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Investigating Large Microscopic Volumes of Lung by Computer-guided 3-D Image Compositing in Confocal Microscopy

Andres Kriete
Institute of Anatomy and Cytobiology, Image Processing Laboratory, Aulweg 123, 35385, Giessen, Germany

There is a long history of experimental difficulties in the quantitative investigation of the respiratory part of lung, because such studies require imaging of extended volumes at high resolution. Structures like the pulmonary acinus, the ventilatory unit formed of several alveolar ducts, extend to a size of 2000x2000x2000 microns in rat and mouse. They can only be analyzed for their structural organization and branching pattern if all structural details of the lungs parenchyma down to the alveoli are completely resolved. Because of these requirements, only one detailed and complete investigation of an acinus is given in the literature, based on a very time consuming manual performed reconstruction of serial sections with a wax plate technique (Boyden 1971). It was suggested and demonstrated for smaller substructures of the respiratory system, that confocal imaging applied to thick sections and digital image processing might be an alternative way for such investigations (Oldmixon and Carlsson 1993). We describe a computer-guided image acquisition procedure in confocal microscopy, which allows a 9-fold increase of the field of view and an extended imaging in axial direction by scanning aligned thick serial sections.

Histological sections of 50-80 micron thickness (HE stained) of a rat lung are the starting point for our investigation. First the sections are previewed with a low magnifying lens of 5x (such as Plan-Neofluar, NA 0.15). The numerical aperture of this lens is not sufficient for a good axial resolution, but offers a wide field of view. These images are aligned for linear shift and rotation and the approximate region of interest is defined through 3-D visualization of the data stack. When transferred back to the confocal microscope, these corrected images serve for alignment of the histological sections. The goal of the next acquistion step is to sample higher resolved volumes. Variation of the scanning offset, i.e. shifting the scanning beam electronically away from the center in all 8 possible directions at a lateral distance of 600 microns, gives 9 reference images. At a zoom factor of 4 they construct a frame of 1800 um side length (data hold for a Zeiss LSM 410). Switching to a 20x magnifying lens (Plan Neofluar NA 0.5), the field of view at zoom factor 1 can be fitted exactly to that of the individual reference images, so that 9 adjacient, non-overlapping subvolumes can be acquired by optical sectioning within one histological section.

We give an example, where 13 histological sections of 70 micron thickness, each scanned at an axial step size of 10 microns, result in a total of 9x7x13=819 images of 256x256 pixels each. Subsequently these images are fused at every level of optical sectioning, achieving a final volume of 768x 768x 91 voxels being equivalent to 1800x 1800x 910 microns. This volume encloses one complete acinus, but exhibits as well structural details like the alveoli.

In order to visualize such a data volume, various image preprocessing steps have to be performed. The sponge-like character of the lungs parenchyma does not give any insight if a volume rendering algorithm is directly applied, but the structures of insterest have to be segmented and enhanced first. In particular, the intensity of all sections has to be equalized by histogram normalization, before an automatic segmentation of tissue and background can be performed. In order to detect connectivities and to differentiate the structure of the acinus of interest from branches of adjacient acini, the binary volume is seeded at the largest visible diameter of the acinus and binary operations are carried out up and down the data stack, labeling connecting structures.

This procedure is repeated until all connectivities are detected and identified. Once the complete structure of the acinus is defined, volume renditions can take place.

REFERENCES

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