Object Localization Procedure for Intratumoral Microvessel Density Estimation

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Abstract: - The intratumoral *microvessel density* (MVD) estimation is a broad used procedure to quantify the appearance and growing of tumors in breast cancer. To determine this parameter, an intelligent system has to localize, as accurate as possible, all the microvessles from the image. After obtaining the optimum methodology for the segmentation of the breast cancer images [3], the next step, described in this paper, consists of: the enhancement of segmented image by linking the parts of the same objects, localize and determine some parameters of each object. The linking procedure is based on morphological operations. Some results and conclusions are presented in the last part of the paper.

Key-Words: - microvessel density (MVD), image enhancement, objects localization, morphological operations

1 Introduction

Even if in this moment the main implication of image analysis is only in assisting pathologists to achieve a difficult diagnosis by objective means [3], it is obviously an increasing trend of using these kind of applications in anatomic pathology field.

Angiogenesis represents the growth of the new tumors from preexisting capillaries and post capillary venules. Tumors depend on angiogenesis for their growth, progression and metastasis [4]. The higher the count of microvessel and larger the surface area of the vessels are, the greater is the probability that tumor cells will enter into circulation. In breast carcinoma, several studies have shown that the level of intratumoral *microvessel density* (MVD) is indeed a significant and independent prognostic-factor.

A quantitative histological technique to assess tumor neovascularization was suggested in 1972 by Bream. More recently, Weidner reported that the angiogenesis could be measured using immunohistochimical methods [7]. Several authors showed that angiogenesis could be quantified by counting microvessel that were highlighted by immunohistochemical staining and demonstrated a good correlation between MVD, distant metastasis and survival in node-negative and node-positive breast cancer patients [8].

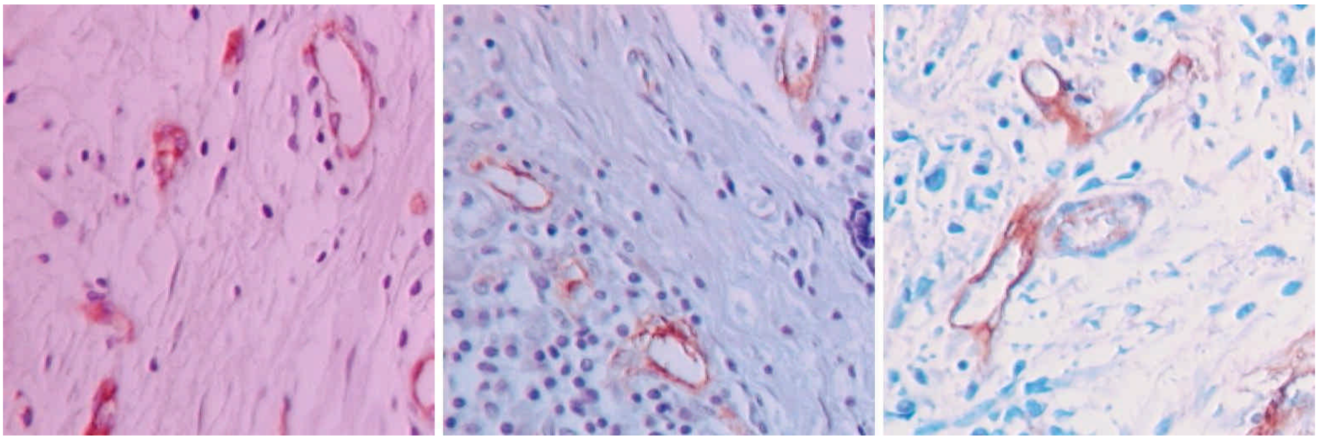


Fig. 1 Samples of breast cancer tissue microscope image

Up to now, the most common method used to evaluate angiogenesis in human breast cancer is based on the identification of intratumoral microvessels by specific markers of endothelial cells, immunohistochimical assays and evaluation of MVD in histological slides [8].
Taking into consideration the importance of these prognostic factors, we decided to implement a digital image processing system able to evaluate the *microvessel density* (MVD) in breast cancer
The first part of this system deals with the segmentation procedure of the images in two main parts: the microvessels (the objects of interest) and the background. We studied several segmentation procedures: based on hue-saturation transform, using ICA transform and classical level-slicing method. The conclusions obtained at the end of this study are:

- the hue-saturation based method gives the best results
- the quality of segmentation using ICA is a bit worse, but very closed to hue-saturation procedure
- the global ICA method is the faster (it needs only a scalar product)

So, even if the results are slightly under the optimum, because we have to process a great amount of data, our preferred method for segmentation step is that based on the global ICA technique.
In this paper we present the results of the second stage, consisting of: segmented image enhancement and estimating of objects' parameters.

2 Segmented Image Enhancement

The medical image database consisting of breast cancer tissue samples was built as follows:

- for each medical case, the pathologist located four areas of high neovasularization, since these areas are more likely to have biological significance;
- for each case, two different digital images with resolution of 1600x1200 pixels, true color (24 bits/pixel), were acquired using a microscope with a magnification of 200:1;

In figure 1 are presented 3 samples of tissue images. As it can bee seen also from this figure, the image samples are of different types, depending on a lot of factors, the most important of them being the microscope (generally speaking, video system) calibration.

Briefly explained, the segmentation procedure consists of 2 main steps:

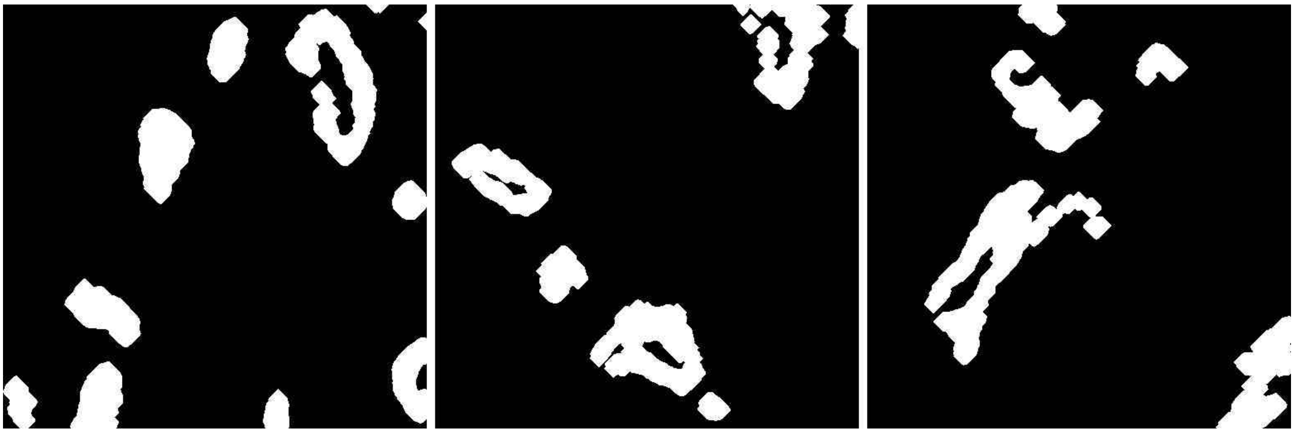
- A microvessel emphasized transform, used also to reduce the initial space dimension; Independent Component Analysis (ICA) techniques was applied to a set of different kind of images to obtained the *global transform*, which means a transform valid to all the images from the database.
- Segmented the second ICA source using an unsupervised approach of LVQ neural network.

In figure 2 are presented the results of segmentation of the samples form figure 1. The results are fairly good, but there are some objects that are split in several parts. This is a serious problem since our final task is to localize and to count the microvessels. It is obviously that without a linking operation, used to improve the quality of the segmented images, our system will give bad results.

To link the parts of the same object we used morphological techniques. We applied a modified *"closing"* operation [8]. The dilation was made in a classical way, using a 7x7 square structuring element. In order not to loose the connectivity of the objects obtained after the dilation, the erosion was replaced with a thinning procedure. This operation can be assimilated with an erosion operation with an adaptive-modified structuring element: when the pixels are in extreme positions or are connection pixels then the structuring element is adaptively changed. The results obtained using this procedure are fairly good, in figure 3 being presented some of their sample.

After passing the linking stage the object extraction was applied. For doing this, a neighbor-searching algorithm was used, its result being a set of coordinate pairs *(x, y)* for each object. The mean vector of each set of values represents the center of each object.
From the anatomic pathology point of view the orientation and a spreading measure of each microvessel is important to be known. For determining these parameters, the auto-covariance matrix for each object was computed. The eigenvectors of the auto-covariance matrix are the principal spreading directions of the microvessel shape and the associated eigenvalues represent a measure of spreading along these axes.

Fig. 2 Results of image segmentation using global ICA procedure



a) Dilation operation

b) Erosion-thinning operation

Fig. 3 *Close* operation used to link the parts of the same object

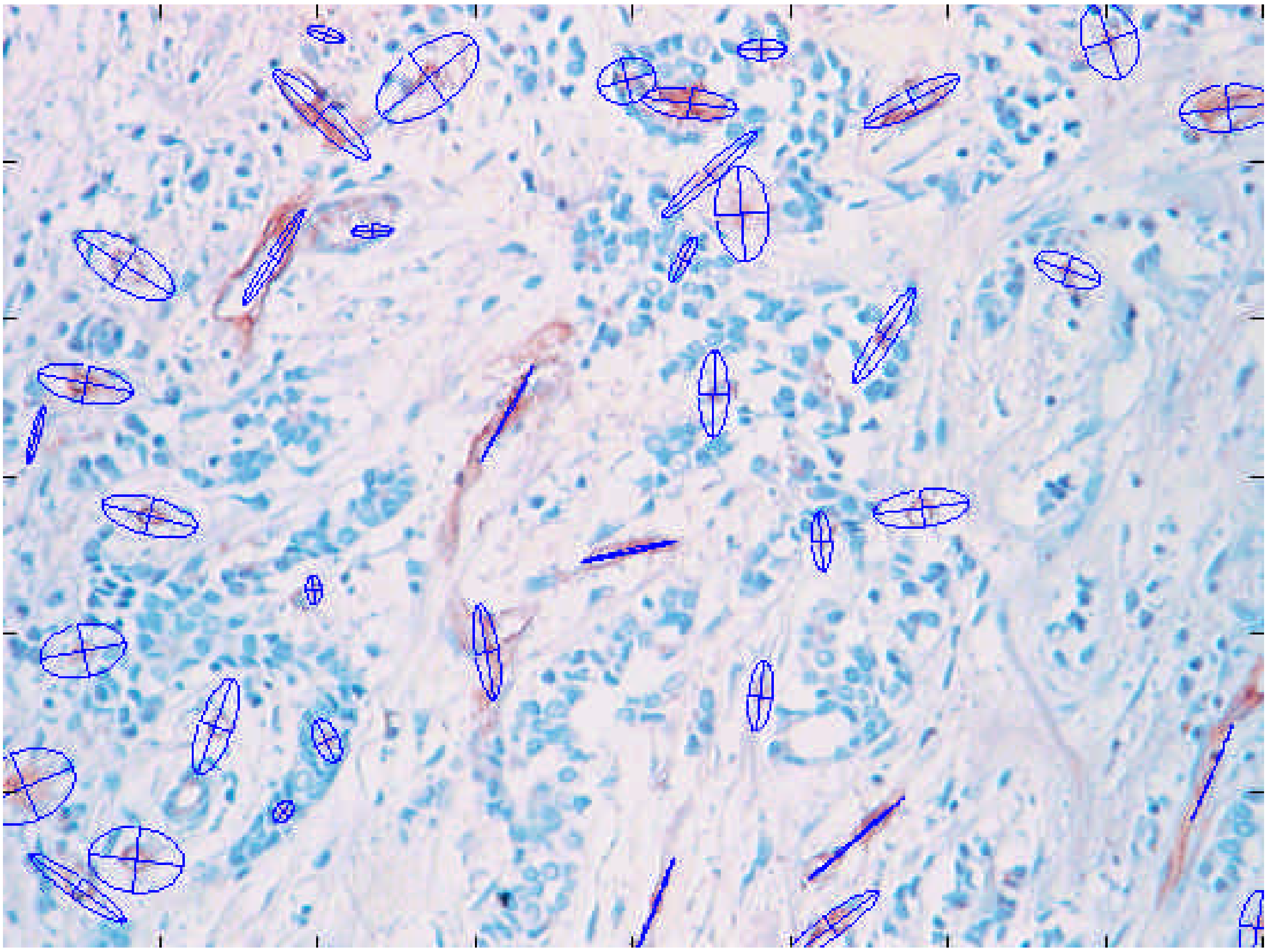


Fig. 4 Result of object localization;
the ellipses describe the orientation and the spreading of the microvessels

3 Results and Conclusions

An automatic system for microvessels density estimation was design. First, the segmentation step was studied choosing the optimum methodology based on a global ICA technique [3]. The second stage, the segmented image enhancement, the localization and the parameter evaluation were discussed in this paper. In figure 4 is presented the result given by the system for an entire 1200x1600 image. In the image the line crossing represents the center of the objects, the lines are the two principals orientation axis of the microvessels and the dimension of the ellipse is proportional with the spreading degree of objects. As it can be seen the result is good, the localization of the objects is correct, the correlation between the real objects and the detected ones is also good.

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