Astrocytoma Brain Tumor Automated Classification Procedure Based on Ki67 Expression

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Abstract. It is known to be a very challenging task for pathologists to perform astrocytoma biopsy analysis especially with the high demand for an accurate and precise analysis. An inaccurate diagnosis might affect the treatment decisions for the patient. Therefore, this study aims to develop an automated procedure for classification astrocytoma brain tumor. The procedure consists of four stages, namely image preprocessing, image segmentation, feature extraction and selection, and classification of Ki67 expression. Initially, the image’s quality was enhanced by using global stretching and unsharp masking filter. Then, the adaptive color thresholding technique was used to segment the Ki67 expression. The process was followed by extracting the features related to the positive and negative Ki67 cells. ReliefF feature selection algorithm was used to select the best features in defining the positive and negative cells. In the classification stage, two classifiers, namely Support Vector Machine (SVM) and Multilayer Perceptron (MLP) were used to differentiate between the positive and negative cells. From 81 samples images, both classifiers yielded promising results, with an accuracy of 98.5% for SVM and 99.6% for MLP respectively.

1. Introduction
A brain tumor is a mass of cells that grow abnormally and multiplied aggressively in the brain. Gliomas are a brain tumor type, where these tumor cells are developed in the glial cells. These glial cells play a supportive role by protecting, nourishing and supporting the neurons. Astrocytoma is the most common glioma tumor which develops from astrocytes cell that support the nerve cells. According to the World Health Organization (WHO), astrocytoma can be divided into four grades, which are grade I (pilocytic astrocytoma), grade II (diffuse astrocytoma), grade III (anaplastic astrocytoma), and grade IV (glioblastoma) [1].

Ki-67 is one of the proliferation marker used to measure cell proliferation activity. This nuclear protein is usually associated with tumor cell proliferation and growth. Ki67 immunohistochemical staining (IHC) is a technique where the antibodies are used to detect specific antigens in a sectioned tissue. During IHC staining process, the tissue specimen will be stained with the Diaminobenzidine (DAB) and counterstained with Hematoxylin (H). As a result, the positive Ki-67 cells (tumor cells) will appear in granular brown colour, while the negative Ki-67 cells (non-tumor cells) appear in blue.

An accurate and correct diagnosis of a tumor tissue biopsy is important since it will determine the grading results and outcome treatment to the patient. However, there are some problems related to the diagnosis of the biopsy slide. The process is very time-consuming as it required the pathologists to count the positive and negative Ki-67 cells manually under a
microscope observation. This might as well cause a huge burden on the pathologists, especially when dealing with a blurry image. Sometimes, to save time, pathologists applied an eye-balling technique in which the percentage of positive Ki-67 cells will be estimated and this estimation does not involve the counting of individual cells [2]. For that reason, this paper proposes an automated Computer-Aided Diagnosis (CAD) procedure for classification the astrocytoma based on the Ki67 expression. This paper is organized as follows. Section 2 will discuss the related works on developing CAD procedure in the medical field. Section 3 will explain in details the methodology of the proposed CAD procedure. Section 4 highlights the results and discusses the performance of the CAD procedure, while Section 5 concludes the paper.

2. Related Works

Today, the development of the CAD procedure has been rising and gaining attention from many researchers, especially for those who work in the medical field application. Fukuma et al. [3] proposed a CAD procedure that involved stages of image segmentation, feature extraction and disease classification for brain tumor histopathological images. The color space of the input images was converted first from RGB to grayscale. Then, the image enhancement and image smoothing techniques were applied to improve the quality of input images. Next, the thresholding method was applied for the nuclei segmentation and then the object-level and spatial-arrangement features were extracted. For classification, the classifiers of SVM and RF were trained using the features datasets and obtained the accuracy result of 98.6% and 99.8% for object-level features and 82.1% and 86.1% for spatial-arrangement features, respectively.

Gupta et al. [4] proposed an automated classification procedure for the MR images of astrocytoma by classifying the images into low grade and high grade. The astrocytoma MR images taken from BRATS database were enhanced using Contrast Limited Adaptive Histogram Equalization (CLAHE) and segmented using basic global thresholding. A total of 38 features were extracted from the images with 22 features were texture features while the other 16 features were shape feature. The procedure made use of a $k$NN classifier for the classification and achieved an accuracy of 93%.

Sanjeev et al. [5] proposed a CAD procedure for the detection and classification of brain tumor MR images by applying three parts in the procedure which are image segmentation, feature extraction and selection and classification. For the image segmentation stage, the images were sharpen using a sharpening filter and a threshold value was set to obtain the density and area of the connected region. A texture features dataset which containing texture parameters were obtained using the Gray-Level Co-Occurrence Matrix (GLCM). The features dataset was reduced using Principal Component Analysis (PCA), and SVM classifier was trained using the dataset to identify and classify the tumor into benign or malignant. The results indicated that the SVM with radial basis function kernel has good performance for the classification of both malignant and benign brain tumor, which is in the range of 60-80% and 50-70% respectively.

Wang et al. [6] developed an automated multiparametric glioma classification procedure by investigating the contributions of multi-parameters from multimodal data. The geometrical (shape, size, circularity) and texture (homogeneity, contrast, entropy) features were extracted after using the watershed nuclei detection algorithm for the image segmentation. The features were later selected using RF-based combined with backward feature elimination and trained into four classifiers which are RF, SVM, Gradient Boosting Decision Tree (GBDT) and $k$NN. The results show that SVM has the highest classification accuracy of 90% while the other three have the same performance with an accuracy of approximately 87%.

3. Proposed Procedure

This section presents all the methods used for the development of an automated classification CAD procedure. The proposed procedure consists of four major stages, which are image preprocessing, image segmentation, feature extraction and selection, and classification of Ki-67 expression.
3.1. Image Acquisition
There were 81 histopathological images used in this study. Since this study is only focusing on the cell classification of positive and negative Ki-67 cells, hence, the number of images used are more than enough to conduct the study. These images were captured and obtained from Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian, Kota Bharu, Kelantan, Malaysia. The sample images were captured under 40x magnification using an Olympus BX51 microscope and Cell^F software that acts as an interface to the digital camera attached to the microscope. Each image was saved in JPEG (*.jpg) format with a resolution of 4140x3096 pixels, as recommended by Cell^F software.

3.2. Image Pre-Processing
This stage is important for removing unnecessary effects such as noises from the image, and adjust or transform the image as needed for further processing [7]. At first, the resolution of the input image was resized by a factor of two, from 4140x3096 to 2048x1536 (4:3 aspect ratio resolution) to speed up the performance of the procedure. The contrast enhancement approach was used to improve the contrast of the images by implementing the function of imadjust in MATLAB. This technique mapped the values of the input intensity image to new values that will enhance the image contrast. By default, 1% of the data is saturated at low and high intensities of the input data thus, increasing the contrast of the image.

In this study, the contrast enhancement was done separately. At first, the procedure will enhance the image of positive Ki-67 cells and followed by negative Ki-67 cells’ image. An additional technique called gamma correction was applied to the negative Ki-67 cells’ image since the image’s contrast was too low. The gamma correction can be conducted by using equation (1);

\[ V_{out} = A \times V_{in}^\gamma \]  

where \( V_{out} \) is the output value, \( A \) is a constant (maximum pixel intensity) and \( V_{in} \) is the input value, raised to the power \( \gamma \). The value of gamma can be any value between zero and infinity and if gamma is less than one, the image will be brighter which indicated the mapping is weighted towards higher output values. In contrast, when gamma is more than one, the image will be darker (mapping weighted toward lower intensity values) [8]. For this study, the value of the gamma was set to a fixed value, which is 0.67. The value was determined by applied different values on various images to have an optimal value. To improve the visibility of the input image, an unsharp masking filter was implemented as it can improve the detail appearance by enhancing a small scale of edge contrast of the images [8].

3.3. Image Segmentation
After pre-processing, the background of the images will later be removed to get the segmented cells for further analysis by using color thresholding. Original color space of Red-Green-Blue (RGB) was converted into Hue-Saturation-Value (HSV) color space because it provides useful information regarding shade, brightness and color. The HSV images were then separated into three components namely hue, saturation and value.

An adaptive color threshold was applied on the images in which different threshold values are selected dynamically over the image, unlike the global threshold which only selected a single threshold value. The purpose of selecting multiple thresholds is because when the intensity of the image or the saturation of color in the image varies throughout the image, the results obtained by a mere single threshold value cannot bring accurate results for the image. Therefore, it is recommended to select multiple threshold values [9]. As mentioned in Section 1, the positive Ki-67 cells are brown-colored while the negative cells are blue-colored. To segment both positive and negative cells, ‘hue’ and ‘saturation’ component was chosen respectively. The threshold values of maximum and minimum were determined using impixel function in MATLAB and the objects that lie outside the threshold values were removed. After segmenting the images, the following step is to remove the existing noises by using the area opening technique. This technique will eliminate any objects that have a smaller value than the defined thresholded value.
3.4. Feature Extraction and Selection
This stage is crucial to transform large input data into a smaller representation set of features to analyze the images after segmentation. In this study, geometrical (shape), texture and color features were used to identify the positive and negative Ki-67 cells. Geometrical or shape features represent the outward characteristics of the cells, while the texture features describe information about the deviation in the intensity of cells’ surface and measure its properties. Color feature is one of the most widely used visual features in image classification as it has many advantages in terms of robustness, effectiveness, implementation complexity, computational simplicity and low storage requirements.

As for feature selection, ReliefF feature selection was used in this study as it is a commonly practiced feature weighing technique that capable of estimating the quality of the features from a given dataset by appointing weights to each of them [10]. The basic idea of ReliefF is that it evaluates the quality of the features by their capability on differentiating instances in a local neighborhood from one class to another, that is the features that provide more to enlarge the distance between different class instance while providing less to enlarge the distance between same class instances are the best features.

3.5. Classification
In the classification stage, the cells will be classified into positive Ki-67 cells, negative Ki-67 cells and unwanted cells. This is to ensure that all cells in the tissue specimens are analyzed and correctly classified by using the proposed procedure. This study implements two types of classifiers, SVM and MLP for differentiating between the positive and negative Ki67 cells. SVM is a classifier that learns a linear separator which will categorize the samples according to their class in the representation space and it is very useful especially for a task with binary classification (e.g., separate positive and negative Ki-67 cells). With the advantage of high accuracy and easy-to-use, this classifier becomes one of the popular supervised machines learning especially for classification problems as it can serve as both binary and multi-class classification.

As for MLP, it is useful for regression and mapping the input data into a suitable set of output data. MLP is a classification procedure that consists of at least three layers of nodes: first layer as the input layer, middle layer as a hidden layer and third layer as the output layer. It works by connecting neuron from one layer to the neurons in the next layer which make them prone to overfitting. In this study, a pattern recognition tool provided in MATLAB was used to train and test the features dataset. The dataset which consists of 3581 data with the selected 13 features was divided into training, validation and testing with a ratio of 70:15:15. 20 hidden neurons were used with a hyperbolic tangent sigmoid transfer function ($tansig$) to calculate a layer’s output from its net input. For performance function, a Levenberg-Marquardt (LM) backpropagation with the function of $trainlm$ was applied due to its advantages of being the fastest backpropagation algorithm in the toolbox.

The training dataset was trained according to $trainlm$ training parameters which have been fixed for maximum number of epochs to train and maximum validation failures as 500 and 100 respectively. Assuming that the network performance on the validation vectors unable to increase or constantly maintained for 100 epochs in a row, the validation vectors will stop the training early and the test vectors are utilized to verify whether the network is generalizing well or not. The learning rate and the number of hidden nodes were optimized to obtain the suitable parameters. The results of the classification between the two classifiers will be compared and the best classifier will be utilized for the classification procedure.

4. Classification Results and Discussion
In this section, the results obtained from the development stages are presented. In this study, 81 histological images of astrocytoma were used.
4.1. Image Pre-Processing
Initially, the contrast enhancement technique was applied by adjusting the intensity values of the images to increase the contrast. Then, unsharp masking filter was applied to the resultant images. Figure 1 illustrates the comparison between the original and enhanced images.

![Figure 1](image1.png)

**Figure 1.** Comparison between the original and enhanced images of positive and negative Ki67 cells

Figure 1(b) showed the enhanced image of positive Ki-67 cells from the original image (Figure 1(a)) with the contrast limit of [0.2 0.8]. Based on the resultant image in Figure 1(b), it showed that the contrast of the positive Ki67 cells was enhanced. Moreover, the texture of these cells also more visible compared to the original image. As for negative Ki-67 cells, the image was obtained by removing the positive cells and was presented in Figure 1(c). A contrast limit of [0.3 0.7] demonstrated the suitable limits to visualize the negative Ki-67 cells. If the contrast limit is too large, it will produce an image with low contrast and the procedure may not able to detect the positive and negative Ki-67 cells. Meanwhile, if the contrast limit is too small, it has too high contrast level and there might be unnecessary artifacts that were enhanced too.

4.2. Image Segmentation
The image was converted into HSV color space and separated into three components: hue, saturation and value before it was segmented. The purpose of this separation is to determine which component is more suitable for the segmentation. Hue and saturation components were selected for segmentation of positive and negative cells, respectively. The color thresholding method was applied by selecting the threshold limits of maximum and minimum using `impixel` in MATLAB. The pixels outside of the threshold limits will be removed and the positive and negative Ki-67 cells were segmented. Figure 2 shows the sample of segmented images of positive and negative Ki-67 cells respectively.

![Figure 2](image2.png)

**Figure 2.** Resultant segmented images of positive and negative Ki-67 cells
4.3. Feature Extraction

After segmenting the targeted objects, the feature extraction stage was applied to extract features of positive and negative Ki-67 cells in order to classify them. In this study, there were overall 21 features that have been extracted from Ki-67 cells, which are 5 features of shape, 4 features of texture and 12 features of color. Table 1 shows the list of features followed by their category.

| Features          | Category | Features     | Category |
|-------------------|----------|--------------|----------|
| Area              | Shape    | Mean of Red  | Color    |
| Eccentricity      | Shape    | Mean of Green| Color    |
| Perimeter         | Shape    | Mean of Blue | Color    |
| Solidity          | Shape    | Mean of Hue  | Color    |
| Circularity       | Shape    | Mean of Saturation | Color |
| Contrast          | Texture  | Mean of Value | Color    |
| Correlation       | Texture  | Variance of Red | Color   |
| Energy            | Texture  | Variance of Green | Color   |
| Homogeneity       | Texture  | Variance of Blue | Color   |
|                   |          | Variance of Hue  | Color    |
|                   |          | Variance of Saturation | Color   |
|                   |          | Variance of Value | Color    |

Table 1. List of Features Extracted

Shape features were extracted using `regionprops` function in MATLAB by using binary segmented images and the extracted features were: area, eccentricity, perimeter, solidity and circularity. As for texture, the features such as contrast, correlation, energy and homogeneity were extracted using Gray-Level Co-Occurrence Matrix (GLCM). In addition, two color spaces have been used in this study for the feature extraction stage which are RGB and HSV color space. Mean and variance were extracted from each color component.

4.4. Feature Selection

ReliefF was used to reduce the dimensions of the features for more efficient classification procedure. As ReliefF utilizes the \( k \)-Nearest Neighbors (\( k \)-NN) concept to rank the features according to the weight of each feature, thus, selection of \( k \) is crucial for ReliefF performance. The value of \( k \) for the algorithm was varied from 1 until it reached an elbow (circa 287). The selected features for ReliefF with \( k \)-value of 287 were divided into five sets and then evaluated 10 times for training, validation and testing with MLP classifier due to its reliability. The selected Set 1, 2, 3, 4 and 5 consists of 6, 10, 13, 17 and 21 features, respectively. Figure 3 shows that feature Set 4 gives the highest accuracy except for the training dataset. Having high accuracies on validation and testing category signified that the features dataset has the best generalization. Set 5 has the highest average accuracy for training but low for validation and testing. This indicated that the feature Set 5 was overfitted. The average overall accuracy started to become uniform after reaching Set 3 which contains 13 features. As the purpose of feature selection is to reduce the dimensionality of features, it was recommended to select the feature datasets which have fewer features but exhibits high accuracy for overall performance. Therefore, Set 4 which has the best generalization with an average overall accuracy of 99.6% was selected.
4.5. Classification Results
The selection of a suitable classifier was analyzed based on classification performance using accuracy, sensitivity and specificity for overall testing accuracy, testing accuracy for positive Ki-67 cells, and testing accuracy for negative Ki-67 cells. Both classifiers have been tuned for their best designed parameters prior to the comparison so that both will perform at their best performance. Table 2 shows the average accuracy, sensitivity and specificity for SVM and MLP. It is obvious that MLP slightly surpassed SVM in all tests and proved that MLP is more suitable for the classification procedure.

| Parameter   | SVM (%) | MLP (%) |
|-------------|---------|---------|
| Accuracy    | 98.5    | 99.6    |
| Sensitivity | 99.5    | 99.4    |
| Specificity | 66.7    | 99.7    |

5. Conclusion
A classification procedure by conducting image segmentation, feature extraction and selection, and classification has been presented. The segmentation allows the elimination of a good amount of unwanted pixels and retained only those pixels characterized to have similar features to the astrocytoma. Although it seems like image segmentation already separate positive and negative Ki-67 cells, there might be artifact mixed with the segmented cells. Therefore, it is an added advantageous to utilise an intelligent classifier for better classification. The results proved that the proposed procedure yields good classification performance. MLP classifier provides slightly better classification performance than SVM with 99.6%, 99.4% and 99.7% for accuracy, sensitivity and specificity, respectively.

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