

Fractal Analysis of Microscopic Images of Breast Tissue

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Abstract: - This paper presents a new method for the detection of abnormalities in microscopic images of breast tissue. Breast cancer is the most common form of cancer among women. The diagnosis is confirmed by the subjective evaluation of tissue samples, that is usually done by the pathologist manually. An automatic system for the analysis of tissue samples would be of great help. The aim of this study was to investigate the feasibility of a new method for characterizing tissue images. The method is based on fractal geometry. Fractals are geometrical entities which are well suited to capture the complexity and the variability of biological structures. We estimated the fractal dimension using the euclidean distance mapping. Reported results show that the value of fractal dimension is a very good feature in separating normal from cancerous tissue images.

Key-Words: - Medical images, breast cancer, fractal analysis, quantitative pathology, classification

1 Introduction

Breast cancer is the most common form of cancer among women. The final diagnostic decision is based on the microscopic examination of tissue samples performed by the pathologist. This analysis is time consuming and requires the visual interpretation of complex images. Automation of this analysis would be of great help to the pathologists, as they have very many images to assess each day.

Considerable research has been dedicated to the problem of computer based quantitative analysis of malignant tumor images. In breast cancer research, two major approaches are considered. Many authors have investigated the classification of malignant tumors using mammograms [1, 2]. In parallel, several studies have been dedicated to images obtained by cytology. Numerous features have been proposed to identify and analyze isolated cells, such size, shape, texture [3, 4]. A very recent review of this approach and its historical development can be found in [5].

The aim our research was to analyze histological samples of intact tissue taken with a biopsy. We are interested in developing methods the can improve the automated classification between cancer and normal tissue. The specific aim of this paper was to investigate the use of fractal analysis to characterize tissue images.

Fractal geometry has been proved very useful for quantifying and simulating the complex patterns encountered in nature that were previously described only qualitatively [6]. Fractals have been applied in many fields, from pure mathematics, to biology, geology and medicine since a long time [7, 8]. As concern applications to breast cancer studies, fractal dimension has been successfully used to quantify chromatin in epithelial cell nuclei [9, 10].

In our study, we proposed the fractal dimension for quantifying the tissue irregularity subjectively observed by pathologists. In fact, as described by pathologists, the cells in normal breast tissue are well organized in regular objects, while the cells in cancerous tissue present high irregularity, because they are growing without control and invading the surrounding tissue. It has been shown a high correlation between human visual irregularity perception and fractal dimension, and thus fractal dimension is a good candidate for quantifying the cell irregularity subjectively observed by pathologists.

Since the introduction of the concept of fractal dimension by Mandelbrot [11], a large number of analysis strategies have been developed to allow the measurement of fractal dimensions.

In our experiments, the method used for computing the fractal dimension of a binary image is based

on the Minkowski “sausage” model and on the Euclidean Distance Transform [12, 13].

2 Image Collection

2.1 Tissue Preparation

The tissue specimens were fixed in 4% buffered formaldehyde, mounted in paraffin and cut into $4\ \mu\text{m}$ thick sections. Immunohistochemical staining was then performed as follows. A primary antibody (Cam 5.2; Becton Dickinson Immunocytometry Systems, USA) was used to bind to the low molecular weight keratin of the epithelial cells. Detection of the primary antibody was performed using Dako ChemMate(tm) Envision(tm) Detection Kit (DAKO A/S, Denmark). For visualization of the binding site, Vector SG Substrate Kit (Vector Laboratories, USA) were used. These procedures stained the epithelium black. The specimens were then stained with the Feulgen reaction which stained the nuclei red.

2.2 Image Acquisition

After preparation, the tissue samples were observed using a Leica DM RXE microscope. A magnification of $10\times$ was used in all experiments. The microscope was connected to a Leica DC 200 digital camera, allowing the images to be recorded onto a personal computer.

Color images were 1280×1024 pixel matrices, with a resolution of $1.03 \times 1.03\ \mu\text{m}$.

2.3 Image Description

From each sample 10 images were acquired. Samples were collected from different patients and classified by the pathologist in four distinct classes. A total of 200 images has been employed in the present study.

For this research we considered two malignant conditions, namely ductal cancer and lobular cancer and a benign condition called fibroadenosis. Images of normal tissue were also acquired. Examples of images of different tissues are shown in Figure 1.

Ductal carcinoma is the most common type of breast cancer, it originates in the cells of the ducts. Cancer that begins in the lobes or lobules is called lobular carcinoma, it is the second most common type of breast cancer.

As described by pathologists, the cells in normal breast tissue are well organized in regular objects,

which are all about the same size; the cells do not invade the surrounding tissue. The cells in the two types of cancers do not form any kind of regular pattern, because the cells are growing without control; the cancerous cells are invading the surrounding tissue. In fibroadenosis, that is a benign condition, the cells are mainly organized into separate objects, but the ducts of the breast are often widely dilated due to the fluid filled cysts.

3 Image Analysis

3.1 Segmentation

The original color images have been transformed to binary images through a segmentation procedure.

First the intensity non uniformities present in the images are corrected using the iterative spline fitting algorithm described in [14].

Then a fuzzy clustering method, the Fuzzy c-Means Algorithm, has been used to separate different colors. This algorithm is an optimal approach that minimizes the objective function in an iterative procedure. It is widely used in image processing, especially for color image segmentation [15].

Using the fuzzy c-means, images were segmented in regions having three different values, based on color. One value has been used for the epithelial cells (black in the original images), another value for the nuclei (red in the original images) and the third value for the remaining structures.

In these images there are numerous isolated nuclei that are not of interest for our study. Therefore a conditional dilation process has been used to remove them [16]. A morphological dilation has been applied to the regions belonging to epithelial cells, with the condition that only the regions containing nuclei that touch the starting regions will be connected.

In the final binary images (see Figure 2), the white objects (value one) are the epithelial cells together with their nuclei.

3.2 Fractal analysis

Fractal dimensions are extremely useful in quantifying the degree of ruggedness of highly irregular objects. Several methods have been proposed to measure fractal dimensions in image analysis to describe irregular objects, by estimating for example the fractal dimension of boundary or of the surface texture.

Some of the methods for computing the fractal dimension of a binary image are based on the

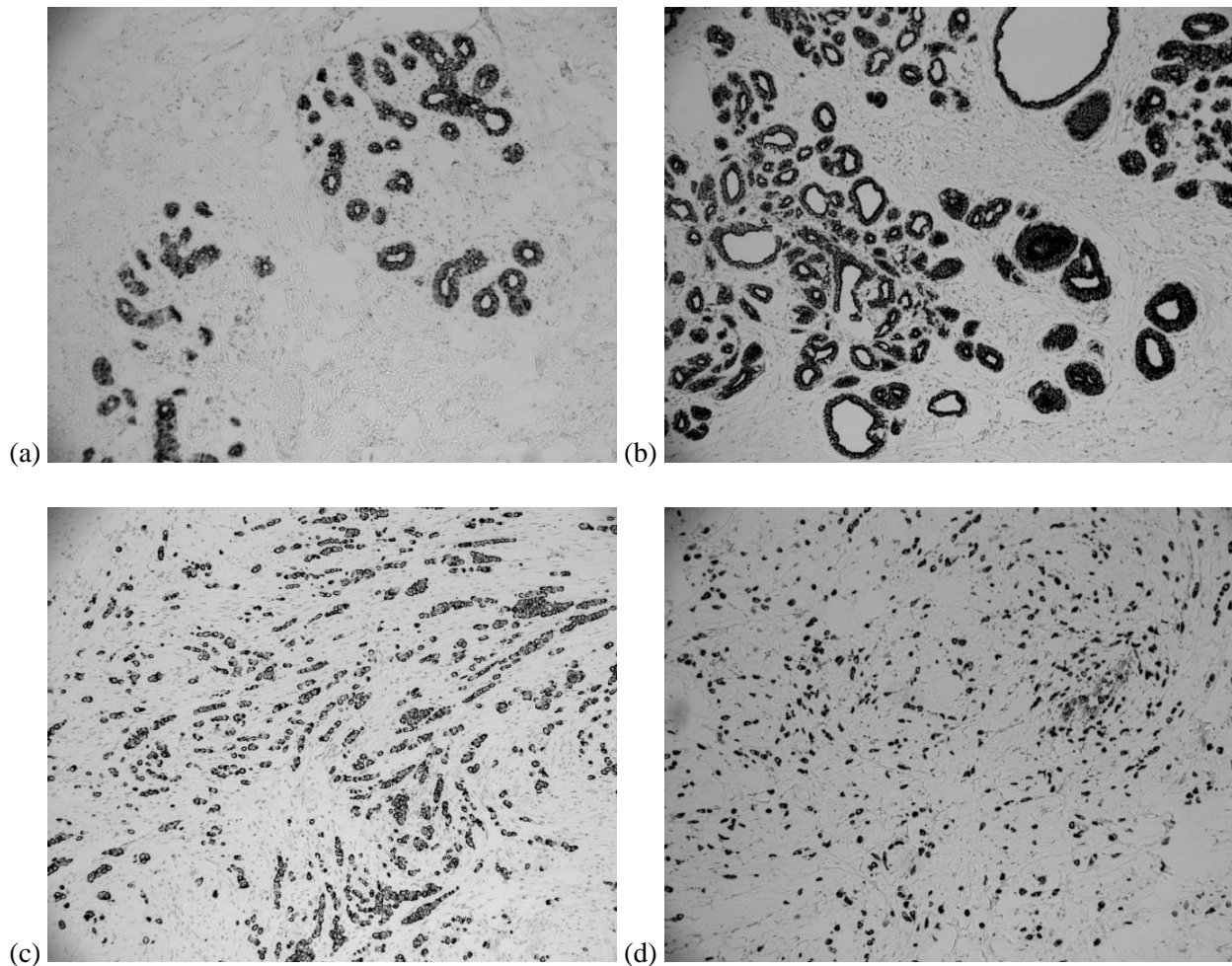


Figure 1: Examples of microscopic image of breast tissue: (a) normal, (b) fibroadenosis, (c) ductal carcinoma, (d) lobular carcinoma.

so called Minkowski “sausage” model. According Minkowski’s logic, circles of finite diameters are drawn around each point of the outline of an object. The area of the ribbon created, usually called covering or “sausage” is measured. This value is then plotted as a function of the circle diameter on the log-log plot (known as Richardson-Mandelbrot plot), and the slope s of the regression line gives the fractal dimension.

Based on a review by Allen et al. [17], the most accurate method to approximate the Minkowski “sausage” model is the Distance Transform method [12]. This is confirmed also by validation tests performed by Bérubé [18]. Therefore, this is the method the we decided to use in our study. Here is briefly described.

A distance transformation mapping converts a binary image (black and white) to a distance image, or distance transform (grey level image). In the distance transform image each pixel has a value measur-

ing its distance to the nearest pixel of a given object within the image [19].

For example, if a single pixel is plotted in the centre of the image, the resulting distance transform of the pixel object will have grey levels ranging from 0, for those pixels at 0 pixels distance from the object, to 255 for those pixels that are 255 pixels or more from the object (for a typical 256 grey level image).

If a shape profile is distance transformed, each outer grey level perimeter corresponds to the outer perimeter of a Minkowski “sausage”, therefore as proposed by Adler and Hancock [12] the distance transform can be used to approximate Minkowski “sausage” logic. It is fast, accurate and sensitive to object complexity.

More than one feature can be present in the image. This method allows the measurement of the fractal dimension of the entire image or individual features.

This method has been applied to the binary images obtained after the segmentation procedure described



Figure 2: Example of segmented image: white objects are epithelial cells and their nuclei.

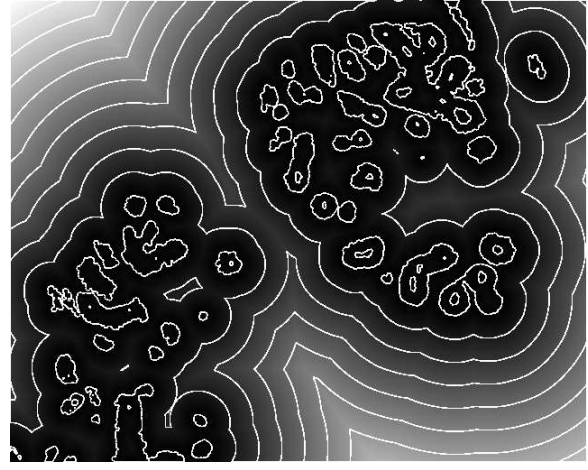


Figure 4: Distance transform with contour plot superimposed

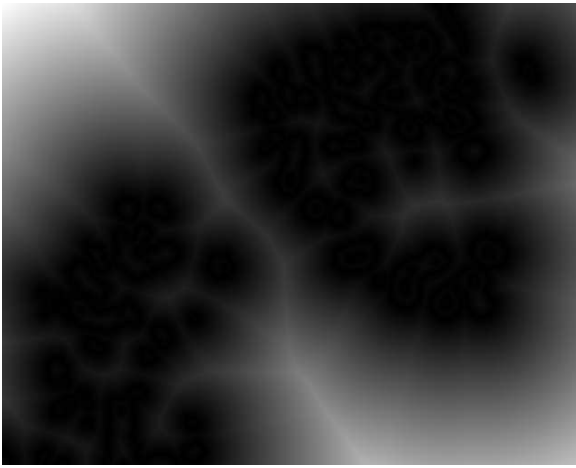


Figure 3: Distance transform

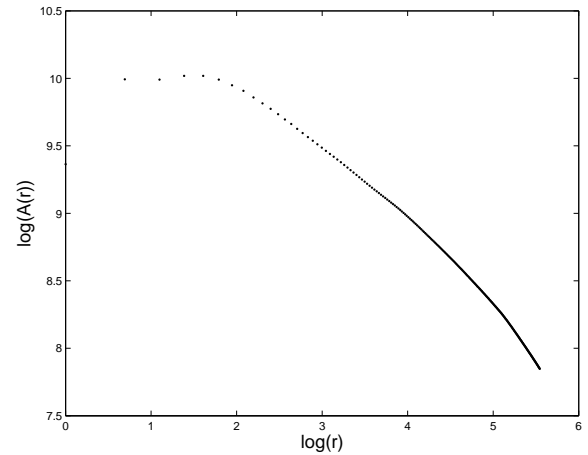


Figure 5: Richardson-Mandelbrot plot

in the previous section.

The distance transform of the image in Figure 2 is shown in Figure 3. For clarity, see also Figure 4 where the contour plot (using isolines every 20 grey levels) is superimposed on the distance image.

Thresholding the image at varying levels of grey produces an isotropic ribbon (or “sausage”) around the outline.

The fractal dimension can be computed by plotting the log of the area of the “sausage” against the log of the width of covering ‘circles’ (approximated by the number of the selected grey levels).

The Richardson-Mandelbrot plot of the values used to compute the fractal dimension, i.e.:

$$\log(A(r)) \text{ vs } \log(r) \quad (1)$$

is shown in Figure 5.

It is possible to observe that a linear relation is satisfied over an acceptable range of values of $\log(r)$.

For this reason we limited the estimation of the regression line to this range.

Finally, from the slope s of the regression line, the fractal dimension F_D has been obtained by the relation:

$$F_D = 1 - s \quad (2)$$

The means and standard deviations of the fractal dimensions for the four groups of images used in this study are listed in Table 3.2.

It can be noted that these values can be easily used to distinguish tissues having different pathologies. The mean fractal dimension is considerably higher in both ductal and lobular carcinoma than in normal tissue. Fibroadenosis mean value is still higher than the value obtained for normal tissue, but closer to this one than to values obtained for the cancerous tissue.

All these differences were obtained with a very high significance level $P < 0.0001$ (One-Way Anal-

Class	fractal dimension
normal	1.6739 ± 0.16718
fibroadenosis	1.8845 ± 0.07874
ductal carcinoma	1.8926 ± 0.05751
lobular carcinoma	1.7134 ± 0.13579

Table 1: Fractal dimensions (mean \pm standard deviation) of the four groups of images

ysis of Variance (ANOVA) test). You can get some graphical assurance that the means are different by looking at the box plot in Figure 6 obtained by the ANOVA test.

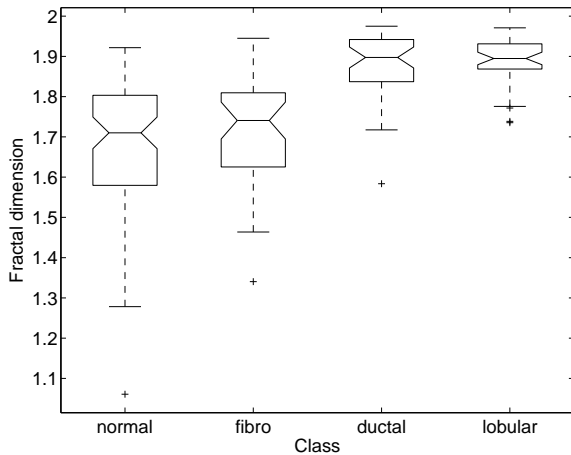


Figure 6: Box plot of the fractal dimensions for the four groups of images

4 Classification

On its own, fractal analysis gave high significant results for the separation between different tissues. However, fractal analysis was combined with feature analysis to improve the classification.

The feature analysis was based on granulometries and it gave a sensitivity of 90%. It has been already described [20].

A multi-layer feed-forward neural network has been used for the classification. The network has one input layer with 5 units, one layer of hidden units and one output layer having 4 units corresponding to the 4 classes. It has been trained with the back-propagation algorithm [21]. The inputs were the features extracted from each image, including granu-

lometries and fractal dimensions. The training was repeated 10 times. In each run a set of 10 images from each class was held out for testing.

Classification results are reported in Table 4.

True class	Classified as			
	normal	fibro	ductal	lobular
normal	85	4	11	0
fibro	70	15	15	0
ductal	8	4	52	36
lobular	2	1	21	76

Table 2: Confusion matrix.

The correct classification into the four classes was not very high, because (as in our previous study) a relative high number of images presenting fibroadenosis (that is a benign alterations) were classified as normal. Moreover several classification errors were made between the two type of cancerous tissue, i.e. some images presenting ductal carcinoma were classified as lobular carcinoma and viceversa. Anyway in all these cases the cancerous tissue was identified.

Therefore, the overall sensitivity was 92.5%. This means that the addition of the fractal dimension improved the correct classification of cancerous tissue from 90% to 92.5%

5 Conclusions

A method has been presented to analyze microscopic images of breast tissues by means of fractal geometry. Color images of tissues sample have been first converted to binary images using a segmentation procedure based on fuzzy clustering algorithms and mathematical morphology. The fractal dimension of binary images has been computed using the euclidean distance mapping. The classification is performed using a neural network.

The encouraging results suggest the fractal dimension can be used as a parameter to assist pathologists in the diagnosis of breast cancer, and potentially in developing techniques for the automatic classification of cancerous tissue.

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