Computer Methods and Programs in Biomedicine (2005) **78**, 75—86

Computer Methods and Programs in Biomedicine

www.intl.elsevierhealth.com/journals/cmpb

Semi-automated image processing system for micro- to macro-scale analysis of immunohistopathology: application to ischemic brain tissue

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Received 11 April 2004; received in revised form 14 December 2004; accepted 30 December 2004

KEYWORDS
Immunohistology;
Classification;
K-means classifier;
Ischemic stroke

Summary Immunochemical staining techniques are commonly used to assess neuronal, astrocytic and microglial alterations in experimental neuroscience research, and in particular, are applied to tissues from animals subjected to ischemic stroke. Immunoreactivity of brain sections can be measured from digitized immunohistology slides so that quantitative assessment can be carried out by computer-assisted analysis. Conventional methods of analyzing immunohistology are based on image classification techniques applied to a specific anatomic location at high magnification. Such *micro-scale* localized image analysis limits one for further correlative studies with other imaging modalities on whole brain sections, which are of particular interest in experimental stroke research. This report presents a semi-automated image analysis method that performs convolution-based image classification on micro-scale images, extracts numerical data representing positive immunoreactivity from the processed micro-scale images and creates a corresponding quantitative *macro-scale* image. The present method utilizes several image-processing techniques to cope with variances in intensity distribution, as well as artifacts caused by light scattering or heterogeneity of antigen expression, which are commonly encountered in immunohistology. Micro-scale images are composed by a tiling function in a mosaic manner. Image classification is accomplished by the K-means clustering method at

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doi:10.1016/j.cmpb.2004.12.005

the relatively low-magnification micro-scale level in order to increase computation efficiency. The quantitative macro-scale image is suitable for correlative analysis with other imaging modalities. This method was applied to different immunostaining antibodies, such as endothelial barrier antigen (EBA), lectin, and glial fibrillary acidic protein (GFAP), on histology slides from animals subjected to middle cerebral artery occlusion by the intraluminal suture method. Reliability tests show that the results obtained from immunostained images at high magnification and relatively low magnification are virtually the same.
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1. Introduction

Immunohistochemistry is a technique for identifying cellular or tissue constituents (antigens) by means of antigen—antibody interactions. The specific antigen becomes visible upon binding to a detectable antibody. Immunostaining has been extensively employed to identify and reveal specific components in specimens and has been an established technique for pathological diagnosis for about 50 years [1]. This technology has also been widely applied to ischemic stroke research. To investigate the mechanisms of injury and to evaluate tissue alterations resulting from damage, healing, or the effects of therapeutic agents in cerebral ischemia studies, it is highly desirable to analyze accurately and quantitatively the immunoreactivities of antigens in different components of the central nervous system. In cerebral ischemia research, current immunohistology studies usually concentrate on micro-scale ($\times$10 or above) quantitative analysis of specified regions in brain tissues. Representative examples of these studies include mapping the topographical relationship between amyloid precursor protein (APP) accumulation and the region of infarction [2]; investigating compensatory and repair mechanisms in ischemia-damaged neurons after transient focal cerebral ischemia [3]; verifying endothelial barrier antigen (EBA) immunohistochemistry declines in zones of ischemic infarction [4]; and quantifying terminal deoxynucleotidyl transferase (TdT) dUPT nick end labelling (TUNEL) positive cells in the CA1 region of the hippocampus [5].

Computer-assisted image analysis has become a necessary tool for extracting quantitative information from histological images. Microscopic images of immunostained histopathology sections are usually obtained by a CCD-type camera that is connected to a computer-controlled image acquisition device. Quantitative analysis of such images aims at partitioning an image into homogeneous groups or regions—a typical application of *image classification*. Many algorithms and methods have been developed in this field [6—8]. Among them, two major approaches are usually applied, individually or in combination, to the analysis of immunostained histopathological specimens. There are a pixel intensity (color)-based approach [9] and a morphological information-based approach [10,11].

The aim of pixel intensity (color)- based image classification schemes is to find a discrimination point, i.e., a *threshold*, if the image is in gray scale; or a discrimination plane, if the image is in color. The criterion to classify an image is to assign pixels to the same class whose intensity values are above (or below) the threshold. If an image has only one threshold, it is also called the global threshold. The optimal threshold or the discrimination plane can be determined by the Bayes' classifier [6,7]. Nearly every image-processing software includes this method, for example, NIH Image (Research Services Branch, the National Institute of Mental Health, Bethesda, MD), Northern Eclipse (Empix Imaging Inc., Mississauga, Ont., Canada), Image-Pro Plus (Media Cybernetics Inc., Silver Spring, MD), Leica Image Processing and Analysis System (Leica, Cambridge, UK) and MCID (Imaging Research, St. Catherines, Ont., Canada). Applications of this method to immunohistology images have been reported [12]. Immunopositive objects, however, often exhibit inhomogeneity of intensity or color [13,14], which result from inconsistent staining processes or scattering of illuminating light. Moreover, immunopositive objects may have various shapes including unwanted artifacts. In addition to the threshold criterion, morphological information, such as object's diameter, perimeter and area, is also exploited as a second criterion to identify immunopositive objects or to identify and eliminate unwanted artifacts [15].

Most image classification applications to immunostained histopathological specimens are successful under high magnification ($\times$10 or above). The difficulty of identifying immunopositive objects (if they exist) is usually overcome by increasing the magnification of the microscopic image. Image acquisition under higher magnification results in a

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