



**HAL**  
open science

## Tridimensional Imaging of human lung early development

Inoue Megumi, Bordeu Ignacio, Hernandez-Garzon Edwin, Yorick Gitton, Couly Gerard, Simons Benjamin, Chedotal Alain

► **To cite this version:**

Inoue Megumi, Bordeu Ignacio, Hernandez-Garzon Edwin, Yorick Gitton, Couly Gerard, et al.. Tridimensional Imaging of human lung early development. Crick BioImage Analysis Symposium 2022, Nov 21-22, 2022. Francis Crick Institute, London, UK., Nov 2022, LONDRES, United Kingdom. hal-04237973

**HAL Id: hal-04237973**

**<https://cnrs.hal.science/hal-04237973>**

Submitted on 13 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

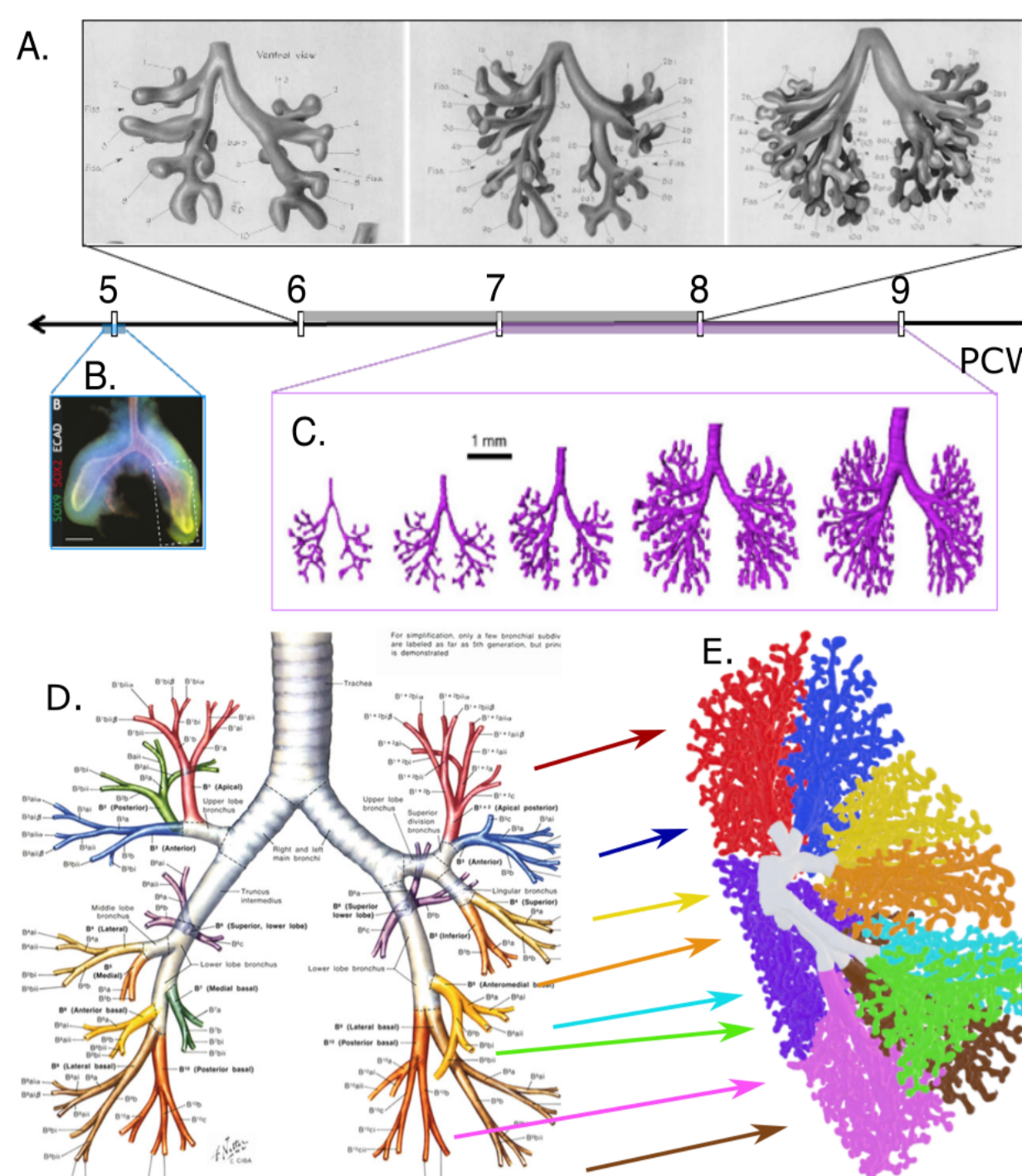
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Branching Morphogenesis in Human Lung Development

Megumi Inoue (megumi.inoue@inserm.fr)<sup>1</sup>, Ignacio Bordeu<sup>2</sup>, Edwin Hernandez-Garzon<sup>3</sup>, Yorick Gitton<sup>1</sup>, Gerard Couly<sup>1</sup>, Benjamin Simons<sup>4,5,6</sup>, Alain Chedotal (alain.chedotal@inserm.fr)<sup>1</sup>

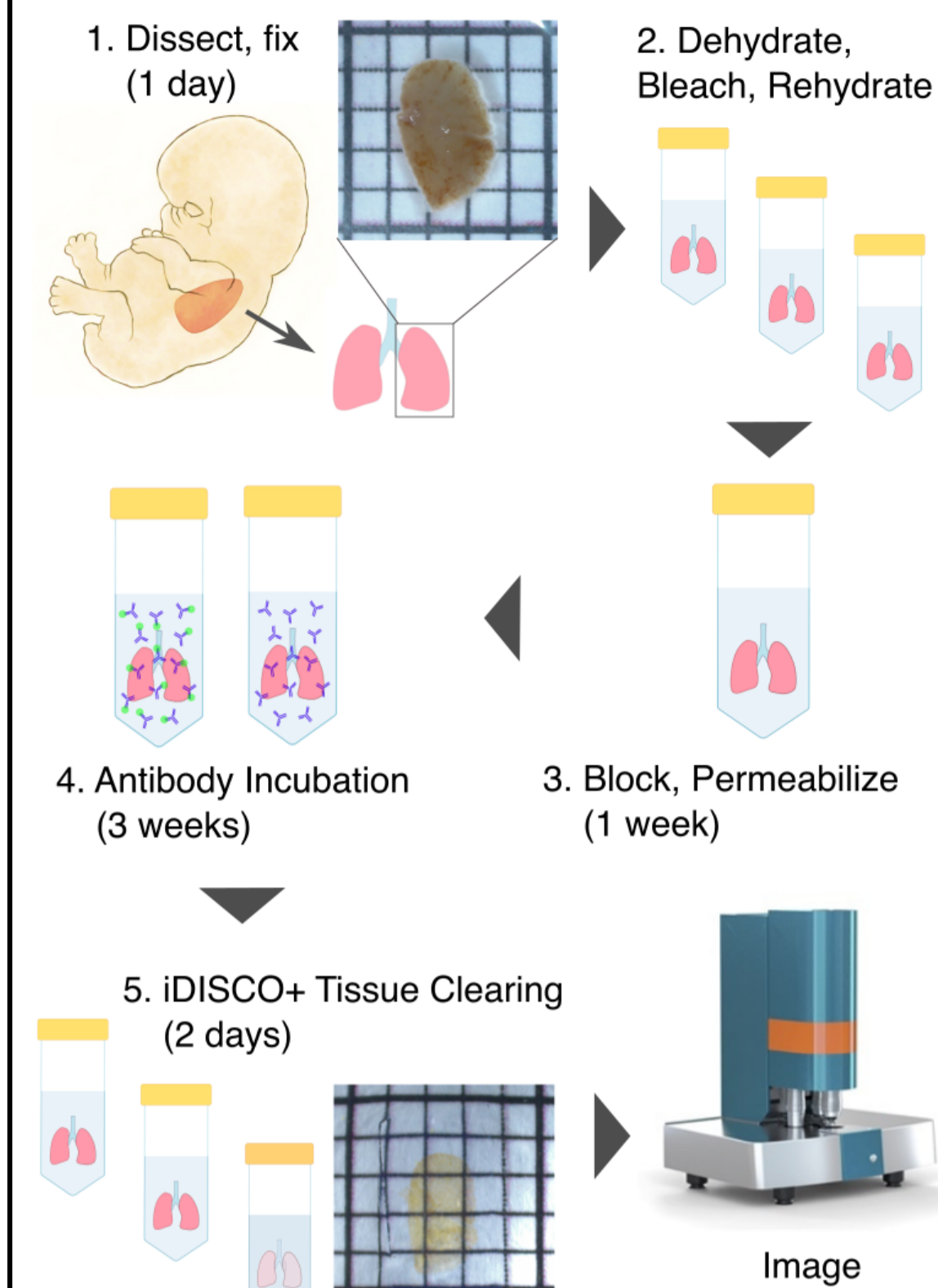
<sup>1</sup> Institut de la Vision, Sorbonne Université, INSERM, CNRS, Paris, France.  
<sup>2</sup> Departamento de Física, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile  
<sup>3</sup> Alcala Bio-imaging Core, CI2A - CSIC, Alcala De Henares, Spain.  
<sup>4</sup> The Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK.  
<sup>5</sup> Cavendish Laboratory, Department of Physics, University of Cambridge, UK.  
<sup>6</sup> Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, UK.

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 (see right, A-C). Here, 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? 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.

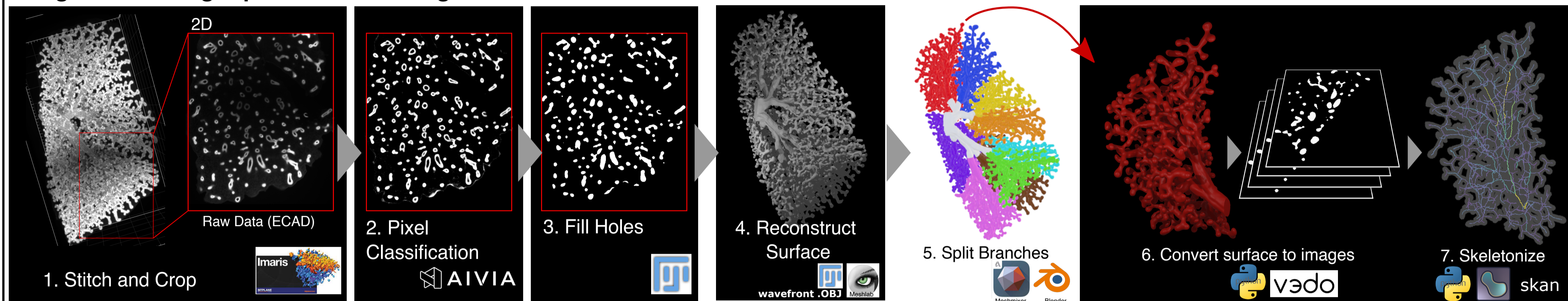


Previous studies of human lung development. A. Wells and Boyden, 1954 Am. J. of Anat. B. Nikolic et al., 2018 Development. C. Fujii S et al., 2020 J of Anat. D. The Netter Collection of Medical Illustrations - Respiratory System. E. Nomenclature mapped to our dataset EH3685-Left.

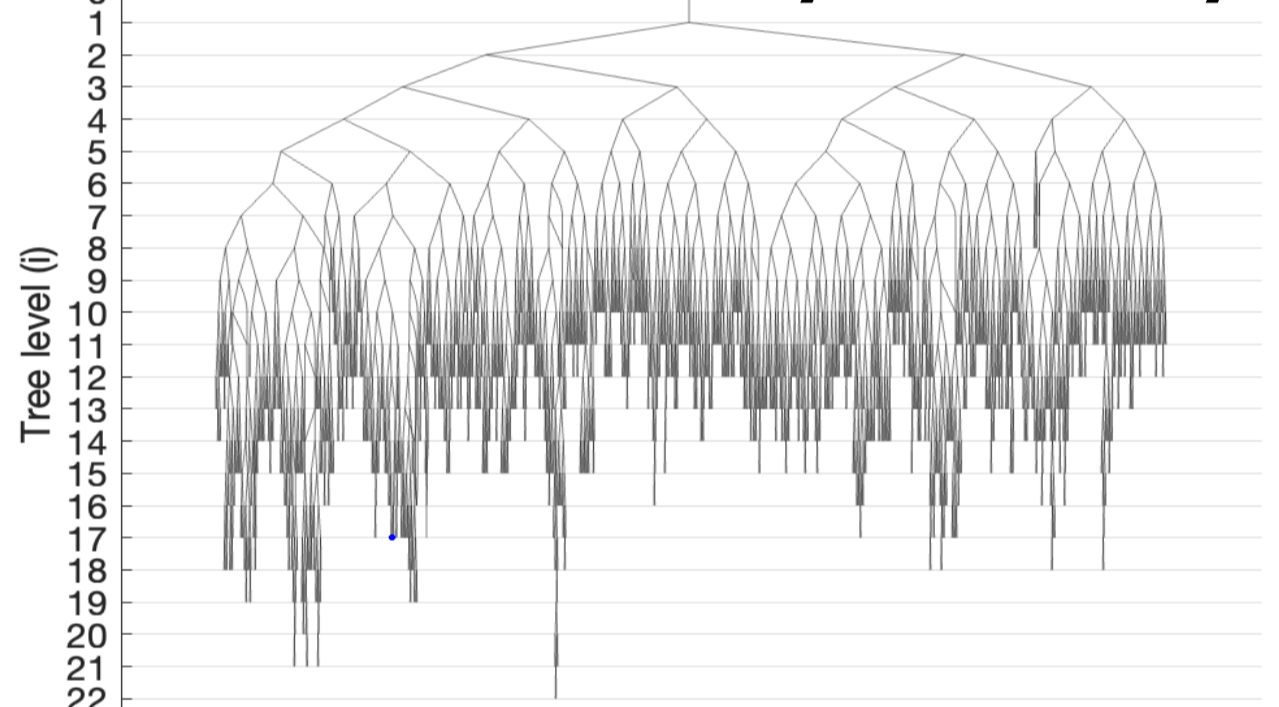
## Sample Preparation



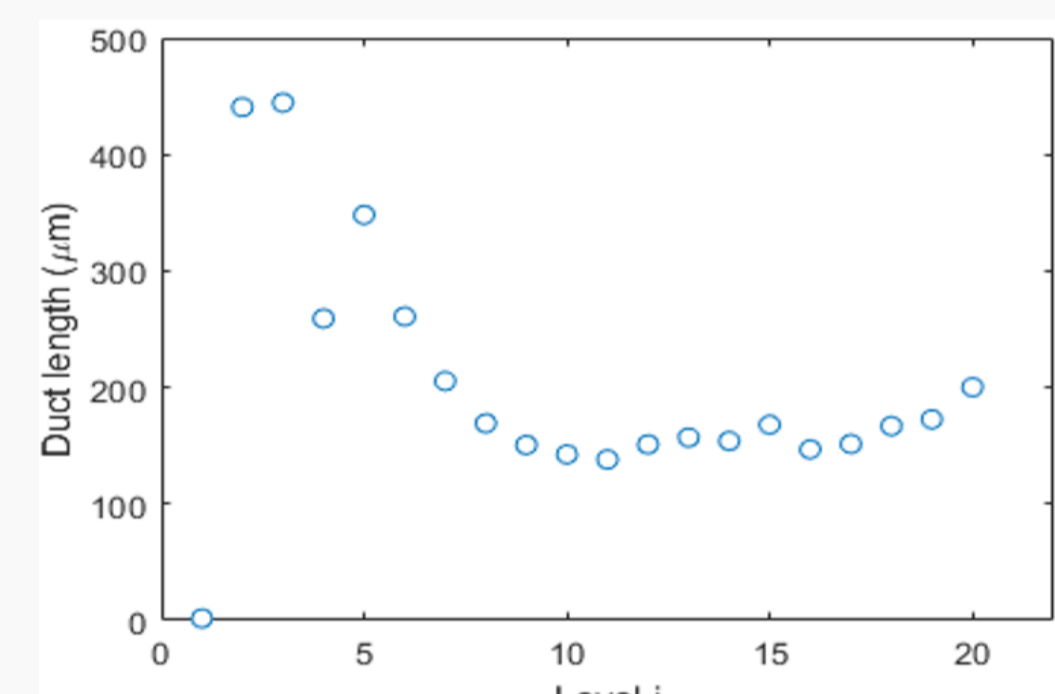
## Image Processing Pipeline - From Image to Branchwise Skeleton



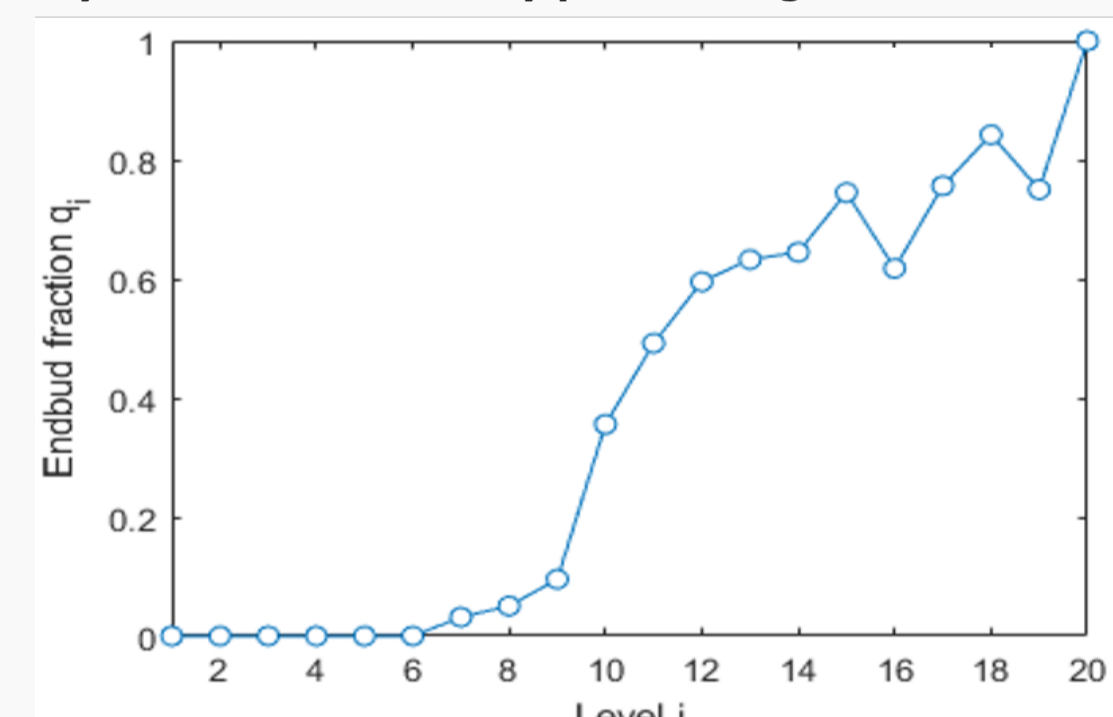
## Airway Tree Analysis for PCW9 Left Lung



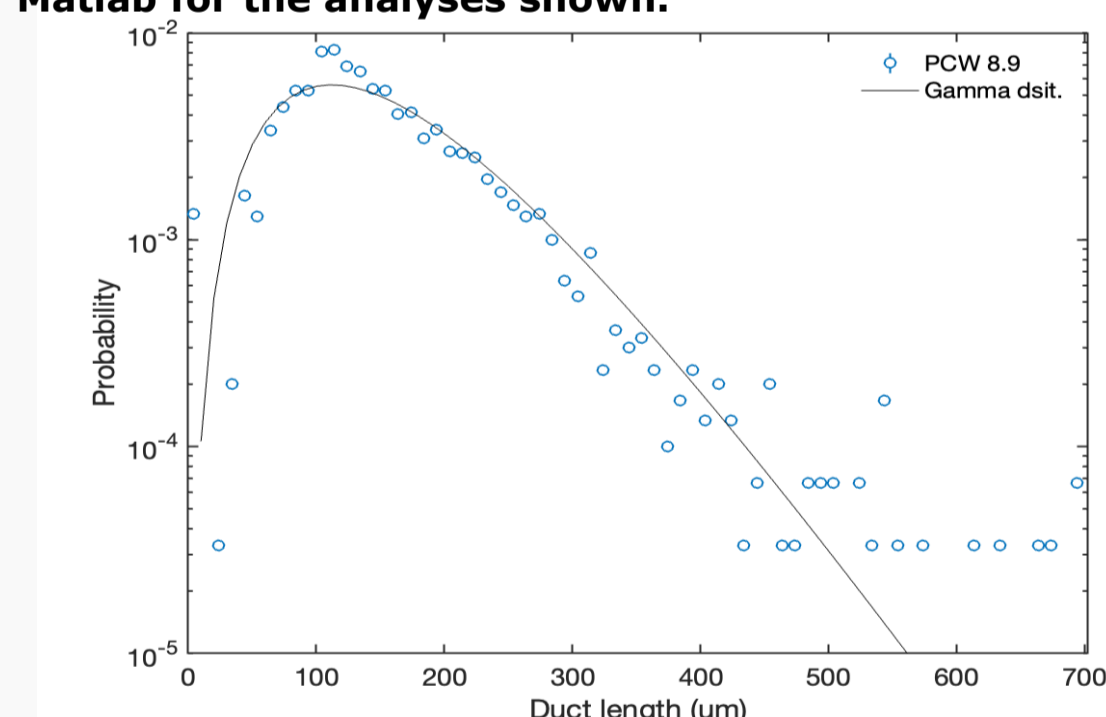
The skeleton is converted into a graphical tree representing the hierarchical network, revealing that tree level reaches a depth of up to 20 levels. Ad-hoc scripts were developed in Matlab for the analyses shown.



Average duct length stabilizes with tree level, which may indicate a stationary pattern of growth.

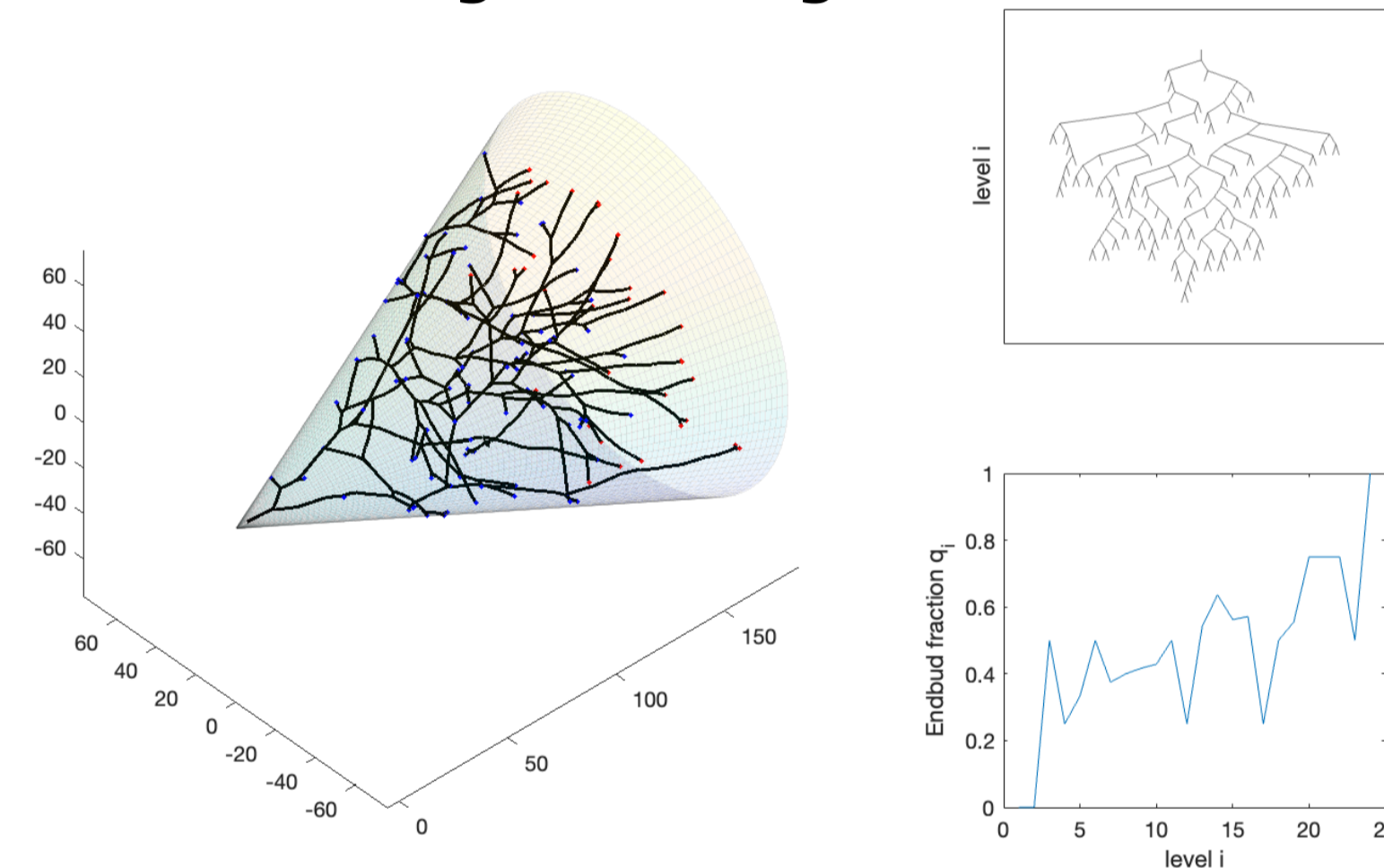


The fractions of terminating endbuds across tree levels show an early phase of dichotomous branching with no termination followed by a phase of terminations.



Duct length distribution reveals a "typical duct length" around 100µm and an exponential-like decay in long duct distribution.

## Towards a mathematical model of human lung branching



We use the statistics of the branching organization to constrain potential models of human lung development based on the dynamics of ductal tips or endbuds. We start with the branching and annihilating random walk model where 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. We find the simplest model, in which the influence of boundary effects is neglected, is not sufficient to explain the complexity of the branching patterns. Instead we consider a model in which branching morphogenesis is constrained by boundary effects (represented as a cone to mimic the 3D space of each bronchopulmonary segment). We will next incorporate correlated branching angles.

## Lung Datasets

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 3D printing and web-based visualizations for our datasets.

