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Applications of 'TissueQuant'– A color intensity quantification tool for medical research

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ABSTRACT

This paper demonstrates the use of TissueQuant – an image analysis tool for quantification of color intensities which was developed for use in medical research where the stained biological specimen such as tissue or antigen needs to be quantified. TissueQuant provides facilities for user interaction to choose and quantify the color of interest and its shades. Gaussian weighting functions are used to provide a color score which quantifies how close the shade is to the user specified reference color. We describe two studies in medical research which use TissueQuant for quantification. The first study evaluated the effect of petroleum-ether extract of *Cissus quadrangularis* (CQ) on osteoporotic rats. It was found that the analysis results correlated well with the manual evaluation, $p < 0.001$. The second study evaluated the nerve morphometry and it was found that the adipose and non adipose tissue content was maximum in radial nerve among the five nerves studied.

1. Introduction

Image analysis plays a very important role in medical research. It has become a powerful scientific tool and offers a whole range of measurements of optical parameters and color characteristics of objects. Not only does it provide a repeatable, accurate and objective evaluation but also makes it fast and convenient for analyzing large image datasets. The need to draw accurate and reproducible conclusions is a fundamental objective in histological and cytological diagnosis. Many different kinds of coloring agents called stains are used to obtain expression of components in a biological sample. Different components present in the biological sample express themselves by taking up different colors upon staining. The shade of color expressed by a particular component also depends on the concentration of the component present at that point. Manual evaluation of such samples introduces the inter-observer and intra-observer variations [1,2]. In the case of a large image dataset, automated analysis is highly desirable. If the algorithm can eliminate the cases which can be classified with high confidence and filter them out, only the remaining cases may be examined by a human expert, which decreases considerably, the tedious task of manual assessment. Such a decision support system can facilitate high throughput and at the same time maintain accurate, repeatable and objective assessment.

Many research groups have made efforts to apply digital image analysis for the quantification of a biological specimen for study of diseases like breast cancer [3–5], lung diseases [6], nephropathy [7], tuberculosis [8], cervical cancer [9],

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atheromatous lesions of human aorta and coronary arteries [10], skin lesions [11] and squamous cell carcinoma [12]. The digital image analysis has also been tried for quantification of stained specimens for medical research in many studies [13–16].

Researchers have approached the problem of staining intensity quantification in four ways. The first approach is evaluation by visual judgment [17] and manual point counting [18].

The second approach is to use general purpose image processing software like ImageJ, Image Pro Plus, Adobe Photoshop, etc. They wrote macros for the repeated execution of a set of commands. Prodanov et al. [19] performed granulometric analysis of synaptic boutons of cultured cells. In this study, the images were acquired on cyan and red channels. Image processing and measurements were performed using ImageJ. Identified boutons were counted and other parameters like area, planar coordinates and equivalent diameters were measured. Allen et al. [20] report development of customized method using Image Pro Plus software to quantify molecular stress in cetaceans. The protein under study was expressed as red pigment in the image. The region of interest was selected using free form drawing tool. A color file was generated which selected the hue–saturation–intensity components that represented the protein expression. All images were processed using the same setting and the color score was obtained as the product of the area and the mean optical density of red channel. Lehr et al. [5] report using Adobe Photoshop to quantify hormone receptors in breast cancer. Magic wand tool from the Select menu was used to choose the entire nucleus, followed by 'Similar' command to select all the stained nuclei. Subsequently, the image was converted to grayscale. Histogram tool from Image menu was used to generate a plot of optical density. Immunocytochemical index was calculated as difference between nuclear staining and background staining. Encarnacion et al. [7] report use of MetaView software for stain quantification. MetaView is a general purpose image processing software. Suitable threshold values for red (R), green (G) and blue (B) components are selected to choose the stained area. The amount of positively stained area gives the measure of the substance under study.

Another approach is to use specialized software like Ariol SL 50, BioQuant Nova Prime, IHCScore, etc. Ariol SL 50 is an automated microscope slide analysis tool, which acquires monochrome images through three bright field filters, uses cell masking templates and applies area analysis. Turashvili et al. [21] report using Ariol software for their study. They used color pick up tool to select membranes with weak positive staining and labeled "1+". Similarly, they selected membranes with strong positivity, labeled them "3+", Counterstained nuclei were also selected with the color pickup tool. Numeric values for colors of positive objects and negative objects, were stored in a color classifier file. These files were used for the automated analysis of the rest of the images. The use of Bioquant Nova for image analysis of immunohistochemically stained specimen has been reported [22]. Bioquant Nova is an advanced image analysis tool specifically designed for biomedical research. The user is required to trace the desired region of interest and threshold the positively stained area and then, the software automatically determines area and light density and calculates percentage coverage. Pauschinger et al. [16] and Soukupova and Albrechtova [23] make use of Lucia software for stain quantification. This makes use of the measure of optical density.

The fourth approach is to develop specialized software to solve the task at hand, based on LabView, MATLAB, etc. The study reported by Rexhepaj et al. [24] describes the development of image analysis algorithm to quantify immunohistochemically stained nuclear proteins. The algorithm was developed using MATLAB 7. The image is converted to CIE Luv color space and threshold value of the component is obtained to select the positively stained nuclei. RGB to HSV transformation was performed to separate the connective tissue. Hu et al. [25] developed image analysis system using LabView based routines. Karaçal et al. [26] report the development of an algorithm in which all the image pixels were converted from RGB to CIE L*a*b* color space. A k-means unsupervised clustering ($k = 2$) was applied on the set of staining ratios from the reference image to identify the positively and negatively stained.

It can be observed that the above mentioned studies attempt to quantify the substance under study, but do not take into account the possibility of the substance being present in varying concentrations. To overcome this problem, we propose to develop an algorithm such that the different shades of a particular color representing positive staining, are assigned different scores. In this paper, we discuss two cases of study in the area of medical research, which requires this kind of staining quantification namely assessment of antiosteoporotic activity and nerve morphometry.

1.1. *Assessment of antiosteoporotic activity*

Osteoporosis is considered an important health problem which is characterized by deteriorating bone structures leading to fragility fractures [27]. At present, the most effective approach to prevent osteoporosis is the hormone replacement therapy. Estrogen, calcitonin, bisphosphonates, ipriflavone, calcium products and anabolic steroids are being used clinically as effective medications [28]. However, these medications are associated with numerous side-effects [29]. Hence there are many attempts still being vigorously pursued to identify new inhibitors of bone resorption that minimizes the necessity for drug therapy. Therefore there is a need to search the natural compounds for the treatment of postmenopausal symptoms with no toxic effects. This study evaluates the effect of petroleum-ether extract of *Cissus quadrangularis* Linn. (CQ) which is a plant used in folk medicine, on osteoporotic Wistar rats.

1.2. *Nerve morphometry*

Axonal regeneration after an injury depends upon the amount of intervening perineurial tissue [30–32]. Poor prognosis of nerve repair in elderly patients may be due to changes in internal structure of the nerve with age. Histological findings of the nonfascicular area can be used as an indicator for the prognosis of nerve repair. We apply the image analysis tool to evaluate the amount of adipose and non adipose tissues in five different nerves namely lateral antebrachial cutaneous