Micro-CT based Stereology

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Aims

Traditionally, structural anatomy is analyzed on physical slices (light or electron microscopy) of an organ using stereology [1]. This method aims for the quantification of structures and their features within an entire organ based on subsampling and counting procedures.

Although stereology led to a plethora of discoveries during the past five decades, this method is very time consuming and therefore is not well suited for studies with large number of samples. The subsampling procedure [2], the physical slicing and the aligning of the slices are by far the most time consuming tasks. In order to overcome these bottlenecks of stereology, we decided to apply stereology on virtual slices obtained by micro-CT.

Method

"Micro-CT based stereology" was applied to rat lung in order to quantify the number of pulmonary functional units (i.e. the acinus). To do so lung lobes were critical point dried, in order to enhance the air-tissue contrast, and scanned (Skyscan 1172, Bruker microCT, Kontich, Belgium) at voxel isotropic side length of 2.5-4 µm [3]. The number of acini was manually counted on the image stacks, obtained from the micro-CT imaging, and extrapolated for the entire lung.

In addition, we develop a novel contrast agent, modified Angiofil (Fumedica AG, Muri, Switzerland), in order to use "micro-CT based stereology" to analyze the vasculature of different organs. Murine skeletal muscles, rat brains, and murine kidneys were perfused using our novel contrast agent, fixed with 4% of paraformaldehyde, and scanned at voxel side length of 0.8, 4.5 and 2 µm, respectively.

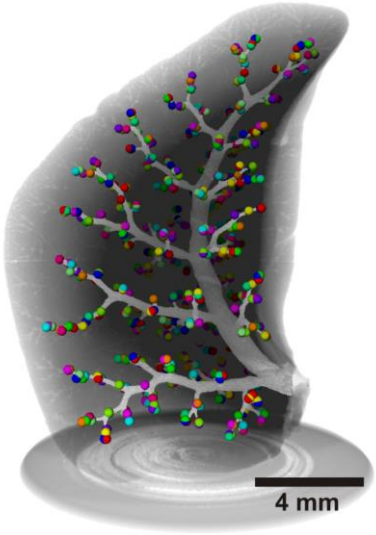

Figure 1. Tree of conducting airways shown inside a right middle lobe (young adult rat). The walls of the conducting airways are shown in light grey and the spheres represent the acini entrances. The grey shadows delimit the lobe tissue and the sample holder. This 3D reconstruction illustrates that the bronchial tree of rat does not follow the dichotomous branching pattern of the human lung. Out of [3].

Results

The micro-CT approach considerably reduces the manpower needed for stereological analysis. For example, we could quantify the number of pulmonary acini (5612 ±547) for an

entire lung within one day [3], whereas traditional techniques demanded several weeks. In addition 3 dimensional models could be reconstructed in order to characterize the conducting airways (figure 1).

Our novel contrast agent was successfully used to perfuse the vasculature of different organs. This enabled us to visualize and detect anatomical macroscopic structures, like the glomeruli (filtering units) of murine kidneys or rat brain tumors. Not only large structures can be visualize with our contrast agent but also the smallest (approximately 5-8 µm) blood vessels (i.e. capillaries). Capillary visualization was achieved on murine skeletal muscle of the hind limb (figure 2). In addition to micro-CT visualization, other imaging modalities (e.g. light or electron microscopy) can be used on the same sample.

Conclusion

Micro-CT imaging is an elegant way to by-pass the slicing and aligning procedures demanded for the traditional stereology. This fastens up the analysis process and allows new kinds of investigations (3D analysis, registration with histology, multimodal imaging, etc…). "Micro-CT based stereology" is an efficient way to reduce the investigation time or to massively decrease the approximation errors due to traditional stereology.

"Micro-CT based stereology" combined with our novel contrast agent will certainly contribute to a better understanding of the vasculature and the angiogenesis of healthy and tumor tissues.

Figure 2. 3D visualization of capillaries in mouse skeletal muscle (soleus muscle). Developed approach with modified Angiofil allows the visualization of blood vessels (panels a & b, red and yellow circles) as well as capillaries (panels a & b, blue arrows) using various imaging techniques (a: light microscopy, b: micro-CT). Micro-CT enables for the segmentation (threshold based) of all kinds of blood vessels and for the 3D visualization (panel c). Scale bars (panels a & b: 50 µm, panel c: 400 µm).

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