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Western Blot Pattern Classification Using Convolutional Neural Networks for Breast Cancer Diagnosis

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Abstract. In Mexico, breast cancer is the leading cause of women's death. This work aims to discriminate between healthy and breast cancer patients based on the band patterns obtained by western blotting using deep learning techniques. This work proposes Convolutional Neural Networks (CNN) to classify breast cancer. CNN reaches 68.24% of the classification rate in three classes (healthy, benign breast pathology, breast cancer) and 81.43% in two class labels (healthy, breast cancer).

Keywords: Breast cancer, convolutional neural networks, western blot, Fourier transform.

1 Introduction

Breast cancer has become a global health problem since it represents the first place in incidences and the fifth place in cancer mortality worldwide [1]. In Mexico, breast cancer is the leading cause of death in women between the ages of 30 and 54, surpassing cervical cancer since 2006, becoming a public health problem and a severe challenge for the health system [2].

Several methods complementing each other as a whole are proposed for its diagnosis. These methods include a clinical breast examination, ultrasound, mammography, and biopsy. However, these methods are ineffective in the early cancer detection, since they aim is to identify the disease. Moreover they are invasive, subjective, expensive, and in sometimes painful [3-4].

In contrast to the traditional methods for breast cancer diagnosis, some other techniques detect tumor particles before the disease develops. In other words, these

Fig. 1. Example of an image containing 15 to 17 nitrocellulose membrane strips obtained from the Western Blot method for specific protein antigens (T47D) in each patient's serum sample.

methods identify the autoantibodies dedicated to recognizing tumor proteins present up to 4 years before the disease detection [5].

For example, Desmetz et al. [6] discriminate accurately between healthy patients and those with early-stage breast cancer, especially carcinoma in situ, by evaluating the autoantibody response to a set of tumor-associated antigens. The result obtained from this work could help in the early detection of breast cancer, especially in women under 50.

Similarly, Romo-González et al. [7] describe a method that corroborates the presence of autoantibodies against tumor cells of the T47D cell line (ductal carcinoma of the breast), allowing distinguishing women with and without breast pathology. In this work, the bands' analysis expressed in the one-dimensional Western Blot images in which the autoantibodies react of the T47D tumor line antigens.

Although the results are promising, the image analysis is very complex, subjective, and time-consuming, taking up to a month to create the binary database. It is because image analysis requires the expert to align the bands of each patient's strips with the Quantity One software from Bio-Rad Laboratories (Fig. 1). As a result, the final bands' identification and their position depend on the expert eye.

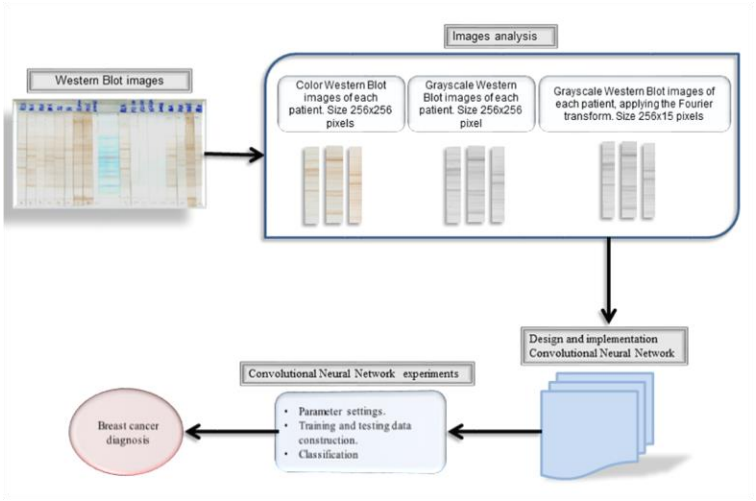

Fig. 2. Proposed Methodology.

A semi-automated protein band analysis system was designed to avoid subjectivity and delays due to image analysis, classifying band patterns by time series [8]. The time series data corresponds to the band's pixel shade variation.

Since time series were different lengths, they were adjusted to the same length with a geometric scaling transformation. Afterward, the K-nearest neighbor algorithm with Euclidean, Mahalanobis, and Correlation similarity distances was used for classifying time series. This method reaches a classification rate of 65.40% with three classes (healthy, benign breast pathology, breast cancer) and 86.06% with two class labels (healthy, breast cancer).

Although the classification rate achieved was high and similar to the expert, the method is considered semi-automatic since an area is subjectively chosen in each strip for the band analysis, resulting in a variation of the time series length.

For this reason, in the present work, we proposed to discriminate between healthy patients, patients with benign breast pathology, and patients with cancer using the bands of Western blot images of antigen-reactive antibodies (tumor line T47D - ductal carcinoma) and convolutional neural networks.

Our primary objective is to reach a classification rate of 84% at least, avoiding subjectivity and analyzing images directly instead of extracting time series from selected areas [8].

2 Methodology

Figure 2 shows the proposed methodology. The employed database contains 149 nitrocellulose membrane strips images with band expression obtained from the Western Blot of autoantibody binding to specific protein antigens (T47D), of which 50 correspond to patients with breast cancer, 50 with benign breast pathology, and 49 to healthy patients.

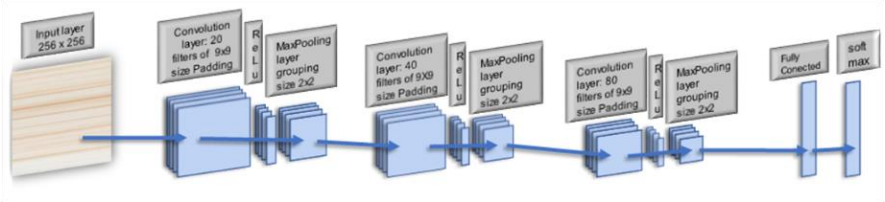

Fig. 3. Convolutional Neural Network Architecture.

These images were provided by the Biology and Integral Health Area of Instituto de Investigaciones Biologicas of the Universidad Veracruzana, following the ethical norms and with the corresponding informed consent of the participants. Furthermore, the protocol was reviewed and approved by the Research Ethics Committee of the Hospital General de México "Dr. Eduardo Liceaga" (DI/12/111/03/064).

Finally, it is essential to mention that this study conforms to the Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (July 18, 1964). The images have been used in 3 ways: 1) Color, with dimensions of 256x256 pixels, 2) Grayscale, with dimensions of 256x256 pixels, 3) Grayscale applying the Fourier transform, with dimensions of 256x15 pixels.

To achieve the classification of western blot bands in healthy patients and cancer patients, a Convolutional Neural Network was trained, which was designed by iteratively and manually adjusting the number and type of hidden layers as well as the parameters of each one of them. The architecture had the following features (Fig. 3):

- Input layer: 256 x 256.
- Convolution layer: 20 filters of 9x9 size Padding.
- Normalization Layer / ReLu Layer.
- maxPooling layer - grouping size 2x2.
- Convolution layer: 40 filters of 9X9 size Padding..
- Normalization Layer / ReLu Layer.
- maxPooling layer - grouping size 2x2.
- Convolution layer: 80 filters of 9x9 size Padding.
- Normalization Layer / ReLu Layer.
- Fully connected layer.
- Classification layer with softmax method for values normalization.

Once the network architecture is defined, the database is divided into the training set (70% of the images) and the test set (30% rest) for the classification task.

Table 1. Experiment 1: Classification with Convolutional Neural Networks with three classes.

Test	Epochs	Kernel	Classification rate	Std dev (-+)	P<0.05 Significant differences
Sánchez-Silva, Acosta-Mesa, & Romo-González, 2018	N/A	N/A	65.40%	N/A	N/A
Color images	20	9	68.24%	62.67% - 73.77%	0.26[1]
Grayscale images.	20	9	66.44%	63.78% - 69.11%	0.03754[1]
Grayscale images applying Fourier Transform	70	3	61.55%	54.76% - 68.34%	0.5964

3 Experiments and Results

Our proposal was programmed and executed in Matlab software. The results described in this section were designed according to two experiments explained below.

Experiment 1. All images were considered with the three classes of the database: healthy patients, patients with benign pathology, and patients with cancer. In the case of the convolution kernels, the kernel sizes used were 3, 7, and 9 coefficients.

As far as the type of data is concerned, three variants were employed:Color images, 70 epochs were tested:

- Color images and 20 epochs were tested.
- Grayscale images and 20 epochs were tested.
- Grayscale images applying Fourier transform, 70 epochs were tested.

Experiment 2. 99 images were used, representing two classes in the database corresponding to healthy and cancer patients. In the case of convolution kernels, the kernel sizes used were 3, 7, and 9 coefficients. As far as the type of data is concerned, three variants were used:

- Color images and 20 epochs were tested.
- Grayscale images and 20 epochs were tested.
- Grayscale images applying Fourier transform, 70 epochs were tested.

[1] Significance values were evaluated using non-parametric techniques.

Table 2. Experiment 2: Classification with Convolutional Neural Networks with two classes.

Test	Epochs	Kernel	Classification rate	Std. dev (-+)	P<0.05 Significant differences
Sánchez-Silva, Acosta-Mesa, & Romo-González, 2018	N/A	N/A	86.06%	N/A	N/A
Color images	20	3	81.99%	77.50% - 86.96%	0.2223[2]
Grayscale images.	20	7	82.33%	74.95% - 89.71%	0.5097[2]
Grayscale images applying Fourier Transform	70	3	86.00%	81.90% - 90.09%	0.351

When the test data satisfied the assumption of normality, Analysis of Variance (ANOVA) was used to evaluate significant differences between more than two groups.

Otherwise, the non-parametric Kruskal-Wallis test was used. Furthermore, the t-student test was used to assess the significant differences between two groups.

On the other hand, the Mann-Whitney test was employed in case the data did not satisfy the normality assumption. If $p<0.05$, the data had significant differences. Both experiments were run ten times, calculating the average, standard deviation, and evaluation of significant differences in the classification percentage obtained in each run. The results are shown in Table 1 and Table 2.

Figure 4 and 5 show the better confusion matrix for both experiments, these allows visualization of the performance of the proposal model. Each row of the matrix represents the instance in the actual class and each column represents the instance in a predicted class.

With this matrix is possible calculate the false negatives, false positives, true negative and true positives values. This allows more detailed analysis than simply observing the proportion of correct classifications (accuracy).

4 Discussion

As show in the tables, we have the comparative results obtained by testing the convolutional neural network with proposed parameters, color spaces in the image, and applying the Fourier transform in the grayscale images.

The best classification rate for the three classes (healthy, benign breast pathology, and breast cancer) was 68.24% in color images (62.67% - 73.77%) and a $p=0.26$ significant difference.

The best classification rate for two classes (healthy, breast cancer) was 86.00% in grayscale images applying Fourier transform (81.90% - 90.09%) with a $p=0.351$ significant difference. For the experiments conducted, we could conclude that exhaustive processing that uses a lot of time and resources is unnecessary, since from

[2] Significance values were evaluated using non-parametric techniques.

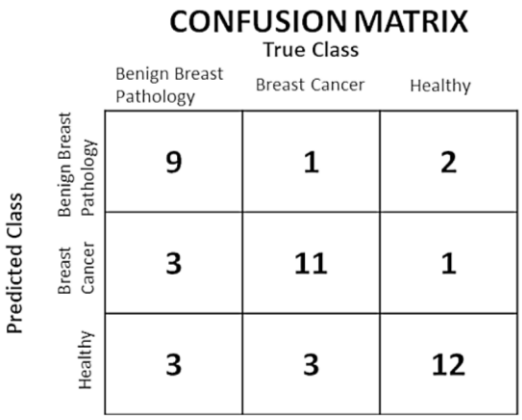

Fig. 4. Confusion Matrix of the Convolutional Neural Networks with three classes.

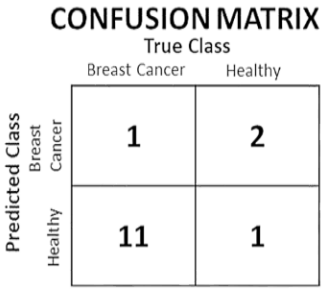

Fig. 5. Confusion Matrix of the Convolutional Neural Networks with two classes.

epoch 20 with color and grayscale images, or in epoch 70 with images applying the Fourier transform, the classification rate remains constant.

Thus, we can conclude image processing performed with convolutional neural networks reduces time and subjectivity compared to those analyses a proteomics specialist would perform with these images.

Our proposal directly classifies the bands of Western blot images of antigen-reactive autoantibodies (tumor line T47D - ductal carcinoma) without a preprocessing stage (delimiting an area to obtain time series).

These results guide us to continue experimenting on how convolutional neural networks allow us to get a better classification rate. On the other hand, artificial intelligence is applied as a support tool to diagnose breast cancer before it manifests itself, leading to better prevention, diagnosis, and treatment of breast cancer.

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