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STUDY OF IMMUNOHISTOCHEMISTRY OF BREAST CANCER

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ABSTRACT

The quantification of biomarkers on immunohistochemistry breast cancer images is essential for defining appropriate therapy for breast cancer patients as well as for extracting relevant information on disease prognosis. This is an arduous and time-consuming task that may introduce a bias in the results due to intra- and inter-observer variability which could be alleviated by making use of automatic quantification tools. However, this is not a simple processing task given the heterogeneity of breast tumors that results in non-uniformly distributed tumor cells exhibiting different staining colors and intensity, size, shape, and texture, of the nucleus, cytoplasm and membrane.

In this research work, we demonstrate the feasibility of using a deep learning-based instance segmentation architecture for the automatic quantification of both nuclear and membrane biomarkers applied to IHC-stained slides. We have solved the cumbersome task of training set generation with the design and implementation of a web platform, which has served as a hub for communication and feedback between researchers and pathologists as well as a system for the validation of the automatic image processing models. Through this tool, we have collected

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https://jmhsr.com/index.php/jmhsr

annotations over samples of HE, ER and Ki-67 (nuclear biomarkers) and HER2 (membrane biomarker) IHC-stained images. Using the same deep learning network architecture, we have trained two models, so-called nuclei- and membrane-aware segmentation models, which, once successfully validated, have revealed to be a promising method to segment nuclei instances in IHC-stained images. The quantification method proposed in this work has been integrated into the developed web platform and is currently being used as a decision-support tool by pathologists.

KEYWORDS: Breast cancer, IHC Quantification, Instance Segmentation, Deep learning, Biomarkers

INTRODUCTION

Breast carcinoma is one of the most common malignancies with the highest mortality rate among women in industrialized countries (Ferlay et al., 2019). Due to the aggressive behaviour of some subtypes and given that the breast is an accessible organ for early diagnosis, breast cancer is a permanent object of study concerning diagnostic methods and treatment. To determine the diagnosis of the disease in breast cancer, some classical clinic pathological features derived from the histological analysis of primary breast cancer samples are used. These features include, among others, tumor size, histological type of tumor, cellular and nuclear pleomorphic, mitotic index and presence of necrosis or vascular inva- sion. However, these parameters on their own are not sufficient to determine a precise prognosis and predictive factors of this complex disease (Zaha, 2014). For this reason, several ancillary techniques, including immunohistochemistry (IHC) and molecular studies, are often used to guide treatment decisions, classify breast cancer into biologically distinct subtypes with different behaviors, and ultimately, serve as prognostic and predictive indicators.

IHC is a general term that covers many methods used to determine tissue constituents (the antigens) with the employment of specific antibodies that can be visualized through staining (De Matos et al., 2010). The detection of antigen-antibody interaction under an optical microscope can be achieved by labeling the antibody with a visual sub- stance, which is combined with a fluorescent or, more frequently, chromogen label, and then performing colorimetric evaluation.

Different pathology guidelines (Duffy et al., 2017; Calvo et al., 2018; Burstein et al., 2019) recommend deter- mining in all cases of breast cancer, in addition to histologic grade, several tumor IHC biomarkers. Particularly, to evaluate the prognosis and establish therapeutic options, the updated guidelines from the European Group on Tumor Markers (Duffy et al., 2017) specify as

Journal of Medical & Health Sciences Review
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https://jmhsr.com/index.php/jmhsr

mandatory the measurement of estrogen (ER)-alpha receptors, progesterone (PR) receptors and human epidermal growth factor receptor 2 (HER2) for all patients with invasive breast cancer, as well as the quantification of proliferation marker Ki-67 for determining prognosis, especially if values are low or high. In short, ER, PR and Ki-67 are nuclear immunohistochemically markers with varying grouping complexity and their quantification requires counting the number of immunonegative (blue stain due to hematoxylin counterstain) and immunopositive (brown stain in bright field microscopy) tumor cells in given regions (Lo´ pez et al., 2008). On the other hand, the criteria for assessing the status of HER2 are based on the intensity and completeness of cell membrane immunostaining and the percentage of membrane-positive cells (Qaiser et al., 2018).

Despite the importance of an accurate evaluation of these biomarkers, their quantification depends on the sub- jective evaluation of staining color and intensity by a trained pathologist. This quantification or scoring is a time- consuming process in which errors are introduced due to intra-observer (variations in a single observer's interpretation of results) and inter-observer (subjective differences in interpretation between observers) variations (Kirkegaard et al., 2006). The quantification process usually involves the selection of hot-spot areas or regions of interest. Then, the staining of hundreds of cells that appear within the selected areas must be visually evaluated to score the IHC-stained preparation, which is cumbersome and error-prone. The subjectivity involved in these two steps makes the inter- and intra-observer variations of the scoring process not negligible, as has been already demonstrated (Leung et al., 2019).

Recently, huge advances in image acquisition devices have enabled histology technicians to scan conventional glass slides to produce high-quality digital slides, also known as whole slide images (WSI). This leads to pathologists moving from viewing glass slides in the microscope to navigating in a digital virtual slide similarly to how one can do in Google Maps (Zarella et al., 2019). It brings many new opportunities that cannot be achieved with traditional microscopes, including digital collaboration, working from remote sites, integration with electronic workflows and, what is relevant in connection with this work, the application of computer-aided diagnosis/prognosis (CAD) support tools based on artificial intelligence computing methods (Farahani et al., 2015). CAD tools are essential in the exten- sion and establishment of digital pathology, given the urgent need to develop systems that support pathologists in their routine tasks, alleviating their workload and addressing issues related to the low reproducibility of diagnostic results.

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
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https://jmhsr.com/index.php/jmhsr

Regarding the automatic scoring of IHC-stained images through automatic methods, there are a variety of com- metrical software that include tools designed for quantitative image analysis. Some examples are ACIS (ChromaVision Medical Systems, Inc., San Juan Capistrano, CA, USA), AQUA (HistoRx, New Haven, CT, USA), Ariol SL-50 (Ap- plied Imaging, San Jose, CA, USA), BLISS and IHC score (Bacus Laboratories, Inc, Lombard, IL, USA), iVision and GenoMx (BioGenex, San Ramon, CA, USA), LSC Laser Scanning Cytometer (CompuCyte, Cambridge, MA, USA), ScanScope (Aperio Technologies, Inc., Vista, CA, USA), SlidePath's Tissue Image Analysis (Leica Biosys- tems, Wetzlar, Germany) and Virtuoso (Ventana Medical Systems, Tucson, AZ, USA) (Rojo et al., 2009). Several of these commercial applications have demonstrated more reproducible and uniform results than manual evaluation and have received approval for diagnostic use by the FDA (US Food and Drug Administration) and CE-Mark for In-Vitro Diagnosis (Garcia-Rojo et al., 2019). However, the majority of the mentioned software relies on conventional image processing techniques based on the detection of hue, saturation and brightness levels (Chlipala et al., 2020), and some even implying the need for the pathologist to establish thresholds prior to processing.

Likewise, multiple IHC quantification works based on conventional computer vision techniques, such as the implementation of morphological transformation schemes (Huang & Lai, 2010), modified watershed algorithms, (Shu et al., 2013; Akakin et al., 2012), local thresholding (CLT) method (Shu et al., 2020) and the spatial color algorithm (SCA) prior to thresholding (Bar- ricelli et al., 2019) for nucleus region detection, can be found in the literature, exhibiting similar limitations. In this regard, new methods for the accurate quantification of IHC-stained images require, on the one hand, to be robust to non-uniformities that may appear between WSI, such as different staining intensity between different labs, background staining, tissue folding, etc. On the other hand, the new approaches need to take into account contextual information, i.e., not only distinguishing pixels according to hue, saturation and brightness levels, but also considering whether the pixels are part of tumor/non tumor cells, artifacts or other structures that should be ignored in the quantification process. This involves the application of techniques capable of abstracting information in a more complex way, and this is where machine learning can offer its great potential.

As evidence thereof, the last decade has seen an increase in research into machine learning techniques applied to the quantification of digital breast cancer immunohistochemistry images

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analysis (Irshad et al., 2013). Generally speaking, these machine learning algorithms can be classified into hand-crafted and non-hand-crafted algorithms (Badejo et al., 2018). The former comprised those methods in which a specialist manually decides which image features are relevant to solve the processing tasks involved in the automatic quantification process. Examples of hand-crafted algorithms applied to IHC images rely on K-means clustering (Al-Lahham et al., 2012), support vector machines (SVM) (Chen et al., 2019a; Markiewicz et al., 2009), and online sparse dictionary learning methods (Xing et al., 2013). The latter, of which the most representative are the deep-learning techniques, learn these characteristics from the data automatically and efficiently, revealing a greater capacity for generalization. First attempts to use deep learning for the quantification of nuclear biomarkers (Saha et al., 2017; Sheikhzadeh et al., 2018; Narayanan et al., 2018), specifically Ki-67, and membrane biomarkers (Vandenberghe et al., 2017), addressed the problem in two steps, first extracting small patches in which the different nuclei appear centered in the image, and then classifying these into immunopositive or immunonegative cells through a deep learning model, which entails a high computational cost of classifying each nucleus independently and the inability to distinguish between tumor and non-tumor cells. Xue et al. (2016) employed a deep learning model to analyze the cell counting task as a regression problem (instead of segmentation and post-counting problem) by generating spatial density prediction maps. Later, several works presented a modified U-Net (Ronneberger et al., 2015) deep learning model for the segmentation of nuclei from bigger patches in nuclear IHC images (Zhang et al., 2020b) and the segmentation of cell membrane immunostaining in HER2 IHC images (Khameneh et al., 2019), avoiding the prerequisite of segmenting isolated cells. The results are more robust and computationally efficient than in previous works, but by solving the problem through a semantic segmentation, the algorithm has limitations in separating the grouped cells. Emerging from the work reviewed, several obstacles to the development of advanced image processing techniques to address detailed marker quantification at IHC images can be identified. The main concern is related to the lack of labeled and publicly available data sets. Labeling a 1000 × 1000pixel size region of interest in an ×40 magnification image may involve the manual annotation of up to hundreds of cells. It is easy to understand the scarcity of databases containing this type of data, given the great effort required to generate them. Moreover, the patterns that can appear on the images can vary greatly depending on the type of cancer and its location, leading to a need for extensive train- ing sets for the training of algorithms, further aggravating the aforementioned problem. In addition, the most

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https://jmhsr.com/index.php/jmhsr

recent described techniques treat the problem of cell segmentation as a problem of semantic segmentation. Semantic segmentation treats multiple objects of the same class as a single entity. However, instance segmentation treats multiple objects of the same class as distinct individual objects (or instances), which is ideal to separate immunopositive or immunonegative cells in cluttered areas.

As far as we know, this work presents the first method for precise and automated quantification of nuclear (ER, PR, Ki-67) and membrane (HER2) biomarkers using the same deep learning model structure that deals with instance segmentation of cells, where cells/nuclei of the same immunotype, although clustered, are unequivocally differentiated.

The major contributions of this paper include:

• Creation of training and test image datasets. We have developed a web-based platform, including a WSI viewer and annotation tool that allows pathology specialists to annotate IHC images, establishing in this way a method-

ology to extract expert knowledge in the form of training data sets, to alleviate the tedious work of manual image annotation and to validate the development of new image processing methods.

• The design of an expert system for the accurate automatic quantification of digital breast cancer immunohistochemistry images through a computationally efficient and robust deep-learning based instance segmentation method capable of tackling the presence of clustered or overlapping cells as well as the presence of stromal cells and lymphocytes which are not subject to counting.

The reminder of this article is organized as follows. First, Section 2 introduces the description of the training and test data sets used in this work, as well as the annotation and decision support tools developed to create the above data sets. Then, the deep learning-based instance segmentation model used for the quantification of IHC images is described. Afterwards, Section 3 presents the experimental results of applying the instance segmentation method to nuclear and membrane IHC-stained images. Finally, Section 4 summarizes the limitations of the research, some concluding observations and future research directions.

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VOL-2, ISSUE-1, 2025
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