Three-dimensional morphometric analysis of the renal vasculature

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Abstract

Vascular topology and morphology is critical in the regulation of blood flow and the transport of small solutes including oxygen, carbon dioxide, nitric oxide and hydrogen sulfide. Renal vascular morphology is particularly challenging as many arterial walls are partially wrapped by the walls of veins. In the absence of a precise characterization of three-dimensional branching vascular geometry, accurate computational modeling of the intrarenal transport of small diffusible molecules is impossible. An enormous manual effort was required to achieve a relatively precise characterization of rat renal vascular geometry, highlighting the need for an automated method for analysis of branched vasculature morphology to allow characterization of the renal vascular geometry of other species, including humans. We present a semi-supervised method for three-dimensional morphometric analysis of renal vasculature images generated by computed tomography. We derive quantitative vascular attributes important to mass transport between arteries, veins and the renal tissue, and present methods for their computation for a three-dimensional vascular geometry. To validate the algorithm, we compare automated vascular estimates with subjective manual measurements for a portion of rabbit kidney. While increased image resolution can improve outcomes, our results demonstrate that the method can quantify the morphological characteristics of artery-vein pairs, comparing favorably with manual measurements. Similar to the rat, we show that rabbit artery-vein pairs become less intimate along the course of the renal vasculature, but the total wrapped mass transfer coefficient increases then decreases. This new method will facilitate new quantitative physiological models describing the transport of small molecules within the kidney.

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Introduction

The three-dimensional (3D) topology and morphology of blood vessels is of interest in many fields of renal anatomy and physiology. The renal artery originates from the abdominal aorta, while blood returns to the inferior vena cava via the renal vein. The renal vessels branch multiple times in a hierarchical manner forming arterial and venous trees. Larger intrarenal arteries and veins are generally paired, and are often intimately associated, with veins often observed to partially wrap a nearby artery (27). Further, a number of renal functional abnormalities can be associated with the vessel-structure. An example is renal artery stenosis, due to the formation of atherosclerotic plaque in the main renal artery, which can lead to increased renal vascular resistance, secondary hypertension and chronic kidney disease (11, 15). It is also thought that abnormalities in the structure of the walls of small arteries in the kidney might contribute to the pathogenesis of essential hypertension (3).

In order to characterize the morphology of vessels, volumetric imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) can be used to generate 3D reconstructions (33), with a resolution approaching micrometers under optimal conditions (37). Intravascular administration of a contrast agent renders blood vessel lumens radio opaque, so the vascular structure can be defined using X-ray CT (20). Initial studies of intrarenal vasculature were limited to volumetric statistics over anatomical regions of interest. For example Garcia-Sanz et al. quantified vascular volume for cortical and medullary regions of rat kidney from synchrotron CT images (12), while for a model of chronic nephropathy, Xie et al. used MRI to observe differences in vascular volume in anatomical sub-regions of the rat kidney (34).

When attempting to computationally model transport in a large number of vessels in a highly branched vascular structure, it is commonplace for vessels to be idealized as a series of cylindrical tubes of varying radii and length (35). However the actual vascular geometry can be crucially
important, since even apparently minor differences in anatomical details of the vascular geometry can have important physiological consequences, for example, modifying blood flow and the advective transport of solutes along the vessels, and modifying diffusive transport through vessels walls.

In the mammalian kidney a critical limitation in our current understanding of small molecule transport arises from the imprecise knowledge of geometric relationships between paired arteries and veins throughout the branched network (13, 14, 19, 20). Specifically, veins are often observed to partially wrap arteries (Fig. 1). It has been previously proposed that this wrapping may facilitate small molecule transport between these vessel pairs (27). But the resulting concave cross-section of veins in the renal vasculature is not captured when models are based on cylindrical tubes, and this can result in incorrect estimation of vessel separation distances and so mass transfer between vessels (19, 20).

In a previous work to more precisely define the renal vascular geometry, Yoldas and Dayan measured the topology and lengths of intrarenal arteries from endocasts of rat kidneys, obtained using an injection-corrosion technique, defining between-animal variations in large renal vessel branching and volumes (36). Sled et al. observed a high degree of similarity in the distribution of vessel diameters within mouse kidneys based on micro-CT images (31). Marsh et al. scanned polymer cast of a rat renal vasculature using low and high resolution micro-CT imaging (23). They measured the segmental lengths of arteries and afferent arterioles, and distances between arteriolar pairs to study vascular organizational patterns in the arterial-nephron network. However, three-dimensional structure of vessels was reconstructed by manual outlining. Most importantly, detailed insights into structural aspects of intrarenal vasculature for a single rat kidney were presented by Nordsletten et al. who combined high and low resolution micro-CT images to construct an (estimated) tubular geometrical model (28). Nordsletten et al. analyzed length, radius, cross-section
area and connectivity based on cylindrical tubes for each discrete order of arterial and venous vessels. Although cylindrical tubes are often useful to approximate hemodynamic vascular models (e.g. defining flow rates and pressure drops along the renal vascular circuit) (18, 30), such an approach averages out potentially important in vivo vessel morphology that influences local renal flow characteristics (e.g. local wall shear stresses). Cylindrical tubes are also inadequate for renal transport modeling. For example the actual structural morphology, peculiar to intrarenal vessels, involves characteristic wrapping of veins around arteries. Precisely defining wrapping is essential to our understanding of the physiological and pathophysiological importance of diffusive shunting between arteries and veins (27), and exchange with nearby tissues. An enormous manual effort was required to achieve a relatively precise characterization of the renal vascular geometry of the rat (19), highlighting the need for an automated method for analysis of branched vasculature morphology to facilitate characterization of the renal vascular geometry of other samples, and other species including humans.

In this paper, we propose a method for measuring the detailed geometric relationships of intrarenal blood vessels using micro-CT images, with a view to using these data in future computational models of renal physiology. The method includes scanning and processing images, manually labeling arterial and venous vascular networks, automatically extracting centerlines for the vascular topology and then automatically defining the morphological characteristics of the vessels. In the current study we used this approach to generate continuous quantitative information for the renal vasculature for a sample volume of a synchrotron CT image of a rabbit kidney. We compute 3D morphometric parameters including crucially important transport parameters such as arteriovenous (A-V) diffusion distance and wrapping fraction identified by Gardiner et al. (14), and compare them with subjective manual measurements. Unlike manual measurements based on 2D images, the automatic 3D morphometric parameter measurements are observed continuously along the vessel network. Based
on vessel radius, vessel separation distance and wrapping fraction, for the first time we estimate the mass transfer coefficient as a continuous function of distances from the origin of the renal artery.

Finally we discuss the implications of the proposed method and provide suggestions for its improvement.

Methods

CT scanning: The kidneys of New Zealand White rabbits were perfusion fixed and the vasculature filled with the radio-opaque silicone polymer Microfil®, as previously described (26). These studies were approved in advance by the Animal Ethics Committee of the Monash University Animal Research Platform. They conformed to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Images of the renal vasculature were generated at the Imaging and Medical Therapy Beam Line at the Australian Synchrotron. Moderate resolution scans were captured by an X-ray source of 32 keV. Scanning was performed by placing the specimen in the path of parallel beam and recording projections at uniform angular increments of 0.1 degrees as shown in Fig. 2A. Cross-sectional images were reconstructed by a (Linear-Ramp) Filtered Back-Projection algorithm using the XLI-XTRACT software (CSIRO, Australia) at a voxel resolution of 15.6 µm in both the object plane and the sample plane. The voxel values were converted from arbitrary units by linear scaling and normalization to the range (0, 255). The reconstructed slices were stored as 8-bit TIFF volumetric images.

Vascular segmentation: Segmentation is the process of assigning image voxels to anatomically or functionally meaningful volume categories. Much of the research in image segmentation has focused on using 2D image segmentation (8). Segmentation methods can be categorized as supervised (manual), semi-supervised (semi-automated) or unsupervised (completely automated). These methods have the potential to be extended to segmentation of 3D images, such as CT and MRI. Here
we implement a new semi-supervised method for 3D image segmentation to reveal physiologically important anatomic details of the renal vasculature.

A multi-seed ‘region growing’ algorithm (2) was implemented for segmentation of arterial and venous voxels. Region-growing works by grouping together voxels with properties similar to the seed voxels, so forming distinct regions (2). Multi-seed region growing takes into account the presence of multiple structures with uniquely labeled seeds. The following summarizes the multi-seed region-growing algorithm we implemented in MATLAB (Mathworks Inc., USA).

Initially, seed voxels are manually distributed in the vascular regions corresponding to distinct types of vessel (in our case, arteries and veins). The image voxels are then subjected to a grayscale thresholding to distinguish ‘vascular’ voxels from ‘non-vascular’ voxels (the threshold chosen depends on image contrast). Seeding and thresholding are the only essential manual steps required of this method. This is followed by the computation of geodesic distances of the vascular voxels from all seed voxels. Each vascular voxel is then designated a vessel-type based on the nearest seed voxel. A volumetric label image is generated which maps the vascular voxels to their identification as either an artery or vein. After smoothing the labeled image with an averaging filter, a 3D vascular geometry is obtained using iso-surface generation by connecting neighboring voxels of the same label (Fig. 2C). The geometry is a tessellated triangular mesh, which defines the vascular surface. Each triangle has a unique size (of the order of voxel resolution) and position which together vary to conform to the local shape of a vessel. The triangular faces share edges with their neighboring triangles forming a mesh in three-dimensional space. The generated mesh is simplified by reducing the number of triangles while preserving the overall shape of the vessels. The key benefit of mesh simplification is that it reduces the computational requirement for processing, analysis and visualization.
Vascular skeletonization: The vascular topology can be defined by a skeleton of vessel centerlines. Vascular centerlines are extracted using a mesh contraction algorithm (6). The algorithm achieves this by iteratively contracting the mesh until all vertices approximately converge to a curved centerline. The centerline is pruned, smoothed and converted to a skeleton as shown in Fig. 2D. A graphical representation of the skeleton allows identification of the branch intersections (nodes), vascular branches (edges) and their connectivity (adjacency). The information contained within an undirected graph \((G)\) of the centerline of the vessels is given in Table 1.

An undirected graph of vessel centerline can be converted to a directed graph \(G = (V, E)\) by defining a reference node which denotes the origin of ‘(fluid) flow’, and finding paths to all neighboring nodes using a graph search (16). The root node can be automatically detected as the vessel with maximum thickness or it can be specified manually. A directed graph of the vessel centerline enables determination of variation in vessel attributes in the direction of blood flow.

Morphometric analysis: We consider a vascular branch \(e_i\), whose luminal surface is characterized by tessellated triangular faces \(s_i\) and a vessel centerline characterized by a series of points \(p_{j,i} = 1,2, ..., m\), along the centerline of branch \(e_i\), which can be analyzed to estimate explanatory variables describing the vascular morphology. The procedure for computation of morphometric parameters is as follows:

Distance from root: The distance of a branch from the root vessel is the length of its shortest path along the vascular tree. It is a hierarchical indicator of a branch in terms of a continuous variable (i.e. distance). The distance from the root is measured with the help of graphical representation of the skeleton. The undirected graph edges are given weights equal to the vessel lengths \((L_i)\). The length of a vessel segment is calculated as the node to node geodesic distance traversed over the vessel centerline, viz,
Then, a graph search algorithm (9) determines the shortest route of each node \(v_k\) from the root vessel node \(v_r\). The distance from the root node is defined as 
\[
L^*_i = \sum_{j=1}^{n-1} \sqrt{(x_{j+1} - x_j)^2 + (y_{j+1} - y_j)^2 + (z_{j+1} - z_j)^2}
\] (1)

Radius: Radius is a well-defined attribute of cylindrical structures. However intrarenal vessels may not always have a circular cross-section. This is particularly true for intrarenal veins, which can be ‘bean shaped’ (14, 25). Nevertheless an approximate feature, indicative of the diameter of an arbitrary vessel can be estimated. The distance of each point along the vessel centerline \(p_j, p_{j+1}, ..., p_m\) to the nearest vascular surface is computed as:

\[
r_j = \min_j \|s_i - p_j\|_2, \quad j = 1, 2, ..., m
\] (2)

so the ‘median radius’ of branch \(e_i\) is given as

\[
\tilde{r}_i = \text{median}(r_1, r_2, ..., r_m)
\] (3)

Diffusion distance: The A-V ‘diffusion distance’ is estimated as the shortest distances from the arterial vessel surface to the nearest venous vessel surface, viz,

\[
d^A_j = \min_i \|s^V_i - s^A_j\|_2, \quad j = \arg\min_j \|s^A_i - p^A_j\|_2
\] (4)

where \(d^A \in \mathbb{R}^m\) are the shortest diffusion distances transferred to the centerline. The superscripts \(A\) and \(V\) are symbols to denote intrarenal arteries and intrarenal veins, respectively. Then, the median diffusion distance along a vessel is given by,

\[
\tilde{d}^A_i = \text{median}(d^A_1, d^A_2, ..., d^A_m)
\] (5)
Wrapping: The intrarenal arteries generally have a circular cross-section. However some intrarenal veins exhibit a ‘bean shaped’ (convex-concave) cross-section (25). The side of the vein facing a nearby artery is concave while the other side of the vein is convex. The extent of wrapping of an artery by a vein can be described by the following three independent parameters; (i) proximity of an artery to a nearby vein, (ii) curvature of the vein surface around the artery, and (iii) mutual orientation of the arterial and venous surfaces.

Within the region of wrapping, the proximity of an artery to its wrapped vein is directly determined by the diffusion distance. Surface curvature quantifies the convexity or concavity of a vessel surface. Gaussian curvature, defined as a product of two principal curvatures, $K = \kappa_1 \kappa_2$ determines the extent of local curvature of a surface (1). It is positive for a convex surface (bulging out), negative for a concave surface (bulging in) and zero for a planar surface.

$$K^A_i = K^V_j, \ j = \arg \min_j \| s^V_j - s^A_i \|_2$$  \hspace{1cm} (6)
where $K^A_i$ is the curvature at a point $j$ on the venous surface $s^V_j$ transferred to the nearest point $i$ on the arterial surface $s^A_i$.

The mutual surface orientation is defined as the scalar product of the arterial surface unit normal ($\hat{n}^A_i$) at point $i$, to the venous surface unit normal ($\hat{n}^V_j$) at its nearest point $j$.

$$\theta^A_i = \hat{n}^A_i \cdot \hat{n}^V_j, \ j = \arg \min_j (s^V_j - s^A_i)$$ \hspace{1cm} (7)
where $\theta_i \in [0,1]$.

Wrapping is defined as the fraction of the arterial surface ($S^A_i$) area within a specified angle and proximity of a concave venous surface.
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\[ W_i = \begin{cases} \frac{1}{\sqrt{S_i}} \left( S_i^A, \tilde{d}_i^A < \tau_d \land K_i^A > \tau_k \land \theta_i^A > \tau_\theta \right) \\ 0, \text{ otherwise} \end{cases} \]  

(8)

where \( \tau_d \in [0, \max_i(\tilde{d}_i^A)] \), \( \tau_k \in [\min_i(K_i^A), \max_i(K_i^A)] \) and \( \tau_\theta \in [0,1] \). The thresholds \( \tau_d, \tau_k \) and \( \tau_\theta \) control the influence of diffusion distance, curvature and mutual surface orientation respectively, on the calculation of \( W \).

The local mass transfer coefficient (MTC), \( k_{jAV} \), is a physiologically important parameter that can be derived from the previously mentioned basic parameters. The local mass transfer coefficient (local MTC) from an arterial branch \( e_i \) to its nearest vein is defined as,

\[ k_{jAV}/D = \sum_j \frac{W_j}{D_j}, j = 1, \ldots, m \]  

(9)

where \( D \) is the diffusion coefficient (taken to be one for convenience), \( W_j \) is the local wrapping percentage (expressed in percentage of the local arterial perimeter) and \( D_j \) is the local diffusion distance. The total local mass transfer of all branches \( e_i \) at some nominated distance from the root artery is:

\[ k_{iAV} = \Sigma k_{mAV} \]  

(10)

where the sum is taken across all branches at the same distance from the root artery.

We validated the proposed estimation method by comparison with subjective expert estimation of three manually measurable morphological parameters, namely the radius, diffusion distance and wrapping fraction. We have recently demonstrated that 2D manual measurements of these parameters from micro-CT images are in good agreement with manual measurements from the same vessel pairs generated from 2D histological images and light microscopy (26). Thus, the aim of the current comparative analysis was to observe the extent to which the techniques for 3D semi-automatic and 2D manual measurement of geometric parameters, from micro-CT, agree with each other.
Statistical methods

All statistical analysis were performed in MATLAB (Mathworks Inc., USA) using the Statistics Toolbox and the function GMREGRESS by Trujilo-Ortiz and Hernandex-Walls (32). It is assumed random errors are normally distributed, and all statistical tests employed a two-tailed \( p = 0.05 \) level of significance. To assess the validity of measurement methods, we employed two statistical analysis methods: (i) Bland-Altman plots (5) and, (ii) a combination of a Pearson correlation coefficient (\( r \)) and regression analysis. As both the manual and the semi-automatic measurements potentially contain random errors of similar magnitude, regression lines were fitted to measurements based on a geometric mean regression (otherwise known as ordinary least products regression) (22). The Pearson correlation coefficient provides an assessment of measurement precision, while the geometric mean regression assesses measurement bias (and so relative measurement accuracy). The degree of fixed bias was assessed from the regression y-intercept and its 95% confidence interval (CI), while the degree of proportional bias was assessed from the regression slope and its 95% CI. If the CI on the y-intercept contains zero, there is no statistically significant fixed bias (null hypothesis 1), while if the CI on the slope contains one, there is no statistically significant proportional bias (null hypothesis 2). Before beginning the regression analysis, we confirmed that the Pearson correlation coefficient is significantly different from zero at the two-tailed \( p = 0.05 \) level of significance (22).

Results

A 3D tissue region of 512×512×512 voxels (i.e. approximately 512 mm\(^3\) of tissue, or 5% of a whole kidney (10), involving around 130 million voxels) was sampled from a single whole rabbit kidney that had been perfusion fixed and filled with Microfil®. A grayscale threshold of 228 was applied to the volume. For segmentation of vessels, a total of 131 seeds (Arteries: 59, Veins: 72) were distributed in the volume. The arterial and venous geometries were composed of 129,875 and 165,583 triangles respectively, with an average triangular face of 325.3 μm\(^2\), none exceeding an
aspect ratio of approximately 30. The total length (i.e. sum of all vessel lengths) of arterial and venous vascular networks computed along the centerline was 56.0 mm and 49.5 mm, and the total vascular surface area was 42.4 mm$^2$ and 53.7 mm$^2$, respectively. For manual estimation by a single observer, many 2D cross-section images were generated from the sampled 3D volume. Each cross-sectional image was positioned at the center of an arterial branch and oriented orthogonal to the vascular centerline to facilitate manual measurement by the expert. A subset of 30 branches out of 47 were selected for manual measurements based on the image quality of the cross-section and the proximity of the artery to a nearby vein. The arterial diameter (microns), diffusion distance (microns) and wrapping fraction (%) were manually estimated for the 30 vessel branches of varying size and structure. All automated measurements were independently computed at each cross-section in a 3D region. The thresholds $\tau_d = 200$, $\tau_k = 0.5$ and $\tau_\theta = 55$ were empirically chosen for computation of wrapping.

There was excellent agreement between automatically and manually measured arterial diameters, with a correlation coefficient (r) of 0.99 (Fig. 3A). Agreement was generally better for larger vessels than for smaller vessels, suggesting some proportional bias (Fig. 3D). Considering the image voxel resolution of 15.6 $\mu$m, it is evident that the definition of the smaller vessels in the sample volume becomes a challenging task. Regression analysis showed that the 95% CI for the intercept does not include zero, suggesting some fixed bias (see Table 2). A closer investigation suggests that the automatic segmentation of vessels underestimates the distance between vessel boundaries, leading to smaller vessel diameter (we note that just two voxels explains nearly all the fixed bias). This fixed bias is confirmed by examination of the Bland-Altman plot (Fig. 3D and Table 3). This had relatively less impact on estimated diameter as the vessel diameter increased, which may explain some of the proportional bias (Table 2). While it was possible to relax the segmentation algorithm’s criteria for...
boundary definition, doing so lead to false segmentation results due to ambiguity in distinguishing close vessel boundaries. Crucially, this tradeoff could be reduced with higher image resolution.

For estimation of the diffusion distance (Fig. 3B) the correlation coefficient (r) is again pleasingly high at 0.98. Given the results for arterial diameter, unsurprisingly the regression analysis again suggests some fixed bias (though now just one voxel explains nearly all the fixed bias). Observation of the Bland-Altman plot for diffusion distances in Fig. 3E suggests that estimates by the two methods are generally better at larger diffusion distances. In agreement with the regression analysis (see Table 2), this is consistent with proportional bias.

The local wrapping of the artery by a nearby vein (expressed in percentage of the arterial perimeter) is presented in Fig. 3C. The correlation coefficient (r) was significantly less (at 0.89) than for the two other measures indicating less measurement precision. The automatic method resulted in under-estimation of the extent of wrapping compared to manual measurements. Nevertheless, there appeared to be no statistically significant fixed or proportional bias in either the regression intercept or slope (Table 2), suggesting that estimates generated by the two methods are in substantial agreement.

To better understand the causes of differences in estimates by the two methods, it is helpful to examine more closely individual cross-sectional micro-CT images. Two groups of micro-CT images were chosen: (i) those where estimated morphological parameters were similar, and (ii), those where estimated morphological parameters were dissimilar. For the majority of artery-vein pairs, there was remarkable similarity in the measures of arterial diameter and diffusion distance generated by the two methods (Fig. 4A-4C). However at some cross-sections the two methods provided disparate estimates. This was most evident for smaller vessels (see Fig. 4D-4F). Fig. 4D shows an example where the diameter is underestimated by the automatic method due to an incomplete labeling of the...
artery because of low image contrast. For Fig. 4E, the automatic method estimates a much shorter
diffusion distance. In this case, this automatic estimate may be valid because there could be a nearby
vein, not visible in the 2D cross-section image. Fig. 4F is an example of disagreement in the
wrapping fraction simply because of differences in method, suggesting an issue with reproducibility.
We note that most of the discrepancies occur with the smaller vessels, which suggests agreement
could be improved with increased image resolution.

Finally we determined how vessel attributes change with respect to the hierarchy of branches and
distance from the root artery in the vascular network within the sample volume (Fig. 5).
Unsurprisingly, proximal vascular branches tended to be both longer and larger than the distal
branches (Fig. 5A). But counter-intuitively, the diffusion distance tended to be shorter for proximal
branches than for distal branches (Fig. 5B). Geometric wrapping was high for proximal to
intermediate branches, becoming low for distal branches (Fig. 5C). The local mass transfer
coefficient was highly variable in larger vessels (e.g. vessel 15 in Fig. 6A), and moderately variable
between large vessels (e.g. compare vessel 24 with vessel 29 in Fig. 6A). However the local MTC
became more uniform for smaller vessels (Fig. 6A). Fig. 6B shows the integral of the local MTC
along the segment, while Fig. 6C shows the integral of the local MTC along the vasculature tree. Fig.
6D shows the (smoothed) total local MTC as a function of distance from the origin of the root artery.
It clearly demonstrates that, within the portion of the vasculature we analyzed, the total local MTC
tended to increase and then decrease with distance along the vascular network.

Discussion

While cylindrical tubes may be useful for some blood flow applications, the actual local geometry is
needed for estimating velocity profiles within vessels, for estimating local wall shear stresses (24) or
estimating red blood cell distribution (7) (e.g. Fahraeus effect). Similarly, the actual local vascular
geometry is required to estimate the diffusive transport of small molecules to and from arteries, veins
and tissues (27). In a previous study (28) the branched vessel structures found in the kidney were
represented as a series of connected cylindrical tubes, but this approximation leads to incorrect
estimates for arterio-venous (A-V) shunting of dissolved gases (e.g. oxygen, carbon dioxide, nitric
oxide, hydrogen sulfide etc.). To illustrate one such problem, in some cases a cylindrical tube
representation results in negative diffusion distances, particularly when vessels were geometrically
wrapped (14). As geometric wrapping is a characteristic feature of A-V renal morphology, such
unrealistic representations of the vascular geometry becomes a substantial problem. In contrast, the
criteria proposed in this paper for measurement of diffusion distance is robust and always positive.
To our knowledge, the current study is the first attempt to semi-automatically quantify vascular
wrapping continuously in 3D, a unique structural attribute of intrarenal vessels. Such data are
particularly valuable for estimating diffusion of gases and other small molecules between arteries,
veins, and tissue.

Renal vascular segmentation offers several challenges:

- Contrast: If the same contrast agent is used for arteries and veins, arteries cannot be
discriminated from veins based on voxel intensity;
- Proximity: As veins are often seen to wrap arteries in the kidney, boundaries between arteries
and veins may be indistinguishable when their separation distance approaches voxel
resolution;
- Size and number: The size and number of vessels varies substantially from the proximal to
the distal points in the pre-glomerular circulation. This poses significant challenges for
sampling, characterization and visualization of these structures.
However, provided there is sufficient image resolution, the method of analysis proposed here can overcome all these challenges and provide quantitative morphological data regarding artery-vein pairs in the renal vasculature. The region growing segmentation algorithm is able to delineate arterial and venous vessels semi-automatically, without the specification of any parameters. The method only requires the input image and initial seed locations. We also found the mesh-based vessel center-line extraction algorithm (see Fig. 2D) to be reliable and robust, in contrast to reports of other image-based (e.g. homotopic thinning) algorithms (21). This image-based skeletonization method was sensitive to noise and resulted in a large number of spurious branches. Furthermore it was computationally expensive, with a processing time of the order of $N^3$, where $N$ is a characteristic dimension reflecting image volume. In contrast, the mesh-based algorithm operated on a smoothed, simplified vascular mesh. This facilitated efficient extraction of the vessel topology, which is of practical importance.

The methods described above for feature extraction from the vascular geometry were validated by comparison with state-of-the-art, but nevertheless subjective, expert estimates of three manually measureable morphological parameters (i.e. diameter, diffusion distance and wrapping fraction). A comparatively simple but robust median distance approach (Eq. 5) was employed to estimate vessel diameter. Though still a single statistic, this is a more realistic criterion compared to estimates based on cylindrical or spherical approximations (29). We found the vessel diameter was well estimated using the median distance measurement, as verified by the excellent correlation coefficient, though there was fixed bias (about 2.0 voxels in size) and some proportional bias. We expect both would reduce with improved image resolution.

Diffusion distances between renal A-V vessels are often small (of the order of 10 to 40 microns). Given the resolution of the reconstructed scans was 15.6 microns, it is unsurprising that the diffusion distances were less accurate than diameter estimations, and the correlation coefficient decreased
somewhat. While fixed bias was detected (about 1 voxel in size), proportional bias probably arose because of increasing diffusion distance with diminishing vessel diameter. The criterion for estimation of wrapping fraction reported here demonstrated no bias, but the correlation coefficient was considerably less than those for arterial diameter and diffusion distance. In other words, the limited precision reduced the ability of the regression analysis to statistically uncover any difference in relative accuracy, though the relative accuracy is probably high given the regression slope is close to one.

The small underestimation of wrapping by the automatic method relative to the manual method can be attributed to several factors including segmentation irregularities. In addition, estimation of wrapping fraction requires the simultaneous application of a relatively complex set of rules. These rules are unambiguously stated mathematically in the automatic method of estimation, and so have the advantage of being completely reproducible, which represents a significant advantage over manual methods (4). These defined rules operating on a 3D vascular model are clearly very different to a subjective expert assessment of wrapping based on measurement of 2D cross-sections extracted from a volumetric image. Therefore, some differences between automated and manual methods are to be expected on this basis alone. Clearly, a comparison of quantities from different dimensional perspectives (2D-3D) is a source of divergence in estimates.

Based on our 3D analysis of the renal vascular morphology of the rabbit, unsurprisingly, we found the proximal vascular branches tend to be longer than the distal branches. But counter-intuitively, we found that the diffusion distance between arteries and veins tends to be shorter for proximal branches compared to distal branches. Both these findings are consistent with those previously reported for the rat kidney (20, 25). These previous studies were based only on an extremely time consuming, manual 2D analysis where the proximal-distal positions along the vascular network were inferred based on vessel diameter alone. Unlike previous automated studies (28), here we were able to report on vessel
wrapping based on 3D morphology. The artery wrapping fraction is high for proximal to intermediate branches, but reduces for more distal branches. Most importantly, we see that the physiologically important mass transfer coefficient is highly variable between, and even along, individual vascular branch segments. We also see that the total local MTC tends to increase with distance along the vascular tree, and then decrease. It is possible that this total local MTC increases again at even smaller vessel sizes (20), but this would require higher resolution images.

Importantly, we note that these observed continuous changes in 3D morphometric characteristics along the vasculature are much less uncertain than those based on 2D morphometric analysis of discrete cross-sections and interpolation between the sections. Although not reported here, there is potential to gain other physiologically useful insights by observing other morphometric attributes in a continuous hierarchical manner using a similar approach, including vascular volumes, branching angles and vascular-tissue surface area.

We note the application of the analysis approach presented here is limited by two major factors, moderate voxel resolution and relatively low vascular contrast, which together affect the performance of inter-vessel and intra-vessel discrimination and subsequent segmentation. We also note that the mass transfer coefficients are only calculated for a sub-region of the kidney centered on the renal artery. This is a limitation of the current analysis, but not of the method proposed for calculating 3D morphology of the arteries and veins in the pre-glomerular vasculature. It is expected that with improved scanning resolution and segmentation of vessels, more accurate estimates will be made possible on whole kidneys based on the method proposed. Finally we mention that if reference images with labeled voxels of arterial and venous vessels are available, then training of learning-based segmentation algorithms could be employed to fully automate the semi-automatic method described above, and potentially further improve accuracy (17).
Conclusions

Detailed vascular anatomy is required for physiological models of blood flow and small molecule transport within the kidney. Obtaining these data is very difficult, requiring enormous manual effort, which creates a serious practical bottleneck in development of computational models of renal function. Further, it is highly desirable to mathematically define the parameter estimation methods, as then precisely how estimates are made is unambiguous, thereby improving reproducibility of the data analysis. To overcome these issues, in the current study we developed a semi-automatic method for obtaining detailed topological and geometric data on the renal vasculature, and for the first time obtained continuous 3D data on vessel diameter, A-V separation and the degree of venous wrapping of arteries, a prominent feature in mammalian kidneys. These 3D data were validated using manual estimates of variables at 2D cross-sections. This revealed high precision, but two of the parameters automatically estimated showed some fixed and proportional bias. This bias is largely explained by moderate image resolution. Obtaining continuous data along the vascular tree using the semiautomatic method enabled the continuous estimation of the mass transfer coefficient for shunting of small molecules (such as oxygen, carbon dioxide, nitric oxide and hydrogen sulfide) between arteries and veins, along the vascular tree, albeit for only a small sub-volume of the kidney of a single rabbit. These data are consistent with the proposition that the local mass transfer coefficient varies significantly between branch segments, and even within branch segments, while the total local mass transfer coefficient increases and then decreases with distance along the vascular tree.

Acknowledgements

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Disclosures

None

References


Table 1: Information from a skeletal graph. A graph is characterized by nodes (branching points or end-points) and edges (centerlines).

<table>
<thead>
<tr>
<th>Nodes</th>
<th>Edges</th>
</tr>
</thead>
<tbody>
<tr>
<td>(v_k) node of intersection</td>
<td>(e_i) edge connecting two nodes</td>
</tr>
<tr>
<td>(e_1, e_2, \ldots) edges common to node (n_k)</td>
<td>(v_s, v_t), source and terminal nodes of (e_i).</td>
</tr>
<tr>
<td>(v_1, v_2, \ldots) neighbors of node (v_k)</td>
<td>(\langle p_1, p_2, \ldots, p_m \rangle) physical coordinates of edge (e_i).</td>
</tr>
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</table>

Table 2: Mean geometric regression analysis of fixed and proportional bias between manual and automatic measurements for the diameter, diffusion distance and wrapping of arterial vessels.

<table>
<thead>
<tr>
<th>Regression Intercept (95% CI), Fixed Bias</th>
<th>Regression Slope (95% CI), Proportional Bias</th>
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</thead>
<tbody>
<tr>
<td>Diameter ((\mu m))</td>
<td>-34.2 (-48.6,-19.9), Y</td>
</tr>
<tr>
<td>Diffusion Distance ((\mu m))</td>
<td>13.0 (5.6,20.4), Y</td>
</tr>
<tr>
<td>Wrapping (%)</td>
<td>1.27 (-2.7,5.2), N</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Regression Intercept (95% CI), Fixed Bias</th>
<th>Regression Slope (95% CI), Proportional Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter ((\mu m))</td>
<td>1.07 (1.02,1.13), Y</td>
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<tr>
<td>Diffusion Distance ((\mu m))</td>
<td>0.74 (0.68,0.79), Y</td>
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<tr>
<td>Wrapping (%)</td>
<td>0.97 (0.8,1.1), N</td>
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CI = confidence interval

Table 3: Bland-Altman plots with means and 95% confidence intervals: analysis of differences between manual and automatic measurements for the diameter, diffusion distance and wrapping of arterial vessels.

<table>
<thead>
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<th>Mean (95% CI)</th>
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<tbody>
<tr>
<td>Diameter ((\mu m))</td>
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<td>Diffusion Distance ((\mu m))</td>
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<tr>
<td>Wrapping (%)</td>
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CI = confidence interval
Figure Legends

**Fig. 1.** The phenomenon of venous ‘wrapping’ of renal arteries. Panels A and B show two examples of cross-sectional micro computed tomographic images of the characteristic partial wrapping of the wall of a vein (V) around the wall of an artery (A) in the renal vasculature of a rabbit. Pie-sectors visually indicate the approximate fraction of the arterial surface wrapped by a nearby vein.

**Fig. 2.** Schematic of the renal vascular image acquisition and processing. (A) Renal sample is scanned by a micro-CT X-ray scanner. (B) Scans are reconstructed to generate a three dimensional volumetric image. (C) Image segmentation is carried out to delineate arterial and venous vessels from the background. (D) Vascular geometry is analyzed to generate a 3D vascular skeleton which is labelled with unique indices.

**Fig. 3.** Comparison of manual and automatic measurements. Panels A-C show pairwise scatter plots of manually and automatically measured parameters (3A arterial diameter, 3B diffusion distance and 3C wrapping fraction). Regression lines of best fit were determined by ordinary least products (i.e. geometric mean regression). \( r \): correlation coefficient. All manual and automatic measurements have \( p < 0.05 \) indicating a significant correlation. Panels D-F show Bland-Altman plots of differences between pairwise manual and automatic measurements against their mean. Points grouped vertically closer to the mean difference line are in better agreement than those more distant.

**Fig. 4.** Comparison of manual and automatic measurements of arterial diameter, arteriovenous diffusion distance and wrapping for sample images. Panels A-C show examples of good agreement between manual and automatic measurements (Panel A reused from Fig. 1B). Panels D-F show examples of poor agreement between manual and automatic measurements. In each panel, an original cross-sectional micro-CT image is presented alongside one with an overlaid arterial and venous
vessel. The morphometric measurements of both automatic and manual methods is also presented for each sample. A: Artery, V: Vein, Dia.: diameter, Dif.: diffusion distance, Wrap.: wrapping.

**Fig. 5.** Variation in morphological parameters with the distance into the arterial vascular network (distance from the origin of the root artery). (A) Vessel size (diameter) (B) Diffusion distance. (C) Wrapping fraction. Each vessel is labelled with a branch identification number. Each branch thickness and color is proportionally scaled to the parameter of interest (i.e. with vessel size, diffusion distance and wrapping fraction).

**Fig. 6.** Mass Transfer Coefficients (MTCs). Branch ID labels same as Fig. 5. (A) Local MTC (Eq. 9) for each vessel branch showing local MTC variation along each branch. (B) Total MTC for each individual branch, by integrating the local MTC along each branch segment. (C) Cumulative total MTC, calculated by summing all the total MTCs for all branch segments shown in Fig. 6A up to a nominated distance into the vascular tree. (D) Smoothed local total MTC, calculated by first approximating the S-shaped curve shown in 6C with a cubic polynomial, and then differentiating to obtain the local total MTC (Eq. 10).
Figure 3

A. Arterial diameter

B. Diffusion distance

C. Wrapping

D. Arterial diameter

E. Diffusion distance

F. Wrapping
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