

3D Bioprinting of Pancreatic Tissue for Biomedical Research

Focus on Vascular Network

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PANCREAS

ENDOCRINE

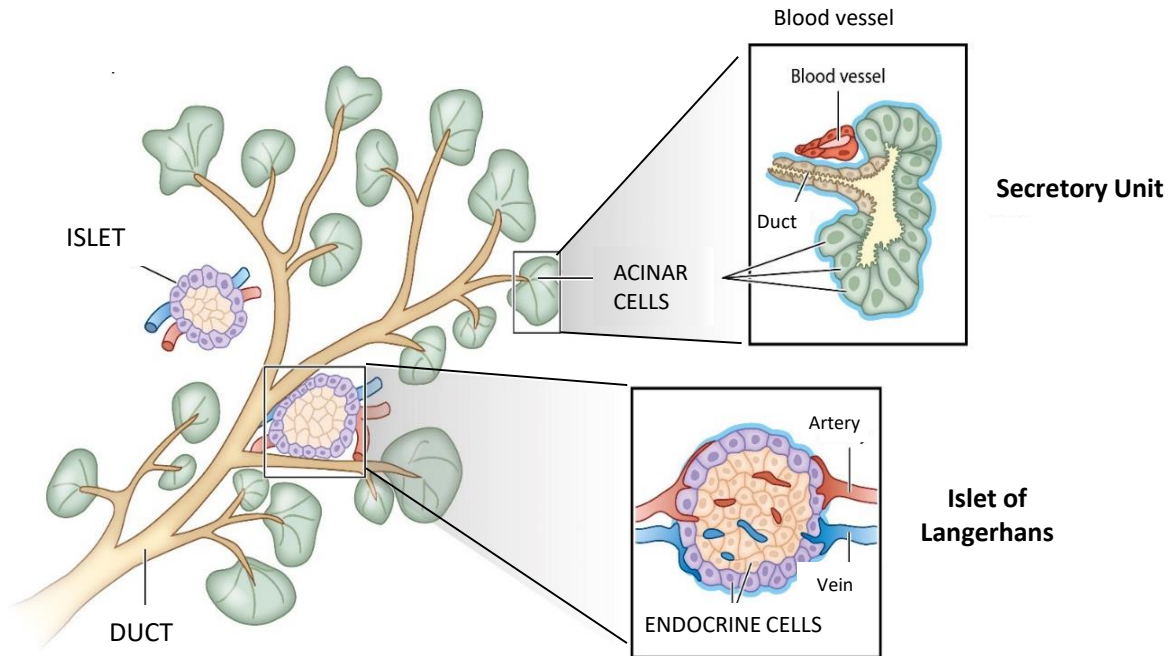
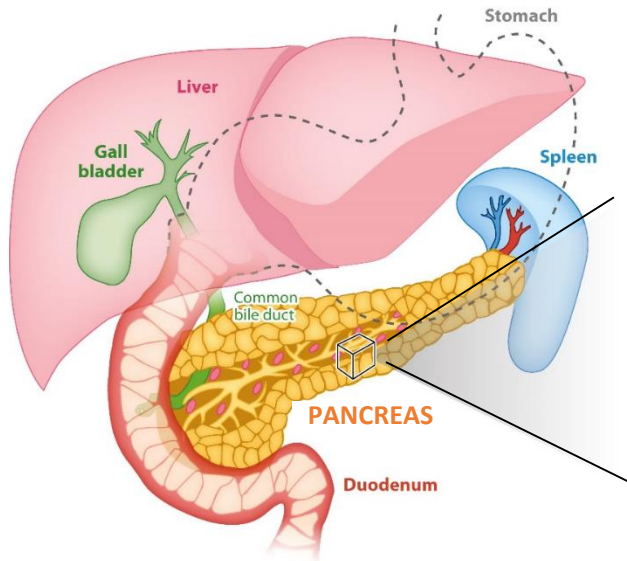
Endocrine cells (α , β , δ , ϵ , PP)

Islets

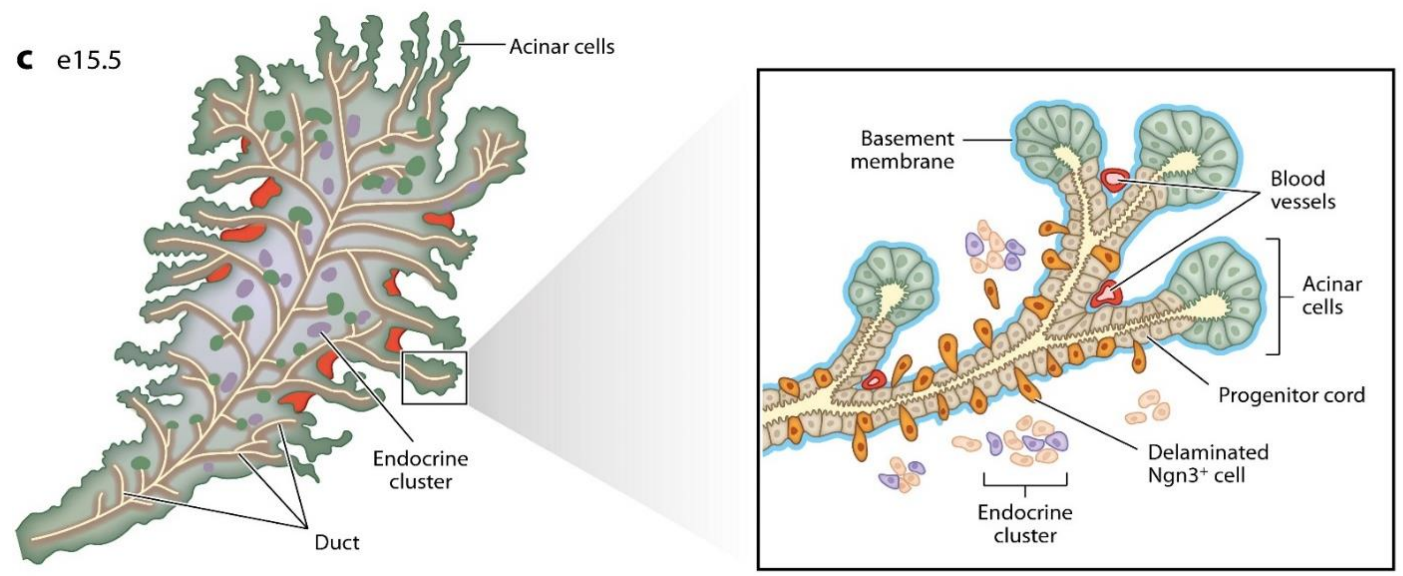
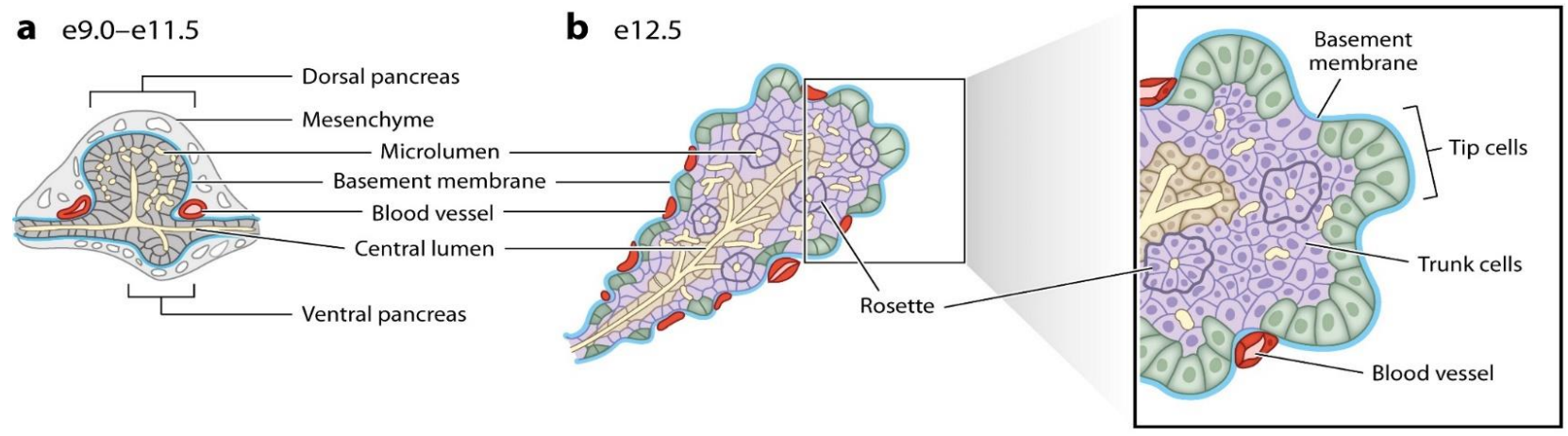
EXOCRINE

Ductal & Acinar cells

Branched Network



PANCREAS ORGANOGENESIS



(Sander et al., 2013)

PANCREATIC DISEASES

Diabetes

422
MILLION
Patients

Pancreatic Cancer

400 000
Death/Year

(IHME, Global Burden of Disease (GBD), 2017)

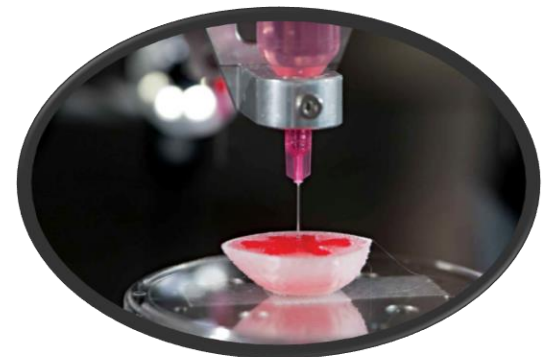
→ Need to better understand the pancreas in order to develop new therapies and medications

Problems

- x In vitro culture are not ideal (2D, homogenous)
- x Limited access to tissues in vivo
- x Animal testing is difficult and burdensome

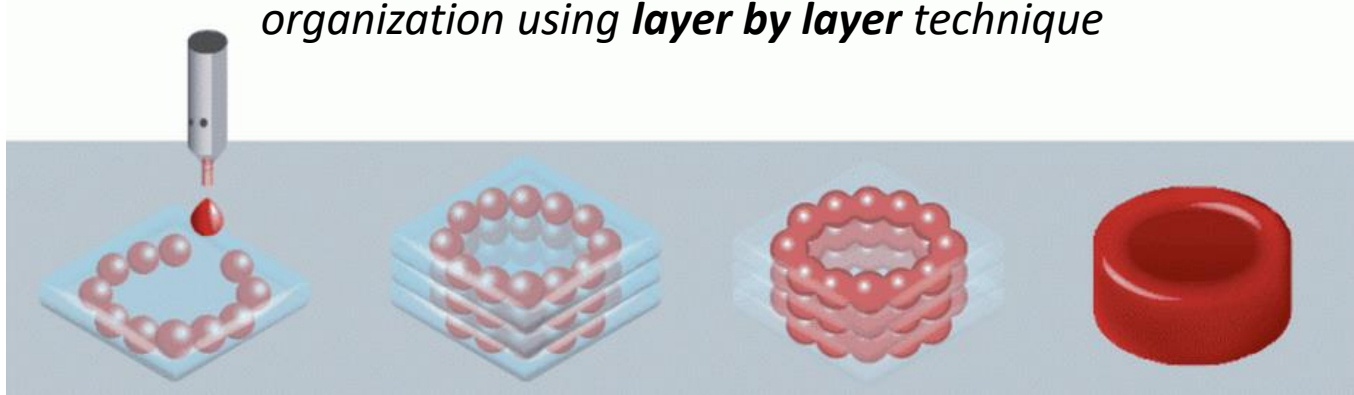
Opportunity

Development of 3D printing technology



3D BIOPRINTING

Computer transfer process for patterning living materials with a prescribed 3D organization using **layer by layer** technique



1986
Stereo lithography

2003
Multicellular spheroids

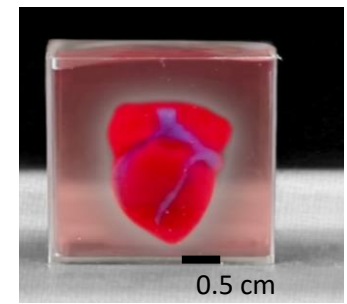
2009
Skin bioprinted

2010
1st Commercial bio-printer

2014
Functional Ear Heart Valve

2019
Heart

1999
Synthetic bladder
1st lab grown organ



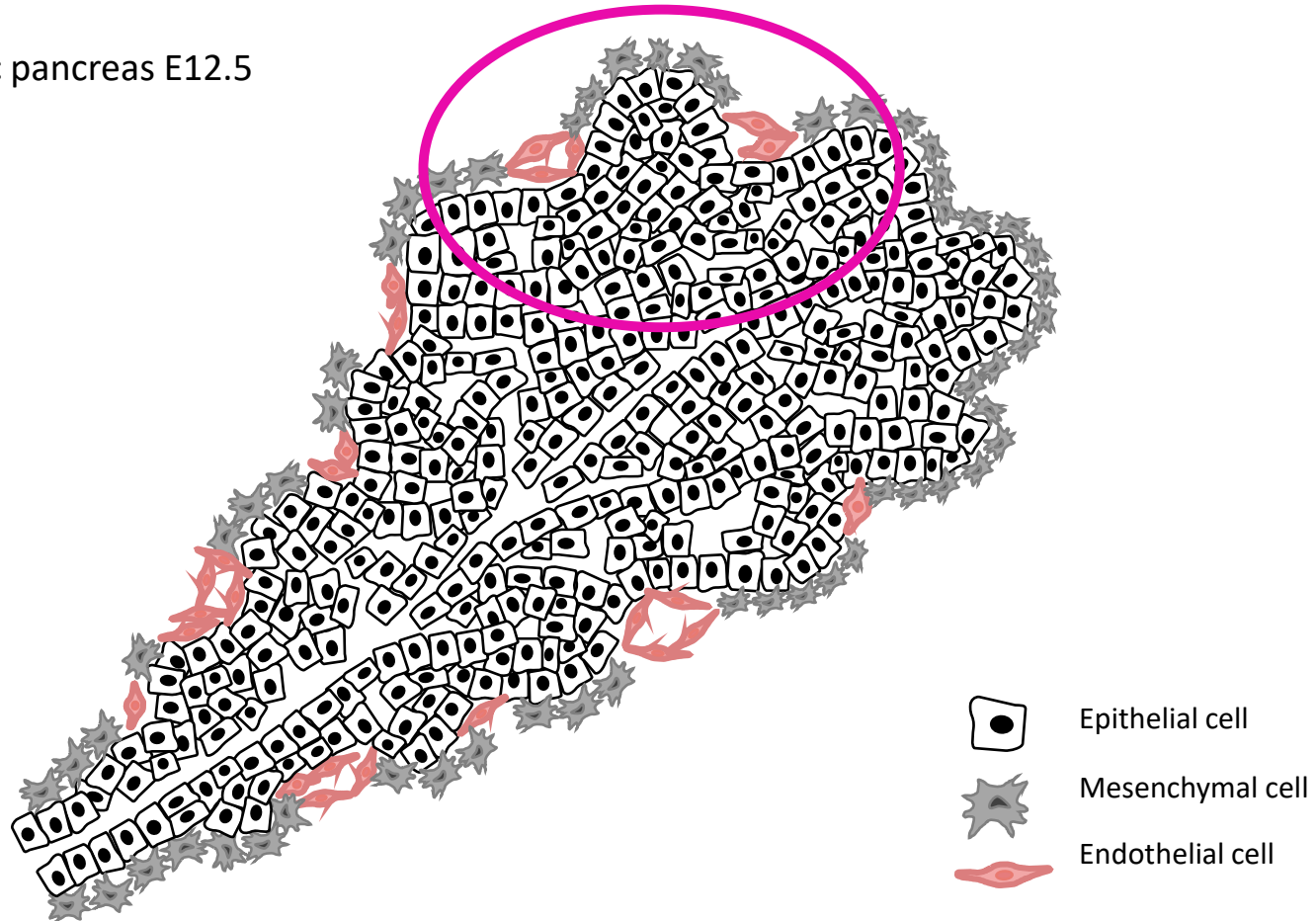
0.5 cm

(Dvir et al)

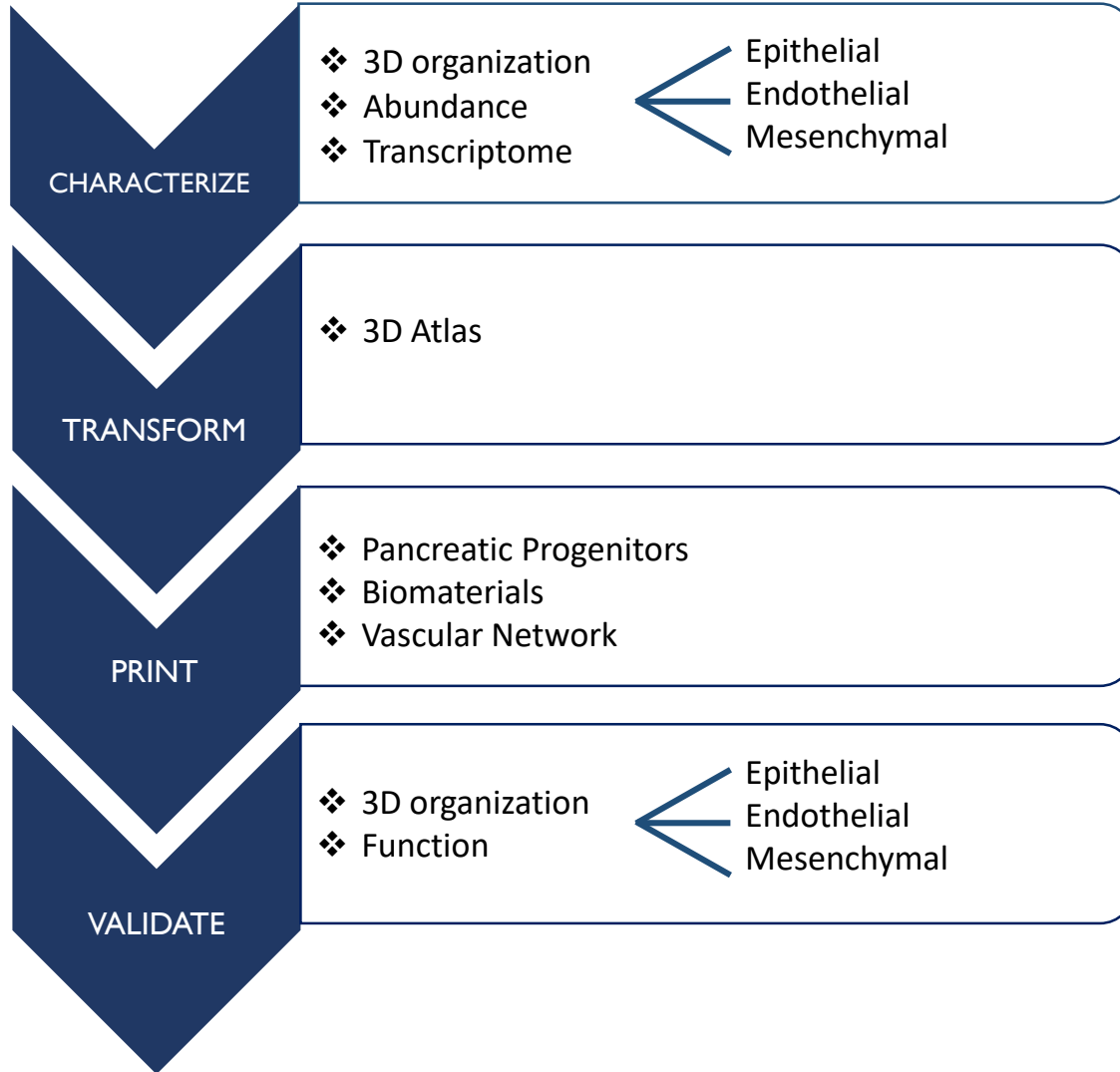
Major impediments : **Biomimicry, Vascularization & Size**

Bioprinting of mini-pancreas taking advantage of the simple 3D architecture & plasticity of **embryonic pancreas**

Embryonic pancreas E12.5

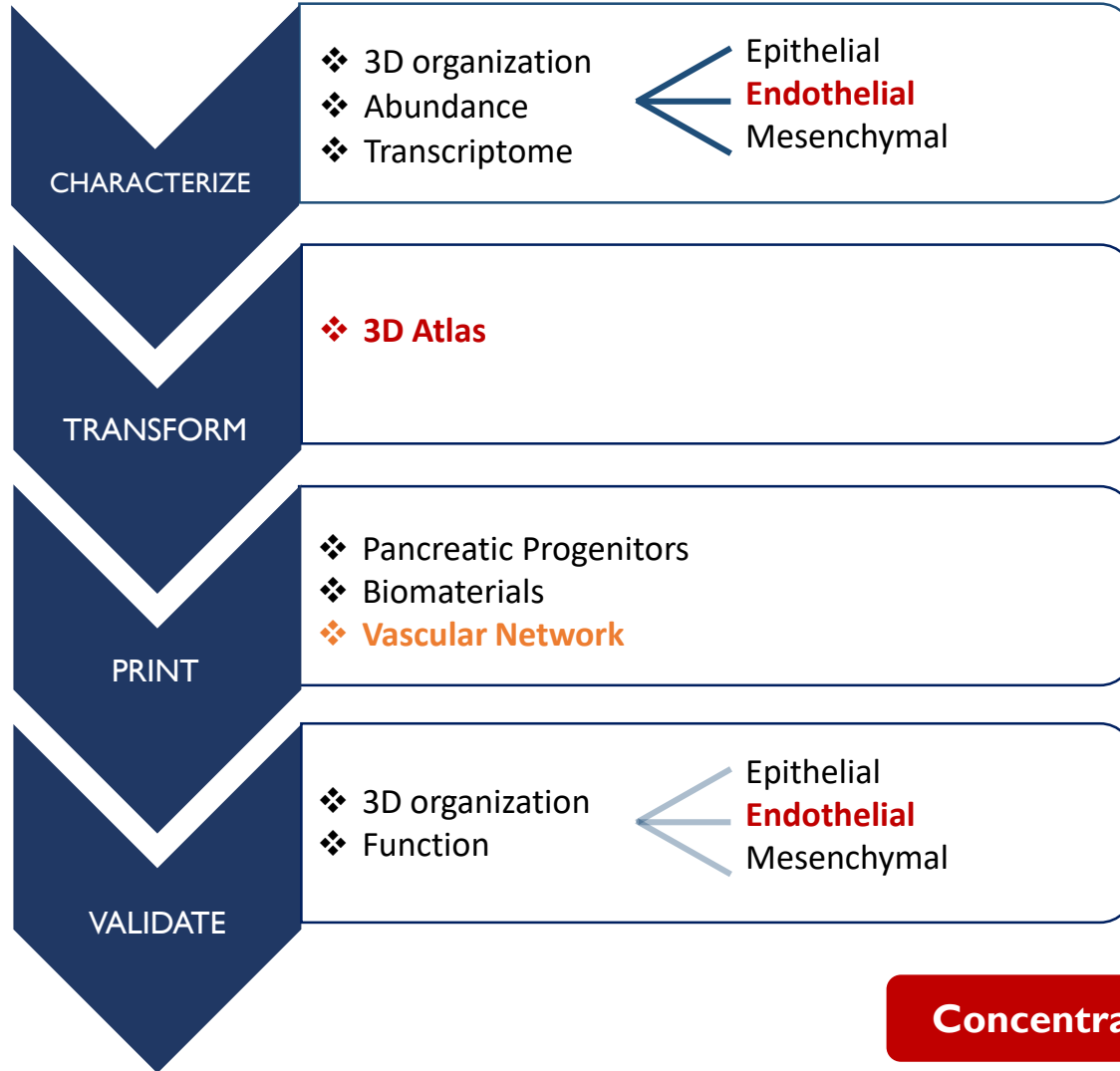


WORK PLAN



- Pan3DP Consortium
1. F. Spagnoli (England)
 2. C. Pierreux (Belgium)
 3. L. Landsman (Israel)
 4. D. Iber (Switzerland)
 5. S. Levenberg (Israel)
 6. F. Guillemot (Poietis, France)

WORK PLAN



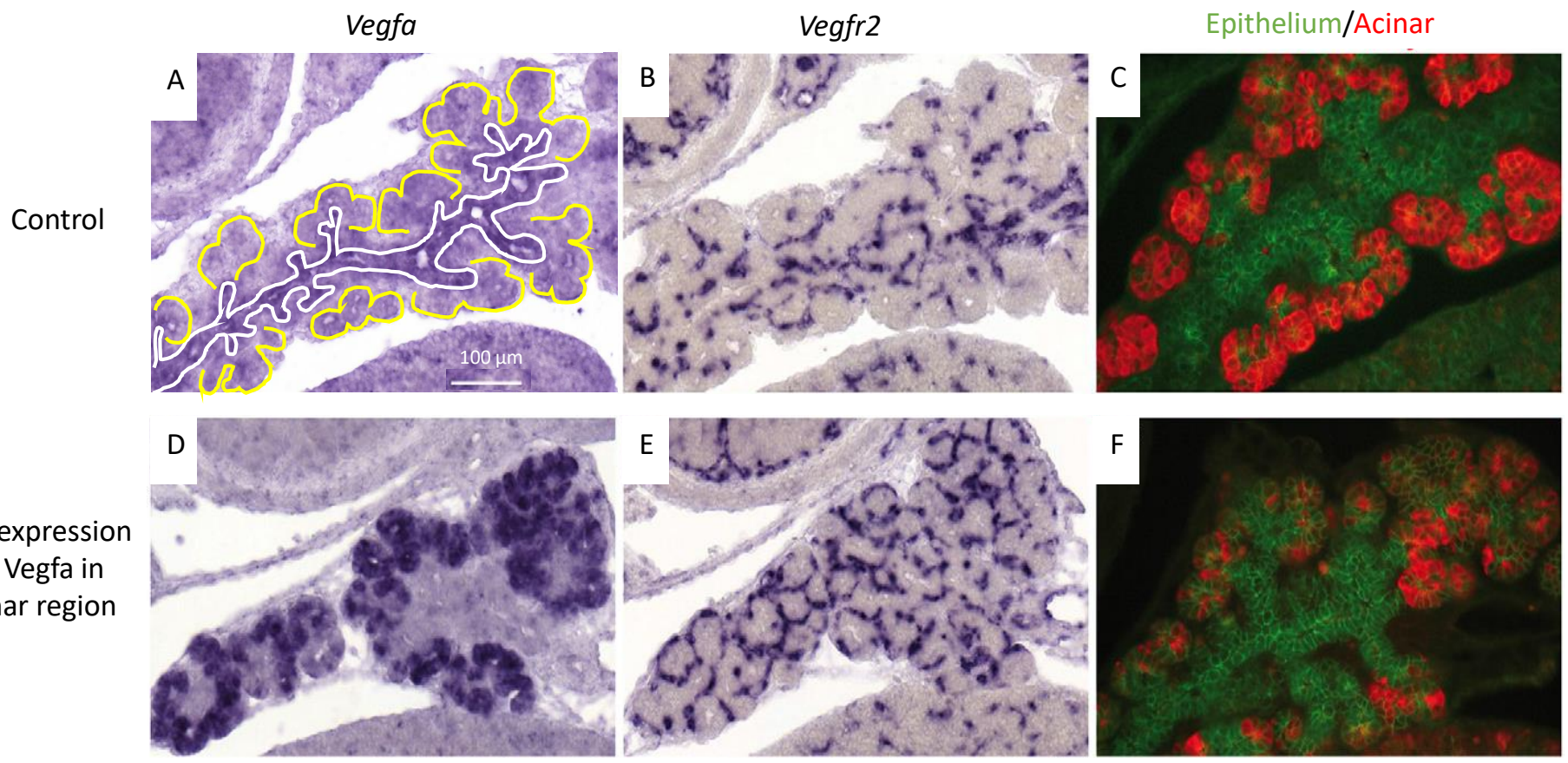
- Pan3DP Consortium
1. F. Spagnoli (England)
 - 2. C. Pierreux (Belgium)**
- Manon Moulis
- Laura Glorieux
 3. L. Landsman (Israel)
 4. D. Iber (Switzerland)
 5. S. Levenberg (Israel)
 6. F. Guillemot (Poietis, France)

OUR GOAL
Concentrate on the vascular network

WHY IS IT IMPORTANT TO INTEGRATE BLOOD VESSELS ?

- Nutritive pipes
- Instructing network : **Quantitative**

Pancreas E15.5



➔ LOCALISATION IMPACT ON CELL DIFFERENTIATION

(Pierreux et al., 2010)

WHY IS IT IMPORTANT TO INTEGRATE BLOOD VESSELS ?

- Nutritive pipes
- Instructing network : **Quantitative**

E12.5

LUNG

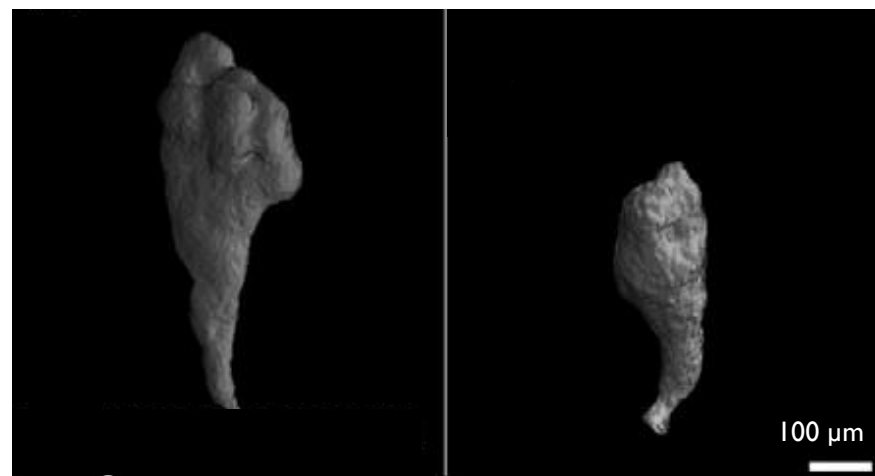
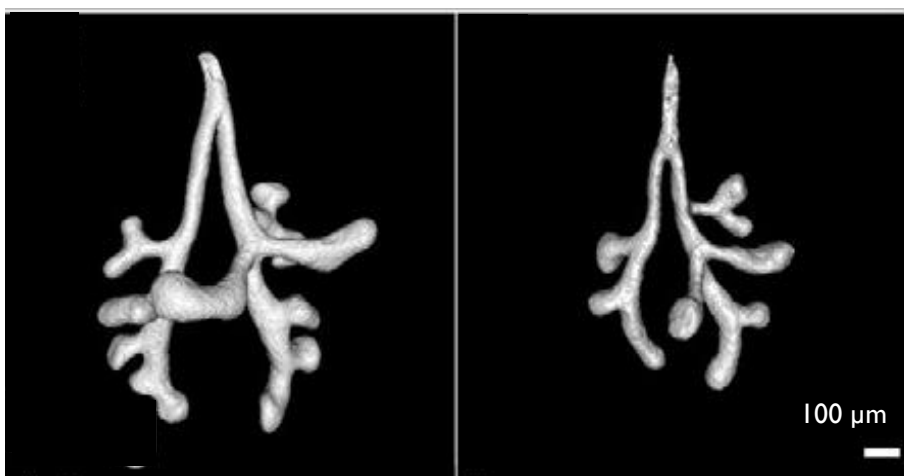
PANCREAS

Control

Abundant vasculature

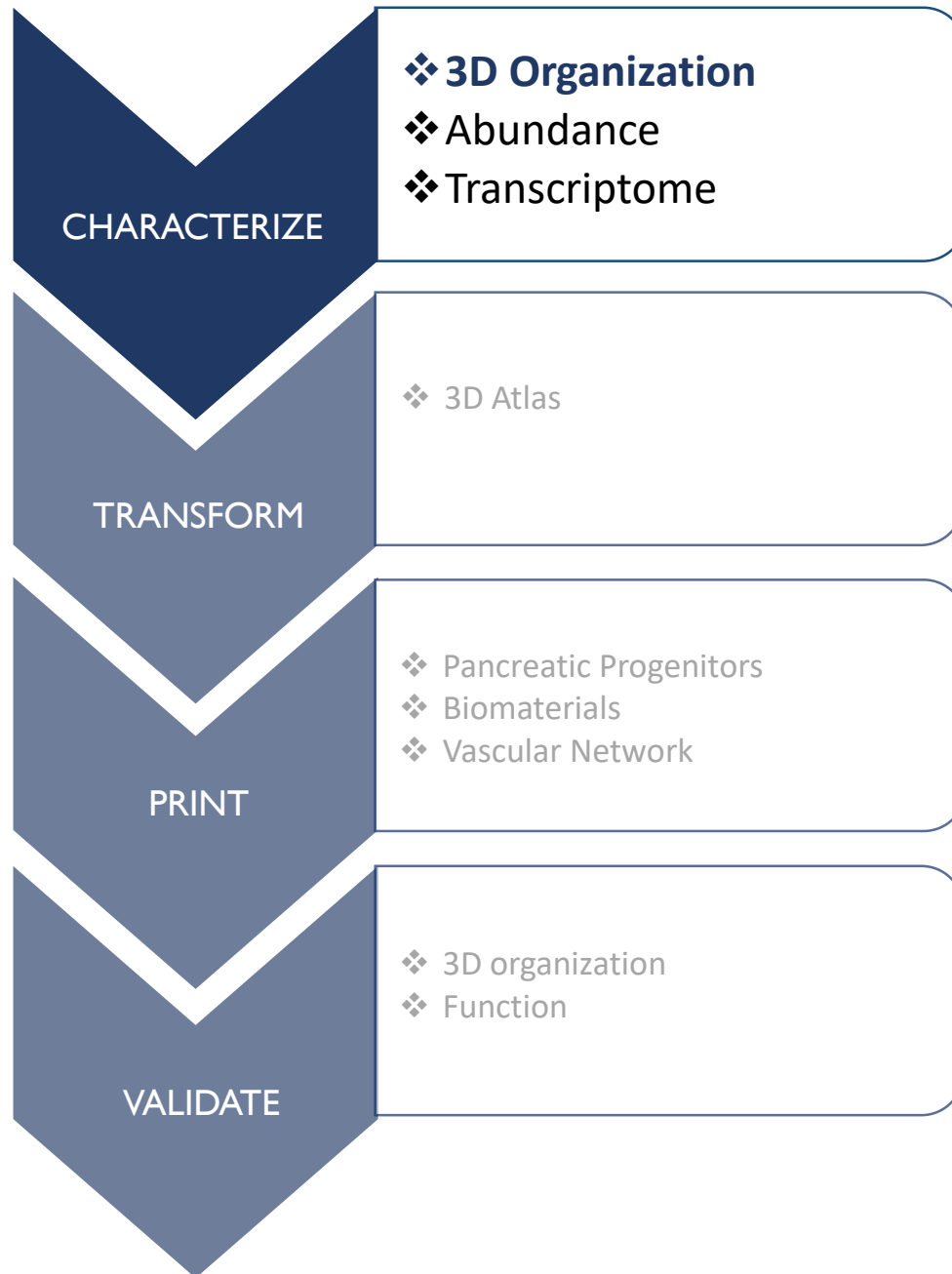
Control

Abundant vasculature



(Sand et al., 2011)

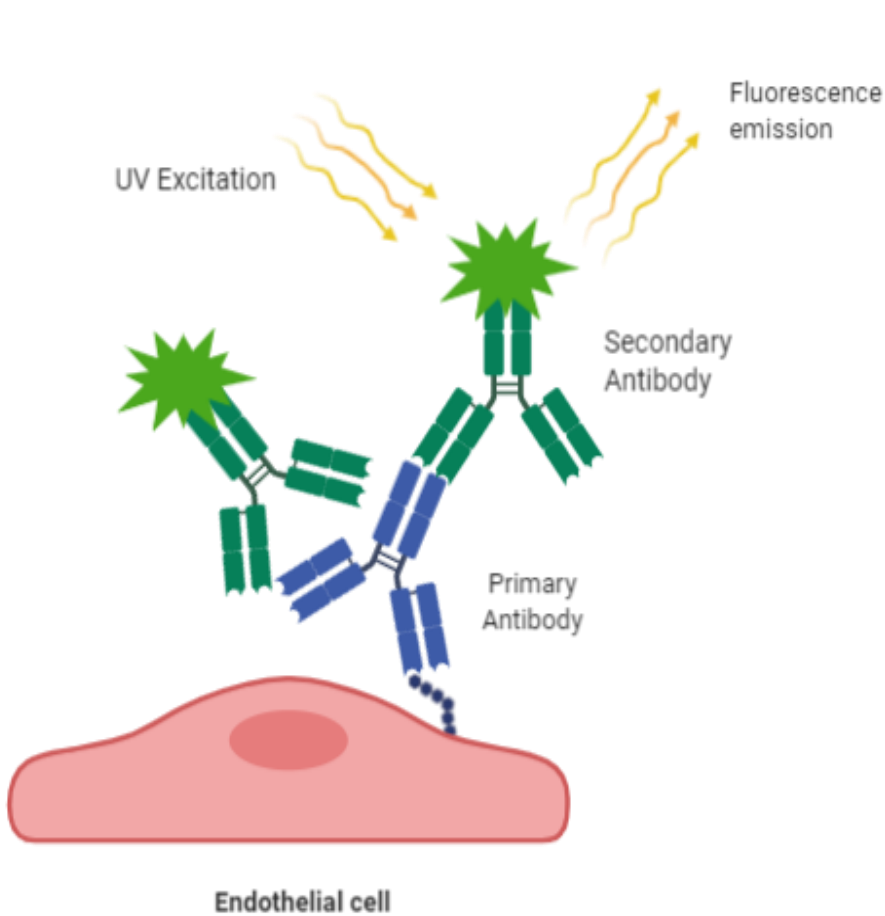
➔ QUANTITY IMPACT ON PANCREAS DEVELOPMENT



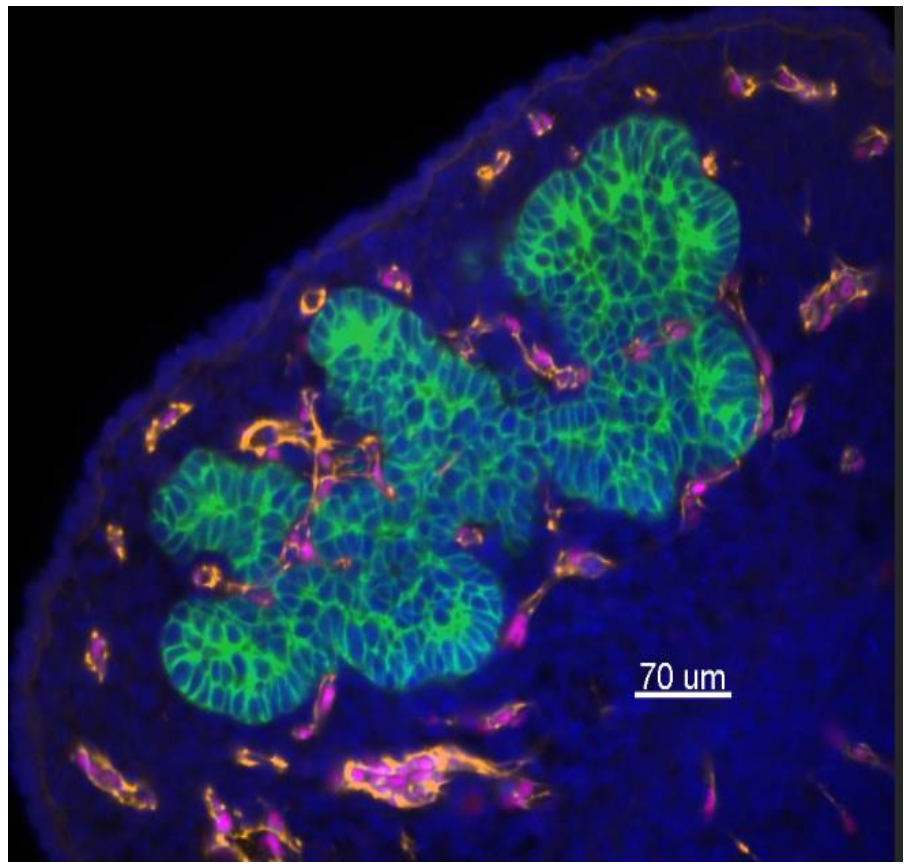
3D ORGANISATION

II. Method

Whole-Mount Immunofluorescence – Clearing – Light Sheet Microscopy



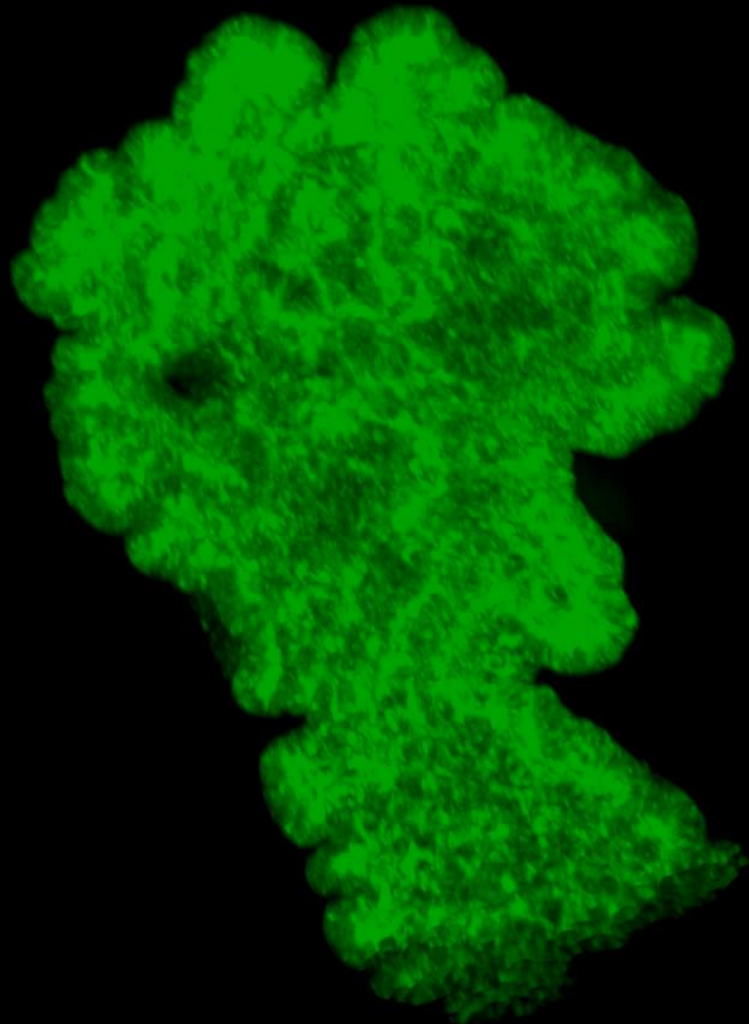
E-Cadherin : Epithelial cells (pancreas)
VE-Cadherin & **ERG** : Endothelial cells (blood vessels)



E-CADHERIN

VE-CADHERIN

ERG



150 um

3D ORGANISATION

III. IMARIS

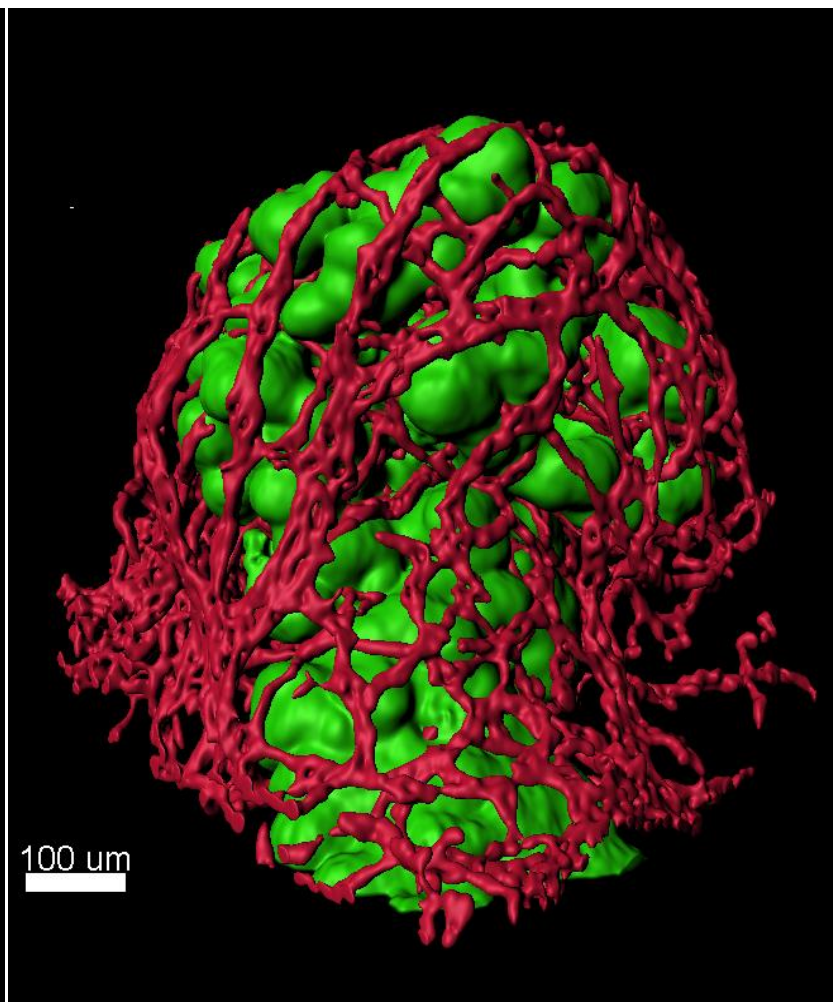
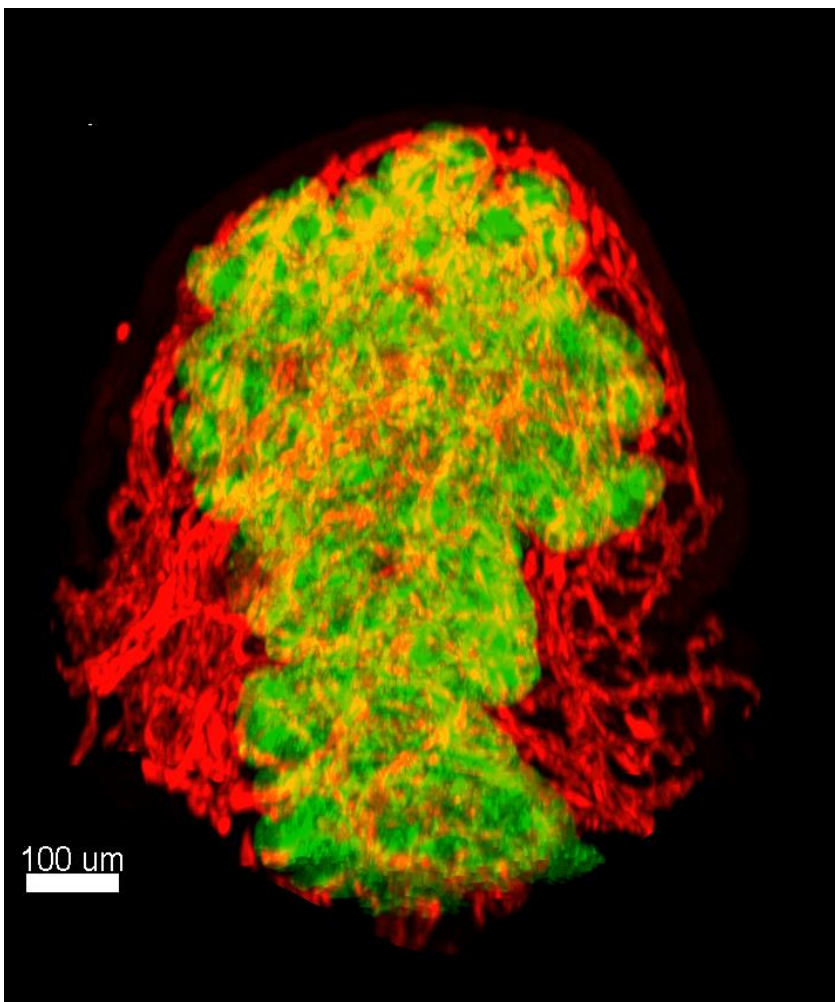
Staining

E-Cadherin
VE-Cadherin



Pancreas
Blood Vessels

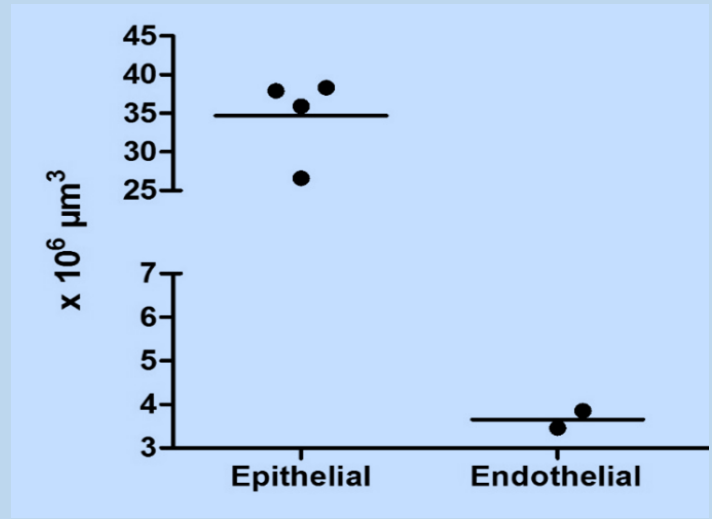
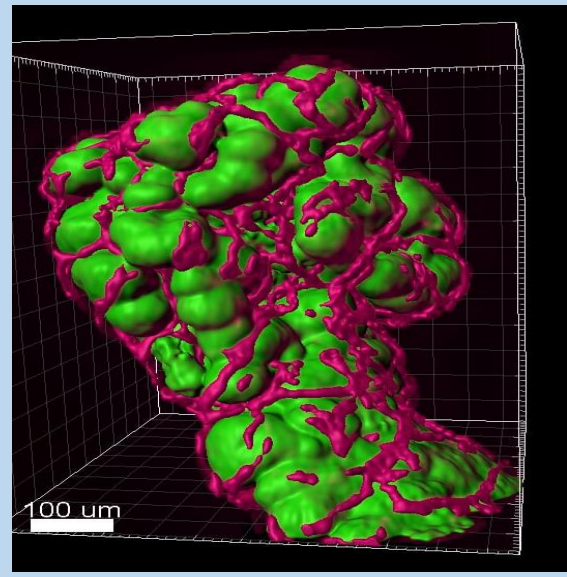
Reconstruction



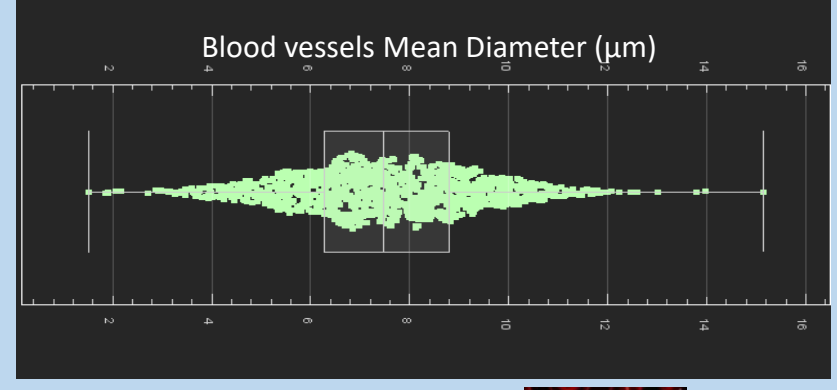
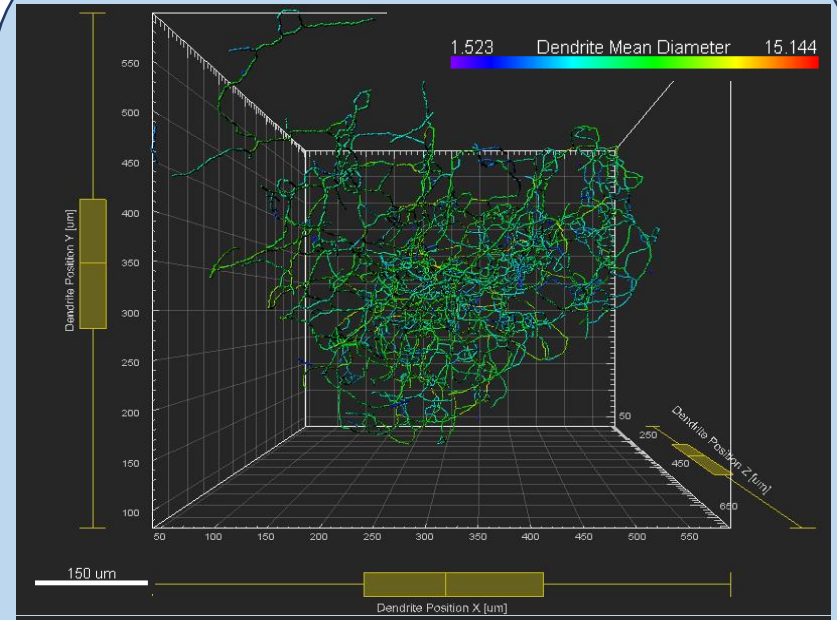
3D ORGANISATION

IV. Analysis

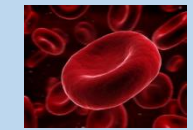
VOLUME



DIAMETER



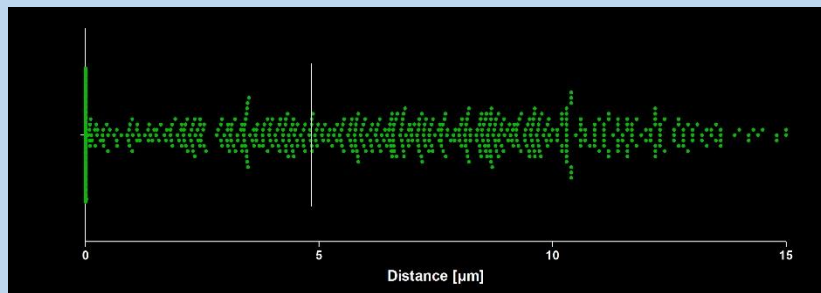
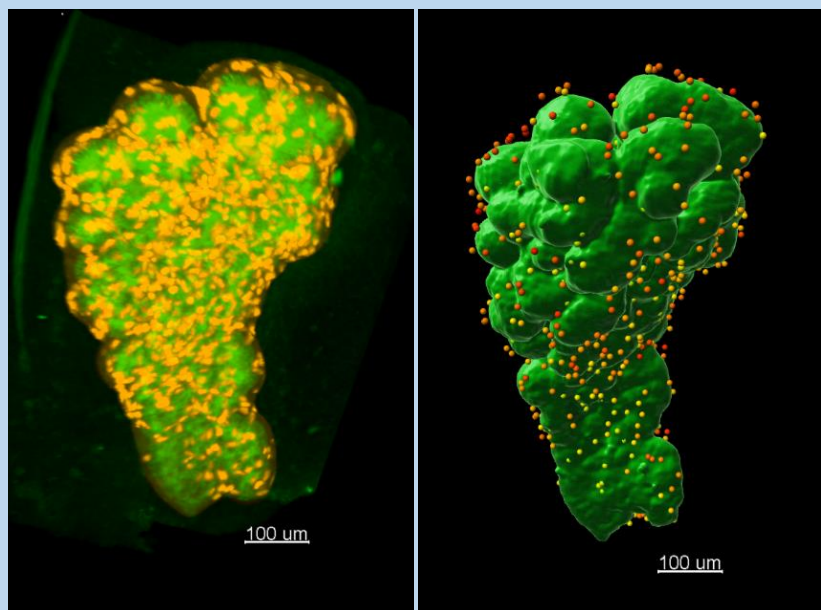
MEAN : 7.5 µm



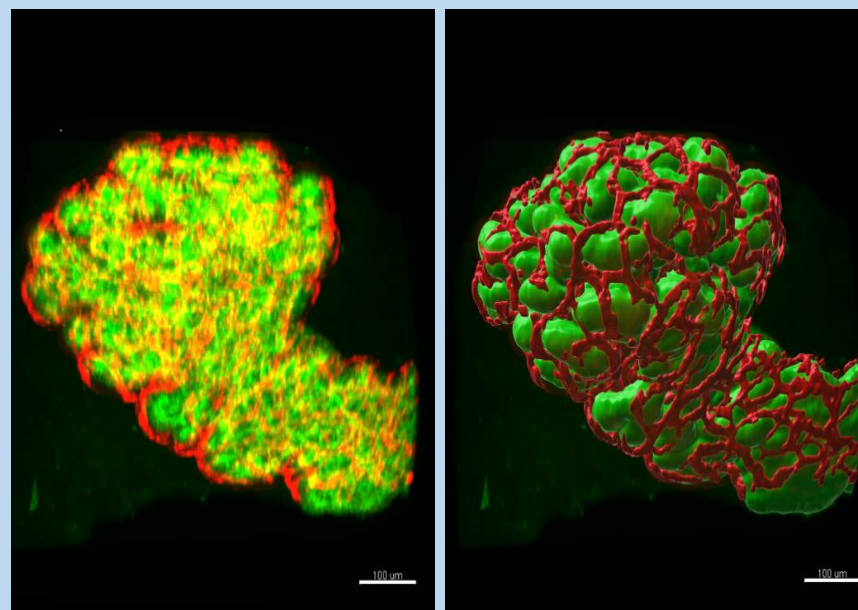
3D ORGANISATION

IV. Analysis

DISTANCE



STRUCTURE

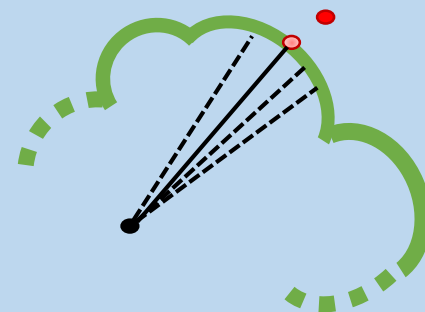


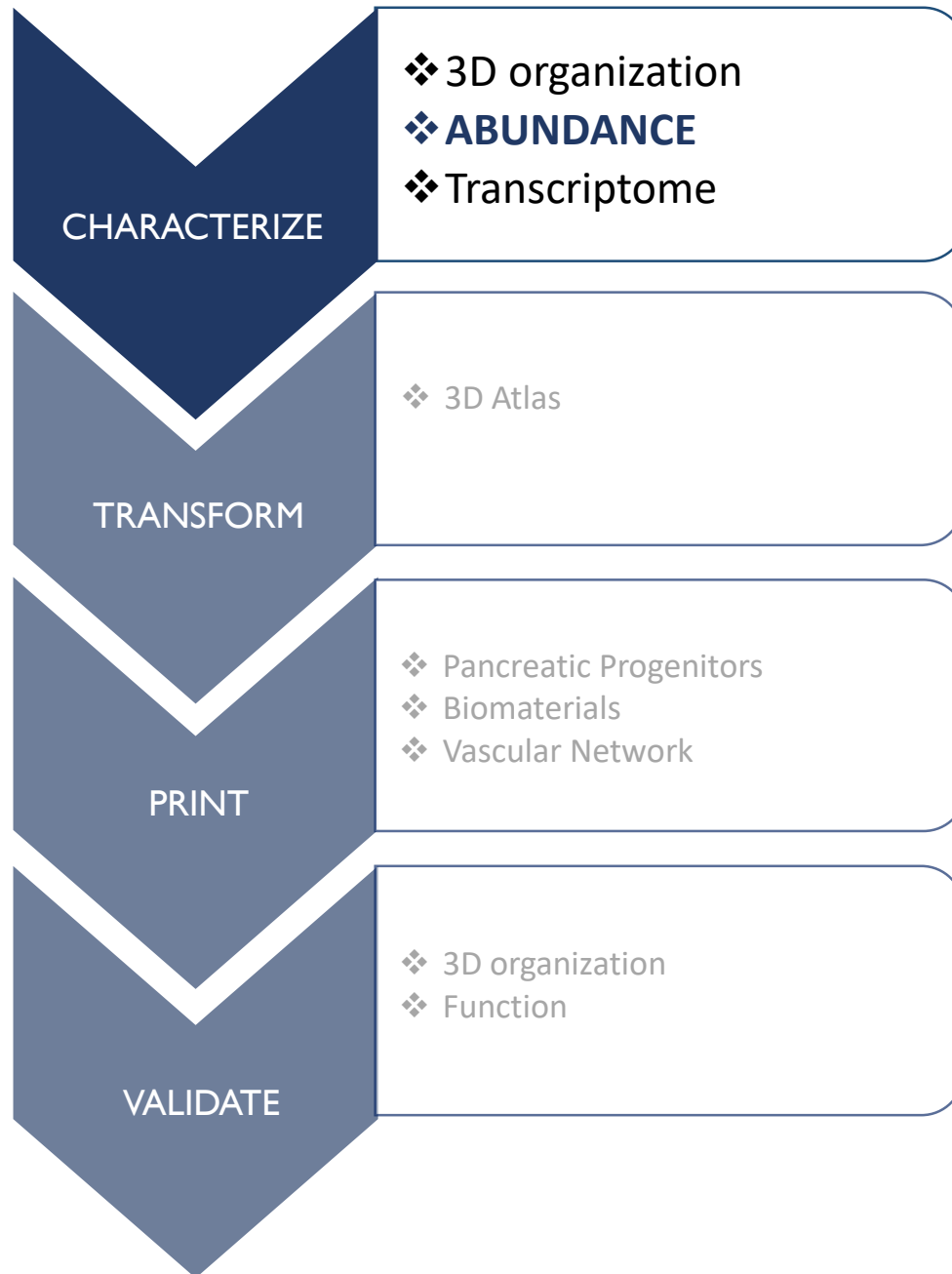
➔ HONEY COMB

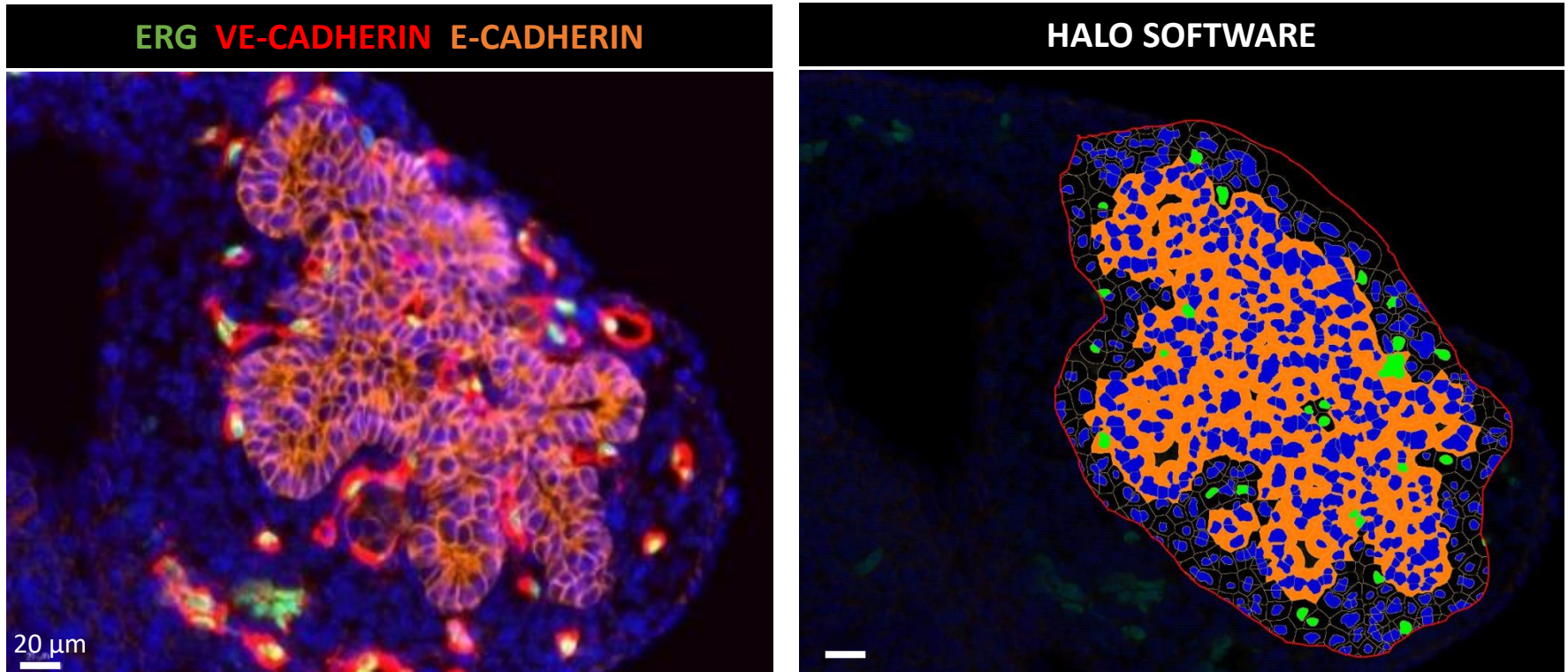
Still in progress

Problematic points :

- Inside surface
- Hollow but on summit



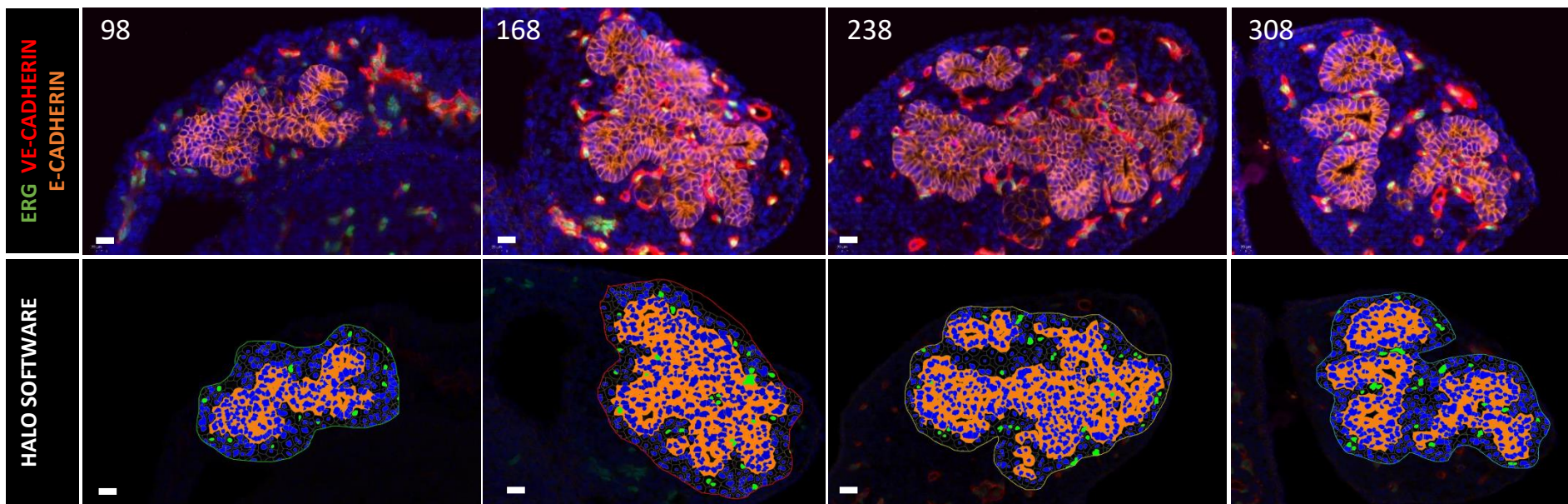


II. Method

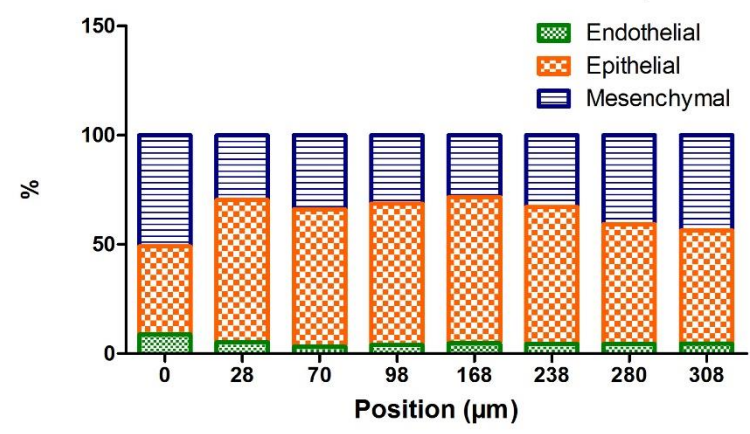
1. Delineate the area (+15μm)
2. Segment nuclei (DAPI)
3. Identify the cell type :
 - Ecadherin → Epithelial
 - ERG → Endothelial
 - No signal → Mesenchymal

ABUNDANCE

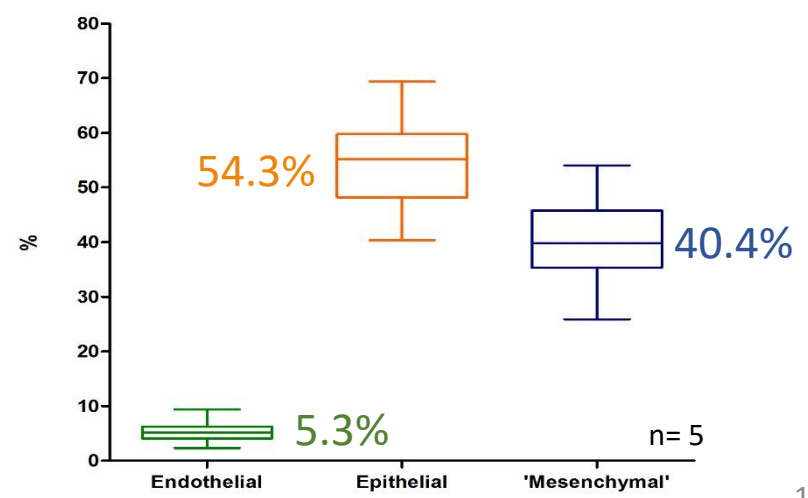
III. Proportion



Proportion of E12.5 pancreatic cells according to their position



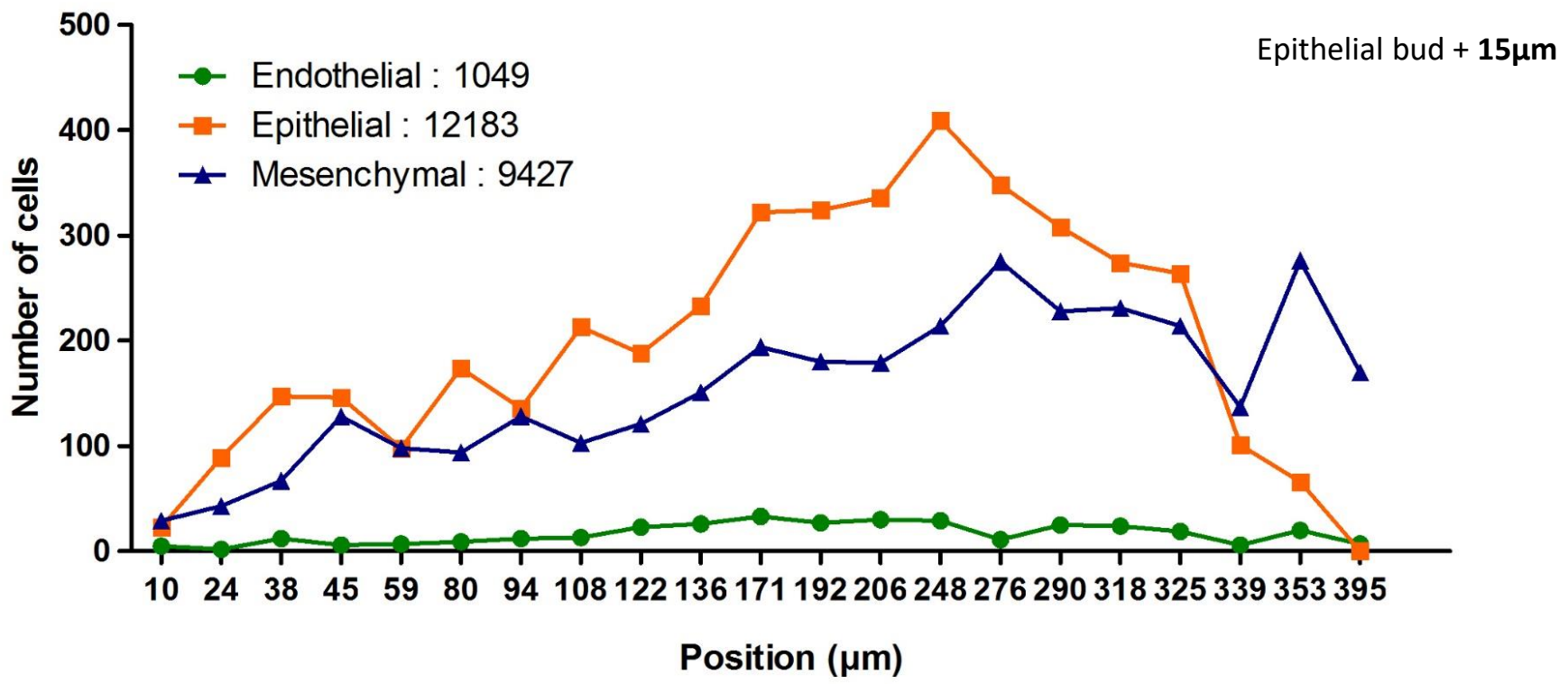
Proportion of E12.5 pancreatic cells



ABUNDANCE

IV. Number

Number of E12.5 pancreatic cells

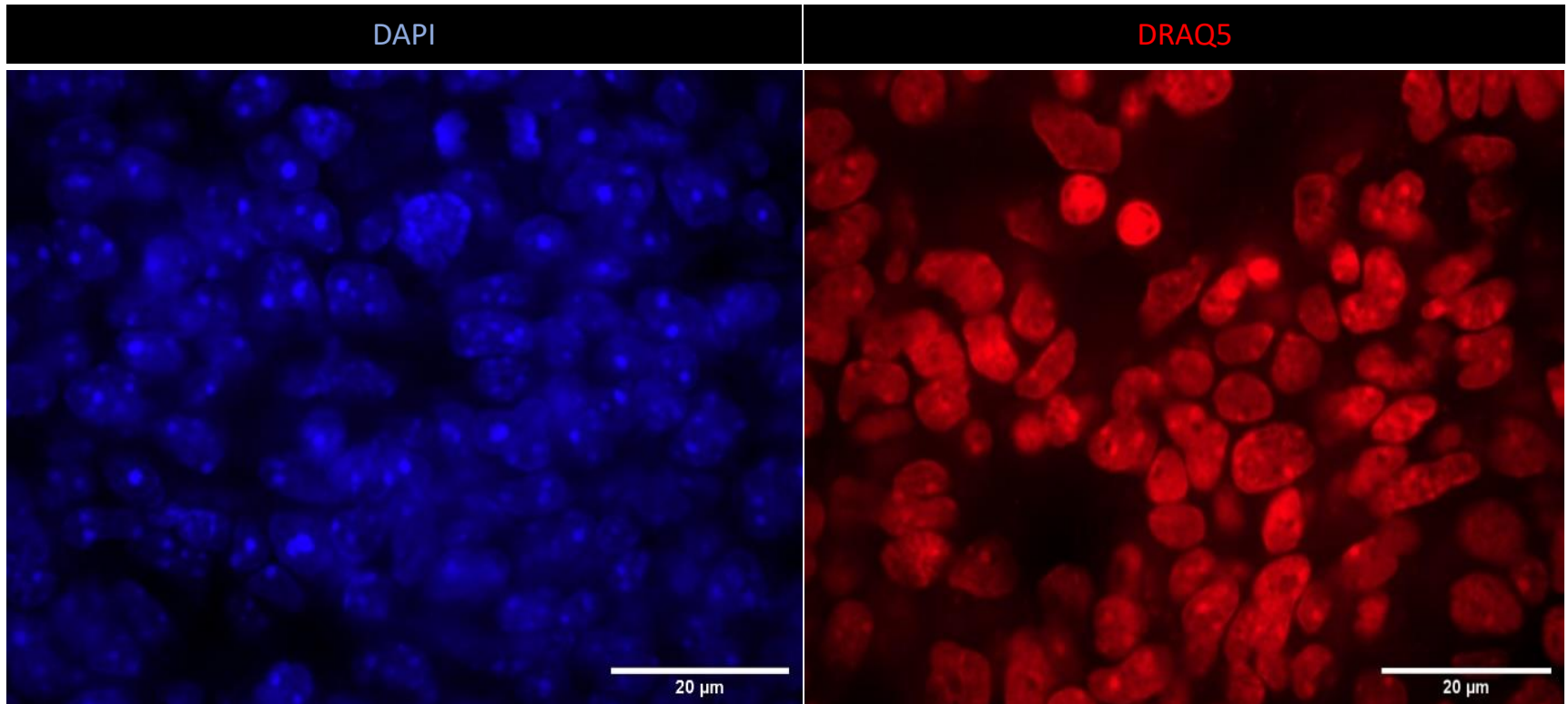


➔ Overestimation from 2D counting

ABUNDANCE

IV. Number : IMARIS

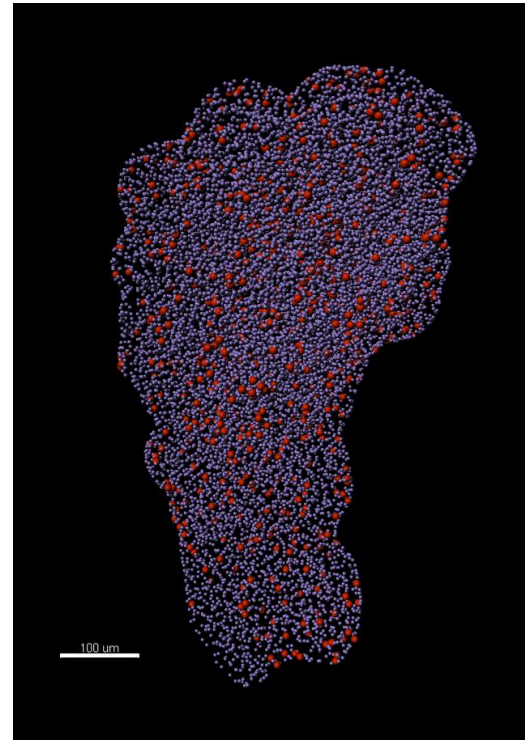
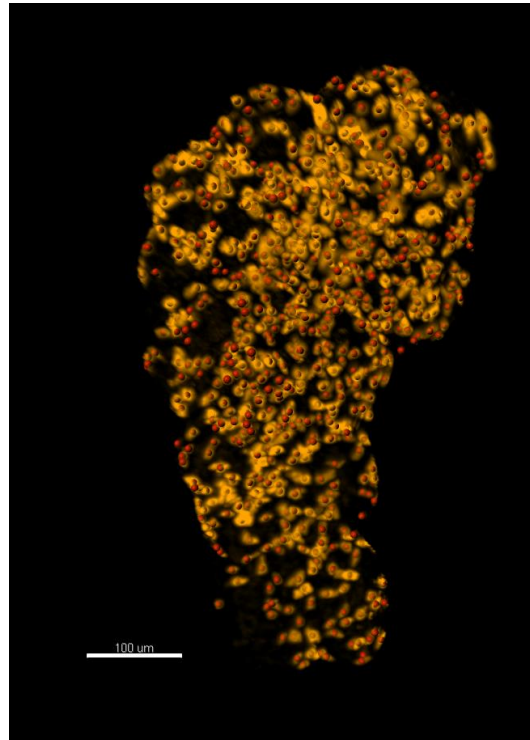
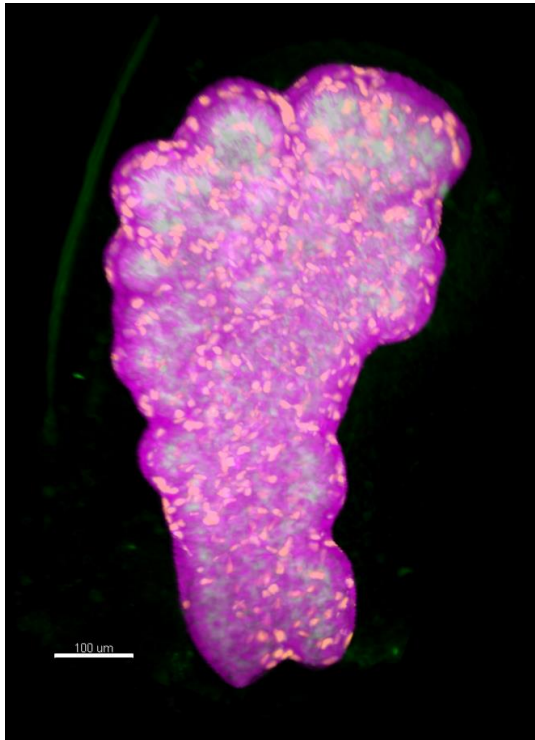
➤ Need a better nuclear staining



ABUNDANCE

IV. Number : IMARIS

E-CADHERIN ERG DRAQ5

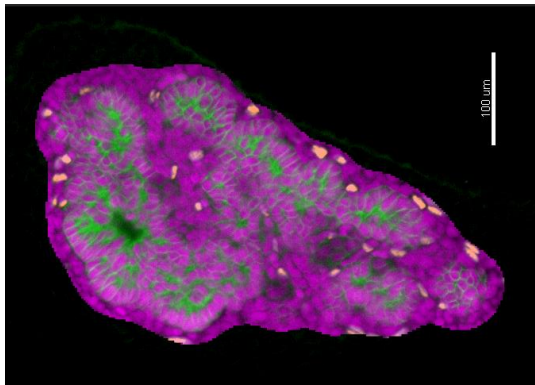


Endothelial cells

All cells

816 cells

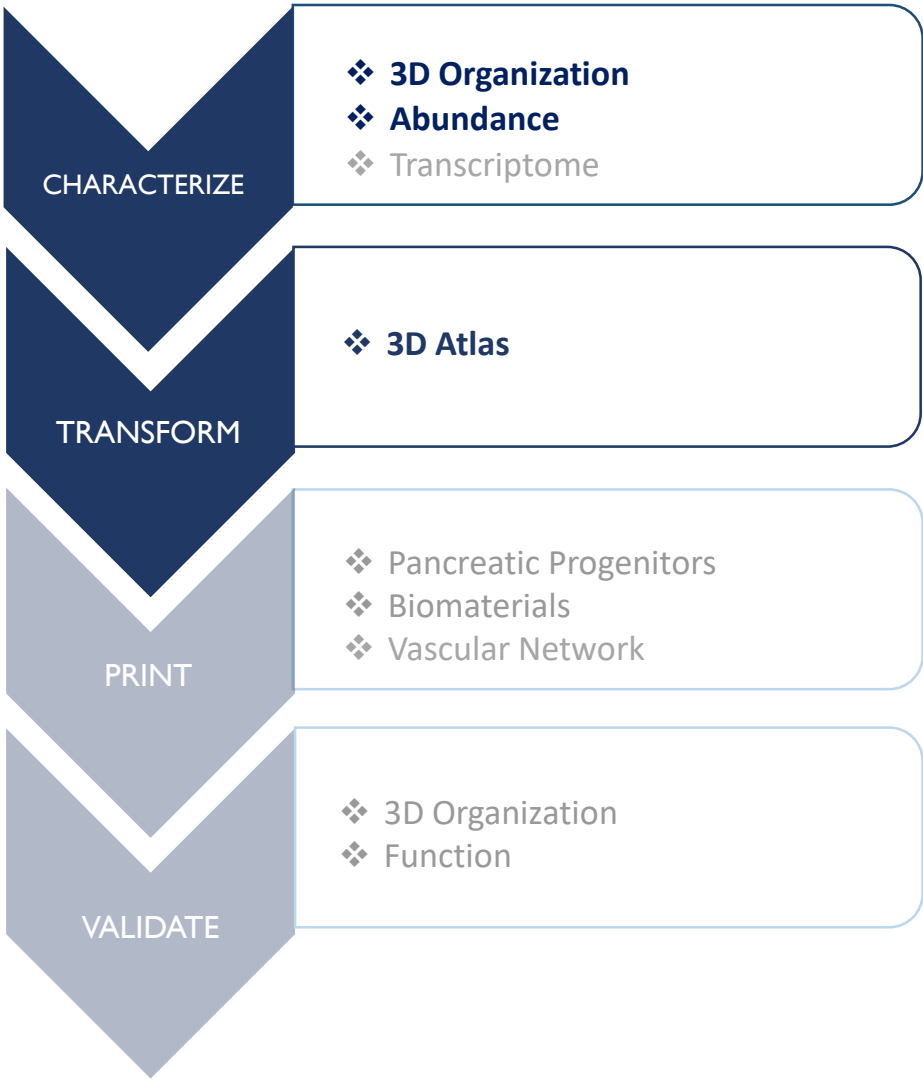
14 200 cells



Epithelial cells??

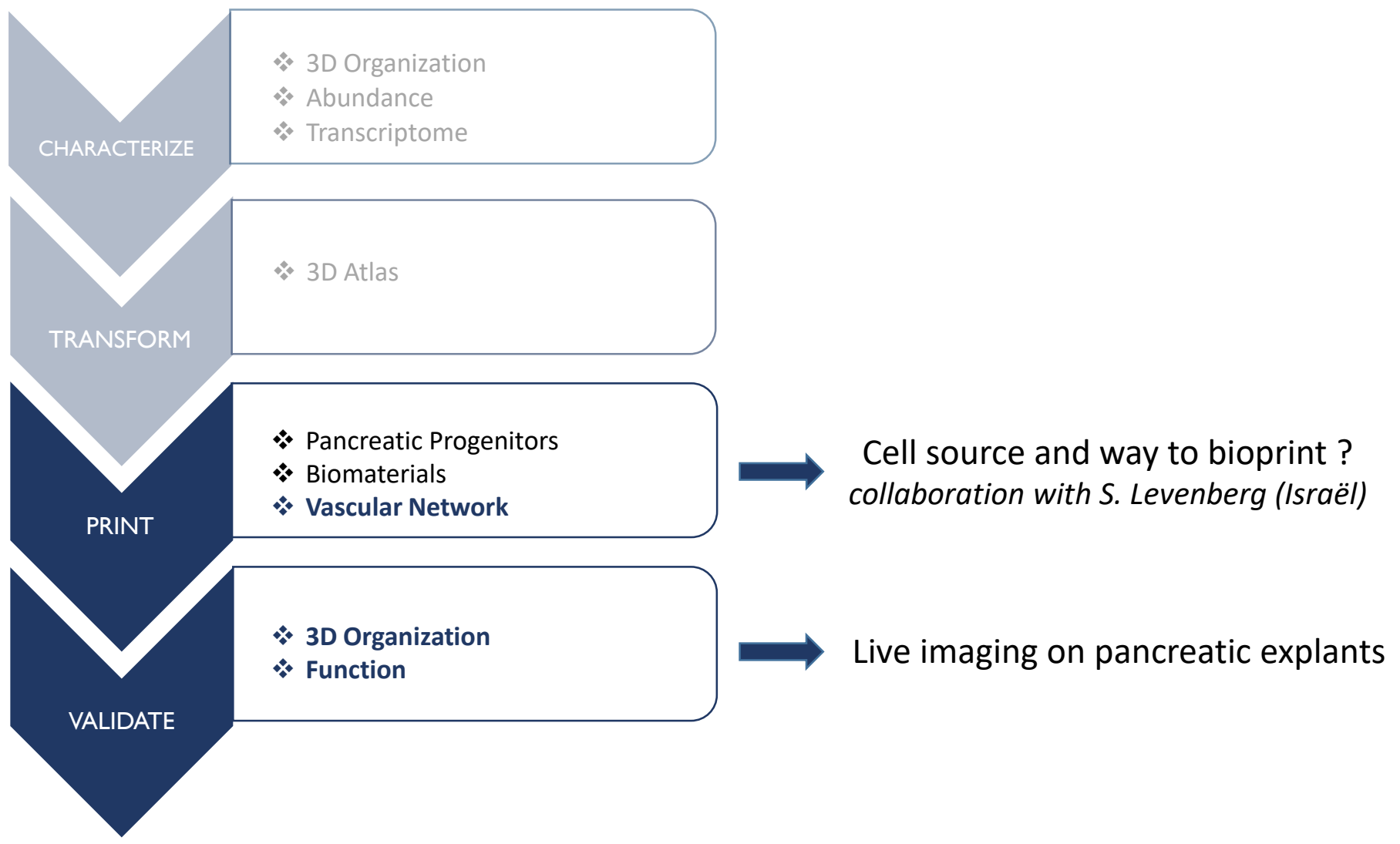
→ Too hard to segment/verify on Imaris

CONCLUSION



1. Honey comb structure
2. Epithelial segmentation

PERSPECTIVES



Thank You For Your
Attention !

