Contributed paper

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Use of two-dimensional matched filters for estimating a length of blood vessels newly created in angiogenesis process

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The complex algorithm for estimating a length of blood vessels' newly created in angiogenesis process has been presented. A filtering method using two-dimensional matched filter is a fundamental process in the presented algorithm. For proper extraction of blood vessels' network more image processing techniques has been used including spatial low-pass filtering, binarisation, skeletonisation, and, developed by the author, algorithms for cleaning vessel network obtained in this process and for removing vessels false detected in the previous steps.

The presented algorithm can be used for fully automatic vessels' length estimating in medical images and can be helpful for angiogenesis process's research.

Keywords: biomedical imaging, digital filters, image processing.

1. Introduction

Angiogenesis is a process of growth of new blood vessels and is an important natural process occurring in the body, both in health and in disease. Angiogenesis occurs in the healthy body for healing wounds and for restoring blood flow to tissues after injury or insult. In females, angiogenesis also occurs during the monthly reproductive cycle (to rebuild the uterus lining, to mature the egg during ovulation) and during pregnancy (to build the placenta, the circulation between mother and fetus).

The healthy body controls angiogenesis through a series of "on" and "off" switches:

- the main "on switches" are known as angiogenesis--stimulating growth factors,
- the main "off switches" are known as angiogenesis inhibitors.

When angiogenic growth factors are produced in excess of angiogenesis inhibitors, the balance is tipped in favour of blood vessel growth. When inhibitors are present in excess of stimulators, angiogenesis is stopped. The normal, healthy body maintains a perfect balance of angiogenesis modulators. In the body attacked by tumour, cancer cells stimulate process of angiogenesis.

Medics experimenting with substance for controlling the angiogenesis process – for stopping and stimulating it. In their research, they need some tools for an automatic process control allowing for estimating blood vessel network length before and after use of the medical substance.

Proposed method is useful for fully authomatic estimating the length of blood vessels newly created in angiogenesis process. It is a combination of well known earlier designed methods such as two-dimensional matched filters [1], low-pass spatial filtering, binarisation, skeletonisation and, developed by the autho, simple algorithms set for cleaning skeleton pictures of blood vessels network and for removing vessels false detected in previous steps.

2. Algorithms

For blood vessels' network extraction, two-dimensional matched filter technique described in Ref. 1 has been chosen. Based on properties of the blood vessels in the considered images the authors assumed that:

- blood vessels usually have small curvatures and thus anti-parallel pairs may be approximated by piecewise linear segments,

vessels' intensity profile can be approximated by the Gaussian curve

$$f(x,y) = A\{1 - k\exp(-d^2/2\sigma^2)\},$$

where d is the perpendicular distance between the point (x,y) and the straight line passing through the centre of the blood vessel in a direction along its length, σ defines the spread of the intensity profile, A is the grey-level intensity of the local background, and k is the measure of reflectance of the blood vessel relative to its neighbourhood.

Because of the above-mentioned assumption, instead of matching a single intensity profile of the vessel's cross section, a significant improvement can be achieved by match-

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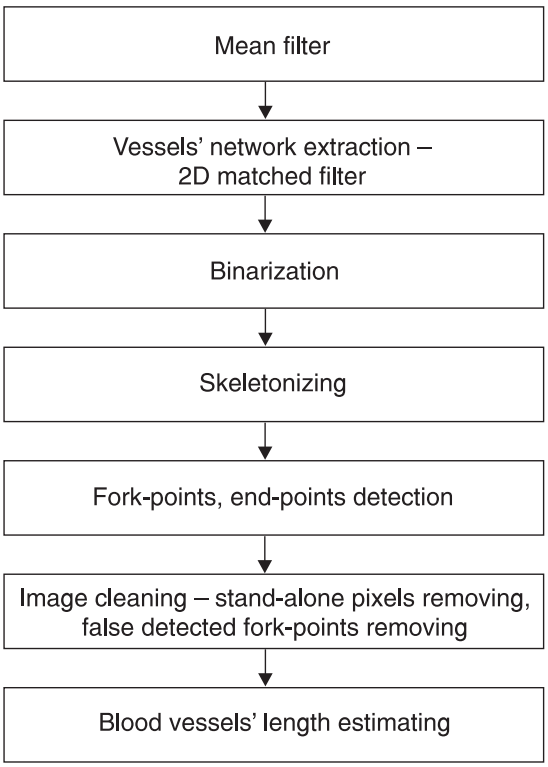

Fig. 1. Schematic diagram of the image processing algorithm.

ing a number of cross sections of identical profile simultaneously. Thus, a kernel can be used which mathematical expression is

$$K(x,y) = -\exp(-x^2/2\sigma^2) \quad \text{for } |y| \le L/2,$$

where L is the length of the segment for which fixed orientation has been assumed. The direction of the blood vessel here is assumed to be aligned along the y-axis and for different orientations the kernel has to be rotated accordingly. Convolution of the generated kernel with a vessel intensity profile function allows deciding if the pixel belongs to the vessel or not.

Before using a matched filter for vessel detection it is recommended to use a low-pass filter for noise removing. Simple mean filter with a 5×5 mask has been used.

Next step after filtering stage is binarisation. A different binarisation methods has been tested and Otsu's method described in Ref. 2 has been chosen as the one giving best results. In short, this method consists in finding optimal threshold for image based on selecting the lowest point between two pixels' classes. First, variance of the two classes is calculated separately (within-class variance) and a total variance for a whole image. The criterion function involves minimising the ratio of the between classes variance to the total variance. Because the criterion function depends on the threshold value we can find the optimal threshold calculating criterion function's value iterative and finding minimum function's value in this way.

Next, it is necessary to make a skeleton picture from a binary image. An algorithm for this operation has been taken from Ref. 3. Then, it is necessary to use some cleaning algorithms for removing false detected, unconnected blood vessels, for removing standalone pixels, and for removing false detected fork points. The applied algorithms based on neighbourhood properties of local pixels'. Schematic diagram of the whole process is presented in Fig. 1.

3. Application and results

Pictures of chicken egg surface with a few weeks old embryos have been used for experiments. A photo of the chicken egg surface is presented in Fig. 2.

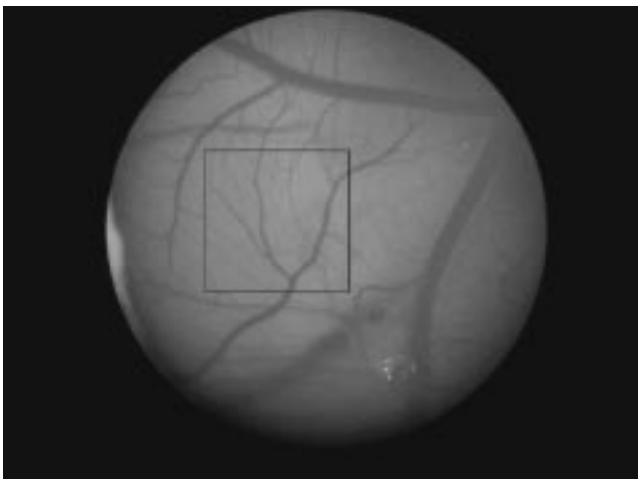

Fig. 2. A photo of chicken egg's. An analysed part of the image is marked with a black line.

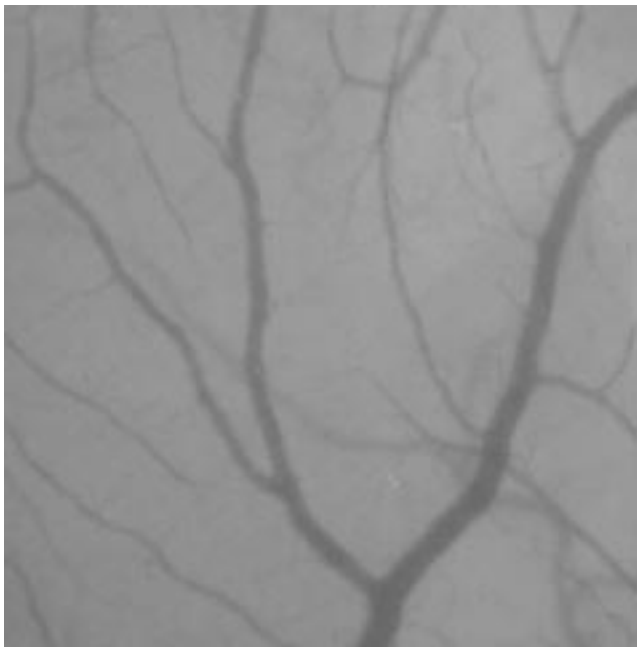

Fig. 3. Zoomed photo of the blood vessels' structure.

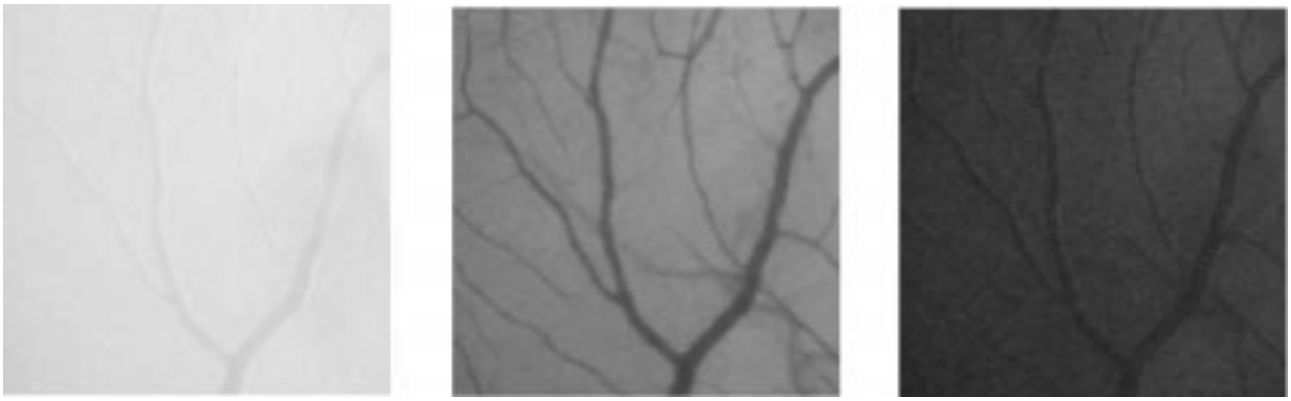

Fig. 4. RGB components of the image (green component in the middle).

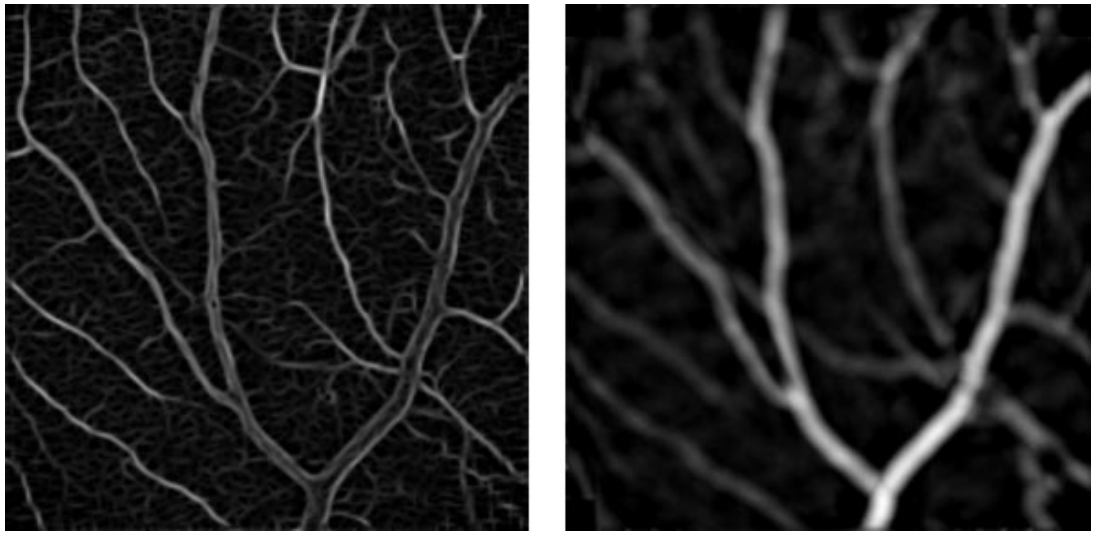

Fig. 5. Matched filter output for the smallest (left) and biggest (right) vessel's diameter.

The pictures have been obtained using digital camera connected to a microscope set. Size of pictures taken in the acquisition process was 3200×2400 pixels and the pixel bit depth was 24 bits. Colour picture has been split into three RGB components and for further analysis only the green component has been taken because of the highest contrast. From this picture a smaller one, including analysed part of blood vessels' network has been cut out and passed for further analysis.

Zoomed picture of the part of blood vessels' network and three RGB components are presented in Figs. 3 and 4, respectively. After smoothing the greyscale picture with the spatial mean filter (5×5 mask has been used) a set of matched filters have been used. A set of filters has been constructed with different kernel sizes because of different blood vessel diameters detected. For every supposed vessel diameter a new kernel has been built with different widths and lengths. A width of the kernel depends on the detected vessel's diameter and the length depends on the assumed fixed orientation length. Because of the need to detect vessels of different spatial orientation, the kernel has to be rotated accordingly. Assuming an angular resolution of 15°, 12 different kernels are needed to span all possible orientations. Corresponding responses from every rotated filter are compared and for each pixel only the maximum response value is to be retained.

A filtering procedure is repeated as many times as the different values of vessels' diameter is assumed. At the end of every filterbank, new output image is generated. The result of application of the matched filter to the image for two assumed vessels' diameters is given in Fig. 5.

For every kernel size its output image is binarised using the Otsu's method. A result of the binarisation process is given in Fig. 6. After that, all binary pictures are added up (binary sum) and new image is generated as a binary representation of the blood vessels network (Fig. 7). Such a solution has been proposed because of the effects of the applied matched filter. It can be seen in Fig. 5 that for small kernel width blood vessels with the bigger diameters are improperly represented in the same way as the thin vessels are (only edges of the vessels are detected). On the contrary, for the bigger kernels, the information about smaller vessels could be lost. For this reason a set of the matched filters followed by a separated binarisation and the final sum image has been proposed.

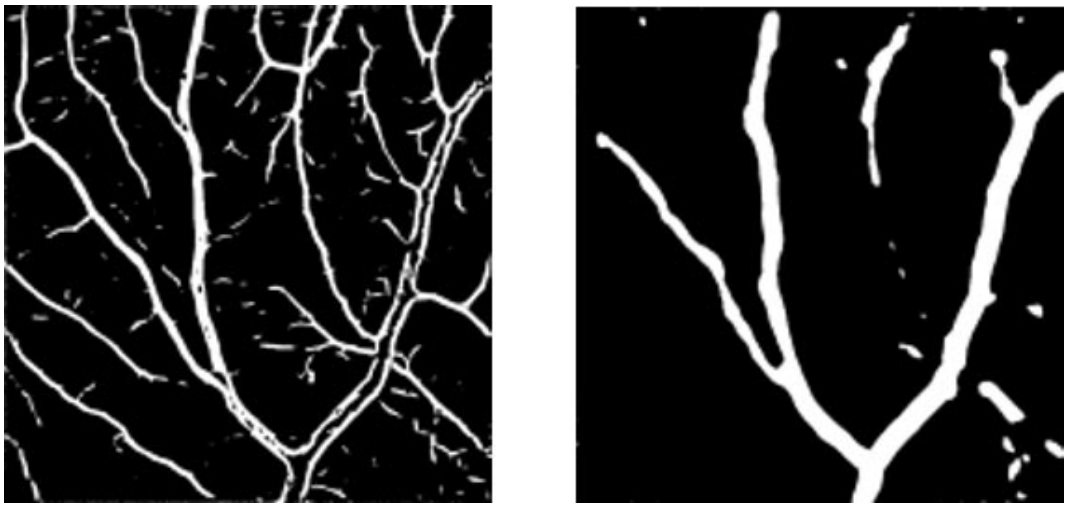

Fig. 6. Binarised filter outputs for the pictures of Fig. 5.

Next step in presented algorithm is picture skeletonising. The goal of the operation is to bring the information about vessels' network topology, ending points' location, fork points, passing over the information about blood vessels' thickness. The result of this operation is presented in Fig. 8. As it can be seen, the marked connection network differs from the expected one. It is caused by the algorithm properties. It marks forks and small vessels that in fact do not exist. Because of that additional operations are needed.

First, all isolated pixels are removed from the picture. Next, all short vessels not connected with the rest of the vesssels' network are removed from the picture either. Every fork point marked in the previous step is tested now if it is a real fork point. For every fork, the point blood vessel width is determined. The algorithm uses information from the binary image presented in Fig. 7 for approximating blood vessel's width at the given point characterised by the properties of the skeleton image. False detected fork points are unchecked and marked blood vessels connected to them are removed.

Two images are given in Fig. 9. One of them is a separated blood vessels structure taken from the image and the other is a corresponding skeleton image after initial "cleaning" procedure.

The final, cleaned skeleton image is used for marking vessels' ending points and fork points. Coordinates of these points are used for estimating the blood vessels length. A vessels geometry is approximated by a piecewise linear segments and a sum of their lengths is taken as a final vessels length. Except of that it is possible to estimate the length of the newly created vessels or the

Fig. 7. Sum of all binary pictures.

Fig. 8. Binary image after skeletonising.

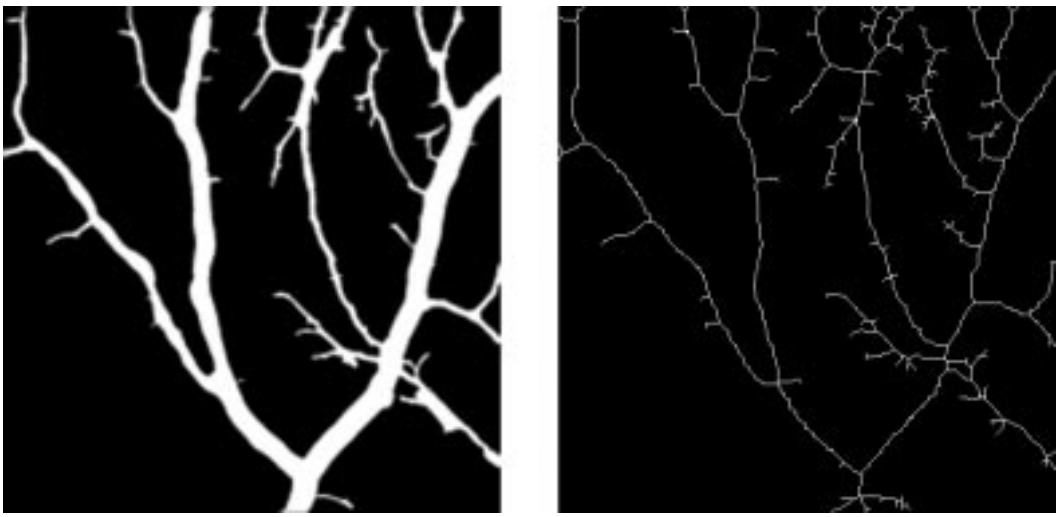

Fig. 9. Separated blood vessels' network (left) and its skeleton representation.

total area of the blood vessels' network in the analysed part of the image.

4. Conclusions

Presented set of algortihms can be used as a powerful tool for fully automatic blood veesels' network or newly created vessels' length estimating. It can be helpful in monitoring of the angiogenesis process stimulated or stopped by the stimulators or the inhibitors accordingly making an analysis of the results easier and faster.

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Molecular Beam Epitaxy
Fundamentals and Current Status

1996. 2nd rev. and updated ed.. XIV, 453 pp. 260 figs., 25 in color,
(Springer Series in Materials Science, Volume 7)
Hardcover. EUR 54.95; £ 38.50; sFr 107
ISBN 3-540-60594-0

Molecular Beam Epitaxy describes a technique in wide-spread use for the production of high-quality semiconductor devices. It discusses the most important aspects of the MBE apparatus, the physics and chemistry of the crystallization of various materials and device structures, and the characterization methods that relate the structural parameters of the grown (or growing) film or structure to the technologically relevant procedure. In this second edition two new fields have been added: crystallization of as-grown low-dimensional heterostructures, mainly quantum wires and quantum dots, and in-growth control of the MBE crystallization process of strained-layer structures. Out-of-date material has been removed.

Jacob Greenberg, The Hebrew University, Jerusalem, Israel

Thermodynamic Basis of Crystal Growth
P-T-X Phase Equilibrium and Non-Stoichiometry

2002. VIII, 249 pp. 126 figs., 28 tabs.
(Springer Series in Materials Science, Volume 44)
Hardcover. EUR 79.95; £ 56.00; sFr 133.
ISBN 3-540-41246-8

This book covers the thermodynamic foundations of inorganic materials science and the controlled synthesis of inorganic materials. A new thermodynamic approach to the non-stoichiometry of crystalline solids, known as vapour pressure scanning, has been developed by the author and is described in detail in this book. It is based on the high-precision experimental determination of the boundaries of the single-phase volume of the solid in the pressure-temperature-composition P-T-X phase space. This approach has been tested on a number of inorganic materials and has been shown to have an unparalleled precision (up to 10-4 at.%) in the determination of non-stoichiometry directly at high temperatures (up to 1200°C). Along with the results obtained by the author and his colleagues, the P-T-X diagrams of other important materials (e.g., III-V, IV-VI semiconductors) are also discussed.

Vitaly Shchukin, Nikolai N. Ledentsov, Dieter Bimberg, Technische Universität Berlin, Germany

Self-organized Formation of Semiconductor Nanostructures

2003. Approx. 250 pp. 70 figs., 10 in color.
(NanoScience and Technology)
Hardcover. Approx. EUR 64.50
ISBN 3-540-67817-4

The main focus of the book are physical mechanisms of spontaneous formation of ordered nanostructures at semiconductor surfaces. These mechanisms lie behind recent breakthroughs in advanced nanotechnology of quantum wire-and quantum dot fabrication. Generic theoretical models are presented addressing formation of all basic types of nanostructures, including periodically faceted surfaces, arrays of step-bunches of equal heights and single- and multi-sheet arrays of both 2- and 3-D islands. Decisive experiments on both structural and optical characterization of nanostructures are discussed to verify theoretical models and link them to practical examples. Experimental tools are described that enable one to intentionally control parameters of self-organized nanostructures, like chemical composition, shape, size, density and relative arrangement of quantum dots and wires.

Boyan Mutaftschiev, Universites de Paris VI et VII, Paris, France

The Atomistic Nature of Crystal Growth

2001. XIII, 368 pp. 98 figs.
(Springer Series in Materials Science, Volume 43)
Hardcover. EUR 94.95; £ 66.50; sFr 157.50
ISBN 3-540-66496-3

Crystal growth and nucleation are treated in the specialized literature in different ways depending on the discipline in question (physics, physical chemistry, chemical engineering) and on the theoretical approaches (atomistic vs continuum approach as regards crystal growth, phase vs chemical concept as regards nucleation). This book relates the different approaches to one another, giving preference to atomistic treatments by the methods of statistical thermodynamics and chemical kinetics. This unified approach also facilitates an understanding of some related phenomena of surface physics, such as adsorption, wetting etc. The book allows research novices and graduate students to get an insight into the physics of the phenomena and to interpret some of the experimental results.

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