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Proceedings

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Analysis of Color Standardization Methods for the Automatic Quantification of IHC Stain in Breast TMA

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Introduction/ Background

IHC biomarkers in breast TMA samples are used daily in pathology departments. This generates large amounts of information, which requires careful analysis [1]. Automatic methods to evaluate positive staining have been investigated since they may save time and reduce errors in the diagnosis that are due to subjective evaluation.

Aims

The aim of this work is to develop a density tool able to automatically quantify the positive brown IHC stain in breast TMA. One of the problems when dealing with stained samples is color variation and distortion [2]. This is due to several factors such as the fixation process, the amount of stain or the digitalization process. One solution to the color variation problem is to apply standardization of reagents and procedures in histological practice. However, stains fade over time and therefore, it is not possible to achieve complete standardization with the current technology. In this paper, different methods for stain normalization have been analyzed and compared in density quantification.

Methods

The methods implemented for stain normalization are based on the use of color distribution by means of dominant color descriptor, scalable and color structure descriptor. These algorithms adjust the color values of an image on a pixel-by-pixel basis so as to match the color distribution of the source image to that of a target image. Then, two main processes were performed to estimate TMA density: a) evaluation of total cylinder area and b) quantification of IHC stained area. For the 1st process, the algorithm distinguishes between normal, broken or distorted cylinders. The 2nd process deals with the evaluation of the positive brown pixels inside the cylinder. The segmentation is based on Lab thersholding together with binary thresholding applied to the H, S and B channels of the HSV and RGB color models. Finally, the tool segments all the positive areas and quantifies the brown density areas.

Results

A dataset of 879 TMA images were used to evaluate the methods. TMAs were prepared with an automatic tissue arrayer (Arraymold tool) composed of 50 holes/TMA with a cylinder diameter of 2mm. Slides were stained using different IHC stains, that is, CD1A, CD4, CD8, CD21, CD57, CD68, CD83, CD123, CK19, OXP3, LAMp3 and S100. The acquisition of the digital TMA images was done with Aperio ScanScope XT scanner at 40x (0.25 µm/ pixel). Afterwards, each cylinder image was individually extracted [3]. The use of color standardization makes the segmentation robust and free of parameter setting.

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Furthermore, the standardization process is able to reduce noise and facilitate the density quantification. The results were compared to manual density quantification by expert pathologists. The tests carried out provided up to 98% agreement when color standardization was applied against 90% without color standardization. The biggest error comes from FOXP3 samples.

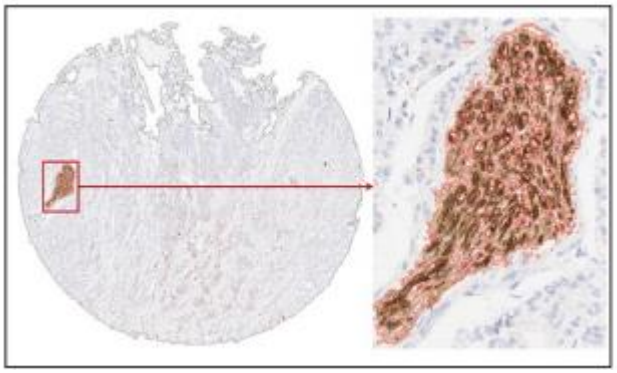

Figure 1: Final result the positive IHC stain areas in red.

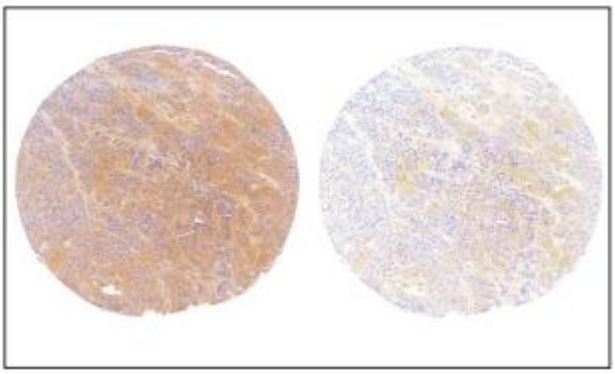

Figure 2: Result of the colour image standardization.

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