Segmentation of numerous tiny objects in very noisy gray-levels images

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ABSTRACT

This paper presents a new approach to isolate tiny objects in very noisy gray-levels images. Our objects of interest consist of blood (micro)vessels present in histological cuts of malign tumors which grow in soft parts of the human body, through a natural process known as angiogenesis. Blood vessels appear in images as small, dark objects of irregular shapes with non-uniform backgrounds. The proposed strategy is based on the application of a conditioned morphological closing, which considers some statistic properties of pixel values explored by the structuring element. The approach gives better segmentation results than those obtained by other two strategies used before and briefly analyzed in this paper.

Keywords: Noise cleaning; Blood vessel segmentation; Conditioned closing; Angiogenesis; statistically based structuring element.

1 Introduction

Noise is a common and serious cause of deterioration of digital images. When high levels of noise are present in an image, it is often nearly impossible to analyze its contents. On the other hand, when efforts are done to eliminate the noise, it is common that some important information disappears together with the noise. Then, obviously, this constitutes a serious obstacle for the future analysis of the image. Hence, eliminating the noise from images without (or with a minimum) loss of the information of interest is of fundamental importance. This task is known in digital image processing as noise filtering, noise cleaning, or noise smoothing. The lower the signal-to-noise ratio in an image, the lesser its quality.

Bad quality of images used in our particular experiments is product of a poor quality digitizer. Images obtained with such a digitizer (6 in total) are unique, and they cannot be created again. It does exist a particular interest by physicians to conserve them. For that reason, in spite of this, we attempted to enhance and segment them, in order to realize a posterior morphometrical analysis.

In this paper we present an efficient approach developed to eliminate the additive and structured noise in images having such numerous irregular tiny objects: the blood vessels. They are successfully isolated from the background. The strategy, oriented to clean the additive noise, includes the selection of the best variant from several procedures composing a sequence of four steps. The last step uses two conditioned morphological closing based on the standard deviation of gray levels in the image, which later is compared with two different numerical values ξ. The first closing uses a ξ value lesser than the second one. Objects obtained from the second closing act internal markers. The combination of markers with the objects resulting from the first closing, offer finally a high quality segmented image.

2 Characteristics of images used in the study

Images used in our experiments represent histological cuts of malign tumors that eventually grow in soft parts of the human body, where blood (micro)vessels are created by tumors to feed themselves. This natural process is known as *angiogenesis*. Two out of six images used in our analysis are shown in Fig. 1. They are colored, but they were transformed to gray-level in our experiments to facilitate the hardcopy reproduction later. Blood vessels appear as small, dark objects of irregular shapes with non-uniform backgrounds. The total area of blood vessels in these pictures is an important indicator for specialists to determine the age (or level of development) this kind of tumor.

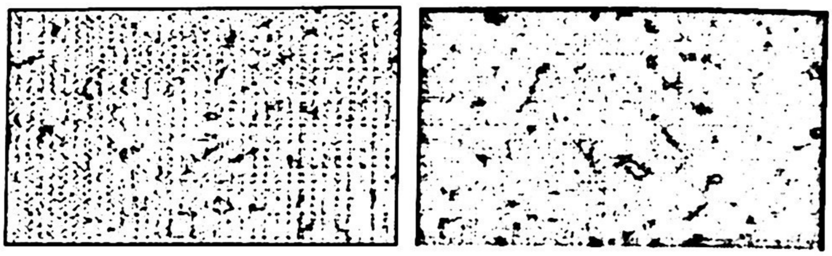


Figure 1. Example of two out of six images used in our procedure.

The presence of noise and the low contrast of images are caused generally by illumination deficiencies. The low quality and sensitivity of the capturing system used to create them contributed also in this sense. Eventually, in the segmentation process of gray-level images, the bad selection of the threshold level in non-elaborated algorithms of binarization produces also some artifacts, similar to tiny blood vessels present in our images. Then, it is necessary to isolate properly the useful objects, in order to calculate later the actual area of blood vessels by morphometrical analysis. In that task an idoneous system described in Rodriguez et al., 1992, is used for that purpose.

Low quality images used in our analysis present additive (uniform), multiplicative (impulsive) and structured (coherent) noises, together with some areas of higher brightness. The intensity and position of the non-uniform illumination source arriving to the mirror of the optical microscope, gives place to the central luminous spot in all images. Originally, images appeared bluish-greenish because of its artificially coloring with methyl green to highlight the vascular formations and thus distinguish more clearly the blood vessels from the background.

Images were observed through a binocular microscope with 200X magnification coupled to a color video camera. Signals from the camera were digitized and sent to the microcomputer. RGB images obtained have spatial resolution of 512 x 512 and 5 bits per pixel for color. For segmentation purposes, color images were transformed to HSI color space, where the intensity gray-level phase of images was selected. Because of the common small size of blood vessels in pictures, it was particularly difficult to distinguish the artifacts (or eventually impulsive noise) from the actual blood vessels.

3 Related topics

In Digital Image Processing (DIP), elimination of different classes of noise in images is commonly required. So far, many articles have been written for this purpose with by using different segmentation methods oriented to such goals (Otsu, 1978; Pun, 1980, 1981; Johannsen-Bille, 1982; Chiralo-Berdan, 1985; Kapur et al., 1985; Wen-Hsiang, 1985). However, elimination of noise in images where the gray level, the size, and eventually the shape of objects is similar to the additive and/or multiplicative noise, have not been frequently found.

Additive noise is usually cleaned from gray-level images by means of low-pass filters and by averaging pixels values during the capture of images, as illustrated in Gonzalez-Wood, 1992, 1996. To eliminate impulsive noise, many methods have been proposed so far, amongst them, the median filter (Huang-Yang, 1978); rank-order filters (Heygster, 1982); and more recently the so-called morphological filters (Maragos-Schafer, 1990). Structured, periodical, or signal dependent noise is commonly eliminated by using the Fast Fourier Transform or other in order to transform images to frequency space, and then interactively suppress undesirable frequencies producing the structured effect. Afterwards, applying the Inverse Fast Fourier Transform (or correspondent) over the filtered image, a cleaned final image is obtained. Examples of this method are encountered in Gonzalez-Wood, 1992, 1996. Thus, in our experiments, periodical noise present in all images was eliminated by means of a narrow band-pass filter in the Fourier frequency domain.

Sometimes in the segmentation process, as a strategy, a (real or false) color image is changed to a gray-scale image and then binarized by thresholding. The main goal of this method is to obtain a binary image with objects highly differentiated with respect to the background. In this case many artifacts are produced, together with the real impossibility to guarantee good segmentation results due to the loss of useful information in the frontiers of the objects of interest. Example of this approach is given in Rodriguez et al., 1999, by using generalized morphological filters and components labeling to overcome in some measure the loss of information.

4 Strategies developed

In order to isolate the blood vessels in images of example, two approaches have been developed in our Group.

4.1. First strategy

Taking into account color models most frequently used in these purposes, the RGB model gave the best results. Amongst the three-color components resulting from this model (red, green and blue), the green component was selected as the best one according to the amount of valid information, probably due to the very particular color characteristic acquired by the histological cuts as a consequence of their preparation in the pathology laboratory. For that reason, all subsequent steps were carried out upon the image on the green channel. Images were segmented by means of the following sequence of procedures (Rodriguez et al., 1998; Alarcon, 1998):

- Filtering the additive noise with a low-pass filter using a 3x3 square mask;
- Using a heuristic combination of a punctual and local thresholding (as in Sharon, 1986), with a modification in the thresholding value. The local method used a window size selected from the experience;
- Finally, applying the median filter.

Results of this sequence of procedures are shown in Fig. 2 for both images taken as examples (Fig. 1). Circles and arrows indicate some clearly observed errors in the segmentation process. They are detected when comparing them with images manually segmented by a physician shown in Fig. 3(b). Errors in percents for all 6 images are shown in Table 1.

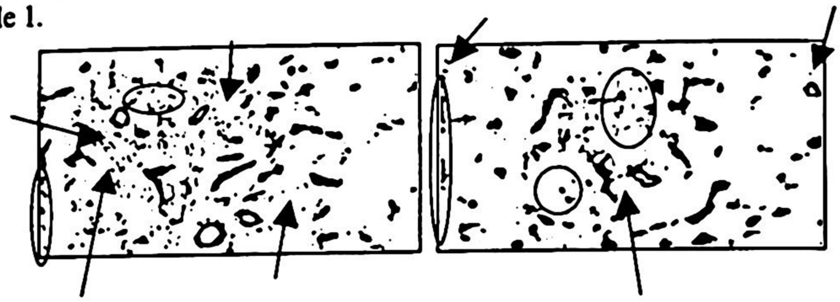


Figure 2. Resultant images from the first strategy.

4.2. Second strategy

In order to eliminate the artifacts still present in noisy images after the segmentation strategy given before (4.1), images were processed later by generalized morphological filters (GMF) and with components labeling (Rodriguez et al., 1998). Results of this procedure are shown in Fig. 3. Main differences (or errors) clearly observed after elimination of artifacts, shown in 3 (b), when compared with images segmented manually by pathologists, shown in 3 (c), are indicated with circles and arrows. Errors in percents are shown also in Table 1.

5 The new approach

The new strategy presented in this paper proposes to deal with the blood vessels and the background in a differentiated form. To achieve this, it was taken into account that the blood vessels in the images have lower gray-levels (darker) than the background. Therefore, the standard deviation respect to the local arithmetic mean (or another measure of central tendency) is greater than that in points of the background, considering windows size bigger than the size of blood vessels.

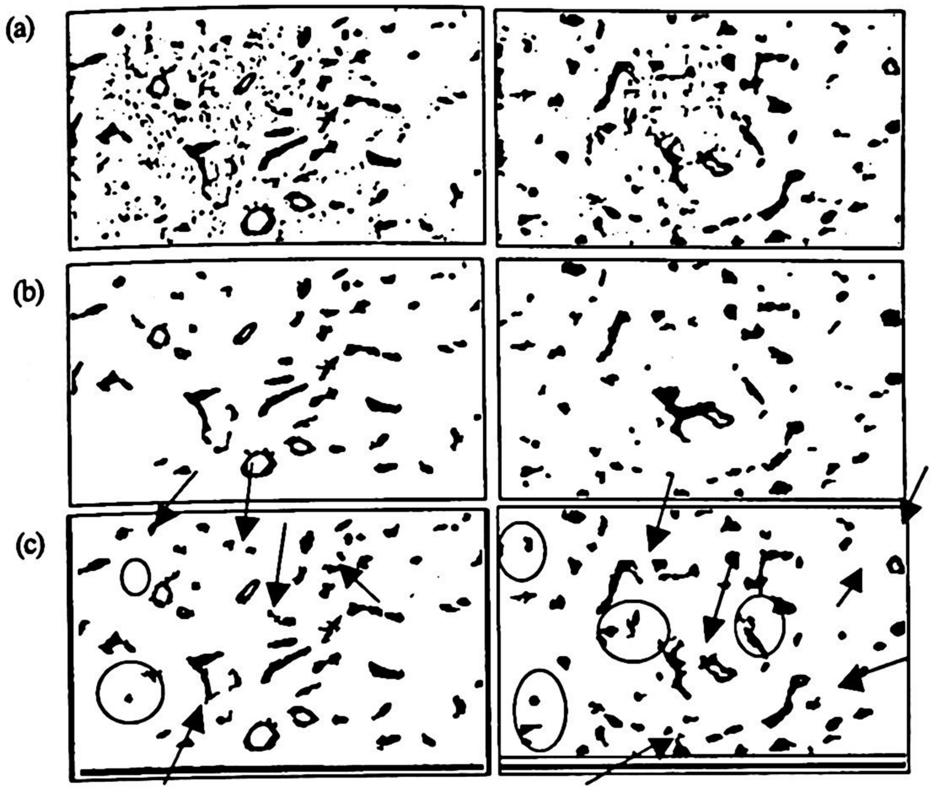


Figure 3. (a) Original images, (b) Images obtained by manual segmentation, (c) Results obtained by using generalized morphological operators and components labeling.

In Fig. 4 (b), the gray level distribution in an arbitrary column of an image is shown. Valleys are related to the lesser (darker) level corresponding to blood vessels in the image. Variations due to the additive noise are clearly observed. When a morphological closing is applied by the structure element shown in Fig. 4.(a) with a "height" equal to 55, the profile shown in Fig. 4 (c) is obtained. As a result, only valleys remain onto a uniform background. This result is produced, because of when the dilation (as a part of closing) is done, pixel values greater than 255 *are limited* to this value. Finally, after the erosion, those pixels acquire the same limited value equal to *(255 – height)*.

In our experiments, the height of the structure element for each image was automatically calculated on a basis of a local processing, considering pixel values within contiguous equal-size rectangular windows, and the calculation of a measure of central tendency M amongst the arithmetic mean, the median and the harmonic mean. Thus, according to this, the *limit* is given by the expression $(255\text{-}M)$.

However, standard morphological filters applied globally to images, affect both the objects and the background. For that reason, new types of morphological filters were defined in our application. In such a way, dilation (or erosion) and closing (or aperture) are done if the standard deviation of pixels values covered by the structure element *(Dev)*, with respect to the measure of the selected central tendency M, is lesser than a given positive integer value ξ. Then, when processed, the *limit* considered for each window of the image was finally calculated by $(255 - M + Dev)$.

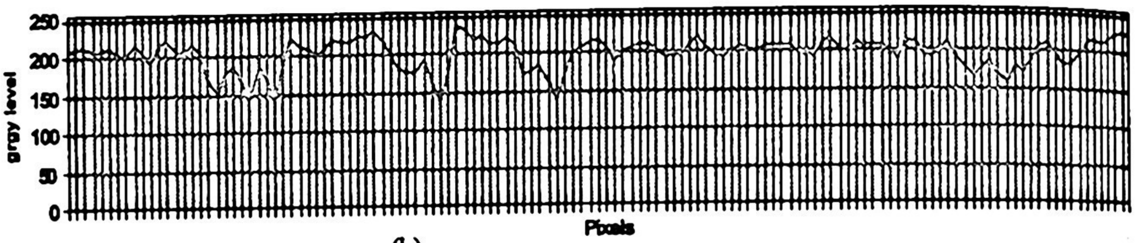
In this sense, a new morphological filter was defined, where the dilation and erosion required by the closing are done only if the standard (or mean) deviation of the pixels values covered by the structure element, with respect to the local measure of central tendency, is lesser than a given value ξ. This special closing we have called *conditioned closing*.

With this in mind, to achieve a homogeneous background, the *conditioned closing* was applied by a specific structure element (rhombus of 5 pixels, obtained from the experience) with gray-levels equal to $(255 - M + Dev)$, where M is the local measure of the central tendency measure selected, and Dev is either the standard (or the mean) deviation. In such a way, the resultant structure element used will have different gray level for each window.

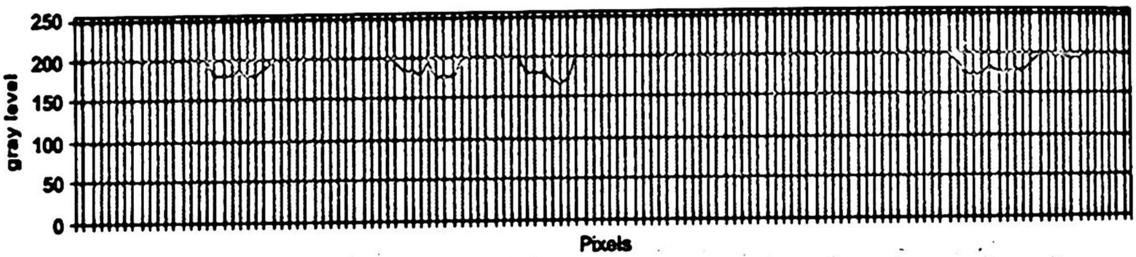
Thus, the new strategy carries out the following four procedures:

$$\begin{bmatrix} 0 & 55 & 0 \\ 55 & 55 & 55 \\ 0 & 55 & 0 \end{bmatrix}$$

(a)



(b)



(c)

Figure 4. (a) Structure element, (b) Pixel values of an arbitrary column of an image, (c) Result of closing with a rhomb with height 55.

- ***Application of standard morphological filters.*** Images were dealt with standard open-close, close-open, close and open filters, each independently, in order to select that with the best performance.

This step is included in order to attenuate gray-levels variations produced by the additive noise, which will influence on central tendency measures to be further calculated.

- ***Windowing.*** Images were dealt locally with two different rectangular windows: 32 x 16 and 64 x 32 pixels.

Due to the non-homogeneous background of images, it is required local processing in order to take into account particular conditions in small areas.

- *Local calculation of different measures of central tendency.* They were calculated locally (for each rectangular window) the arithmetic mean, the harmonic mean and the median.

This step is required to calculate the appropriate gray-levels of the structure element, locally referred to each window, and the standard deviation respect to the measure of the particular central tendency measure selected, required later by our modified *conditioned closing.*

- *Application of conditioned closing. Conditioned* morphological closing by a rhombus of 5 pixels of side and gray-level *(255 - M + Dev)* is applied *Dev* represents either the standard deviation or the mean deviation.

In this step, two values of a positive integer magnitude ξ were selected from experience for comparison. Firstly, a little value of ξ to obtain an image with blood vessels still together with artifacts, but in a homogeneous background. Later a greater value of ξ to obtain an image without artifacts, but with internal markers in places where the blood vessels are located. By using the marker image, the blood vessels are finally isolated from the previous image. Thus, a clean (binary) segmented image has been accomplished in a straightforward form.

In our case, the best variant selected in each respective previous step was the following:

- Opening filter,
- 32 x 16 window,
- Median, as a measure of central tendency,
- Modified (*conditioned*) closing using a standard deviation, with a numerical value $\xi = 10$ to get the cleaned image, and $\xi = 30$ to get the marker image.

Images obtained though this sequence, are shown in Fig. 5 for two images of example. Fig. 6 shows resultant images obtained for six images considered.

It is well known that, due to the lack of ground truth, quantitative evaluation of a segmentation algorithm is difficult to achieve. Our alternative has been to use manual-segmentation results as ground truth, which although it is not perfect, provides a useful indication. Error are given in two metrics: false target count (FTC), that is, those background pixels that are wrongly segmented as target by the algorithm, and false non-target counts (FNTC), that is, those pixels that are in but fail to be segmented as the target. In our case, calculation of respective errors considering all images analyzed gave as result values lesser than 1,19% and 1,73%, respectively. Some differences with respect to images manually segmented are indicated in Fig. 5 with circles and arrows. Comparison of our results with the original ones shows that, qualitatively, certainty is enough high.

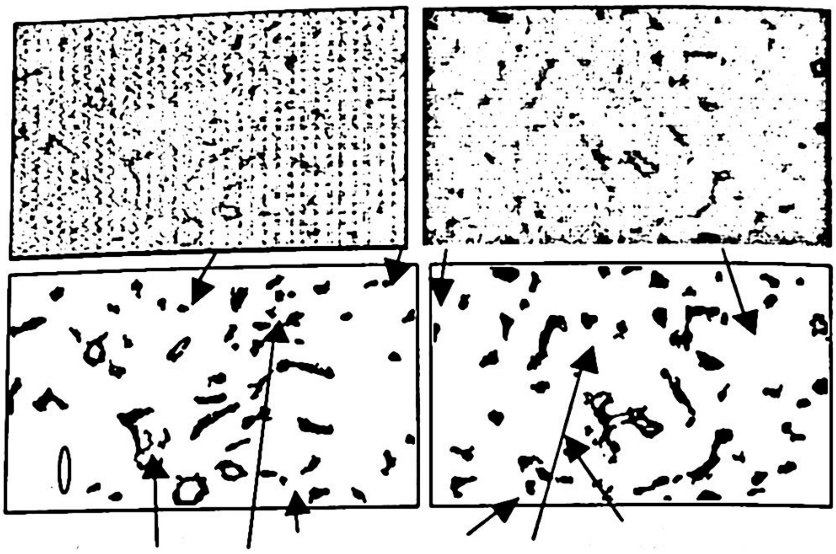


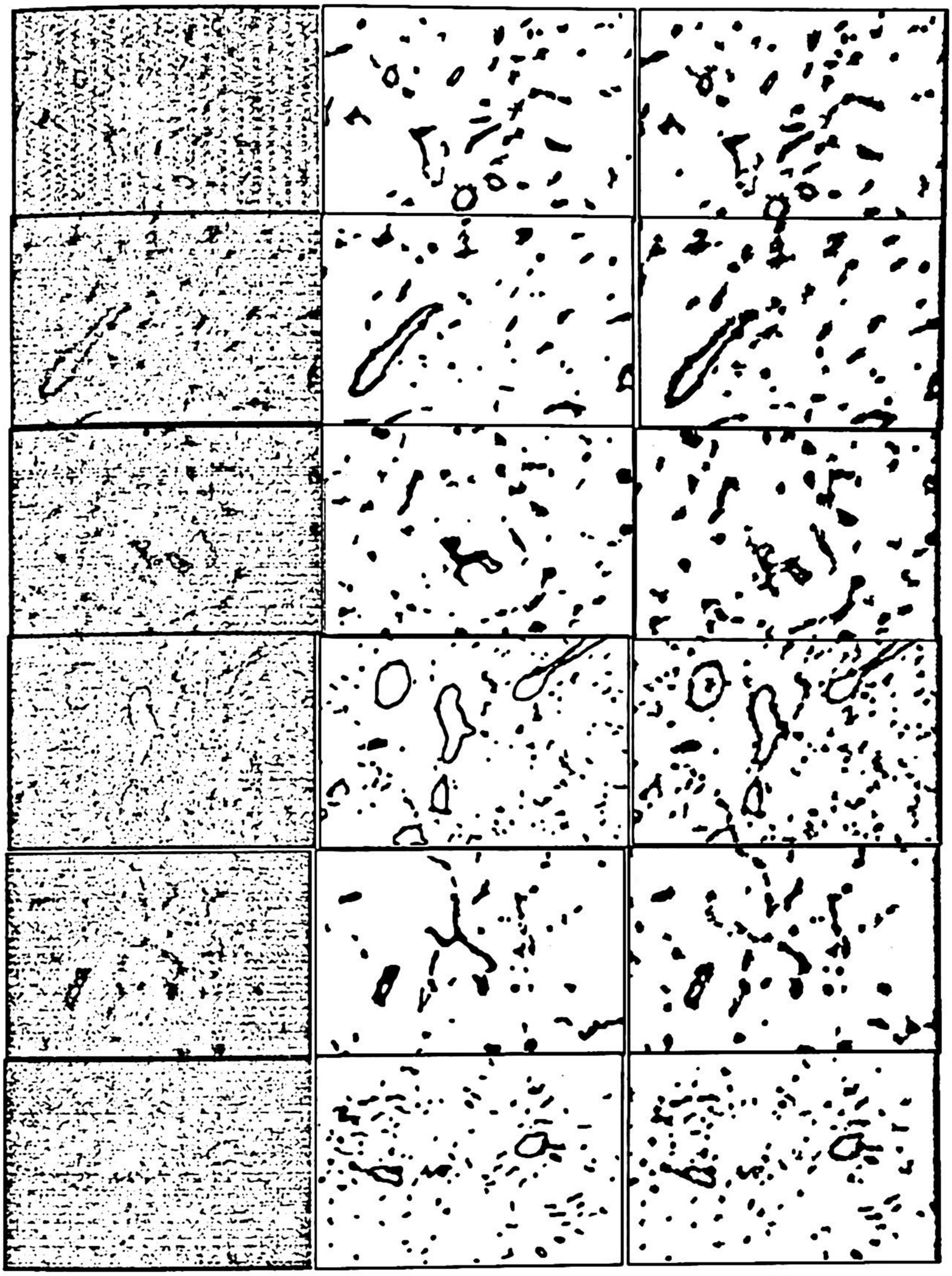
Figure 5. Images obtained with the new approach.

6 Errors analysis

Numerical evaluation of errors in percents is shown in Table 1, obtained with both previous strategies and the new approach when they are compared with images manually segmented.

Image	1st strategy (4.1)		2nd strategy (4.2)		New approach	
	FTC	FNTC	FTC	FNTC	FTC	FNTC
1	0.76	6.15	1.16	2.29	0.67	1.24
2	1.09	6.36	1.49	3.31	1.05	1.57
3	0.91	5.99	1.17	1.86	0.63	1.54
4	1.49	4.82	1.79	2.67	1.19	1.29
5	1.41	7.20	2.25	3.62	0.52	1.73
6	2.13	5.25	3.19	3.12	0.58	1.12

Table 1. Percents of false target count (FTC) and false non-target count (FNTC) errors, for images segmented with first and second strategies and with the approach proposed. Higher values of error are highlighted.



(a) (b) (c)

Figure 6. Final results of segmentation for all six images. (a) Original images, (b) Images manually segmented, (c) Images segmented with the new approach.

7 Conclusions

Comparing results obtained with strategies described in 4.1 and 4.2, with respect to our new approach described in epigraph 5, it is demonstrated that the new approach gives best results. Comparison is carried out with respect to the manual segmentation of blood vessels shown in Fig. 3 (b). The best variant of the sequence proposed, guarantees the best segmentation of blood (micro)vessels present in our particular very noisy images. Errors calculated between 1,19% and 1.73% for target and non-target counts, respectively, for all images, indicate this assessment. Additionally, resultant images were obtained in a relatively more straightforward form.

8 References

Alarcón, T., 1998. Segmentación de imágenes en colores. Su empleo en el estudio de la angiogénesis. Tesis de Maestría en Ciencias, ISPJAE.

Chiralo, R. P., Berdan, L. L., 1985. Adaptive digital enhancement of latent fingerprints. Proc. of IEEE 20.

Gonzalez, R. C., Woods, R. E., 1992. Digital Image Processing. Addison-Wesley.

González, R. C., Woods, R. E., 1996. Tratamiento Digital de Imágenes. Addison-Wesley / Díaz Santos.

Heygster, G., 1982. Rank filters in Digital Image Processing. Computer Graphics and Image Processing 19, 148-164.

Huang, T. S., Yang, G. J., 1978. A fast two dimensional median filtering algorithms. IEEE Transactions on Acoustic, Speech, and Signal Processing, ASSP-27 (1), 13-18.

Johannsen, G., Bille, J., 1982. A threshold selection method using information measures, Proceeding of 6Th International Conference On Pattern Recognition, Munich, Germany, 140-143.

Kapur, J. N., Sahoo, P. K., Wong, A. K. C., 1985. A new method for gray level picture thresholding using the entropy of the histogram. Computer Graphics and Image Processing, 29, 273-277.

Maragos, P., Schafer, R. W., 1990. Morphological System for Multidimensional Signal Processing. Proceeding of the IEEE 78 (4), 690 - 708.

Otsu, N., 1978. A threshold selection method from gray level histogram. IEEE Trans. Systems, Man and Cybernetics SMC-8, 62-66.

Pun, T., 1980. A new method for gray level picture thresholding using the entropy of the histogram. Signal Processing 2, 223-237.

Pun, T., 1981. Entropic thresholding: A new Approach. Computer Graphics and Image Processing 16, 210-239.

Rodriguez, R., Alarcon, T., Castellanos, I., Wong, R., Sanchez, I., 1998. Color segmentation. Its use in the study of angiogenesis. Part I, Journal of Vascular Research 35, Sup.3.

Rodriguez, R., Castellanos, I., Alarcon, T., Navarro, R., Felipe, E., Sanchez, L., 1999. Elimination of noise via morphological filters and components labeling. Its use in the study of angiogenesis. Research Report of ICIMAF No. 99-83.

Rodríguez, R., Fernández-Britto, J. Wong, R., Campos, R., 1992. MADIP, Morfometría Analítica y Digitalización en Patología. Revista Cubana de Investigaciones Biomédicas, 11(2), 126 – 128.

Sharon, A. S., 1986. ANGY: A rule-based Expert System for Automatic Segmentation of Coronary Vessels From Digital Subtracted Angiograms. IEEE Transactions on Pattern Analysis and Machine Intelligence PAMI 8 (2), 188 - 199.

Wen-Hsiang, T., 1985. Moment-Preserving Thresholding: A new approach. Computer Graphics and Image Processing, 29, 377-393.