Tissues, Pathology, and Diagnostic Microscopy

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Quantitative micro-structural assessment of animal models of human lung disease

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Each day a human being inhales and exhales more than 10,000 litres of air into and out of the lung. The parenchyma of the lung consists of a very large number of terminal air bubbles (alveoli) separated by tissue septae which contain a dense network of blood capillaries. At the end of a deep breath, more than 80% of total lung volume is air, about 10% is blood, and only the remaining about 5-10% is tissue. The main function of the lung is gas exchange, in particular the uptake of oxygen from the ambient air into the pulmonary capillaries. The lung´s functional capacity is determined by its structure. Efficient oxygen uptake requires a large alveolar surface and a thin air-blood barrier for diffusion. Within the human lung, the exchange surface for the diffusion of gases is distributed over about 300 to 500 million alveoli and is as large as 120 - 140 m² (nearly the size of a tennis court). At the same time, the thickness of the exchange barrier is only about 2 µm (50 times thinner than a sheet of air-mail stationary).

Several lung diseases are characterized either by a loss of surface area (e.g. emphysema) or by a thickening of the barrier (e.g. intraalveolar edema or fibrosis). These alterations can be induced in animal models of human lung disease which can then be studied under defined experimental conditions. In order to make statistically valid comparisons between experimental groups, lung structure has to be measured to provide quantitative (morphometric) data. The methods of choice to obtain such data in microscopy are those of stereology, a set of unbiased sampling and measurement tools derived from stochastic geometry. The principles of design-based lung stereology have been summarized and adopted as an official research policy statement of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) [1].

Different animal models require the use of different stereological designs, which have to be integrated in the planning phase of the study. Specific steps include the mode of lung fixation, tissue sampling and processing, preparation of sections, microscopic technique, and analysis parameters. Specific recommendations for various animal models of human lung disease have been given recently [2,3].

Microscopic techniques (including light and electron microscopy, immuno-EM, electron tomography) in combination with stereology are essential to characterize lung structure in health and disease. A particular strength of stereological methods is the fact that they can be applied to any imaging dataset, including non-destructive imaging techniques which can visualize whole fixed small animal lungs with alveolar resolution ex vivo (e.g. micro-CT [4,5] and scanning laser optical tomography (SLOT) [6]). This combination allows for a comprehensive quantitative lung phenotype analysis. Here, we give an overview on our recent work on the microscopy-based stereological analysis of the normal and disordered lung.

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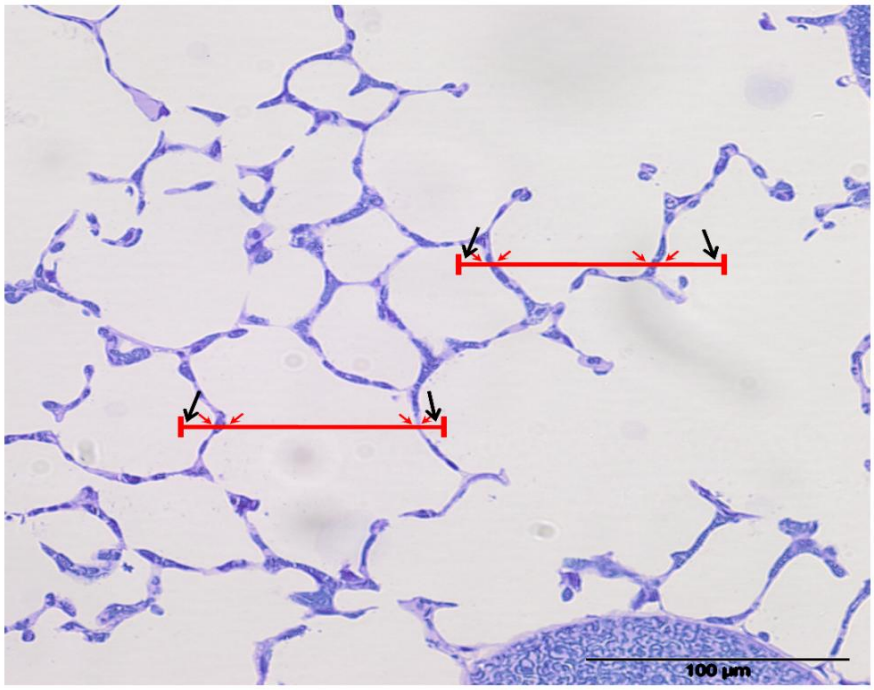

Figure 1. Estimation of alveolar surface area by counting of intersections (red arrows) between test lines and alveolar septae.

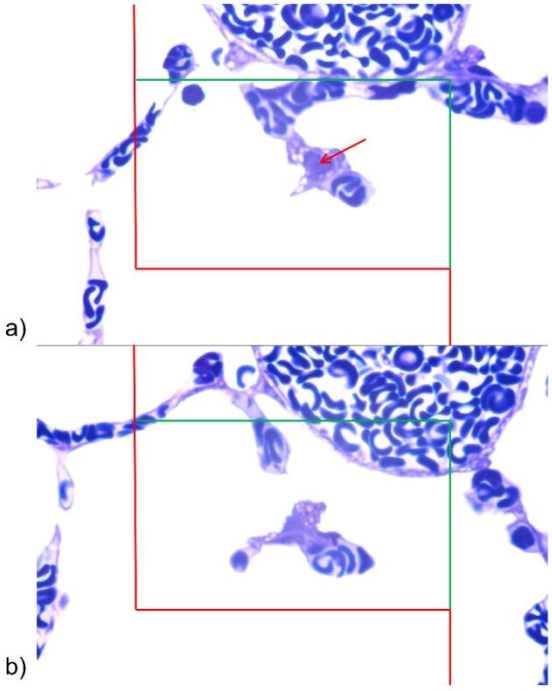

Figure 2. Estimation of alveolar epithelial type II cell number by counting of nucleoli (red arrow) in a disector.