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QUANTIFICATION OF THREE-DIMENSIONAL VASCULAR PATTERNS IN RENAL GLOMERULI

Elzbieta Kaczmarek

Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306-6000, USA

ABSTRACT

A method is developed for quantitative characterization of 3D capillary networks. Volume data was obtained by stacking up confocal epifluorescent images of renal glomeruli with a Zeiss CLSM. To provide the 3D data set, a region of interest containing an entire glomerulus was extracted from the volume data. A reconstruction of a capillary network with nodes representing transversal profiles of capillary lumens is made by applying a node-branch model of 3D vascular patterns. This network is used to derive the connectivity of the 3D vascular structure and the length and number of capillaries.

Key words: capillaries, confocal microscopy, glomerulus, image processing, 3D reconstruction.

INTRODUCTION

Observing vasculature of a renal glomerulus we find that the glomerular capillaries are derived from the afferent arteriole, which divides into several primary capillary branches. These branches are all connected and form one very complicated network. A quantitative characterization of capillaries has to relate their number, connectivity and total length to the space they supply as a reference volume. Unfortunately, quantification of capillaries on two-dimensional transverse sections alone suffers from a fundamental shortcoming: the true spatial capillary geometry is not considered. A capillary network should be described in 3D space. Therefore, the aim of this paper is to present a method for quantitative characterization of 3D branching patterns based on the path length distribution in networks.

MATERIAL

Rat kidneys were perfusion fixed in neutral formalin overnight and then washed in phosphate

buffered saline (PBS), sectioned on a vibratome into 100 or 200 micron sections, and stored in PBS until use. Each section was placed between two layers of a fine nylon mesh and inserted into a biopsy casette. The casettes were immersed in deionized water (dH_2O) and the sections washed thoroughly for 30 minutes. They were then stained with eosin. The eosin stain was done in aqueous solution of 0.025% eosin with 2 drops of glacial acetic acid per 50ml stain. The sections were stained for one or two hours. To wash out excess stain, the sections were placed in several changes of 95% ethyl alcohol until the alcohol remained clear. The sections were then dehydrated and cleared in two changes of absolute alcohol, 10 minutes each, one change of a 1:1 mixture of alcohol and xylene for 20 minutes, and one change of xylene for 20 minutes. The stained, dehydrated sections were removed from the casettes and placed in a watch glass and covered with a mixture of 1:1 immersion oil and xylene for 15 minutes, then drained and covered with a mixture of 2:1 oil and xylene for another 15 minutes. Finally, the sections were transferred to glass slides and mounted in immersion oil, coverslipped and sealed with nail polish. Two different immersion oils were used, with refractive indices of either 1.596 or 1.612.

3D IMAGE ACQUISITION, PROCESSING AND SEGMENTATION

Stack of epifluorescence images through the entire glomerulus was obtained using a Zeiss confocal scanning laser microscope. The light source was an Argon ion laser at 488 nm. An oil-immersion objective (40x/NA 1.3) was used to ensure a high confocal resolution. To obtain a better signal-to-noise ratio each line was scanned 8 times and averaged. Each confocal image of 256 x 256 pixels and 256 gray levels was obtained by nonlinear contrast adjustment during optical sectioning and averaging 8 image inputs (recorded in 1s each), to reduce noise. The distance between two subsequent light optical sections was approximately 0.5μm.. The final 3D image was thus made in a 256 x 256 x 256 region of interest (ROI) at the same resolution between sections and between pixels in sections. This image was then transferred to a Silicon Graphics workstation for image processing and graphic visualization.

To increase the visibility of capillaries image contrast has been expanded by edge sharpening gray scale convolution on the ROI and/or rank operations. Usually 7 x 7 top hat operator and sharpening filters have been used to enhance brightness of lumen's boundaries, and median filter to reduce noise. Capillary networks are not compact objects. Therefore, filtering in plane and stacking up enhanced images gives better results than uniform filtering in the three directions. Afterwards capillary lumens were extracted by thresholding from the 3D volume. Rendering of capillary surfaces has been made by using Advanced Visualization System on Silicon Graphics Work Station. An example of isosurface of glomerular capillaries is shown in Figure 1.

NODE-BRANCH MODEL OF 3D VASCULAR PATTERNS

Applying node-branch model of 3D vascular patterns we can now reconstruct capillary networks. Each node of the network corresponds to the center of a capillary profile. Centers were extracted using the center-of-gray algorithm based on finding the location of the "average" luminance interior to the profile's boundary. Nodes were connected to each other by network branches if and only if transversal capillary profiles on two consecutive sections were overlapping. This network can be used to derive the number and length of capillaries.

The Euler-Poincaré characteristic $N_3(G)$ is a basic measure of a network connectivity. In 3D space

$$N_3(G)=n_0-n_1+n_2-n_3 \qquad (1)$$

where $n0$ is the number of nodes, $n1$ is the number of edges, $n2$ is the number of faces and $n3$ is the number of volume units. In our 3D node-branch model however we do not consider faces and volume units, thus $n2=n3=0$.
The Euler number $N_3(G)$ could be enumerated without any assumption about the isotropy or shape of capillaries.

Let us denote a node-branch network as a graph G. The common neighbor distribution of a graph on $n0$ nodes is a sequence $(v_0, v_1, v_2, \ldots, v_{n0-2})$, where v_i denotes the number of pairs of nodes with i common neighbors (Buckley and Harary, 1990). In any graph G the mean number of common neighbors is

$$\mu_N(G)=\sum_{i=0}^{n_0-2}\frac{i v_i}{\binom{n_0}{2}} \qquad (2)$$

To assess total capillary length in a glomerulus we can calculate Euclidean distances between network nodes.
Let dij be the distance between nodes i and j in a connected graph G. The mean distance of a connected graph G is the average of the distances between pairs of nodes in G, and can be calculated as:

$$\mu_D(G)=\frac{\sum_{i=1}^{n_0} d_{ij}}{n1} \qquad (3)$$

When new capillaries eventually join the existing capillary network, then the connectivity number changes by one, and thus the Euler number makes sense as an estimator of the number of capillaries

$$N(cap)=N_3(G) \qquad (4)$$

Total length of capillaries can be estimated as

$$L(cap)=0.5\mu_N(G)*\mu_D(G)*n1 \qquad (5)$$

and the average capillary length can be derived easily from the following formula

$$l(cap)=L(cap)/N(cap) \qquad (6)$$

IMPLEMENTATION

Applying node-branch model these capillaries have been transformed into graphs (networks) representing connections between overlapping lumen's profiles in pairs of consecutive optical sections. An example of stereo pair of glomerular capillary networks is shown in Figure 1. Colors of branches have been assigned based on Euclidean distances between nodes in 3D system of coordinates. Nodes representing sequences of overlapping capillary profiles in consecutive sections have the same color. This edge coloring allows us to recognize glomerular lobules. To exemplify differences between properties of capillaries, their total number and length with average capillary length are presented in Table 1. Glomeruli B, C, D have similar number of capillaries, but capillaries in smaller glomeruli B and D are much longer than in bigger glomerulus C. This suggests significant differences in amount of blood they supply, and probably both axial pressure differences between the ends of individual capillaries within glomeruli, and significant pressure differences between the afferent and efferent arteriole. These results cannot be generalized. They are presented to give ideas how structural properties of capillary networks can be useful for understanding and interpretation of functions of renal glomeruli.

Table 1. The observed values of glomerular volume V, capillary number N(cap), capillary length L(cap) and average length of capillary l(cap) in example glomeruli (eosin stained).

Glomerulus	$V[mm^3 * 10^3]$	N(cap)	L(cap) [μm]	l(cap) [μm]
A	2293.4	65	3270	50.3
B	1971.3	47	6332	134.7
C	2776.9	52	4323	83.1
D	1629.8	50	7126	142.5

DISCUSSION

The capillary network of a renal glomerulus is one of the most complex structures to analyze, quantify, and reconstruct. Until now, most of the publications on 3D studies of glomerular capillary networks show serious problems with segmentation of capillaries (Shimizu et al., 1988; Nyengaard and Marcussen, 1993; Preston et al., 1995). That is why 3D reconstructions have come mainly from manual segmentation of capillary lumens on each of serial sections. Very recently, Preston et al. (1995) presented the 3D reconstruction of the human renal glomerulus based on 3D filtering. This was done at the expense of eliminating small capillaries. Hand traced 2D boundaries of capillaries were interpolated to 3D volume by using Ξ filters (Preston, 1991). These filters work very well for cutaway view of the stack of capillary sections.

The glomerular capillaries are derived from branches of the afferent vessels. Each branch generally establishes separate lobules, usually three per glomerulus. However, tufts with two, four or five lobules are also encountered (Shea, 1979; Yang and Morrisson, 1980; Winkler et al.,

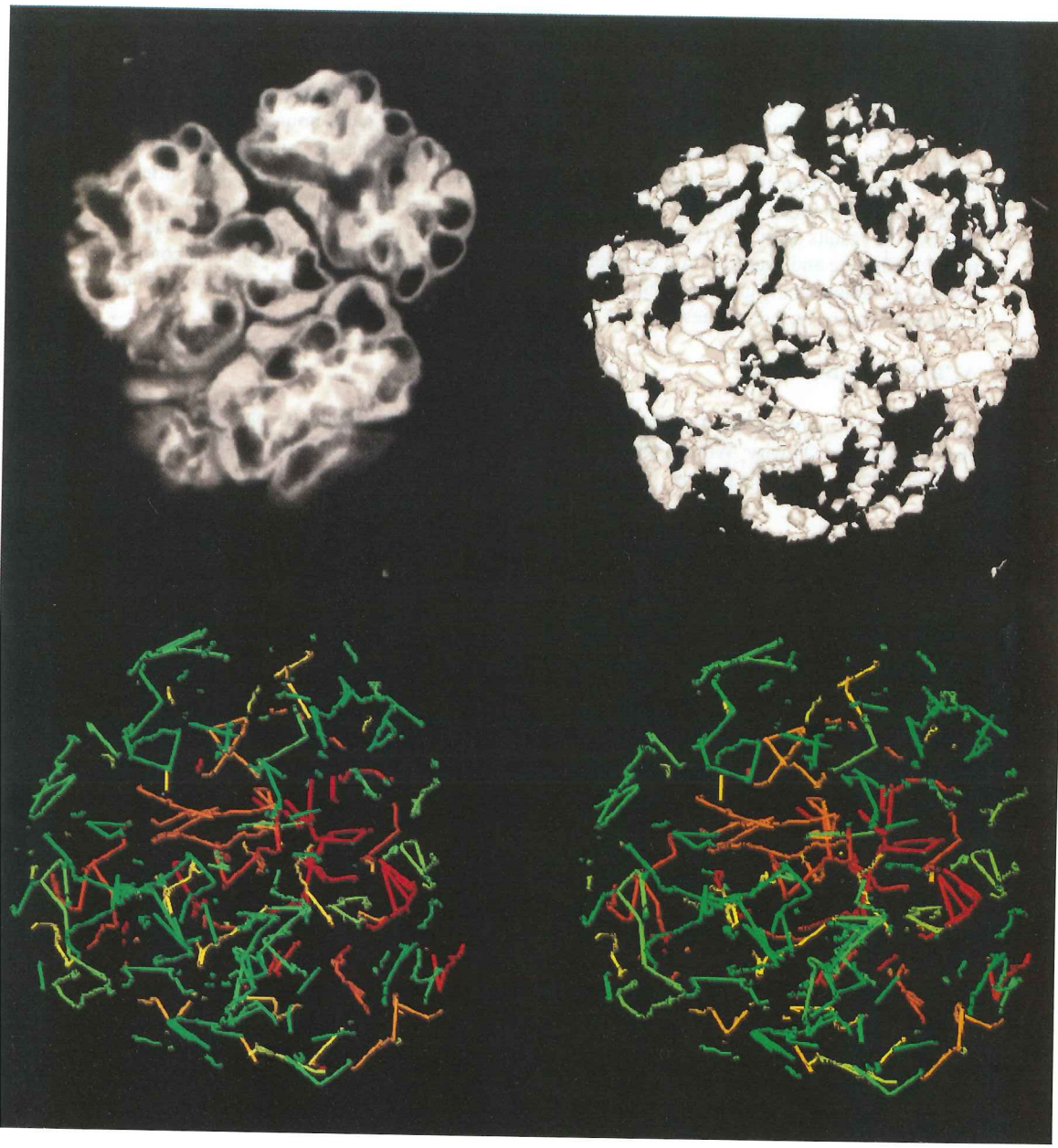

Fig. 1. Stereo pair of a three-dimensional network (bottom) representing capillaries rendering (top right) from a three-dimensional data set containing an eosin-stained glomerulus (top left).

1991; Nyengaard and Marcussen, 1993). Within the lobules capillaries form a network. The lobules are not strictly separated from each other. Winkler et al. (1991) have found connections between them ranging from only one to six connections between neighboring lobules. The spatial model of a capillary network presented by Preston et al. (1995) is simplified to connections between lobules and loses topological structure of capillaries within lobules.

Capillary networks presented in this paper were obtained from profiles segmented by image processing techniques with no preliminary hand tracing of lumen's boundaries. Before the segmentation, hand work has been made only to remove the "environment" of the glomerulus and process glomerular profiles exclusively. It seems that an automatic contrast adjustment during image acquisition might eliminate manual segmentation, reduce the time of image processing and improve the results significantly. Solution of this problem is not trivial, however. An alternative is truly 3D filtering and segmentation appropriate for branching patterns with non uniform contrast instead of traditional image processing techniques. However, solutions of these problems are not trivial and require further studies.

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