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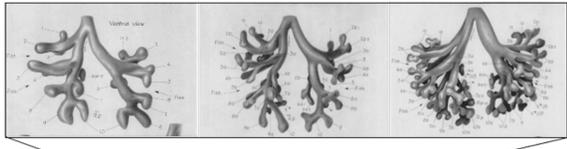
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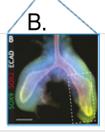
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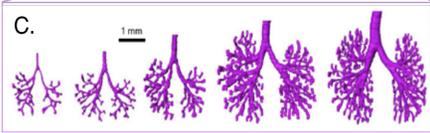
Branching Morphogenesis in Human Lung Development

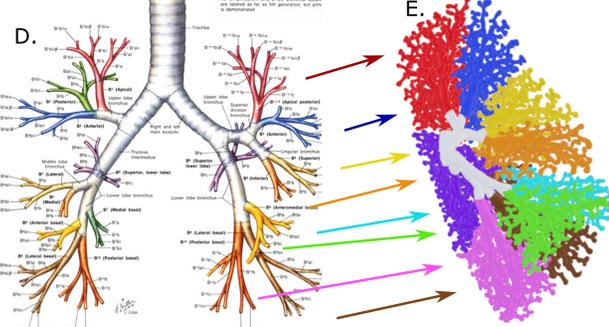
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A. 

B. 

C. 

D. 

E. 

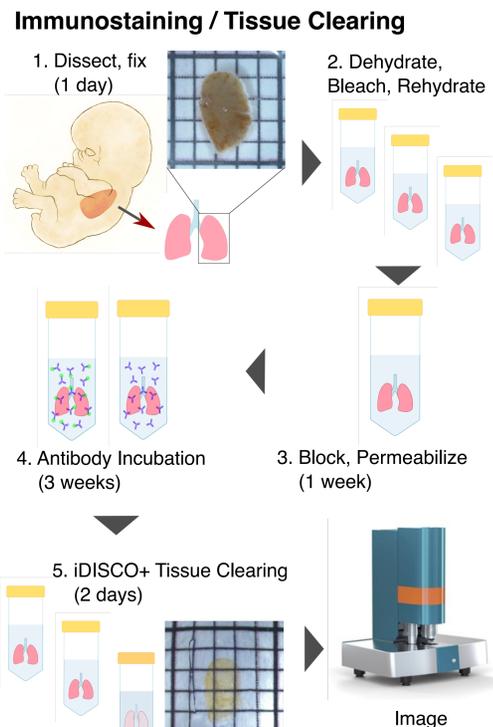
Introduction

Organs such as the lungs, kidneys, and mammary glands develop by a process of branching morphogenesis into intricate tree-like structures. Human lung development begins as an outgrowth of the foregut endoderm at around 4 post-conceptual weeks (PCW) and continues into toddlerhood. Previous studies of human airway development have been restricted to the first few weeks due to technical limitations (A-C). In this project, we study the airway tree in the left and right lungs between 5-12 PCW. What is the statistical, morphological, and cellular organization of the branching epithelium in early human lung development, and how do these characteristics change over time? It has been argued that human lung development follows a genetically predetermined stereotypical phase followed by a more stochastic, space-filling phase, but in-depth studies have not been feasible due to technical limitations in the resolution and computational power required to reconstruct such fine and complex structures. We use whole-mount immunostaining, tissue clearing, and light-sheet microscopy to acquire 3D image datasets of the airway epithelium. Previous studies of adult human lungs have resulted in a nomenclature system of bronchopulmonary segments (D) that provides a reference frame to label our data (E). We have developed an image processing pipeline to extract a “skeleton” of the branching tree by anatomical bronchopulmonary branch segments. To elucidate the balance between stereotypic and stochastic influences on the large-scale architecture of the human airway, we study the statistical features of the branching network across a critical period of branching morphogenesis. These statistical characterizations will inform a mathematical model of the large-scale organization of the 3D branching network, which will be used to develop insights and predictions in the processes that regulate ductal branching, elongation, orientation, arrest and termination that can be tested against data. Altogether, this study will provide both a reference atlas of the developing human airway tree, and insight into mechanisms that drive branching morphogenesis in human lungs.

Previous studies of human lung development. A. Wells and Boyden, 1954 Am J of Anat. B. Nikolic et al., 2018 Development. C. Fujii S et al., 2020 J of Anat. D. The Netter Collection of Medical Illustrations - Respiratory System. E. Nomenclature mapped to our dataset EH3685-Left.

Immunostaining / Tissue Clearing

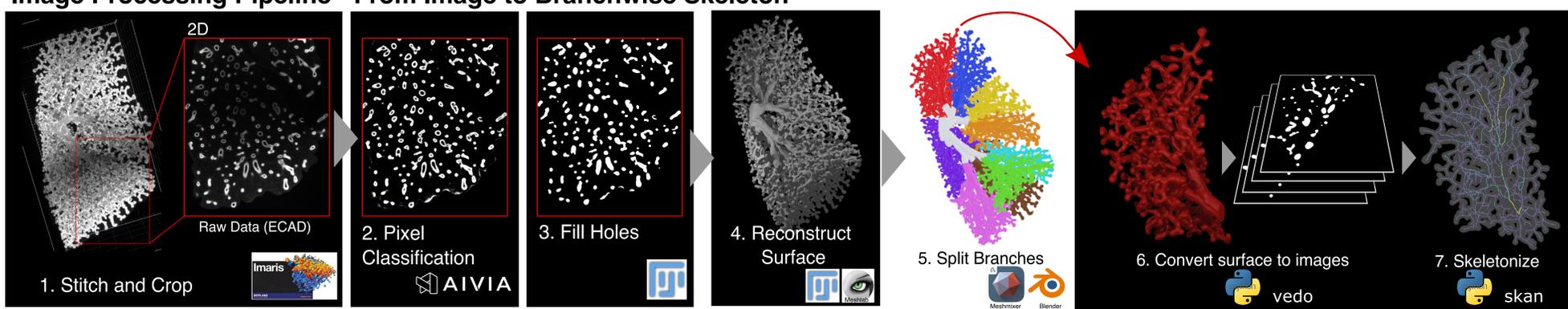
1. Dissect, fix (1 day)
2. Dehydrate, Bleach, Rehydrate
3. Block, Permeabilize (1 week)
4. Antibody Incubation (3 weeks)
5. iDISCO+ Tissue Clearing (2 days)



Image

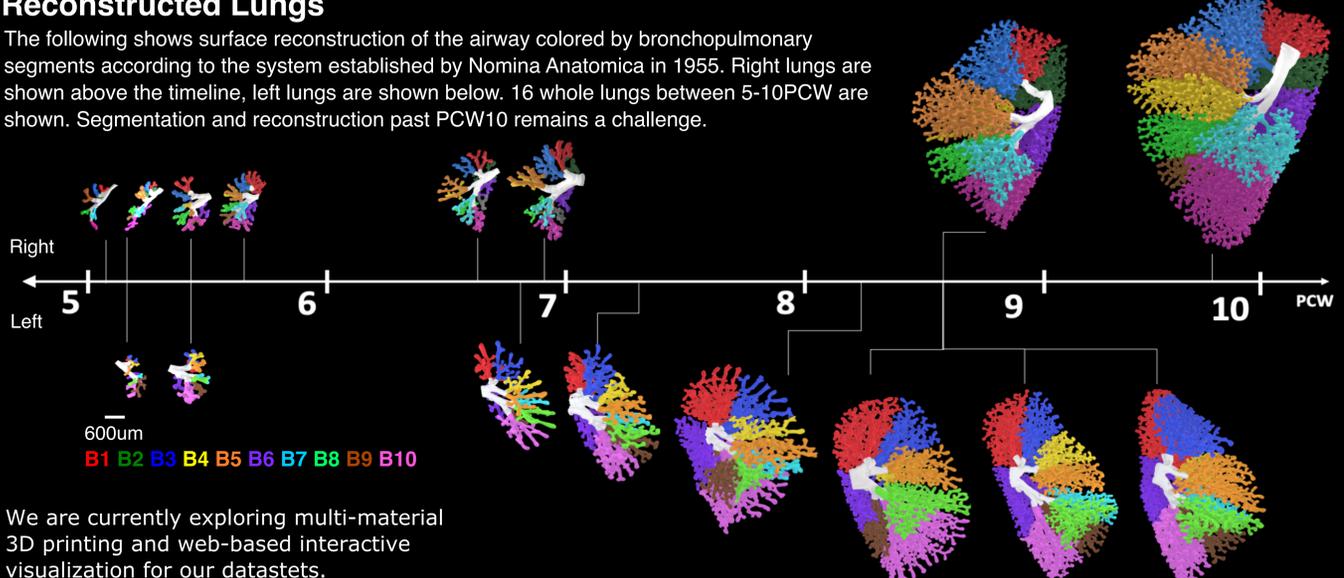
Image Processing Pipeline - From Image to Branchwise Skeleton

1. Stitch and Crop
2. Pixel Classification
3. Fill Holes
4. Reconstruct Surface
5. Split Branches
6. Convert surface to images
7. Skeletonize



Reconstructed Lungs

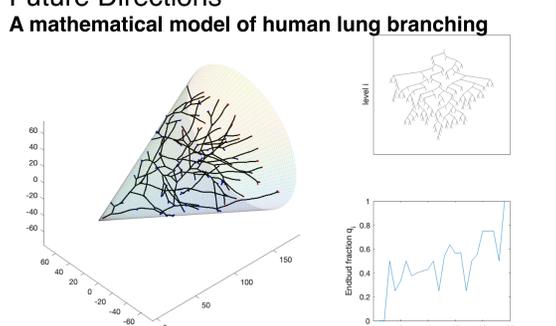
The following shows surface reconstruction of the airway colored by bronchopulmonary segments according to the system established by Nomina Anatomica in 1955. Right lungs are shown above the timeline, left lungs are shown below. 16 whole lungs between 5-10PCW are shown. Segmentation and reconstruction past PCW10 remains a challenge.



We are currently exploring multi-material 3D printing and web-based interactive visualization for our datasets.

Future Directions

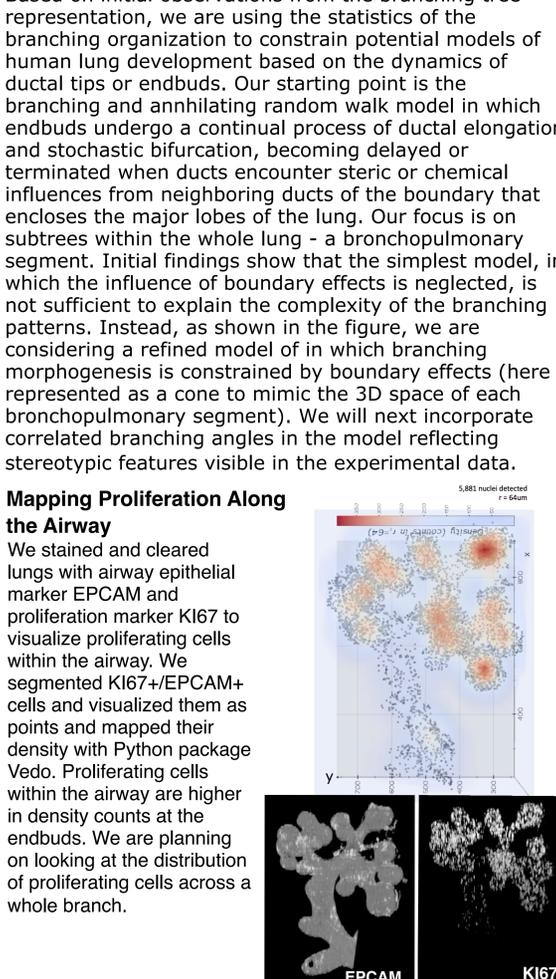
A mathematical model of human lung branching



Based on initial observations from the branching tree representation, we are using the statistics of the branching organization to constrain potential models of human lung development based on the dynamics of ductal tips or endbuds. Our starting point is the branching and annihilating random walk model in which endbuds undergo a continual process of ductal elongation and stochastic bifurcation, becoming delayed or terminated when ducts encounter steric or chemical influences from neighboring ducts of the boundary that encloses the major lobes of the lung. Our focus is on subtrees within the whole lung - a bronchopulmonary segment. Initial findings show that the simplest model, in which the influence of boundary effects is neglected, is not sufficient to explain the complexity of the branching patterns. Instead, as shown in the figure, we are considering a refined model of in which branching morphogenesis is constrained by boundary effects (here represented as a cone to mimic the 3D space of each bronchopulmonary segment). We will next incorporate correlated branching angles in the model reflecting stereotypic features visible in the experimental data.

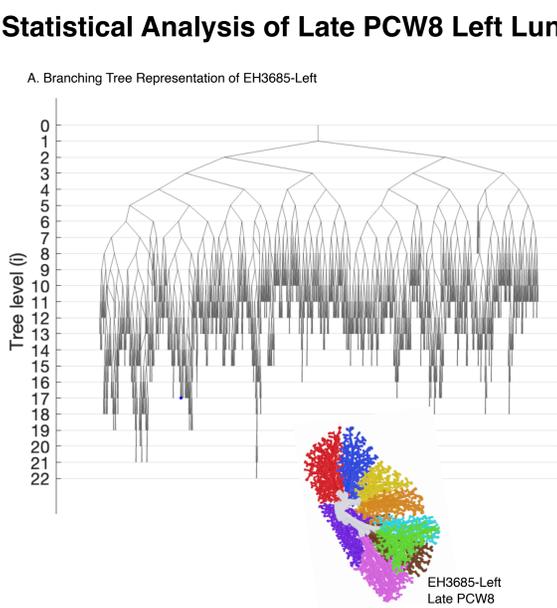
Mapping Proliferation Along the Airway

We stained and cleared lungs with airway epithelial marker EPCAM and proliferation marker KI67 to visualize proliferating cells within the airway. We segmented KI67+/EPCAM+ cells and visualized them as points and mapped their density with Python package Vedo. Proliferating cells within the airway are higher in density counts at the endbuds. We are planning on looking at the distribution of proliferating cells across a whole branch.

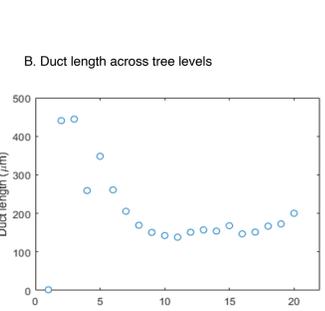


Statistical Analysis of Late PCW8 Left Lung

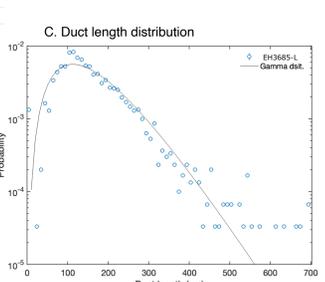
A. Branching Tree Representation of EH3685-Left



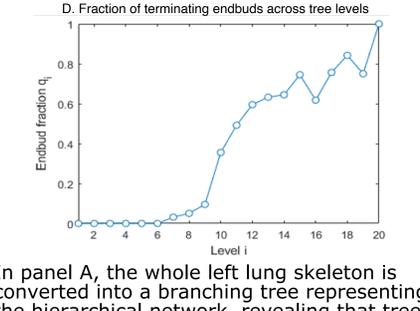
B. Duct length across tree levels



C. Duct length distribution



D. Fraction of terminating endbuds across tree levels



In panel A, the whole left lung skeleton is converted into a branching tree representing the hierarchical network, revealing that tree level reaches a depth of up to 20 levels. Panel B shows that the average duct length stabilizes with tree level, which may indicate a stationary pattern of growth at these stages of development. Panel C shows the duct length distribution, revealing a “typical duct length” of about 100um and an exponential-like decay in the distribution of long ducts. Panel D shows the fraction of terminating endbuds across tree levels, showing an early phase of dichotomous branching with no termination (up to level 8), followed by a phase in which ductal tips are terminated or delayed (level 8 and beyond).