SEMI AUTOMATED MITOSIS DETECTION IN HISTOPATHOLOGICAL IMAGES OF BREAST

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ABSTRACT: Confirmation of clinical breast cancer is done histopathologically on microscopic slides taking into consideration cell modifications, architecture modifications and mitotic cell index. This mitotic cells count (multiplying cells) is a separate index represented by counting the number of multiplying cells figures in at least 10 high power magnification microscopic fields (20x, 40x). It is a time consuming and demanding method, with low reproducibility, so it is suitable for an automated or semiautmated (computer aided) method. We have developed a method for mitosis figure identification using a detector based on a k-Nearest Neighbor algorithm applied on apixel-based feature detection. The results came with high sensibility, but with low specificity.
KEYWORDS: *mitosis detection, computer aided diagnosis, semi automated mitotic index.*

1. INTRODUCTION

Breast cancer is the second leading cause of cancer death for women ([R+10]). Its incidence increases substantially and continuously while the mortality rate remains high despite earlier detection and advances in therapeutic care.
Mitotic cell index (MI) is one of the three main aspects that are used for confirmation of clinical breast cancer. Confirmation is done by pathologists on microscopic slides (histological stained breast cancer tissue sections) or scanned slide images (digital image of the specimen). The other two aspects taking in consideration on diagnostics, beside MI, are cell and architecture (tissue) alterations.
Mitosis is the process by which a eukaryotic cell separates its chromosomes from the cell nucleus into two identical sets, in two separate nuclei www.en.wikipedia.org/wiki/Mitosis. It consists of several stages: prophase, metaphase, anaphase and telophase. All of the stages have distinct pattern characteristics, starting with nuclear membrane disintegration in late prophase and ending with two distinct nuclear figures in the thelopase. To make it even harder to summarize, atipic mitosis appear in malignant cells (larger, with more than two centroids).
The importance of the MI quantification is of a real interest in breast cancer ([DWB04]), but it is mentioned in cancers with other localization like prostate carcinomas ([D+11]) and esophagus carcinomas ([S+96]).
On the other hand taking into consideration the level of agreement (Choean's Kappa statistics, returning k, a 0 to 1 range number, where 0 means independent decision and 1 perfect agreement) between pathologists on the MI the mean interval for mitotic grade is 0.45 to 0.67 ([M+05]), considered to be moderate. This is a real problem itself.
As mentioned before ([DHC98]) the motivation for an automatic method for the MI is to minimize this time-consuming, repetitive and non-reproducible task ([M+05, H+10]) demanded of experienced pathologists.
Several automatic methods based on image analysis have been proposed. The majority of these methods employs cell segmentation, followed by statistical analysis ([S+12]).

2. OBJECTIVE

The main objective of our current work is to develop a detector with high sensitivity and acceptable specificity capable of finding mitotic figures in histopatological slide images. The result of its detection will be presented to the pathologist who will make the final decision.

3. MATERIALS AND METHODS

3.1 The Dataset

The slides are stained with hematoxylin and eosin (H&E). In each slide, the pathologists selected 10 high power fields (HPF) at 40X magnification with a size of 512 × 512 µm2 (that is an area of 0.262 mm2), which is the equivalent of a microscope field diameter of 0.58 mm. A detailed dataset description can be found at the dataset webpage: http://ipal.cnrs.fr/ICPR2012/?q=node/7

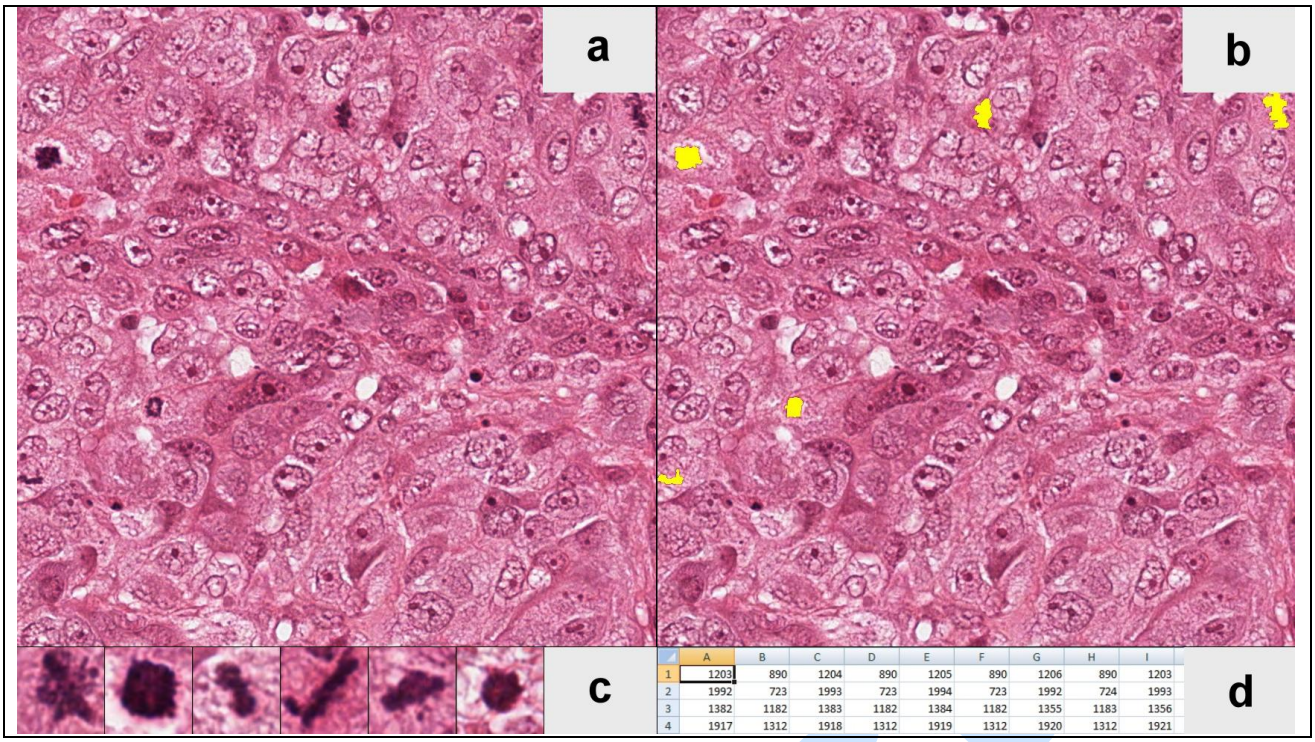

Fig. 1 The Dataset: a - original image; b - manually annotated image; c - mitotic figure examples; d - comma separated values of the mitotic cells position from the annotated image.

3.2 Dataset Observations

There are some aspects that can reduce computational work. Hematoxylin is a chemical that interacts with the nuclear components making them look dark blue on standard microscopes. In mitosis the nuclear chromatin is doubled (more), there are lots of proteins that are preparing the process (denser) and from some stage the nuclear membrane disappears making it look bigger an with irregular borders. Since pixel-based algorithms tend to make the best scores in nucleus and mitosis detection ([G+09]) computational work will be enormous if applied for each and every pixel in the image. So the search spaced must be reduced. We are looking for, relatively large, dark blue objects with irregular shape.

3.3 Search Dataspece Reduction

Having the manually annotated interest objects from the dataset we were able to calculate the histogram of the pixels composing the mitosis (Fig. 2) and compare it with the histogram of the rest of the images (Fig. 3).
Comparing the two histograms we can clearly see that the mitosis are darker – as we expected – (closer to zero, where zero is black and 255 is white) than the hole images. This means we are able to exclude as many pixels as possible by using a variable threshold set to eliminate pixels from the images, but to keep at least one pixel from each mitosis.

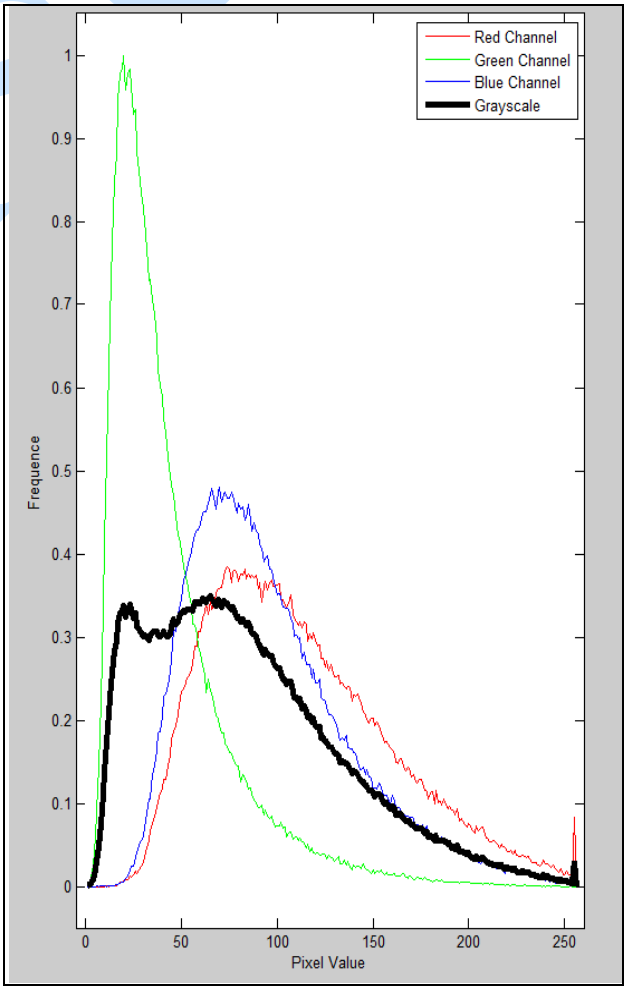

Fig. 2. All Mitosis Histogram: red, green, blue channels and grayscale.

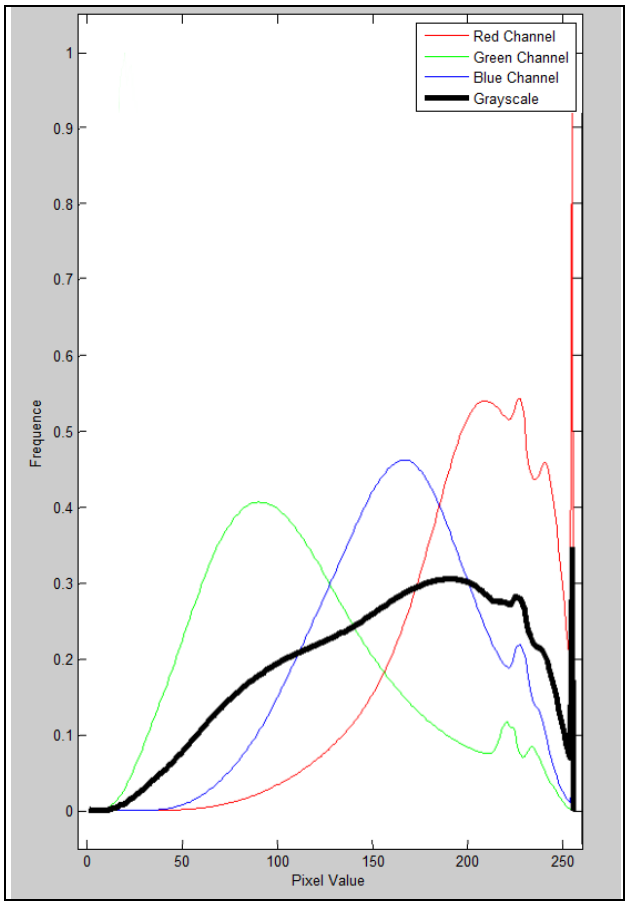

Fig. 3. All Images Histogram: red, green, blue channels and grayscale.

Searching for a good threshold we discovered that there is a specific grayscale intensity level from where all mitoses have at least 1 pixel selected (Fig. 4). This optimum value is grayscale intensity of 60. We can select any pixel after this intensity, but we considered the level of 65 should be taken in consideration in order to avoid any mitosis exclusion.

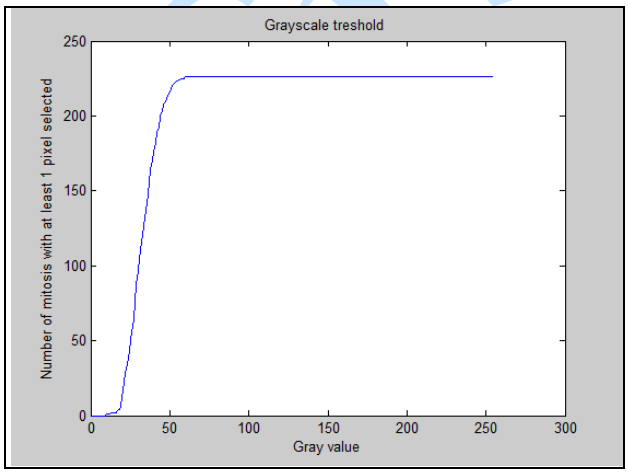

Fig. 4. Variable threshold on grayscale. The plot shows that for a threshold value smaller than 60 each mitosis has at least one pixel selected.

As a result of the thresholding the total number of pixels of interest was reduced to less than 1% (0.7622%). We can see that the threshold selected points within the mitosis (Pic. 5A), but also selected points outside the mitosis (Pic. 5B).

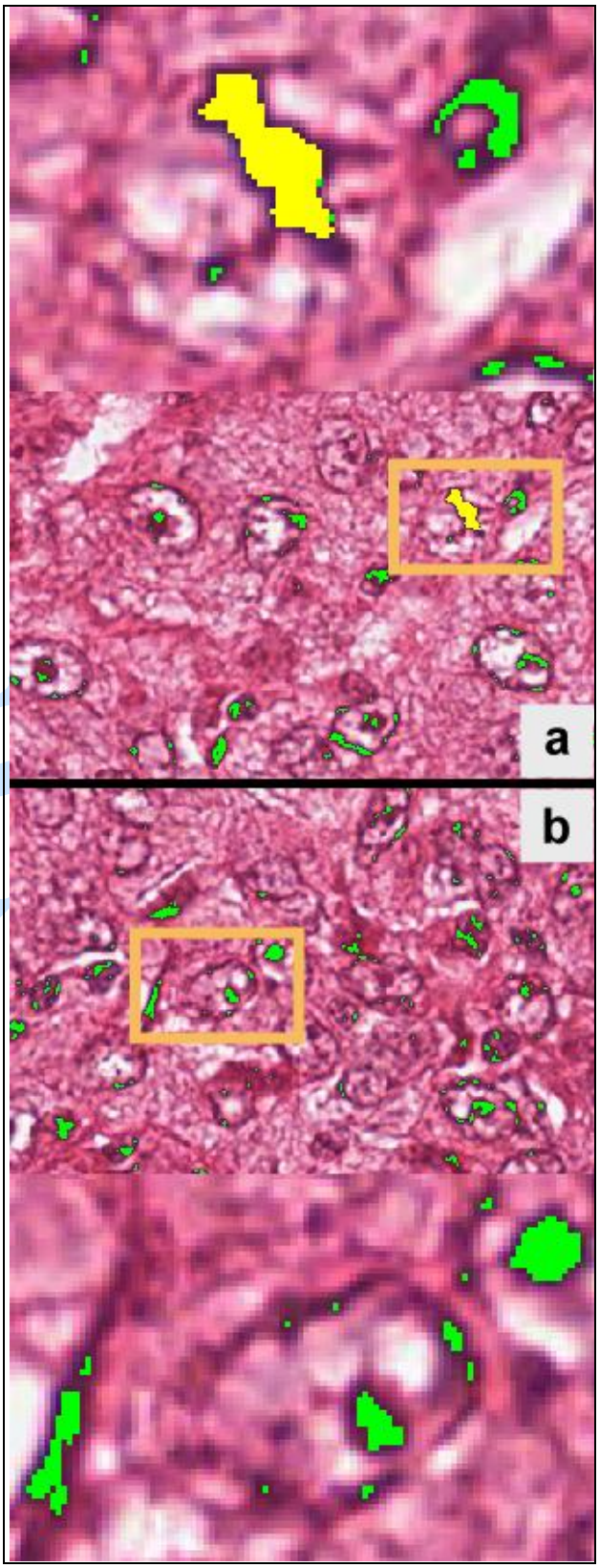

Fig. 5. Threshold selection – pixels witrh grayscale intensity below 65. a - mitosis pixel selection; b - non-mitosis pixel selection.

Each pixel group (possible mitosis) was represented by its centroid. Thus the search space was reduced further.

3.4 Data Selection

Since the largest mitosis was 60 pixels in length a radial (circular) image, centered in each centroid, with a radius of 30 was selected. Linking each pixel from the border with the center of this image, and selecting the intensities of the crossed-over pixels a new image was created, this time a square image (Fig. 6). These images have the advantage that they are square have the same size and if viewed as a repeatable part of a bigger image they are invariant to rotation.

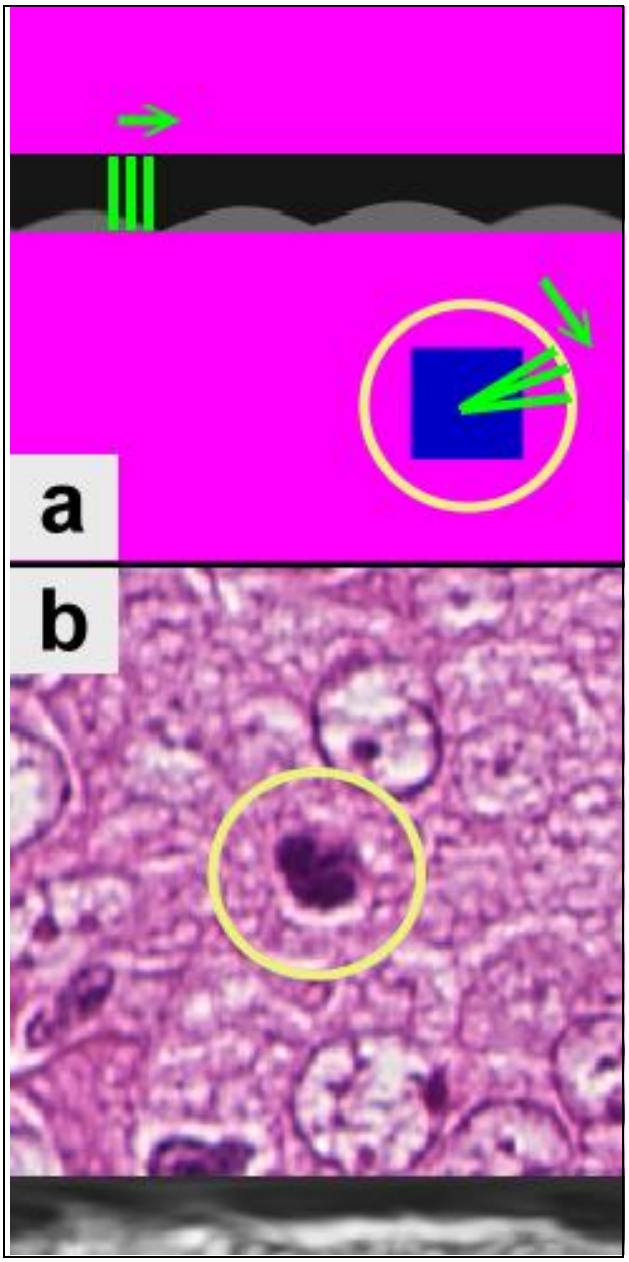

Fig. 6. Comparison Image Generation. A – theoretical aspects with square image result; B – real database example with te resulted image (on the bottom).

3.5 Mitosis Detector

For the final decision the detector took in consideration several texture features (Contrast, Correlation, Energy, Homogeneity, Entropy) described in ([Ser11]) for the images obtained in section 4.3 and the size of the object (number of pixels with intensity lower than 65). A k-Nearest Neighbor (k-NN) algorithm with the value of k (the number of neighbors to be considered) was used to compare each possible mitosis with the annotated mitosis figures from the dataset. The distance (Euclidian) for acceptance was set to 13% (several values have been tried, best result was selected) for the k-NN and for the size of the object the acceptance value was 100 pixels (the size of the smallest annotated dataset mitosis).

3.6 Pathologists Intervention

The marked possible mitoses, resulted from running the detector, have been presented to 2 Pathologists. Both labeled the each picture as mitosis or non-mitotic figure, independently.

4. RESULTS AND DISCUSSION

The overall detection rate was 100%, so the sensitivity of the method was 100%, but the specificity was about 10%. For each of the detected mitosis there were about 9 none-mitosis figures selected as mitosis (false positive) (Fig. 7).

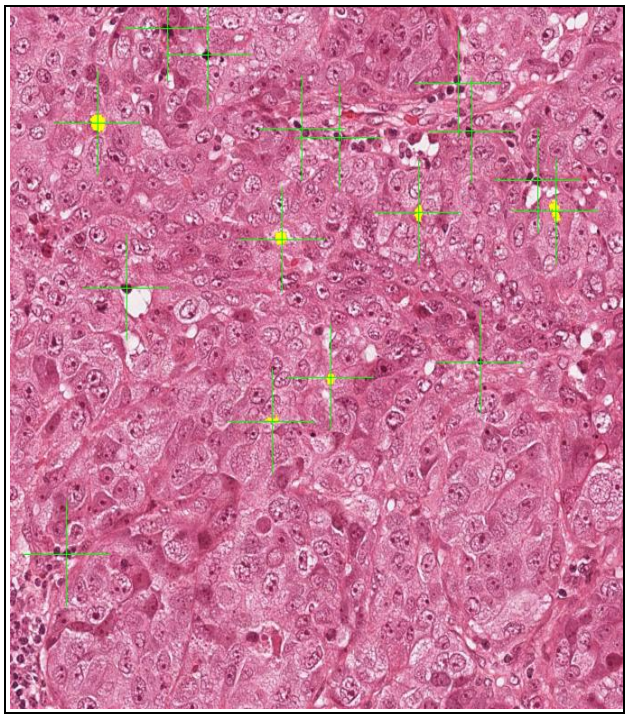

Fig. 6. Detection Result: all of the mitosis figures have been detected (also marked in yellow), but many non-mitotic figures were marked as mitosis.

Though all of the mitosis were correctly detected the two Pathologists excluded some figures, one of them marked 5 of them as non-mitotic figures and the other marked 7. More than that, both of them marked 3 non-mitosis figures (the same) as mitotic.
Though there were more than 2000 images to be labeled the two Pathologists agreed that this was easer work than searching for the mitosis themselves on each image.

5. CONCLUSIONS

We have obtain a semi-automatic mitosis detector capable of pointing out from a histopathlogical scanned image the mitotic figures with a sensibility of 100%, but with a specificity of only 10%.
Though the specificity was at very low rates the two Pathologists who verified the diagnostics agreed that the presented method was helpful and that it was less demanding than the conventional method.
From their false positive and false negative mitotic figures labeling we concluded that MI is a real problem with uncertain visual definition among pathologists.

6. ACKNOWLEDGMENTS

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