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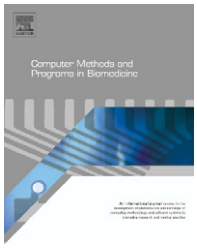

Automation process for morphometric analysis of volumetric CT data from pulmonary vasculature in rats

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ABSTRACT

With advances in medical imaging scanners, it has become commonplace to generate large multidimensional datasets. These datasets require tools for a rapid, thorough analysis. To address this need, we have developed an automated algorithm for morphometric analysis incorporating A Visualization Workshop computational and image processing libraries for three-dimensional segmentation, vascular tree generation and structural hierarchical ordering with a two-stage numeric optimization procedure for estimating vessel diameters. We combine this new technique with our mathematical models of pulmonary vascular morphology to quantify structural and functional attributes of lung arterial trees. Our physiological studies require repeated measurements of vascular structure to determine differences in vessel biomechanical properties between animal models of pulmonary disease. Automation provides many advantages including significantly improved speed and minimized operator interaction and biasing. The results are validated by comparison with previously published rat pulmonary arterial micro-CT data analysis techniques, in which vessels were manually mapped and measured using intense operator intervention.

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1. Introduction

There have been several technological developments in medical imaging scanners in recent years, leading to substantially increased capabilities. Such advances continue to benefit medical researchers and doctors, and the patients they serve. There now exist 'sub-second multi-slice' CT scanners and post-acquisition reconstruction schemes capable of generating large three-dimensional (3-D) data sets detailing the anatomy of various organs [1–3]. Researchers also utilize micro-CT scanners to generate a detailed structural and functional understanding of small animal and biological systems. Current scanners allow high spatial and temporal resolution enabling the study of the etiology and progression of disorders in greater detail than was previously possible. Many of these new imaging methods give rise to very large datasets. For example, in our studies, a single micro-CT volume/image of the rat pulmonary vascular structure is more than 400 MB, resulting in significant challenges in analyzing data efficiently. Applying a manual or semi-automated protocol to detect vessels, map their 3-D coordinates, measure lengths and diameters requires extensive operator interaction. In practice,

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this limits the measurements to a subset of the entire vascular tree. These impediments provided the motivation to develop an automated technique for faster, more complete morphometric analysis.

One of the primary objectives of our group has been to develop a structural and functional understanding of pulmonary vascular remodeling (PVRem). PVRem is defined by cellular and structural changes in the normal architecture of the walls of pulmonary vessels, which typically lead to an increase in the pulmonary vascular resistance. The increased resistance results in an increase in the intravascular pressure, defined as pulmonary hypertension (PH), which then perpetuates the remodeling. Thus, PVRem involves a vicious cycle, which eventually leads to cor pulmonale and death. In order to design therapies, a fundamental understanding of the development of PVRem is important. To approach this goal, our lab studies the pulmonary arterial structure in the rat using micro-CT images obtained over a range of constant intravascular pressures.

We have previously developed a robust operator guided manual technique for morphometric analysis that combines manual and semi-automated methods [4]. In this previous operator guided manual technique (*OGMT*), an operator laboriously indexed slice-by-slice through the image volume and mapped the 3-D coordinates of the medial axis at anatomical landmarks along the main pulmonary arterial tree. After the coordinates of the main trunk were mapped, a Matlab (MathWorks, Natick, MA) algorithm was used to load the image volume and the coordinates of the medial axis inlet and outlet for each vessel segment. The algorithm then calculated segment length and determined the midpoint of each segment from which to extract a two-dimensional (2-D) slice of data orthogonal to the medial axis. The operator was required to manually adjust the slice data to ensure it contained the appropriate, orthogonal vessel cross-section, mask any adjacent objects that were included in the slice, and then monitor the vessel fitting and diameter estimation procedure to determine the diameter at each segment midpoint. In this study, the process of mapping the vascular tree, slice extraction, background masking and vessel fitting has been completely automated and consequently produces a more comprehensive and efficient morphometric interpretation of the data. Additionally, the proposed algorithm significantly reduces the operator effort and allows measurement of the entire vascular tree while at the same time increasing the consistency of analysis by virtually eliminating operator decision support. Using the algorithm presented in this study, effectively the only operator decision required involves identifying the main arterial trunk, a processed used primarily to compare retrospective data. Although many authors have reported automated segmentation of vasculature [5–11], including work focused on pulmonary vessels [12–17], the work reported here is the first to our knowledge that uses hierarchical vessel ordering and an automated optimized multidimensional, constrained nonlinear fitting technique to estimate vessel diameter. Because our segmentation and ordering isolates and maps the entire pulmonary arterial tree, measurement of the complete tree could explicitly and readily be performed. However, the specific algorithm we presented here restricted measurement to the main pulmonary trunk so that the data could be directly applied to our previously developed morphometric models as well as be compared with existing data that was manually measured.

2. Methods

To develop the automated vessel detection and measurement application (AVDMA), we implemented multiple software tools. C programming language and Tool Command Language (Tcl) and the accompanying graphical user interface Tool Kit (Tk) software were used in combination with A Visualization Workshop (AVW) library of image processing functions. Development was performed under the Linux operating system. Analyze®, a biomedical image analysis software, and the AVW library of functions was licensed through the Biomedical Imaging Resource (BIR) at the Mayo Clinic [18–20]. AVW libraries form the basis of the commercially available software Analyze®, which means that the functionality of Analyze® can be replicated in its entirety and customized programs created using algorithms defined by problem specific applications.

2.1. *Biologic data for design and validation*

Data acquired for other ongoing studies was used in the design and validation procedures of the AVDMA. In experiments approved by the local VA Institutional Animal Care and Use Committee (IACUC) and animals treated in accordance with 'The Guide for the care and use of laboratory animals', Sprague–Dawley (SD) rats, weights ranging from 250 to 300 g, were either exposed to chronic hypoxia (CH, 10% O_2) or normoxia (21% O_2) for 21 days. Their lungs were then excised and the arteries filled with a radio-opaque contrast medium (perfluorooctyl bromide or perflubron) and scanned with the Keck functional imaging center's microfocal X-ray CT system. This is a custom build imaging system composed of a Fein-Focus-100.50 X-ray source (3 μm focal spot), a North American Imaging AI-5830-HP image intensifier coupled to a Silicon Mountain Design SMD 1M-15 CCD camera, and a New England Affiliated Technologies specimen micromanipulator stage mounted on a precision rail with position information provided by Mitutoyo linear encoders. The X-ray source voltage was set to 41 kV and current to 140 mA. The lungs were rotated in the X-ray beam at 120°/min and planar images captured at 1° increments to obtain 360 images (512 × 512 pixels) over 3 min. For the first scan, a hydrostatic intravascular pressure was set to 30 mmHg (Pa1), and then the pressure was successively lowered to 21, 12 and 5.4 mmHg (Pa2, Pa3, Pa4, respectively) to obtain data at four discrete arterial pressures. The Feldkamp cone-beam reconstruction algorithm [21] was applied to planar images to obtain isotropic reconstructed volumetric datasets, 497 × 497 × 497 voxels in dimension. Further description of the CT system, data acquisition methods, animal models, lung preparation and imaging techniques have been described previously [4,22,23]. Although the algorithm has been developed for our models of pulmonary hypertension in isolated rat lungs, its utility is not limited to non-clinical data sets. The algorithm can be applied to clinical data in which the pulmonary vascular lumen has sufficient contrast from its surrounding. The recursive portion