Accuracy of Microvascular Measurements Obtained From Micro-CT Images

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Abstract—Early changes in branching geometry of microvasculature and its associated impact on the perfusion distribution in diseases, especially those in which different branching generations are affected differently, require the ability to analyze intact vascular trees over a wide range of scales. Micro-CT offers an excellent framework to analyze the microvascular branching geometry. Such an analysis requires methods to be developed that can accurately characterize branching properties, such as branch diameter, length, branching angle, and branch interconnectivity of the microvasculature. The purpose of this article is to report the results of a study of two human intramyocardial coronary vascular tree casts in which the accuracy of micro-CT vascular imaging and its analysis are tested against measurements made through an optical microscope (used as the “gold-standard”). Methods related to image segmentation of the vascular lumen, vessel tree centerline extraction, individual branch segment measurement, and compensating for the non-ideal modulation transfer function of micro-CT scanners are presented. The extracted centerline accurately characterized the hierarchical structure of the vascular tree casts in terms of “parent–branch” relationships which allowed each interbranch segments’ dimensions to be compared to the optical measurement method. The comparison results show a close to ideal 1:1 relationship for both length and diameter measurements made by the two methods. Combining the results from both specimens, the standard deviation of the difference between measurement methods was 19 μm for the measurement of interbranch segment diameters (ranging from 12 to 769 μm), and 172 μm for the measurement of interbranch segment lengths (ranging from 14 to 3252 μm). These results suggest that our micro-CT image analysis method can be used to characterize a vascular tree’s hierarchical structure, and accurately measure interbranch segment lengths and diameters.

Keywords—Centerline extraction, Hierarchical descriptor, Modulation transfer function, Point spread function, Vascular branching geometry, Vascular imaging, Vascular tree.

INTRODUCTION

The state of microvasculature can reflect and/or be the cause of pathophysiological changes in organ function. Analysis of 3D tomographic images can be used to characterize the branching geometry of the microvasculature—but due to the huge logistic problem, automation of such an analysis is essential. At a basic level of investigation it is sufficient to examine the density or overall pattern of vasculature, but a more detailed analysis of the microvasculature, which deals with the tree’s hierarchy as well as its physical (and fluid dynamic) properties, provides quantitative insight into its capacity to transport blood throughout an organ. In particular, how the spatial heterogeneity of perfusion distribution relates to the branching geometry of the microvasculature, is an important question which has been addressed by various investigators.^{3,14,22,29}

The measurement of length, diameter, and the branching angles and connectivity relationship between interbranch segments lie at the foundation of almost all vascular bed characterizations. For example, using segment diameters and interbranch segment relationships, wave reflection effects in a right coronary artery were studied⁴⁷ and a model of a branching tree was developed.²⁵ To measure these properties of the vasculature, a technique with a high degree of accuracy is needed that can deal with both the wide range of dimensional scales and the inherent complexities of the microvasculature. Although many attempts have been

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made to automate such image analysis processes, a major problem has been the lack of accurate and precise measurements (or a “gold-standard”) against which such programs could be evaluated. Qualitative parameters are generally used to establish the utility of a newly developed image analysis algorithm. In this sense, the fact that a vessel was found or not found, the extracted centerlines were smooth or not smooth, a noise “blob” was removed or not removed, these are the measures used to justify the effectiveness and accuracy of a particular method. The gold-standard used in this study is the optical measurement of the branches of intact casts of vascular trees, with each vascular segment mapped out and measured using micrometer translation and rotation.⁴⁶ Based on these optical measurements, the tree’s hierarchical structure in terms of interbranch segment connectivity, individual branch segment lengths, and individual branch segment diameters becomes “known”. This study evaluates the accuracy with which 3D micro-CT image data can convey this information of vascular branching geometry, as compared to direct optical measurement of vascular trees.

The optical measurement method is thought to be more accurate and is therefore used as the gold standard for testing the measurements obtained from micro-CT. Casts, on which the gold-standard is based, are frequently used for measuring vascular branching networks.^{39,46} However, such a method typically loses information pertaining to the interconnectivity of the branch segments due to the fact that often the casts are physically broken in order to measure the physical dimensions of interbranch segments. Statistical analysis approaches are then used to say something general about the characteristics of that vascular bed.³⁷ The method of optical measurement is very time-consuming, labor-intensive, and highly inefficient. Therefore, the automated method analyzing micro-CT images is of great benefit. In work previously presented by Zamir,⁴⁶ more than a year was spent doing measurements of the vascular tree from a right coronary artery, and a few months were spent on *h61* and *r14*, the two human coronary artery casts used in this study. Also, because the measurements are made from a cast of the vascular tree, the hand measurement method is somewhat destructive in the sense that the vascular bed is isolated from the perfused tissues, which are destroyed in the process. Hence, the method of casting is not applicable to the tissue of a living specimen. The cast was used solely to check the accuracy of our approach and could be thought of, rather, as a test phantom (i.e., a structure of “known” dimensions).

Using the automated analysis of micro-CT images, instead of optical measurements of a cast, saves time and allows analysis of highly complex tree structures.

More important, using micro-CT images of *in situ*, opacified microvasculature, instead of an isolated cast, offers the opportunity to extend the technique to *in vivo* analysis. While this article actually uses the micro-CT images of the casts, this was done only for the purpose of establishing the accuracy of the image analysis method.

Quantifying the impact that the vascular branching geometry has on blood flow distributions still involves uncertainties attributable, in part, to measurement inaccuracies, as well as the fact that measurements are obtained from somewhat distorted (due to casting and/or imaging process) representations of the vascular tree, or the fact that the representations are a “snapshot” taken from a dynamic geometry (due to cardiac pulsation and/or dynamic smooth muscle tone). In addition, the micro-CT imaging process introduces blurring due to the modulation transfer function (MTF) of the scanners imaging system,¹⁵ and partial volume effects due to pixelization of the image data.⁸ Variance in the gray scale of the resulting image also depends on the tomographic approach used (e.g., number of angles of view, cone vs. parallel beam, etc.¹⁰). The effect of blurring disproportionately affects the accurate determination of small vessel diameters. In any real imaging system, the MTF is non-ideal in that the MTF values drop at higher spatial frequencies. This results in the apparent broadening of small vessel diameters, as well as decreases of the CT-value found in opacified small vessel lumens. To account for this, a method was developed that incorporates both a vessel lumen’s local gray-scale value, and the fact that the gray scale of the luminal contents should always have a fixed value.

Previous image analysis methods contain significant problems, including large computer memory requirements and long run-times,^{9,42} post-processing manual tree editing procedures and problems dealing with small vessel measurements,^{30,45} among others. The present micro-CT analysis differs from others^{18,24,43} in that it has an objective basis for evaluation of the image analysis approach’s accuracy and thereby can provide added insight into the accuracy of the vessel lumen centerline, and interbranch length and diameter measurements. The extracted luminal centerline provides information regarding the interconnectivity of the branch segments and is also used for the measurement of interbranch length and diameter. Our centerline extraction method contains modifications of existing methods—modifications which were found to improve such centerline properties as basic centeredness (i.e., how well the centerline is actually centered within tortuous paths), the accurate representation of bifurcations, and automatically finding vessel tree endpoints.

Finally, having created an accurate measurement method, we will be able to study questions such as vessel tree branching patterns. This includes the relationships between parent and its branch segments (parent is the root segment that bifurcates into two branch segments) and whether the branching pattern as a whole is responsible for, or reflects, the spatial distribution heterogeneity of perfusion. Another is the question of how well a micro-CT scan of a vascular tree, which is a snap shot in time, represents the dynamic tree geometry. For instance, how important is the diameter change caused by the pressure pulses resulting from the contraction of the heart? This question can only be answered by doing *in vivo* scans at many time points within a single cardiac cycle. However, *in vivo* we do not have a way of determining the accuracy of measurements, and thus an in depth comparison, as performed in this study, is aimed at assessing that accuracy.

METHODS

Gold-Standard

The casting method used was discussed in more detail by Zamir and Chee⁵⁰ and is here more succinctly described. The casts were produced using a partially polymerized methylmethacrylate plastic (Batson's No. 17 corrosion-casting compound: Polysciences Inc., Warrington, PA, USA), adding the red color dye for observation. This initially freely flowing liquid was injected through the main coronary ostia, maintaining

a pressure between 100 and 120 mmHg. The vascular microcirculation was reached within the first few minutes. Within half an hour setting occurred, resulting in a rigid cast of the vascular lumens. The myocardial tissue was digested by immersing the heart in a 34% potassium hydroxide solution for several hours and then rinsing in warm water. This process was repeated as necessary, with the end result being an accurate replica of the coronary vasculature.

Displayed in Fig. 1 (left) is a micrograph of the cast of *h61*, which is a subsegment (or piece) of the entire vasculature. A detailed analysis of each of two casts was carried out by optical measurements of individual branch lengths and diameters depicted in micrographs. The samples were placed under the microscope and the vascular segments were mapped and measured utilizing micrometer translational and rotational movements.⁴⁶ The tree's hierarchical structure was labeled in (j,k) notation (see Figs. 9.2.3–9.2.4 of Zamir⁴⁸) as illustrated in Fig. 2. Under this notation, ' j ' is the level (or generation) and ' k ' is the sequence within that generation of the individual branch segment. Also, under this labeling scheme, the larger diameter branch receives the odd ' k ' sequence label (i.e., the larger diameter branch of (1,1) is (2,1), and the smaller diameter branch is labeled (2,2)).

For these measurements, the bifurcation point was defined as the point at which the extrapolated centerlines of the parent and its two branches meet. If a centerline was curved then it was replaced by its tangent at the bifurcation point. Diameters were measured at three places along a vessel segment and an average

FIGURE 1. Left panel: microscope digital photograph of specimen *h61*. Right panel: maximum intensity projection of reconstructed micro-CT image.

FIGURE 2. The j,k notation used for labeling the vessel segments in a tree structure, adapted from Zamir.⁴⁸ In general if j, k are the coordinates of a parent segment, then the coordinates of the two branches are $j + 1, 2k - 1$, and $j + 1, 2k$, respectively, the latter being the branch with the smaller diameter. In particular, if the root segment is labeled as 0,1, then the labels of its two branches will be 1,1 and 1,2, respectively, the latter being the branch with the smaller diameter.

was taken. These methods pertaining to the measurement of vessel segment diameter and vessel bifurcation representation are also discussed in Zamir and Brown.⁴⁹ The segment length was taken as the length of the centerline connecting the proximal and distal bifurcation points of the segment (or an endpoint of the terminal vessel segment). If the centerline (i.e., branch segment) was curved then it was divided into smaller sub-segments and the chord lengths of each sub-segment were added. Finally, matching the individual segments in the micro-CT image to the schematics from these optical measurements allowed for the definitive, branch-to-branch, comparison of micro-CT measurements of the individual branch segments' lengths and diameters.

Micro-CT Imaging

Micro-CT images of the two casts were used as the volume data sets for analysis. Figure 1 (right) is the maximum intensity projection of *h61*, visualized with the Analyze software package.³⁰ The micro-CT imaging methods that were used are discussed in more detail elsewhere.¹³ In brief, the vascular cast was mounted on a computer-controlled rotating stage so that an X-ray projection image was generated on a CsI crystalline plate, which converted the X-ray into a light-image, at each of 360° angles of view around 360°. This image was optically projected onto a CCD imaging array which converted the light intensity on each of the 1024×1024 24 μm on-a-side square pixels in the array to an electronic signal proportional to the light (X-ray) intensity. The data, once recorded, were subjected to a modified Feldkamp cone beam reconstruction algorithm⁷ to generate a 3D volume data set. The 3D image consisted of up to 1024^3 cubic voxels, each 18 μm to a side, with gray scale equal to the X-ray attenuation coefficient in units of 1000 cm^{-1} .

Segmentation

A binary file was first computed from a thresholding of the gray-scale 3D micro-CT images. Nearly all of the background (i.e., not cast) image noise could be filtered out through the technique of thresholding because the signal (i.e., the gray scale of the cast material) and the background were divided into two "well-separated" distinct gray-scale populations. The vascular trees were extracted from this image by means of a region-growing segmentation method to include those voxels, with gray-scale values above the thresholded range, that were connected to the vessel tree's root which was initially identified by visual inspection. In order to include small vessel segments, a low CT-threshold of 400 (much lower than half the max value found in large vessel lumens) was chosen in order to include vessel segments that have a reduced peak gray-scale value caused by blurring. The region-growing method resulted in a single connected tree structure for both specimens, *h61* and *r14*.

Centerline Extraction

The segmented volume data set was used as the input for the extraction of the vascular tree's centerline or "skeleton". This is the first step in defining the vascular tree topology. The extraction of the vascular tree centerline allows for the simplification of the complex tree structure from which the branch segment location within the tree, and its connection to the other branch segments, is conveyed.²³ In the case of *h61* and *r14*, this graph is a binary tree in that one parent bifurcates into two branch segments. Using locations along the centerline within the segmented image, the tree's hierarchical nature, branch lengths, and branch diameters can subsequently be determined.

Our centerline extraction method utilizes the fast marching algorithm,³⁵ which simulates the propagation of a wave. This algorithm begins at a source, in this case a single voxel at the root of the tree, and emanates a wave in all directions. This propagating wave is governed by a weighting map which specifies how fast the wave can travel at each voxel it touches as the wave radiates outwards. The weight incorporates details of a vessel voxel's distance to the closest segmented surface. The weight function is such that the wave propagates only inside the lumen of the tree. As the wave passes over each voxel, how long it took the wave to get to that voxel is recorded. Therefore, this arrival time is a function of both the distance a particular voxel is from the source of the wave front, as well as the weighting function that the wave front experiences along the way.

In $\mathbb{R}^3$ we begin with a potential function $F(x)$ that weights vessel tree voxels according to their distance from the vessel wall. The weighted geodesic distance (later referred to as the “arrival time function” under the fast marching algorithm’s framework) between two points $x_0, x_1 \in \mathbb{R}^3$ is given by²⁷

$$U(x_0, x_1) = \min_{\gamma} \left(\int_0^1 \frac{\|\gamma'(t)\|}{F(\gamma(t))} dt \right), \quad (1)$$

where γ is the geodesic curve with $\gamma(0) = x_0$, $\gamma(1) = x_1$, and $\|$ represents the Euclidean norm (i.e., the vector length). In the case that $F(x) = 1 \forall x$ then the integral in Eq. (1) is simply the length of γ and U is the Euclidean (straightline) distance between x_0 and x_1 . To obtain the arrival time function (weighted geodesic distance), we adapted the Eikonal equation³⁵:

$$\|\nabla U\| = \frac{1}{F}, \quad (2)$$

where U is the arrival time function and F corresponds to the weight function or the “speed” of the front progression (we defined F to be the square of the distance map, as discussed later). This equation is solved by computing the arrival time function U at location (x, y, z) using the arrival time value of its neighbors.

Due to the discrete nature of the gradient ∇U in the voxel-based representation of the vessel tree by micro-CT imaging, the corresponding discrete solution to Eq. (2) is given by²⁶

$$\left[\begin{array}{l} \max(D_{xyz}^{-x} U, 0)^2 + \min(D_{xyz}^{+x} U, 0)^2 + \\ \max(D_{xyz}^{-y} U, 0)^2 + \min(D_{xyz}^{+y} U, 0)^2 + \\ \max(D_{xyz}^{-z} U, 0)^2 + \min(D_{xyz}^{+z} U, 0)^2 \end{array} \right]^{1/2} = \frac{1}{F_{xyz}}, \quad (3)$$

where D represents the forward and backward differential operators from standard finite difference notation, as in

$$\begin{aligned} D_{xyz}^{-x} U &= \frac{U_{x,y,z} - U_{x-1,y,z}}{h_x}, & D_{xyz}^{+x} U &= \frac{U_{x+1,y,z} - U_{x,y,z}}{h_x} \\ D_{xyz}^{-y} U &= \frac{U_{x,y,z} - U_{x,y-1,z}}{h_y}, & D_{xyz}^{+y} U &= \frac{U_{x,y+1,z} - U_{x,y,z}}{h_y} \\ D_{xyz}^{-z} U &= \frac{U_{x,y,z} - U_{x,y,z-1}}{h_z}, & D_{xyz}^{+z} U &= \frac{U_{x,y,z+1} - U_{x,y,z}}{h_z} \end{aligned} \quad (4)$$

where (x, y, z) in the above equations represent the 3D location indices of a particular voxel, and h_d is the voxel spacing in the corresponding (d subscripted) direction. The zeros and min-max operators cause only voxels, whose arrival time value has already been computed, to be considered when solving

Eq. (3). An easily implemented “upwind” approach³¹ was used:

$$\left[\begin{array}{l} \max(D_{xyz}^{-x} U, -D_{xyz}^{+x} U, 0)^2 + \\ \max(D_{xyz}^{-y} U, -D_{xyz}^{+y} U, 0)^2 + \\ \max(D_{xyz}^{-z} U, -D_{xyz}^{+z} U, 0)^2 \end{array} \right]^{1/2} = \frac{1}{F_{xyz}}, \quad (5)$$

During the fast marching algorithm, voxels pertaining to the vessel tree may be thought of as having one of the following labels: “frozen” (the arrival time value has been determined for the voxel), “narrow band” (an arrival time value has been computed for the voxel, but the value may not be the voxel’s final arrival time value), and “far” (the arrival time has not been computed for the voxel). The algorithm starts from a seed voxel (vessel tree root) and calculates the arrival time outwards, away from the seed voxel. The initial seed voxel is “frozen” and distances to neighbors are computed according to Eq. (5). The voxels that are computed are now a part of the “narrow band” voxel list. At each step of the algorithm the “narrow band” voxel having the smallest arrival time becomes “frozen” (removed from “narrow band” list) and the neighbors’ arrival times are updated as long as they are not already “frozen”. The algorithm’s speed is greatly increased through the use of heap structures (such as heap-sort³⁴) that can be used to efficiently keep track of “narrow band” voxels having the minimum arrival time value. The use of heap-sort gives the fast marching method a computational cost of $\mathcal{O}(N \log N)$, for N voxels within the vascular tree.

One particular difficulty that was overcome was automating the determination of the vascular tree’s endpoints (i.e., ends of terminal branch segments). By first propagating a wave with no weighting other than the binary representation of the object, allowed the vascular tree’s voxels to be labeled according to their path distance (through the vascular tree) from the vessel tree root. Labeling the background as zeros, the voxel distances were grouped into regions at relatively the same distance (e.g., by normalizing the individual voxel distances to the largest distance and then multiplying by a constant in order to cluster voxels into groups), and the endpoint locations were therefore regional maxima (i.e., the grouping of voxels with higher distance values than their neighbors). Each regional maximum cluster was then searched for a single voxel having the greatest arrival time value. Above a constant multiplier value of 100, the number of endpoints found was observed to plateau (i.e., the same number of endpoints was found) signaling convergence of the endpoint finding process.

The arrival time function, as determined through the fast marching method, can also be thought of as a

type of geodesic distance (i.e., the shortest path over a curved surface, in this case the “surface” represented by the spatial distribution of the arrival time value of surrounding voxels) from any voxel to the wave front’s source voxel (thus finding the geodesic curve γ from x_0 to x_1 in Eq. (1)). Propagating a second fast marching wave to map the object in terms of the arrival time (weighted by a squared distance map), geodesic paths (vascular tree centerlines) were extracted by means of solving a back propagation equation⁴ (e.g., gradient steepest-descent) of the form:

$$\frac{dC(t)}{dt} = -\nabla U, \quad (6)$$

where $C(t)$ is the parametric curve that is used as the object’s centerline (with $C(0) = x_1$), and U is the geodesic distance (arrival time function).

Once the path was extracted, any voxel with a path passing through it was labeled. A weighted comparison between voxels was then used to remove voxels that were not necessary to keep the vascular tree (via one of 26 contiguous neighbor voxels) connected. Taking the extracted paths into a voxel-based representation allowed for later delineation of individual branch segments.

In summary, there were two fast marching waves that were propagated in order to extract the vessel tree’s centerline. The first wave acted on a binary representation (i.e., a weight function that was constant throughout the tree). The arrival time function that was found for this first wave was used for determining endpoint locations. The second wave that was propagated acted on a distance map representation of the vascular tree, a map which had its values squared in order to increase the weighting at the center of vessel lumens. This arrival time function was used to extract the centerline paths from each endpoint to the root (i.e., solving Eq. (6)).

Finally, the process of labeling branch endpoints and bifurcations was automated and individual branch segment voxels (as determined by the extracted centerline) were ordered in a connected voxel list as a pre-processing step to subsequent measurements. This allowed branch segments to be distinguished, labeled, and analyzed individually. The process of automatically labeling the micro-CT vascular tree’s centerline in (j,k) notation allowed individual branch segments to be compared with the optical measurements. Due to the fact that some branch segments were physically lost before the micro-CT scan was performed, this matching process was performed visually to account for the lost segments.

Vessel Branch Measurements

Once the vascular tree’s centerline was extracted and labeled, measurements of the individual branch

segment’s length and diameter were performed. The measurement of an individual branch segment’s length was determined by moving from one voxel to the next in the list of ordered centerline voxels, calculating the Euclidean distance between contiguous voxels, and summing the distances, as in

$$L = \sum_{i=1}^{i=n} \sqrt{(x_i - x_{i+1})^2 + (y_i - y_{i+1})^2 + (z_i - z_{i+1})^2}, \quad (7)$$

where n is the number of voxels of the branch segment, i is a reference to the voxel at location (x, y, z) , and L is the computed length.

Ideally, the length of the branch segment is given by the line integral

$$L(C) = \int_C ds, \quad (8)$$

where ds is the infinitesimal element of length measured along the curve C . The method used here (Eq. (7)) is essentially the “sample-distance” method⁶ where geometrically the curve C is being approximated by the connected polygonal curve whose vertices are the branch segment’s centerline voxels. Another method, that of simply counting voxels,^{30,36} can significantly underestimate interbranch segment length. Curve fitting or smoothing (e.g., a moving average filter) could also be performed and would likely be beneficial for characterizing such properties as branch segment tortuosity in order to remove voxel-based artifacts.

Measurements of vessel diameters have received much more attention than length measurements.²⁸ In numerical modeling of such properties as vessel resistance there is a much stronger dependence on vessel diameter than on vessel length. According to the Hagen-Poiseuille equation,³⁸ the resistance to flow is proportional to length over the diameter to the fourth power, and thus an error of vessel diameter results in a far larger functional impact. Previous methods for measuring vessel diameters can be grouped into four main categories¹¹: derivative-based, threshold-based, densitometric, and model-based techniques.

To measure the diameter, a gray-scale threshold-based method was developed. For each centerline voxel of an interbranch segment, the nearest non-vessel voxel (nearest-neighbor transform) was determined. A line profile was then computed that started from this non-vessel voxel, intersected the centerline voxel, and ended once another non-vessel voxel was reached. The profile length was calculated for two different gray-scale thresholds (1300 and 1500) about the FWHM value (max gray scale ≈ 2800) and an average was taken. The average length of this profile (as computed

for each centerline voxel) was used as an individual interbranch segment's diameter. Since a far lower threshold than the FWHM of the casts was initially chosen in order to extract small vessel segments, small vessel diameters were corrected as discussed below.

Compensating for Micro-CT MTF

To correct for the MTF of our scanner we also analyzed the effect of vessel blurring in order to correct for the over-estimation of small branch segments. Figure 3 depicts CT image brightness line profiles through different diameter branch segments. The ideal profile would be a square wave. As vessel segment diameter decreases, a significant decrease in the peak gray-scale value, as well as broadening of the profile relative to the vessel diameter, was observed. The point spread function derived from the MTF was a quasi-Gaussian blurring function with a $30\text{-}\mu\text{m}$ standard deviation⁴⁴ (0.07 mm FWHM). Displayed in Fig. 4 is a schematic of two different diameter input square waves, or line profiles (dashed line), along with the line profile that occurs in the image due to blurring (solid line). Large diameter vascular segments' diameters are accurately represented by their FWHM profile; however, small segments are not. As the vessel's diameter decreases, the output signal becomes broader (with respect to the true diameter) and the peak output signal decreases. This concept is further elaborated on in Fig. 5. Displayed in the top panel is a plot of the theoretical peak gray-scale values' dependence on vessel diameter, and the bottom panel displays the FWHM of the output signal, with respect to the input (ideal) vessel's square wave profile. The experimental results show that vessel diameters below 0.1 mm were significantly overestimated due to blurring, which matches our theoretical prediction. These branch segments' diameter measurements were corrected by fitting a Gaussian distribution function to the line

FIGURE 3. Line profile of varying diameter branch segments. The y-axis is in the units of 1000 cm^{-1} . Larger diameter segments retain to a great extent the ideal square wave profile. As the branch segments decrease, significant reduction in peak gray scale is observed.

profiles and then computing the input square wave (segment diameter) that was convolved with the imager's blurring function to give such a profile. This was derived on the basis that the area under the blurred image is equivalent to the actual input square

FIGURE 4. An illustration of the image blurring process. The MTF of the micro-CT scanner is a Gaussian blurring function. This blurring function is convolved with the vessel's true cross-sectional profile (dashed line), resulting in the blurred output (solid line). For large diameter vascular segments (left), the FWHM of the line profile accurately characterizes the true diameter. For small vessel segments (right), equating the area under the blurred profile to that of an equal area square profile, with peak gray scale height equal to that found in large vascular lumens, allows for the accurate measurement of small vessel segment diameters.

FIGURE 5. A plot of the theoretical gray-scale values' dependence on vessel diameter (top panel), for three different scanner blurring function widths. Vessel segments with small diameters will have a significantly reduced peak gray-scale value. This is also dependent on the MTF of the imaging system. The FWHM of the output signal with respect to the input (ideal) vessel's square wave profile is also shown (bottom panel). Large vessels' diameters can thus be accurately measured but small vessels' diameters will be overestimated. This deviation occurs at about 0.1 mm, with increasing severity as the vessel segment diameter decreases.

wave's area. These line profiles were extracted as discussed previously, using the nearest-neighbor transform (nearest non-vessel voxel) computed from the original vascular tree segmentation (i.e., the segmentation used to extract the centerline).

RESULTS

Compared with the months spent analyzing the casts by optical measurements, the micro-CT scanning, reconstruction, and image analysis each took a few hours to complete. The centerline extraction and individual branch measurement algorithms, fully automated, ran in under 10 min for both specimens (non-optimized code). Figure 6 displays the computed centerline superimposed on the segmented image of the vascular tree (*h61*), and Fig. 7 depicts zoomed-in views of two particularly critical areas. These areas highlight the ability of the centerline extraction method to follow the centerline paths within tortuous branches, as well as distinguish between branch segments that have some degree of overlap due to blurring. Note that the tree

FIGURE 6. Extracted centerline shown superimposed on the volume rendered display of the vascular tree *h61*. Arrows indicate regions highlighted in zoomed-in views displayed in Fig. 7.

endpoints are followed all the way to their ends, there are no false branches (“whiskers”) within the larger vessel lumens, and the centerlines are well centered within the lumens. In the case of physically missing large branch segments (i.e., broken), the extracted centerline still retains the information pertaining to the remaining ‘stump’ and thus the tree hierarchy is well preserved. Figure 8 depicts the extracted centerline along with the semi-transparent 3D volume rendering of *r14*.

Shown in Fig. 9 are the results for individual branch segments’ diameters and lengths in the form of a regression plot comparison for *h61*. In the top panel (diameters), the corrected and non-corrected results are given. The non-corrected result agrees with our expectation, that small vessel diameters are over estimated. This is also represented theoretically in the bottom panel of Fig. 5. In the case of the diameter measurements, the standard deviation of the difference between optical and micro-CT measurements ($13\ \mu\text{m}$) is within the theoretical voxel resolution of the micro-CT images (voxels are $18\ \mu\text{m}$ to a side). By developing a method to correct for the over-estimation of branch segments with small diameters, an improvement of the regression analysis resulted. Also, the method is valid over a wide scale-range, with diameters spanning $12\text{--}338\ \mu\text{m}$, and lengths spanning $14\text{--}3159\ \mu\text{m}$. Figure 10 displays the same regression analysis performed for specimen *r14*. The closer to ideal 1:1 relationship for the diameter regression analysis of *r14* shows that the developed micro-CT measurement method is accurate for an even larger range of interbranch segment diameters.

To further quantify the individual branch segment comparison, the results for specimen *h61* were plotted in a Bland–Altman comparison¹ which assumes that neither method necessarily gives the correct measurement but shows how well the two measurement methods compare. Figure 11 shows these results, along with the mean value and 95% confidence intervals for the difference between measurement methods, which is calculated as $\text{mean} \pm 2 \times \text{standard deviation}$. The mean diameter difference is $8\ \mu\text{m}$ (micro-CT measured slightly smaller than optical measurements) with 95% confidence interval -34 to $+18\ \mu\text{m}$. The mean length difference is $85\ \mu\text{m}$ (optical measurements measured slightly smaller than micro-CT) with 95% confidence interval -171 to $+341\ \mu\text{m}$. Combining the results from both casts, the standard deviation of the difference between measurement methods was $19\ \mu\text{m}$ for the measurement of interbranch segment diameters (for 60 segments between 12 and $769\ \mu\text{m}$ in diameter). Also, the standard deviation between measurement methods was $172\ \mu\text{m}$ for the measurement of interbranch segment lengths (between 14 and $3252\ \mu\text{m}$ in length). The

FIGURE 7. Zoomed-in views of the vessel tree displayed semi-transparently, with the corresponding extracted centerline. Left image displays path centeredness along a particularly tortuous branch segment, as well as the ability of the centerline extraction method to follow segments to their endpoints. Image on right depicts the ability to delineate overlapping branch segments, and also extract small terminating segments.

FIGURE 8. The centerline extraction method also has the ability to handle complex vascular structures as is shown here with the centerline superimposed on the volume rendered display of the vascular tree *r14*.

FIGURE 9. Regression plots for individual branch segments diameter (top) and length (bottom) measurements of specimen *h61*, by the micro-CT (*y*-axis) and optical (*x*-axis) measurement methods. Solid black line depicts linear least squares fits to the data. Dotted lines are 1:1 values. The corrected (black x's) and not corrected (gray squares) diameter measurements are both shown in the top panel. Vessel segments with diameters below 0.1 mm were measured much larger by micro-CT. These small segments measurements were significantly improved by compensating for the imager's MTF.

FIGURE 10. Regression plots for individual branch segments diameter (top) and length (bottom) measurements of specimen *r14*. Solid black line depicts linear least squares fits to the data. Dotted lines are 1:1 values.

larger difference for the case of individual branch segment lengths stems from a few issues. Probably the largest factor is that the definition of a “bifurcation point” is somewhat subjective during the optical measurement. The designated location of this intersection will affect all three branch segments’ length measurements (i.e., parent and two branches). This error resulting from different bifurcation points is reduced if we consider multiple sequential segments (i.e., a single path through the tree). Comparing the longest path through the tree (i.e., a summation of the individual branch segment lengths) resulted in a percent difference between the optical and micro-CT measurements of only 5%. This was calculated from

$$D = 100\% \times \frac{|O_l - M_l|}{O_l} \quad (9)$$

where D is the percent difference, O_l is the length measured by optical measurements, and M_l is the length measured by micro-CT.

The introduction of the small vessel correction allowed small vessel diameters to be accurately measured. In the studies of Hoozeveen *et al.*¹² and Sato *et al.*,³² approximately 1.5 voxels was determined to be the limit of accuracy for the estimation of vessel radii. For our current investigation of specimen *h61*, 12 out of the 43 individual branch segments are smaller than this value for their optically measured diameter (i.e., the optical method measured a diameter below 54 μm). The proposed correction method allowed for a significant increase in the accuracy of small vessel diameter

FIGURE 11. Bland-Altman method¹ comparison analysis for both interbranch segment diameter (top) and length (bottom) of specimen *h61*, by the micro-CT (M_d and M_l , respectively) and optical (O_d and O_l , respectively) measurement methods. Dashed black line is the mean offset between measurement methods and dotted lines are the 95% confidence intervals for the difference between measurement methods.

measurements, improving the average percent difference from 129% for the non-corrected measurements to 38% for the corrected measurements. Also, for the non-corrected diameter measurements, an average difference between the micro-CT measurement and the optical measurement was 1.68 voxels. For the corrected measurements this value was reduced to just 0.58 voxels, meaning that we were able to measure the diameter of small interbranch segments to within approximately half a voxel.

DISCUSSION

The use of the “fast marching” method for centerline extraction is an active research area.^{5,9,40,42} The advantage of this method for our purpose is that the flow pathways are of particular interest in our application. Here, the focus is on finding the centerline paths through the vessels as opposed to two other skeletonizing methods: (i) iterative thinning^{17,19–21,41} (i.e., peeling away surface voxels) and (ii) mesh contraction^{2,33} (i.e., shrinking). Thinning methods suffer from whiskers and shortening of branch segments (a consequence that is accentuated for thicker branch segments). Shown in the left panel of Fig. 12 is the result of applying morphological thinning to *h61* as the means of forming a skeleton. Major problems of note are that larger diameter segments, are significantly truncated (centerline does not extend to branch segments’ ends), overlapping vessels are not well

FIGURE 12. Left panel (black background): The result of applying morphological thinning to *h61*. Major problems include the truncation of larger diameter segments (centerline is shorter), overlapping vessels are not well delineated, loops are formed, false branch segments are generated (whiskers), and centerline is very noisy and does not follow curved segments well. Right panel (white background): The result of applying a mesh contraction method to specimen *h61*. Major problems include strange bifurcation representations by the centerline, poor overlapping segment delineation, and problems identifying small vessel segments (particularly broken segments). In addition, mesh contraction requires conversion from a voxel-based representation (into a mesh-based representation) and also requires many update steps for centering and correcting skeletons.

delineated, loops are formed, false branch segments are generated (whiskers), and the centerline is very noisy and does not follow curved segments well. The other method of mesh contraction requires conversion from a voxel-based representation into a mesh-based representation, and also requires many update steps for centering and correcting skeletons. Shown in the right panel of Fig. 12 is the result of applying a mesh contraction method to specimen *h61*. Major identifiable problems include strange bifurcation representations by the centerline, poor overlapping segment delineation, and problems identifying small vessel segments (particularly broken segments).

It is well known that by using a weighting function that is a constant value inside the object (i.e., the object's binary representation) for the fast marching wave propagation, the "shortest path" will cut corners and will not be centered (i.e., the path will be the shortest "Euclidean" geometric distance). It has been shown⁵ that applying a weight function corresponding to the distance map within an object caused the paths to retain some amount of centeredness in binarized images of brain vessels. The weight function assigned to each vessel voxel was a value corresponding to the inverse of that voxel's distance to its nearest non-object voxel neighbor. The result was that the fast marching wave propagated more quickly at the vessel lumen's

centers (i.e., the wave front reached those voxels faster), so that the extracted "geodesic" path was constrained from cutting corners.

In addition to a single path centeredness of microvasculature, the path centeredness within bifurcations is very important because measurements of branching angle and lengths are significantly affected on this scale by the accuracy of the branching geometry. Dependent on branching angles and the cross-sectional eccentricity of the bifurcation, stronger weight functions may be necessary in order to cause extracted centerlines to pass through the true junction point. The drawback of using stronger weight functions is an increase in the sensitivity to the surface irregularities (usually due to noise in image gray scale) of the vessel tree, as well as possible numerical inconsistencies which are problematic during the sub-voxel precise gradient-descent step. A discrete gradient-descent along a voxel's 26 contiguous-voxel neighborhood increases this problem at bifurcations, and therefore requires even stronger weighting functions.

The developed centerline extraction method is fully automated and is computationally efficient, overcoming computationally expensive fast-marching-based methods seen in such work as in van Uiter and Bitter⁴² where fast marching wave propagations are calculated for each endpoint that is determined, and in

which there is a criteria set on branch lengths in order to exit the iterative algorithm. In the case of *r14* this would have resulted in up to 60 fast marching waves (vs. the two proposed in this study), while potentially still missing shorter branch segments. Also, for our present application, such methods as using gradient vector flow⁹ to define the object's centerline is unnecessary as the vessel pathways are highly tubular and thus the less computationally expensive distance map is beneficial. To compute the gradient vector flow field for *h61* took 40 min as opposed to the 13 s spent computing the distance map. Additionally, for large vessel trees the gradient vector flow field becomes very memory-intensive since it relies on the use of three cardinal direction gradient maps, typically at double floating point precision. Due to many factors such as (i) the high level of image analysis throughput of our lab, (ii) that our intention is to apply our analysis methods to far larger image volumes, and (iii) the fact that no major benefits in the extracted centerline were observed when a gradient vector flow map was used, a faster, less memory intensive method will be highly beneficial at little to no expense in the subsequent data's accuracy. However, it should be noted that such a weighting map (such as the gradient vector flow field) is useful for irregular pathways, or pathways deviating greatly from circular luminal cross sections, as it has the ability to distinguish the center of mass of such cross sections as an oval or rectangle (for instance), whereas a distance map may have a string of voxels, all at the same value.

Using the binary representation of the object for automated endpoint determination resulted in the endpoints being followed to the correct ends, and was far less sensitive to the noisiness (i.e., roughness) of the vascular tree's surface, compared with a similar method. This similar method, termed the level set graph,⁹ utilizes a distance map weighted representation of an object to determine endpoints. This method, we found, did not converge on a specific number of endpoints and was also highly sensitive to the chosen cluster size. In the case of a distance map weighting, the number of endpoints was not found to converge, meaning that increasing the "constant multiplier" resulted in more and more endpoints being (falsely) identified. These were found to exist on the wall of interbranch segments, not necessarily at the ends of the vascular tree. Intuitively this makes sense since the distance map weighting introduces a strong gradient moving away from the center of vessel lumens toward the wall.

In regards to vascular segment diameter measurements, a detailed study was performed by Hoffmann *et al.*¹¹ that compared various techniques for determining vessel diameters. These techniques included

derivative-based, threshold-based, densitometric, and model-based methods. The study was performed on simulated images, as well as a vessel phantom, composed of distinguished cylindrical objects (i.e., non-bifurcating structures). The main conclusion was that no technique perfectly characterized vessels over the full size range. However, large vessel segments with diameters larger than the FWHM of the imaging systems blurring function (or simulated blurring function) were found to be accurately measured (errors of ≈ 0.1 mm) using an averaging of the first and second derivatives of vessel line profiles. Theoretically the second derivative method is guaranteed to always overestimate vessel diameters, whereas the first derivative as well as the FWHM accurately characterizes the input square wave's width (for large diameter vessel segments). Our method of measurement accurately measures large diameter vessel segments, and is fully automated. Also, small diameter branch segments were accurately measured by using local gray-scale information to take into account for the imaging system's blurring properties.

The micro-CT method is a far faster analysis method that limits the effects of manual error introduced by the tedious, optically based measurement method used in this study and others. Also, the micro-CT method extracts 3D information (such as bifurcation points), which differs from the 2D information contained in optical measurements. Since the accuracy of the micro-CT method of analysis has been characterized, showing favorable results, we are able to validate alternative methods of analysis,¹⁶ and will be able to compare the branching geometry of different vascular structures (e.g., the portal vein, hepatic artery, and biliary tree of the liver).

CONCLUSIONS

An important tool for providing information pertaining to a vascular tree's contribution to various diseases and organ malfunctions is the image analysis of microvasculature. In contrast to the imaging of organ tissues, which typically focuses on topology and variable intensity within that organ, the imaging of vasculature requires a focus on vascular branching architecture. The goal of this study was to highlight some key difficulties in this area of analysis such as imaging resolution, segmentation, centerline extraction, as well as the measurement strategy of individual branch segment's lengths and diameters. The comparative results presented are positive and suggest that a method of characterizing microvasculature tree structures can be accomplished by micro-CT, which is beneficial since the time scale of such an analysis is far

faster and less prone to errors than a tedious method, such as the optical measurement of individual branch segments (i.e., the gold-standard used in this study).

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