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Branching Morphogenesis in Human Lung Development

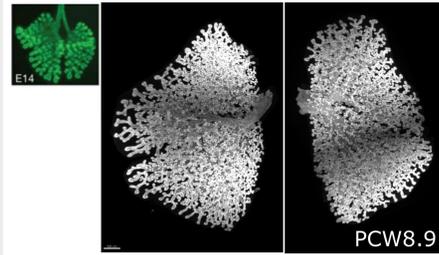
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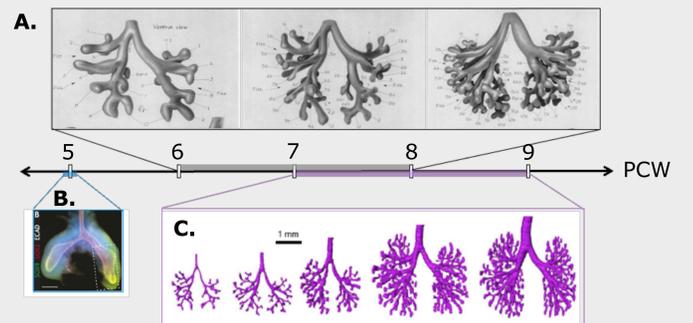
Abstract

The lungs, pancreas, mammary glands, and kidneys contain an intricate network of epithelial tubules that tremendously increase their capacity to exchange molecules. In human lungs, about 480 million alveoli occupy the distal tip of these tubules and are the ground for gas exchange. In branching morphogenesis, tubular trees are built by successive rounds of extension and ramification accompanying organ growth. Human lung development begins with the formation of a lung bud from the foregut endoderm at about one month of gestation. Then, branching morphogenesis forms the basic architecture of the airways during the pseudoglandular phase between 5-17 post-conceptual weeks (PCW). Using immunostaining, tissue clearing, and light sheet imaging, we follow human lung development in 3D through the period of 5-12 PCW to answer the questions: What are the statistical, topological, and cellular organizations of the branching epithelium in early lung development? What determines the structure of the branching airways?

Introduction



Left: Mouse lung at E14 (from Metzger et al., 2008 Nature). Right: Right and left human lung from one specimen in our datasets at PCW8.9, Scale: 500um.

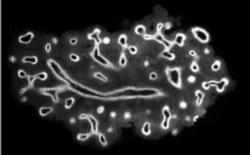


Previous studies of human lung development: A. Wells and Boyden, 1954 Am J of Anat; B. Nikolic et al., 2018 Development; C. Fuji S et al., 2020 J of Anat.

In the mouse, the branching program of lung development has been studied and shown to be stereotypic - they follow a genetic blueprint. Three modes of branching have been proposed to occur in pre-determined sequences: (1) domain branching, (2) planar bifurcation, and (3) orthogonal bifurcation (Metzger et al., 2008 Nature). While stereotypy is undeniable in the first few generations, there has been controversy on the persistence of genetic regulation in later generations and the roles that local signals play. Human lungs differ considerably from mouse in scale, anatomy, and branching patterns. Previous studies in human lung development include studies using wax reconstructions in the 1950s and more recently, those using uCT. However, technical limitations have prevented extensive studies that detail the fine architecture of the branching airways past the earliest stages of branching.

Pipeline

Acquire Image



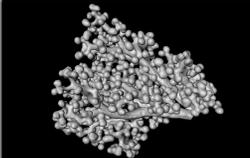
Segment Image



Fill / Edit



Reconstruct 3D



Mesh Edit



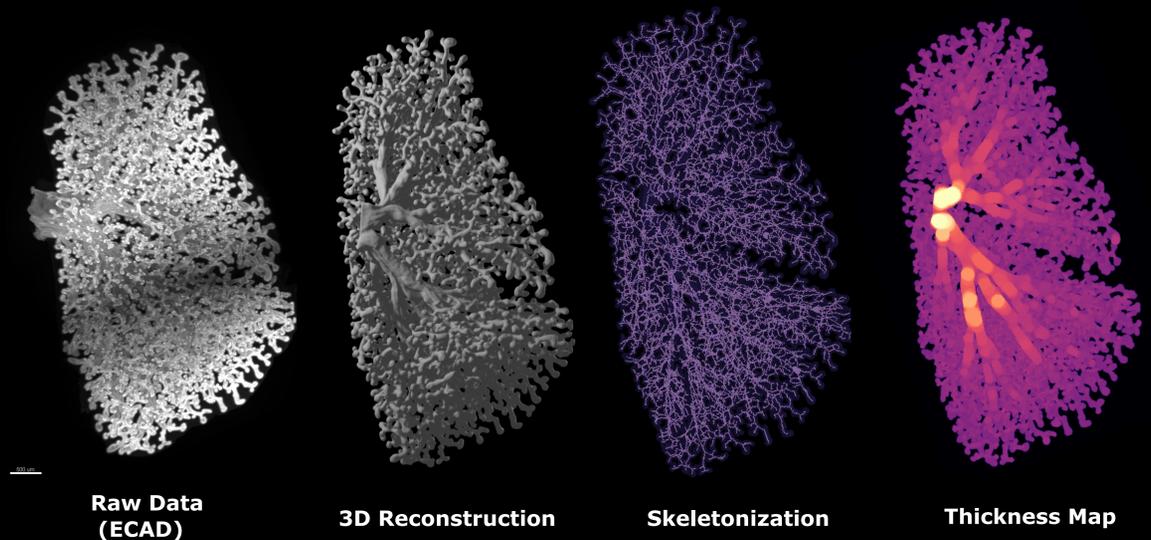
Branch ID

Skeletonize

Anatomical Model

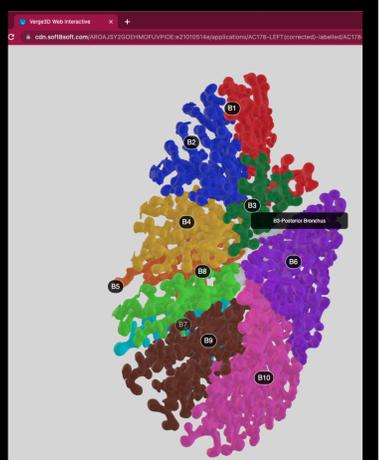
Quantify

Skeletonization



Process of skeletonization from image. PCW8.9 Left Lung with 4776 branches, 2382 end-points. Scale bar: 1000um.

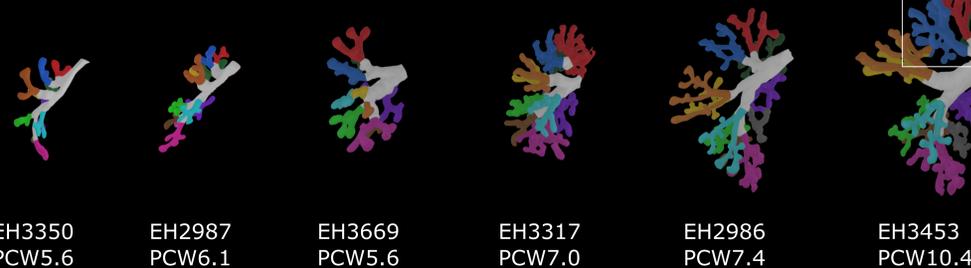
Anatomical Models



Colored anatomical models can be labelled and exported to a web browser for interactive viewing (Shown: left lung at PCW7.5)

Right Lungs

Legend: B1 B2 B3 B4 B5 B6 B7 B8 B9 B10



Reconstructed and colored right lungs PCW5.6 - 10.4, labeled with primary branch numbers from the Boyden system (B1 - B10).

Table shows branching patterns of the right upper lobe commonly found in healthy adult human lungs, the ID of specimens we found with these branching patterns, and the rate of the pattern in % for healthy adult humans. *Reference: Chaye B. et al., 2001 Radiographics

Pattern	ID	Adult Rates*
B1, B2, B3		30%
B1+B2, B3	EH2987, EH2986	26%
B1+B3, B3	EH3669, EH3453	16%
B2+B3, B1	EH3350, EH3317	6%
B2+B1b, B3+B1a		22%

Future Directions

We have been able to apply iDISCO and light sheet imaging to reconstruct the architecture of the developing human lung from PCW5-9. Currently we are extending this pipeline up to PCW12. This method allows us to overcome previous limitations to reconstructing the lungs in 3D and describe their morphology with unprecedented resolution. Following reconstruction, we are able to identify the primary branches and the lung segment it gives rise to which allows us to create anatomical models of lung development. Currently we are creating a library of 3D lung models through PCW5-12. Quantifications from these models will become the basis for a mathematical model to describe the mechanism of branching morphogenesis in human lung development.

Acknowledgements

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