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3D fluorescence imaging of the lung

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The lung is difficult to analyze histologically since most lung harvesting techniques result in a collapse during preparation. Conventional histological methods provide only 2D planar images that are lacking the 3D dimensional information. However, serial sections to get a 3D image may lead to artifacts. Therefore, high-resolution 3D imaging of whole mount tissue will make a big difference to visualize complex structures such as immune cell distribution, cancer cell accumulation and vasculature in the lung, which are difficult to investigate in 2D sections.
The aim of this thesis was to: 1) establish 3D imaging protocols of lung based on two methods: optical tissue clearing (OTC) and expansion microscopy (ExM); 2) apply the ethyl cinnamate (ECi) based OTC method for lungs; 3) establish and modify the ExM protocol with perfused normal lung tissue and further extend it to paraffin blocks and metastases and 4) figure out the best way to do 3D imaging in lung research.
The results of this thesis can be summarized as follows: 1) The retrograde perfusion combined with trachea ligation technique can be used in mice. As compared with the normal trans-cardiac perfusion technique, this approach is much easier in handling and results in a better maintenance of lung morphology. At the same time, it was possible to harvest all of the perfused organs from mice. 2) ECi based OTC can be applied for the 3D imaging of lung vasculature. 3) MHI148-PEI, a cationic near infrared fluorescent agent, is an ideal dye to stain lung vessels during perfusion. Thus, no incubation with an antibody was needed. 4) ExM combined with an antibody immunofluorescence staining protocol was established for high resolution 3D imaging of the lung. By doing so we revealed the 3D structure of alveoli and vasculature in the lung. 5) We also demonstrated that ExM was applicable to paraffin embedded lung tissue blocks. Thus, 20 years old paraffin embedded lungs could be imaged in 3D allowing the staining of the vasculature. 6) By ExM, we could image the microvasculature in lung metastases as well as neutrophil cells from 10 years old paraffin blocks. It was noted that different tissue types (metastasis versus normal lung) expanded differently after expansion.
In this thesis, we established a retrograde perfusion combined with a trachea ligation technique for the lung providing a better maintenance of lung morphology. Furthermore, new 3D imaging protocols were introduced, optimized and established for the lung allowing new analyses of thick fresh lung slices and lung tissue from paraffin blocks.