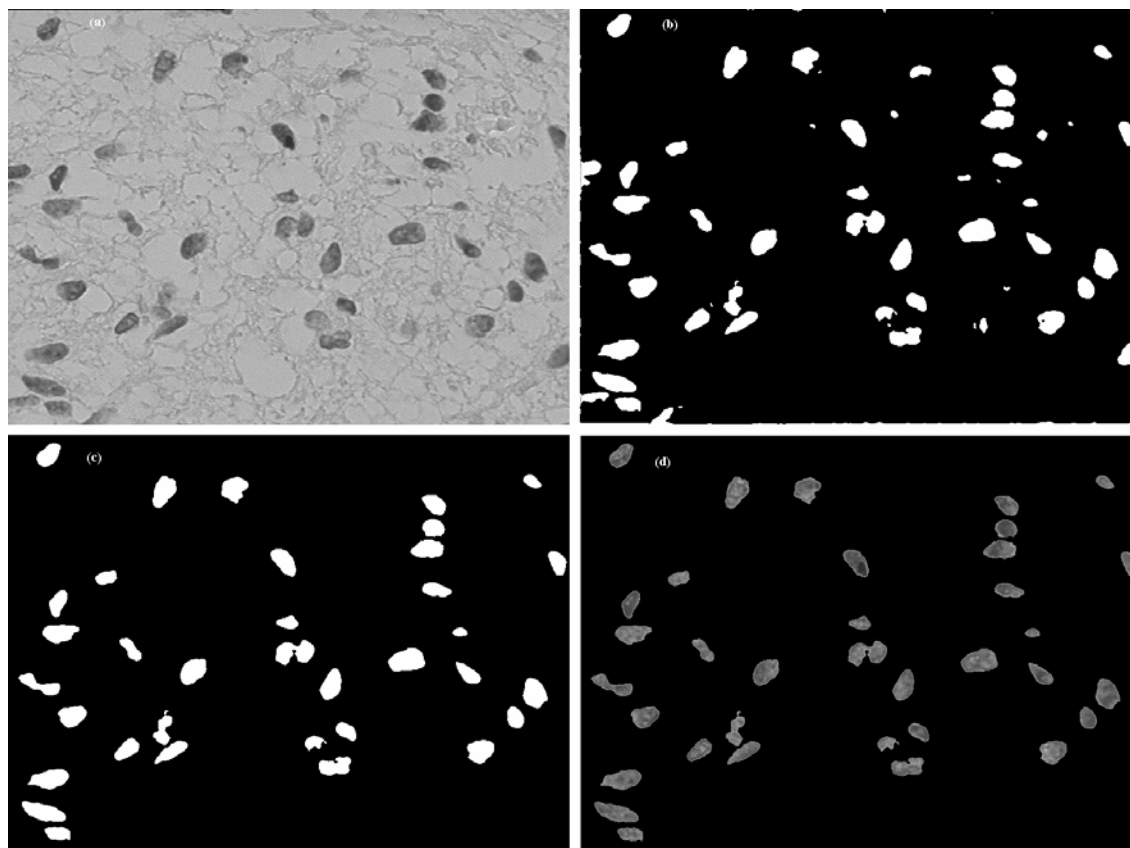


Figure 1. (a) Original image, (b) Result of PNN clustering, (c) Enhancement of nuclei regions by morphological and size filtering processing, (d) Resulting segmented nuclei by superimposing figure 1a to 1c

Diagnosis	Low grade (grade I-II)	High grade (grade III-IV)	Suspicious cases (grade II-III)	Classification Results (accuracy)
Low grade	37	2	2	90.2%
High grade	1	43	3	91.5%
Suspicious cases	0	2	10	83,3%
Overall accuracy				90.0%

Table 1: Truth table demonstrating classification results for the 100 cases of astrocytomas

#### 4 DISCUSSION

In this study, a method for the automatic segmentation and classification of H&E stained images of astrocytomas was introduced by developing a novel PNN clustering algorithm and employing the WHO grading system. The method gave high rates in correctly discriminating low from high grade tumours and ‘suspicious’ cases of intermediate grade with overall accuracy 90.0%. These results are promising, compared to those presented in literature for automatic grading systems that utilize the WHO scheme but more demanding staining procedures, such as 55% in [2], 66% in [3], 88% in [4]. In contrast to previous studies [1-5], the proposed method was designed to additionally specify ‘suspicious’ cases, which are very important, considering that some of these cases might need re-examination [1]. In our dataset, the histopathologist characterized 12 cases as ‘suspicious’ cases (between low and high grade). The PNN algorithm correctly identified 10 of the 12 ‘suspicious’ cases with accuracy 83,3%. The remaining two cases were classified by the algorithm as high grade. These 2 cases were low-grade tumours that recurred after 1 and 2 years from first diagnosis and surgical removal.

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