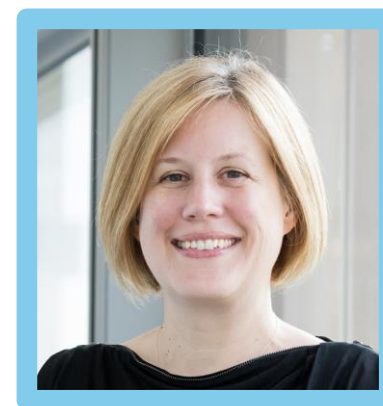


IMPERIAL

A Nanopathology Platform for Prediction and Early Detection of Disease in Kidney Transplant Rejection

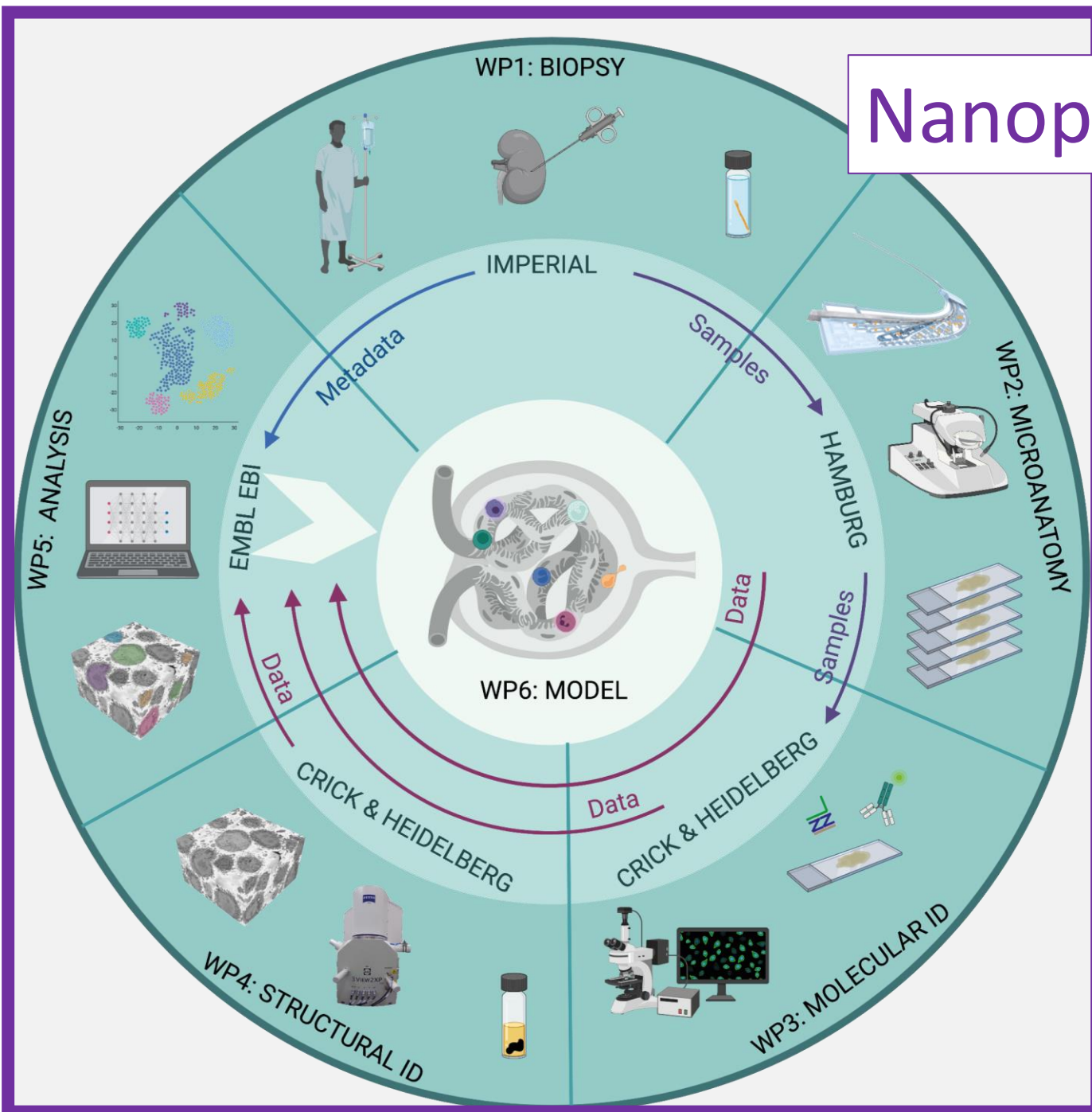
Dr Candice Roufosse
Reader in Renal Pathology
Imperial College London
25/06/2025 – ACEM Ghent

Nanopathology



Lucy Collinson

IMPERIAL





**Medical
Research
Council**

Multimodal Research Across Scales
To Understand Human Disease

Problems

Human tissue fixed in formalin + methanol
Human tissue embedded in paraffin
Human tissue snap frozen
Human tissue fixed in glutaraldehyde
2D

Aim

Enable high resolution volume imaging + molecular characterisation for clinical research

Strategy

Design a new nanopathology pipeline

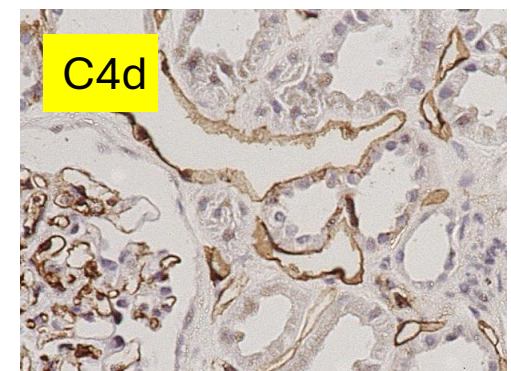
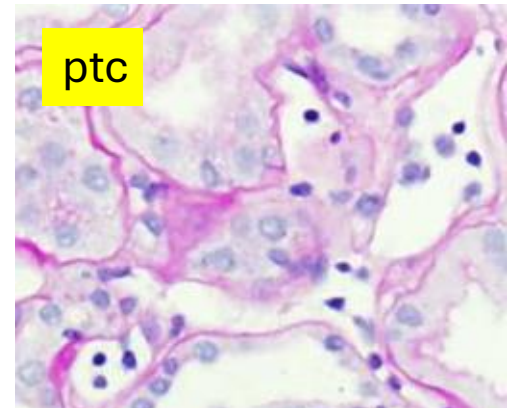
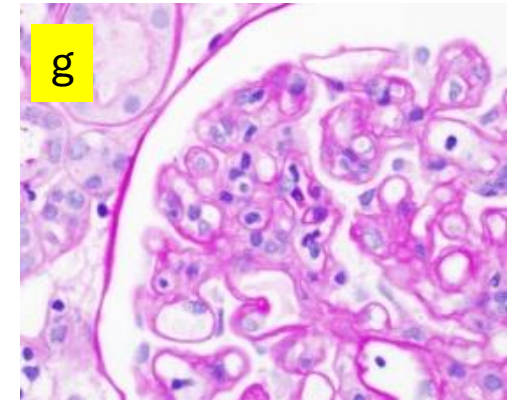
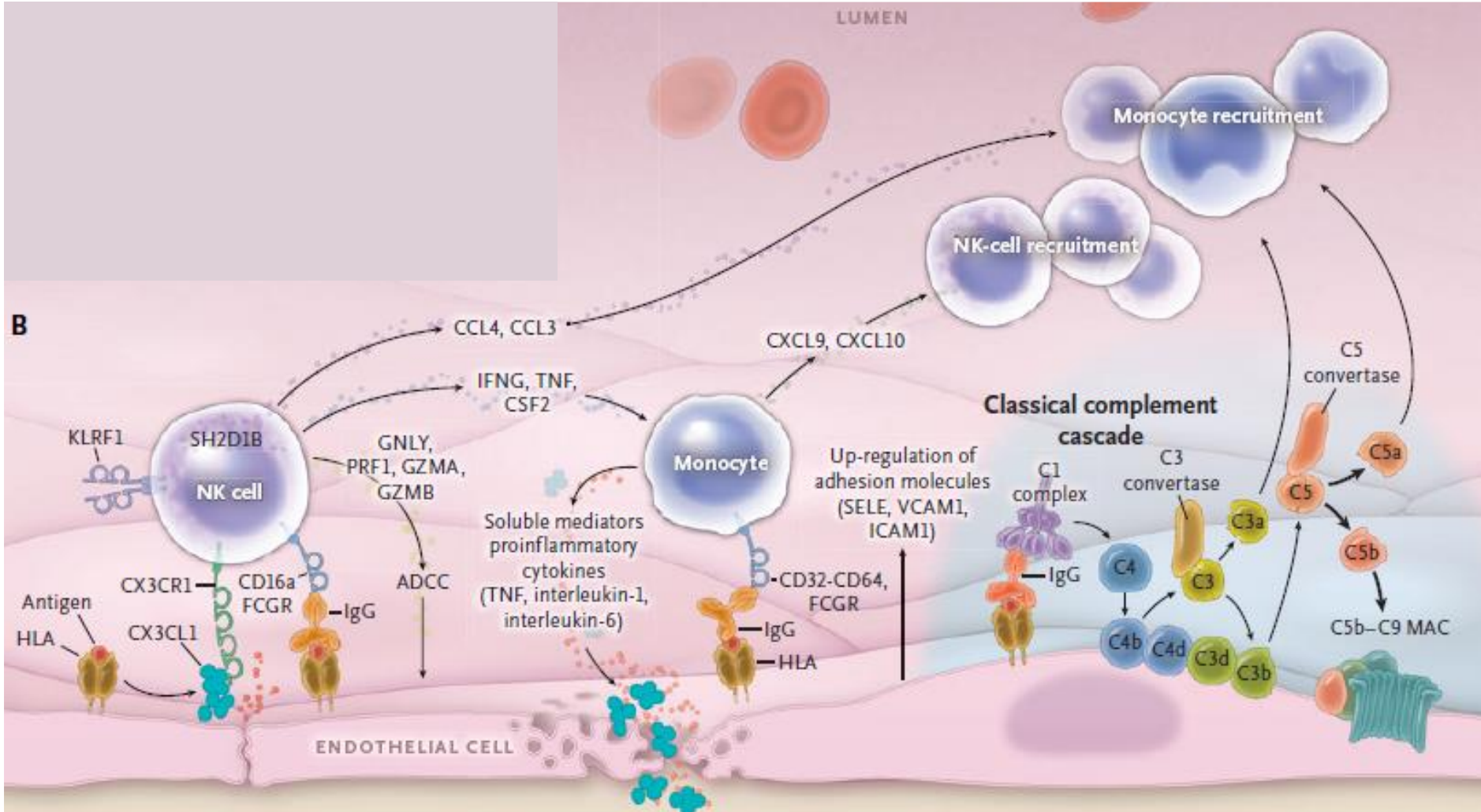
Technique

Correlative and Multimodal Imaging

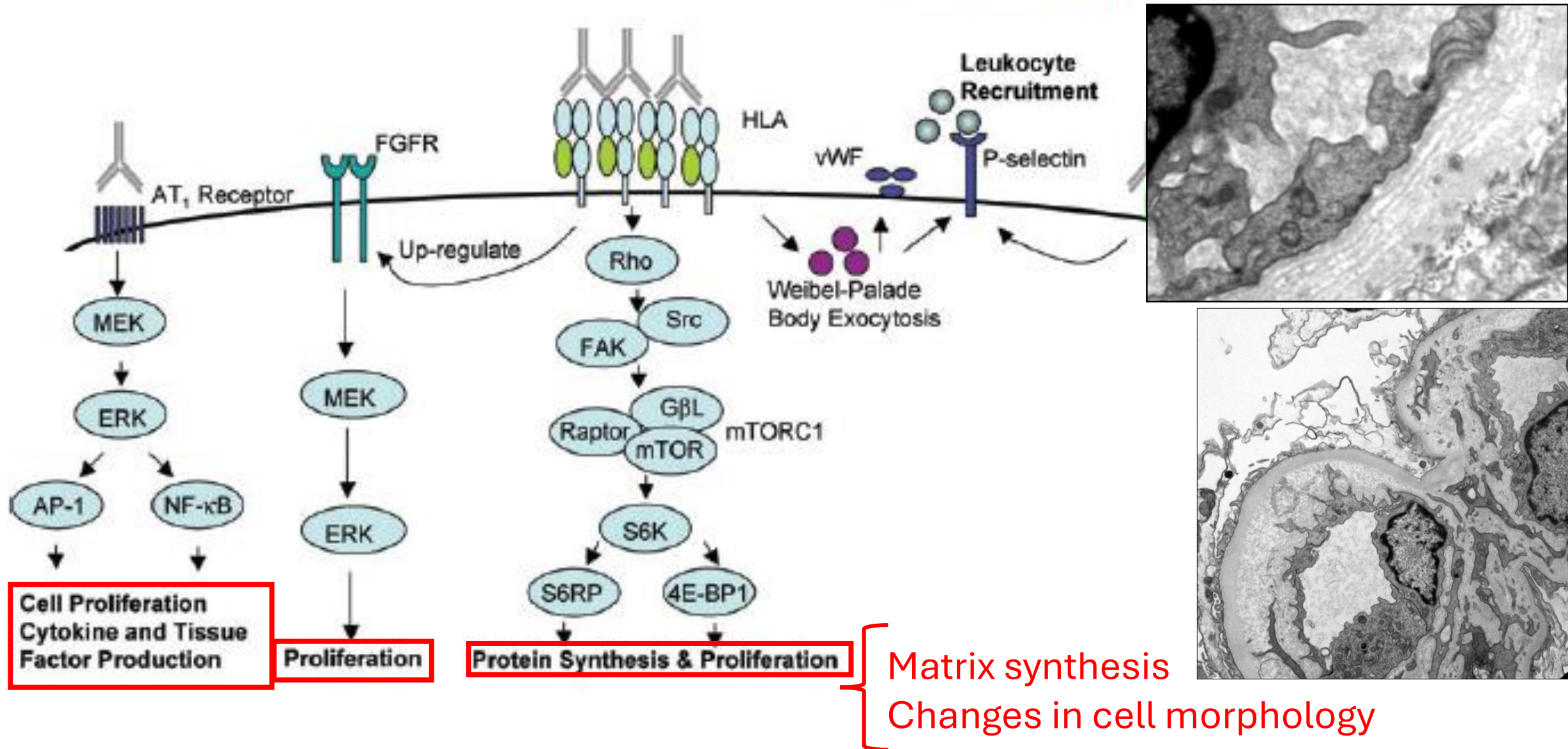
Opportunity

Can high resolution 3D imaging reveal new diagnostic features at the nanoscale?

Antibody-mediated rejection and Microvascular Inflammation in Kidney Transplants



Effect of Antibodies on Endothelium

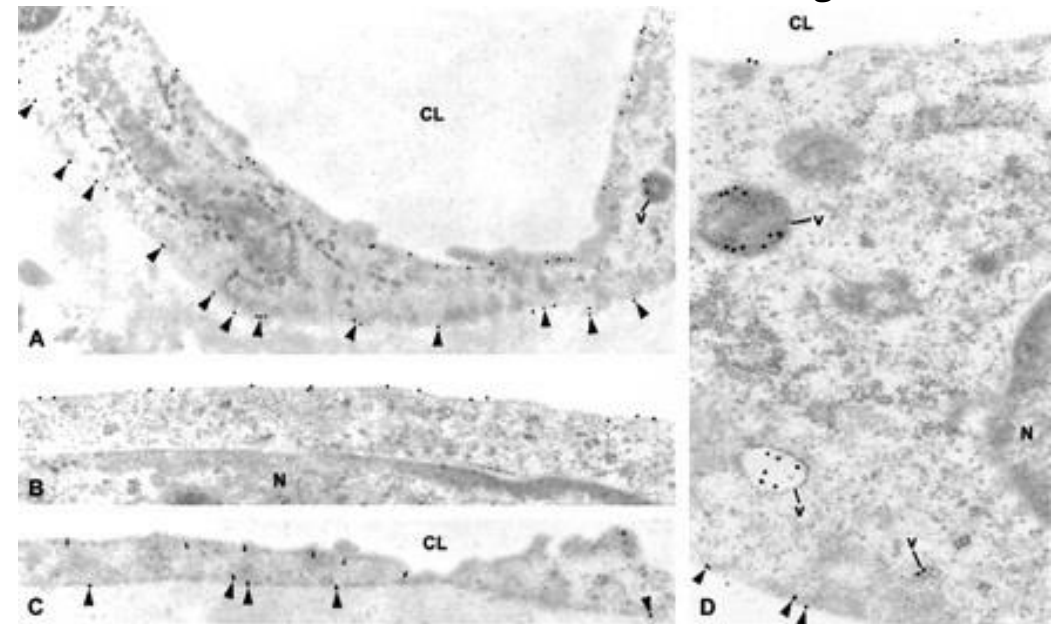
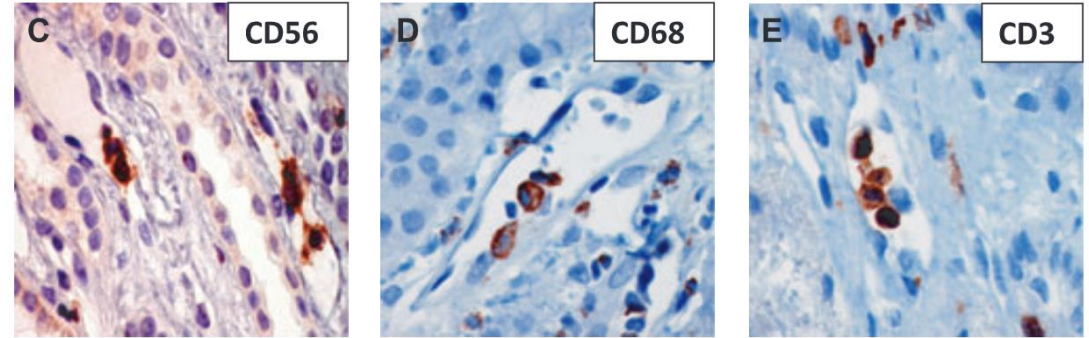


Microvascular inflammation

- Associated with poor outcomes
- Associated with antibodies against the donor
- But poor response to antibody removal as a therapy
- And competing potential explanation for MVI
 - “Missing self” NK cell activation
 - T cell-mediated injury
 - Innate allorecognition by monocytes

Imaging techniques for microvascular inflammation in human

- Low-resolution/low plex with spatial info (IHC)
- High resolution, no (or little) molecular info (immunogold EM)
- High plex no spatial (sc/snRNAseq)
- Spatial proteomics and transcriptomics



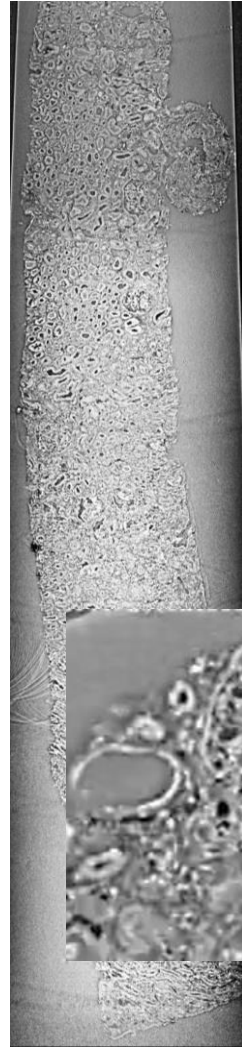
Hidalgo AJT 2010

Regele JASN 2002

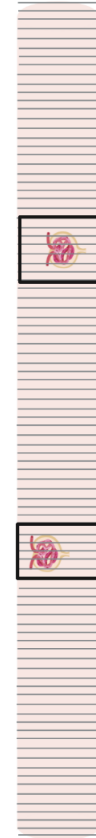
Cross-scale imaging combined with molecular labelling



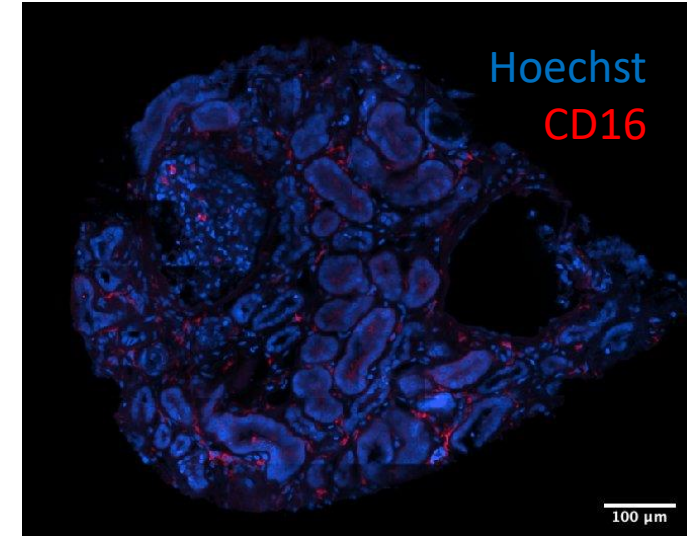
PFA-fixed tissue surplus to diagnostic needs



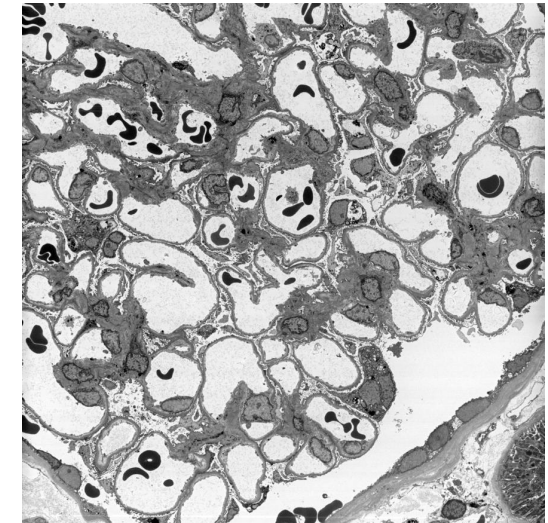
High Throughput X-ray
Tomography (HiTT)



Targeted vibrotome
sectioning



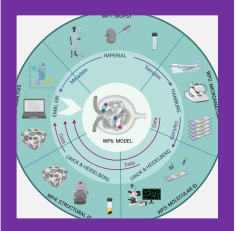
Protein labelling



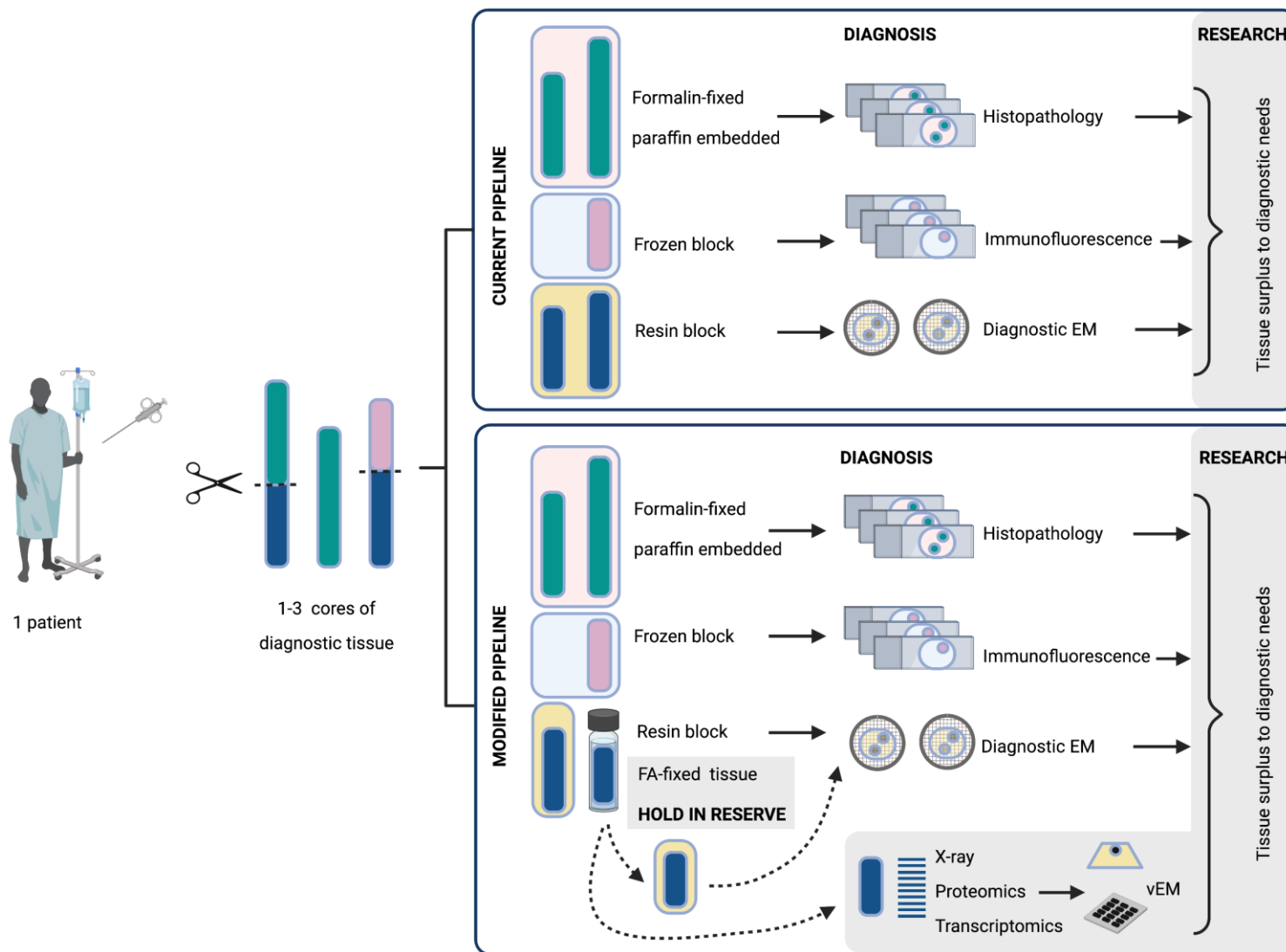
Targeted volume EM acquisition

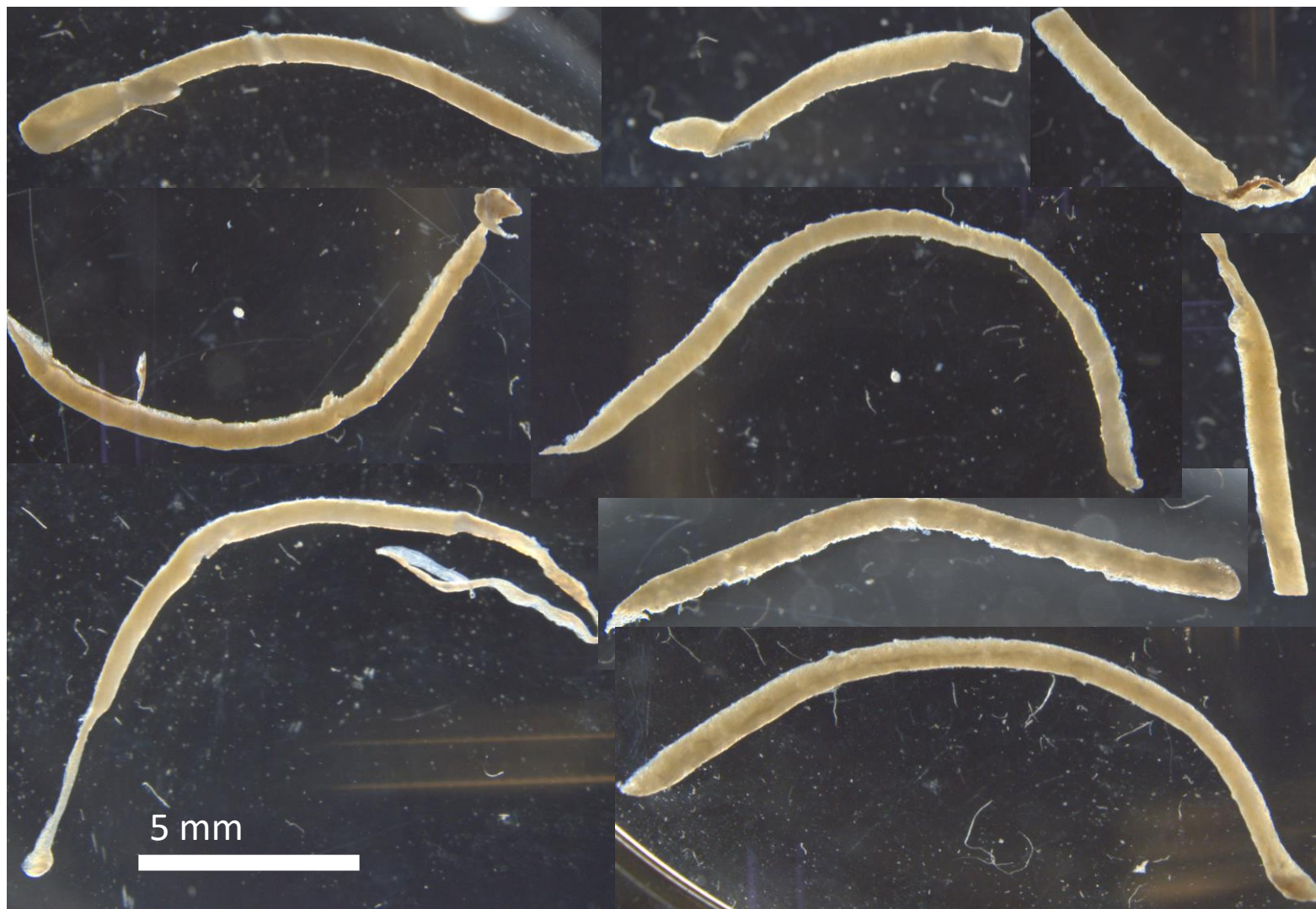
Unanswered questions issues in microvascular inflammation

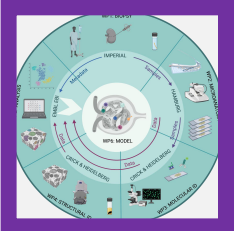
Biological feature	Quantitative readout
Distribution of blood-borne immune cells in the kidney microvasculature	X-ray and/or vEM - Cell counts and positions within glomerular and peritubular capillaries
Morphological phenotype of blood-borne immune cells and of endothelium in the kidney microvasculature	Instance segmentation + learned feature vector on vEM images
Molecular phenotype of blood-borne immune cells and of endothelium in the kidney microvasculature	CLEM and RNAscope FISH/spatial transcriptomics
Interactions between different types of immune cells and between immune cells and endothelium: cell-cell contacts and polarisation	Instance segmentation + learned feature vector on vEM images



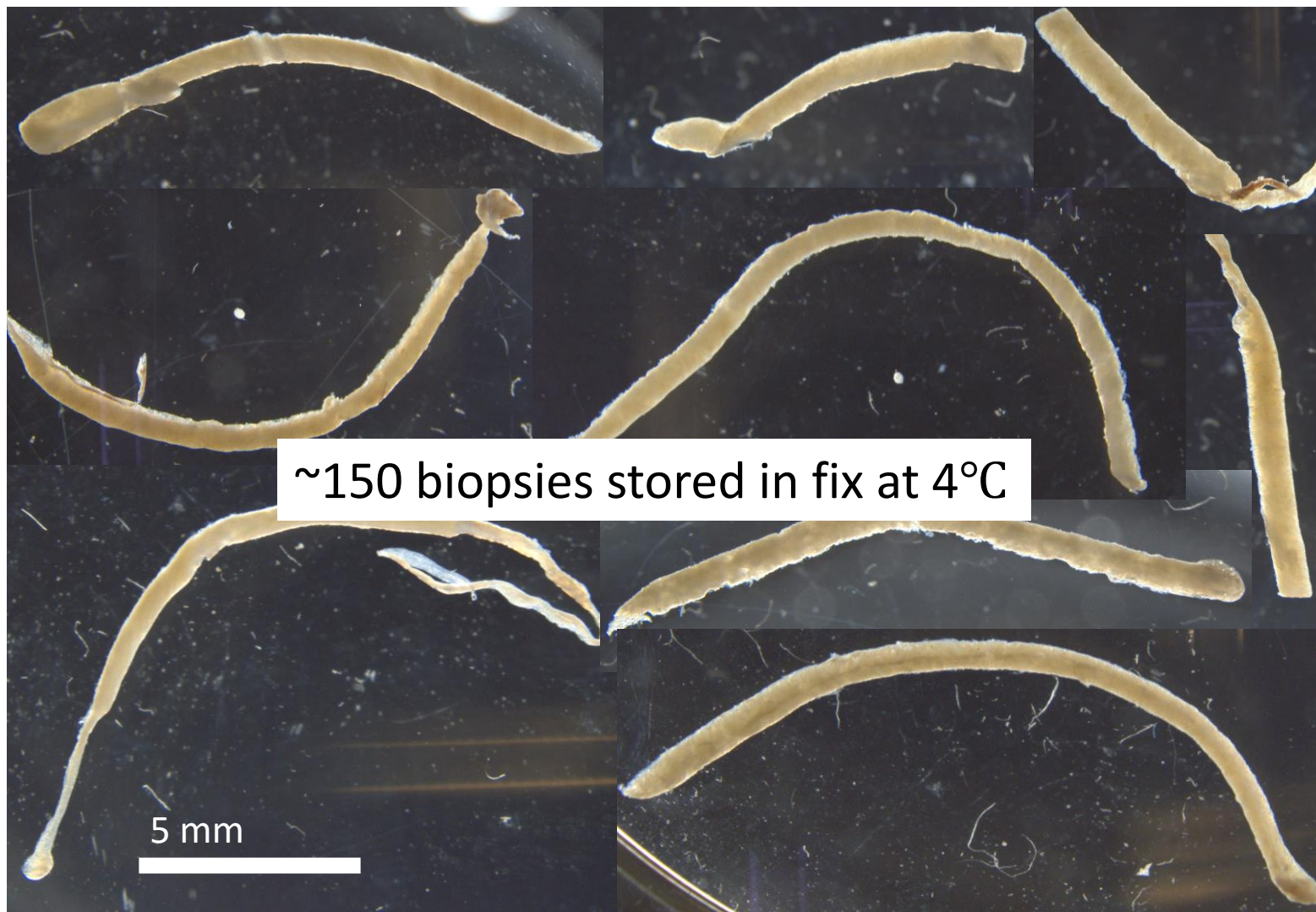
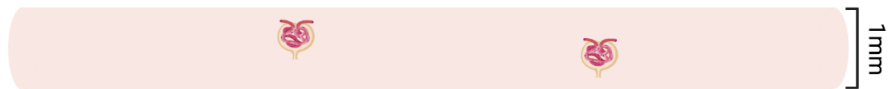
Preservation of human tissue for high resolution imaging







Preservation of human tissue for high resolution imaging

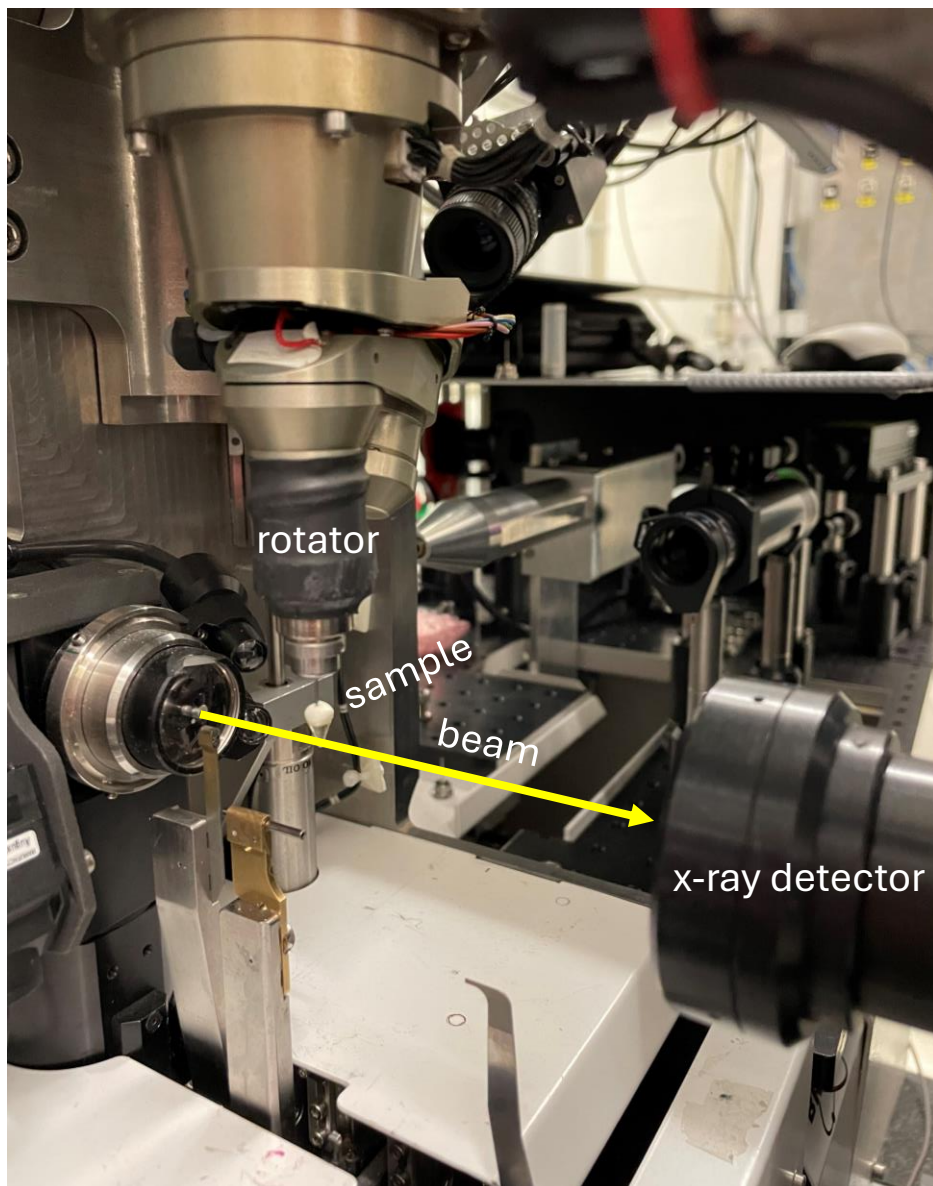
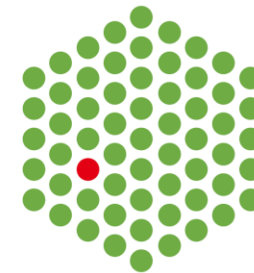


High throughput tomography (HiTT) Imaging (X-ray imaging)



Whole Sample Full Overview

EMBL



Phase contrast based X-ray imaging at the PETRA III
synchrotron in Hamburg

Ideal for low-absorbing samples such as soft tissues

Optimised for speed in both data collection and
reconstruction.



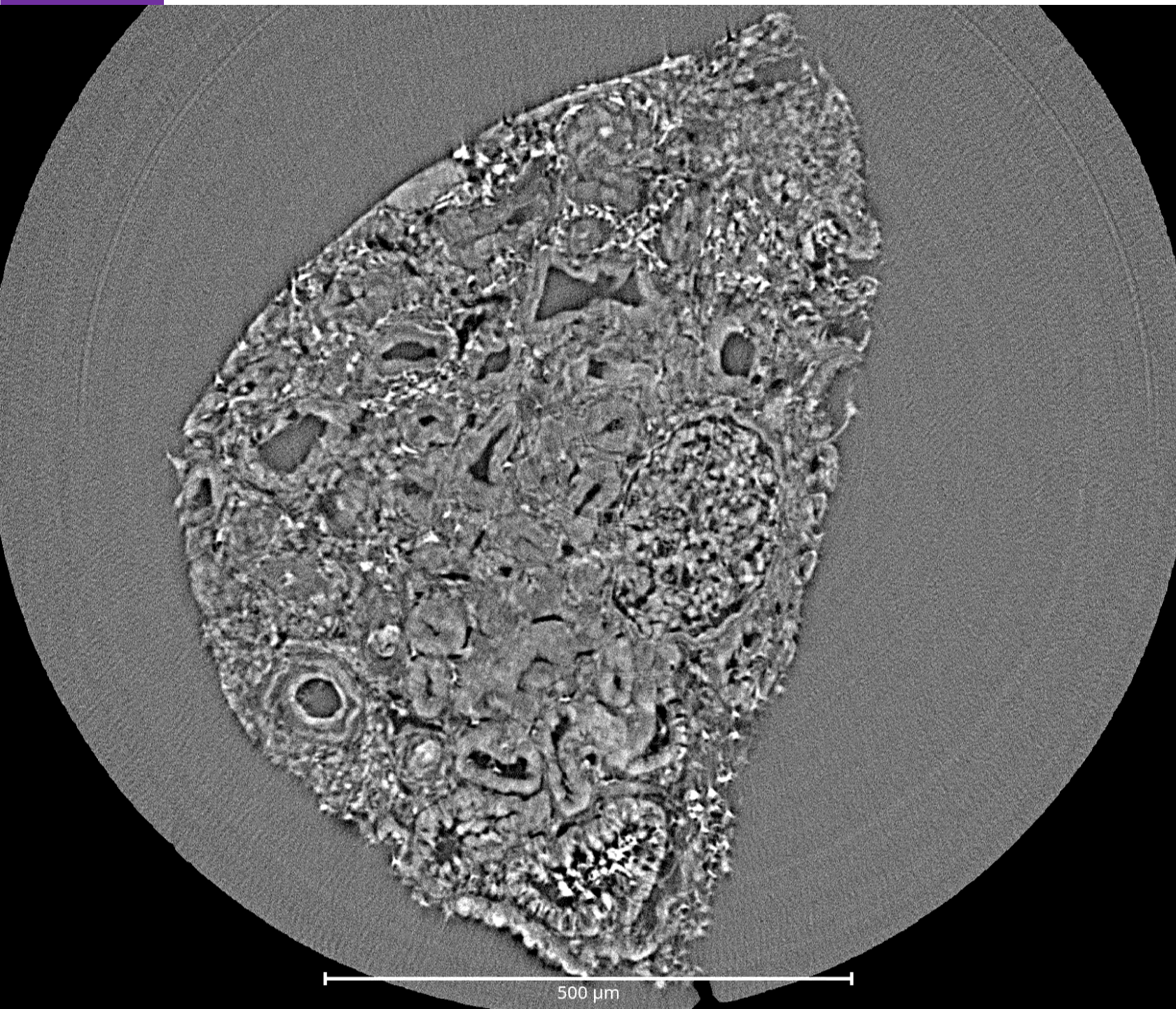
Liz Duke



Jonas Albers



Mat Lawson



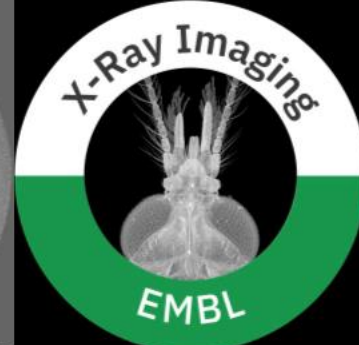
Liz Duke



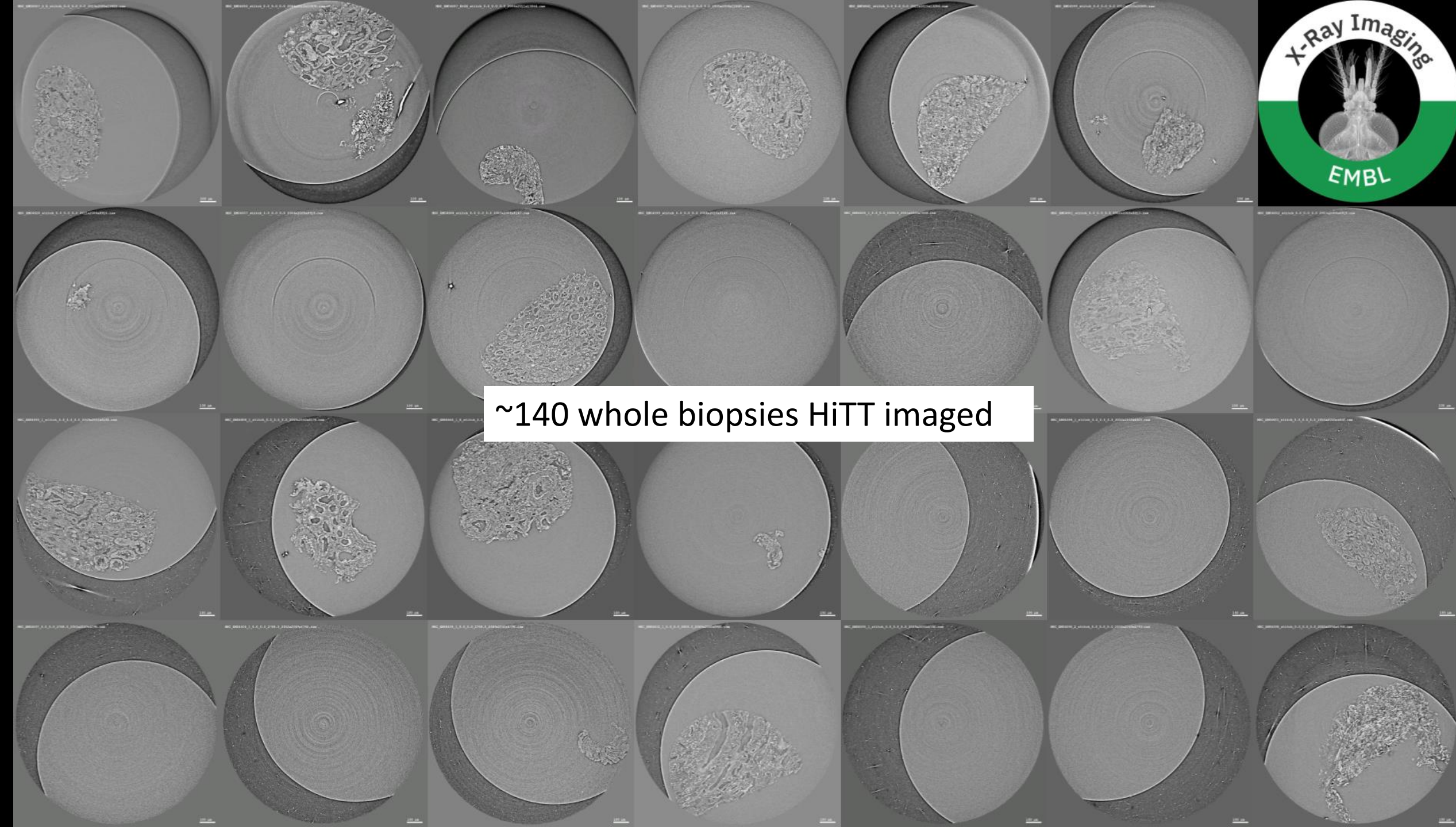
Jonas Albers



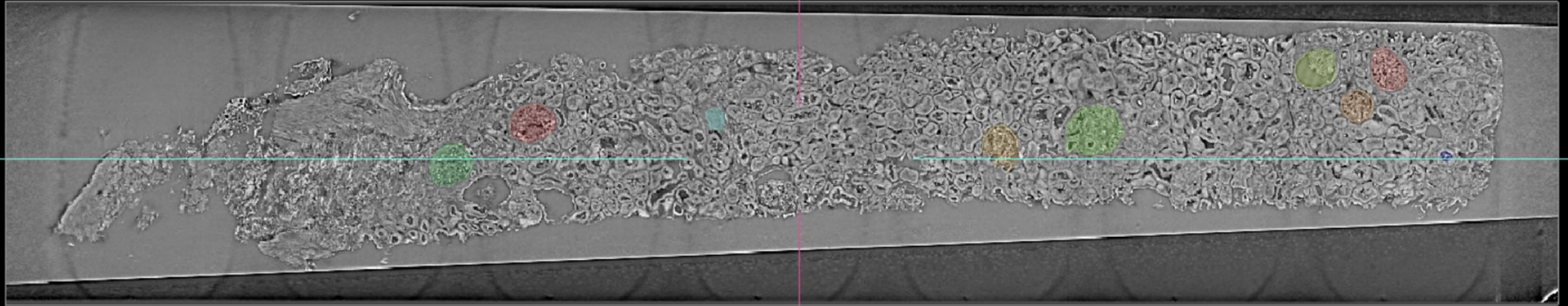
Mat Lawson



~140 whole biopsies HiTT imaged

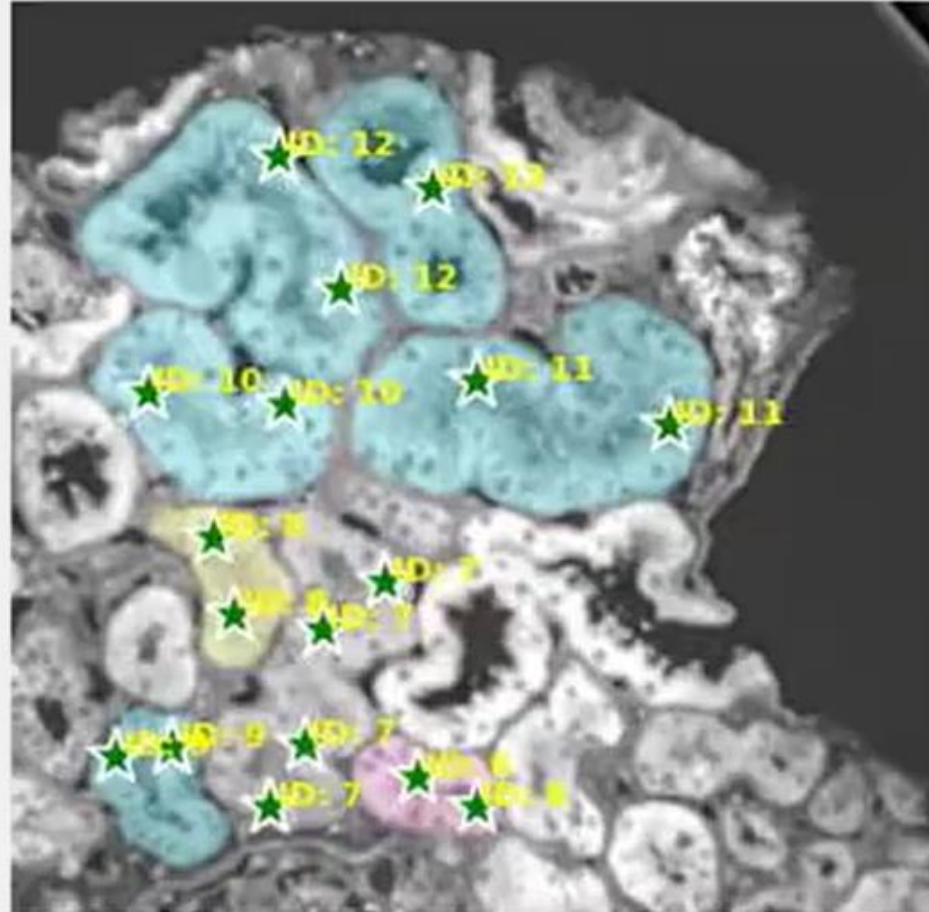


Wet sample HiTT images – segmentation

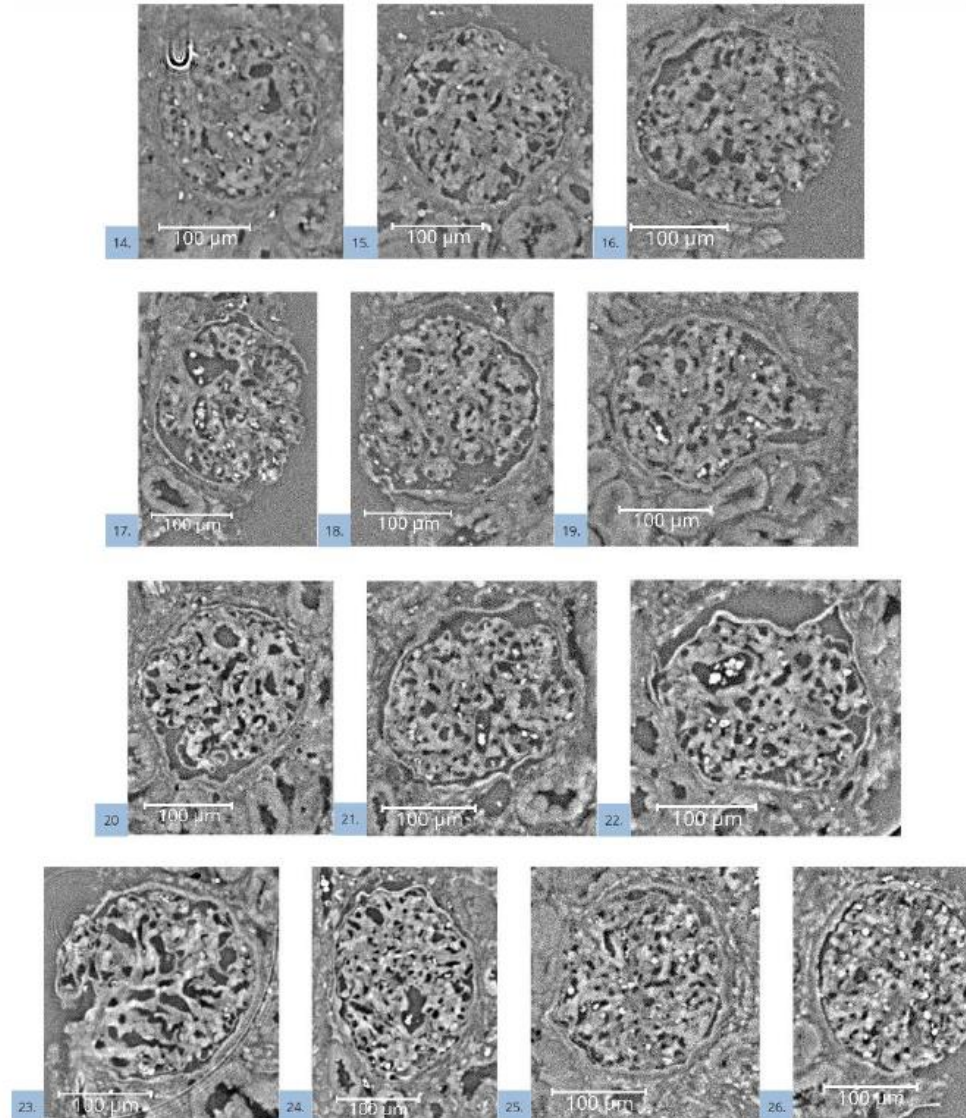


SAM2 segmentation of resin-HiTT

Frame 100 (right click to add prompt points)



Wet sample HiTT - classification



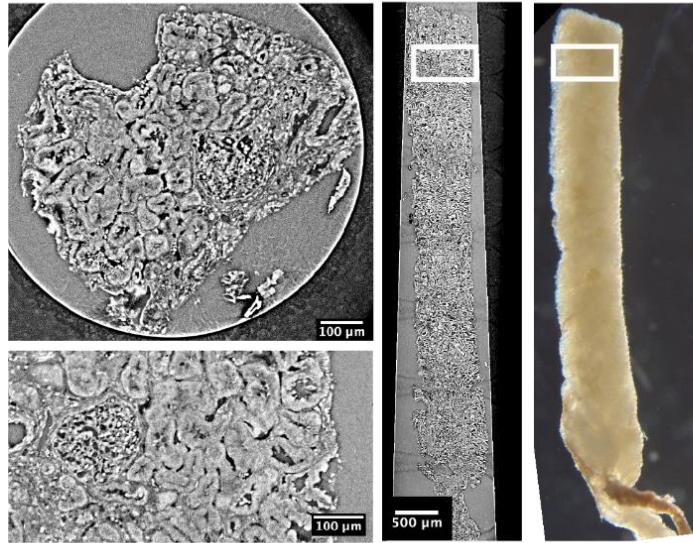
Virginie Uhlmann



Xiaofan Xiong



Alana Burrell



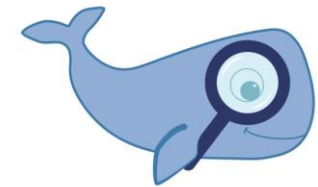
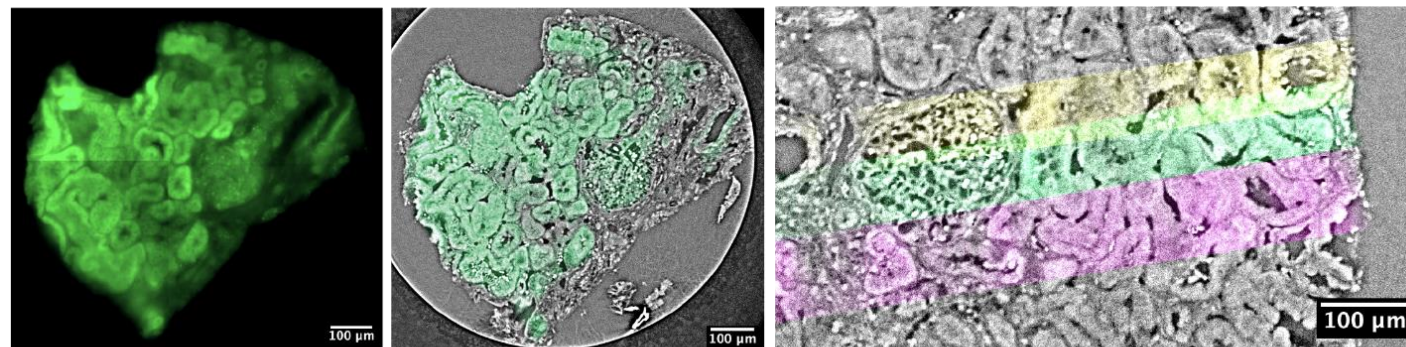
Wet sample HiTT images to find
Regions of interest (ROI) for downstream analysis



24-well with slices

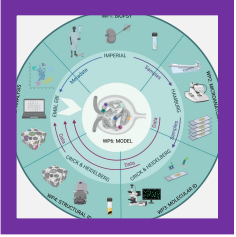
3 x vibrotome sections (~60 mi)

- Sizing for 3D EM
- Molecular cell ID



MoBIE

MultiModal Big Image Data
Sharing and Exploration



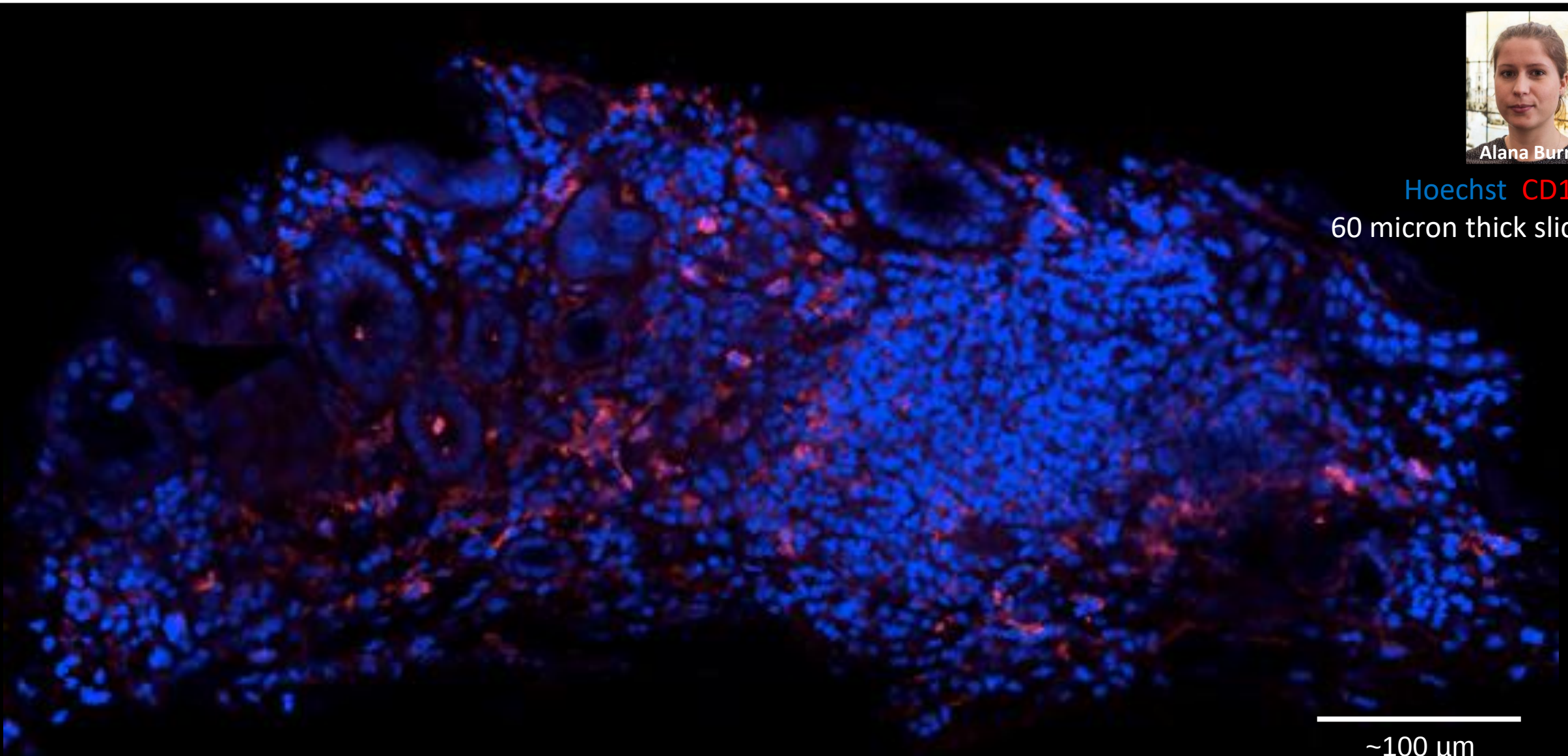
Molecular labelling without permeabilisation

3 Vibratome slices, immunolabel without permeabilization, image with fluorescence microscopy



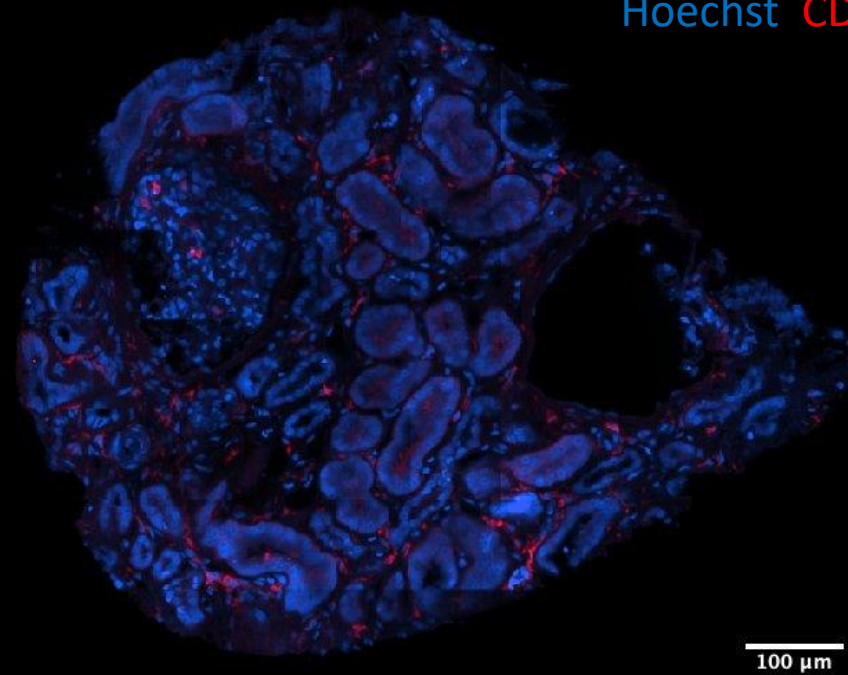
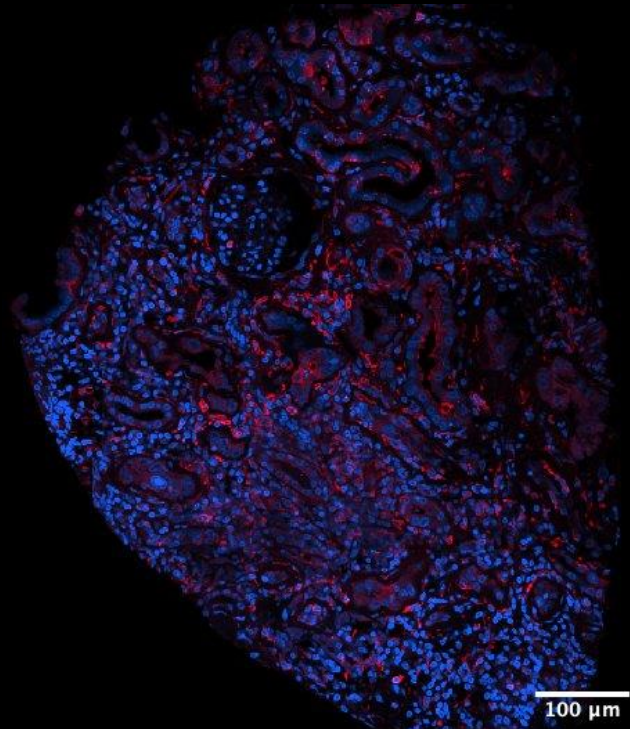
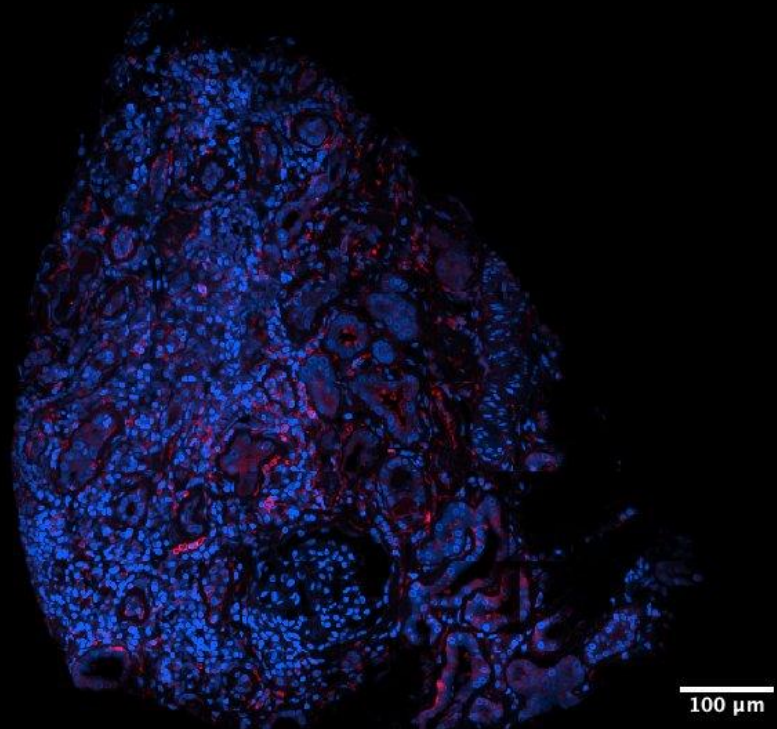
Hoechst CD16

60 micron thick slice

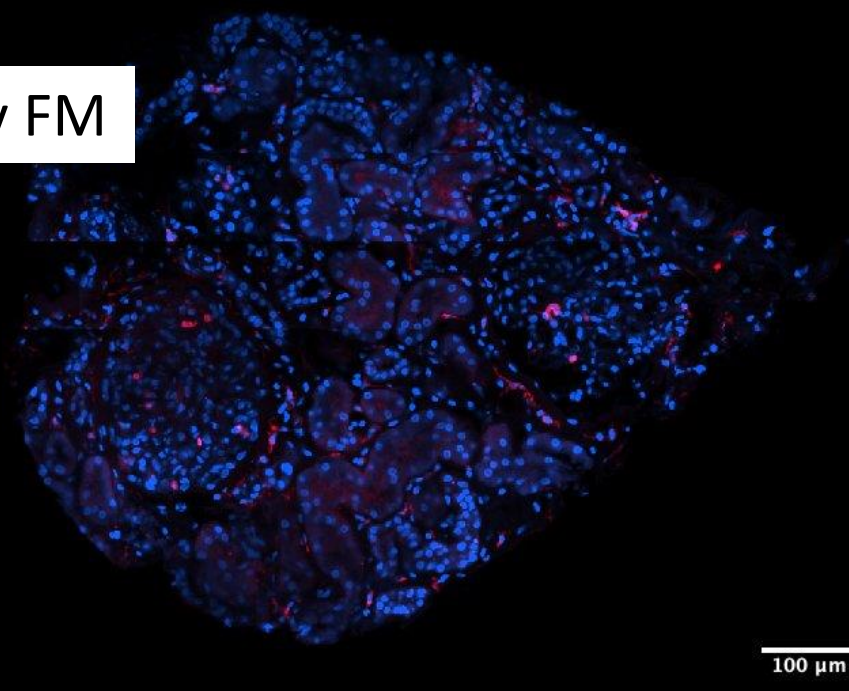
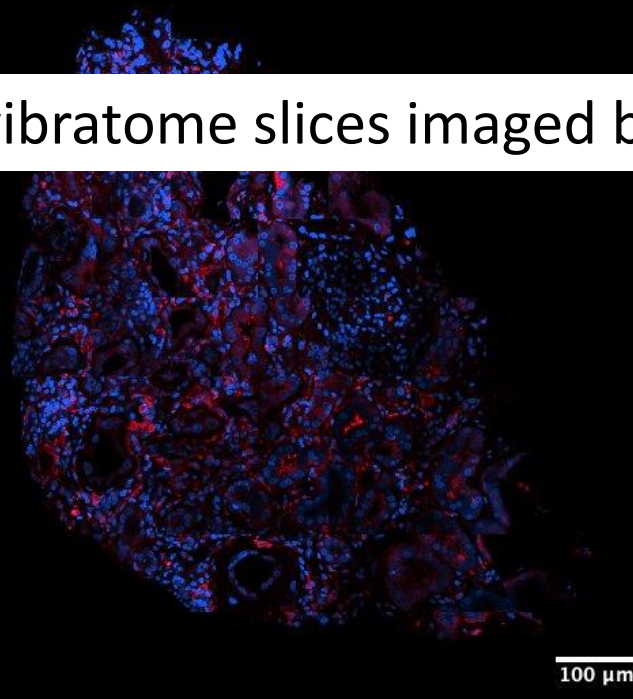
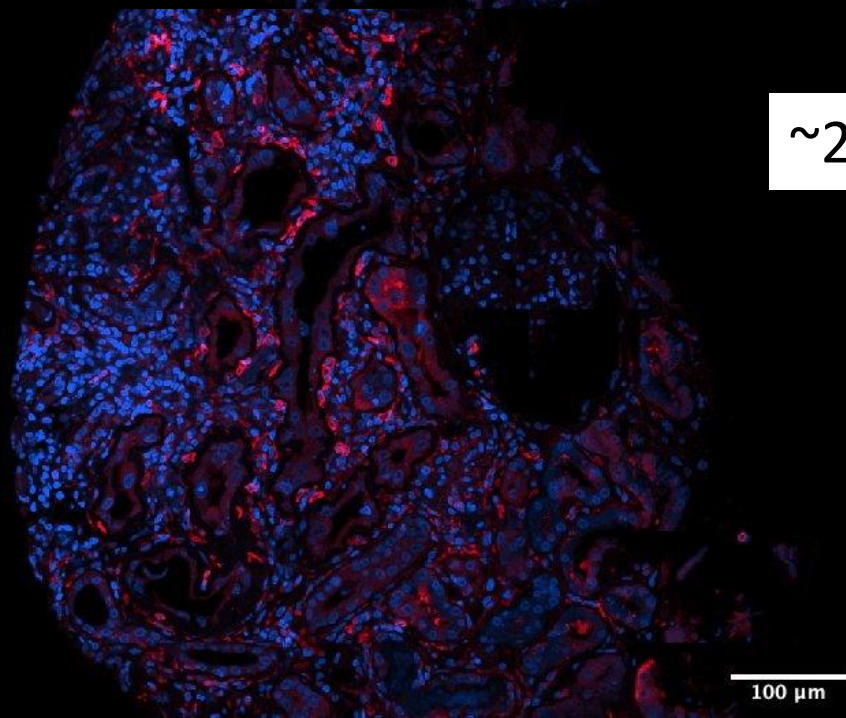


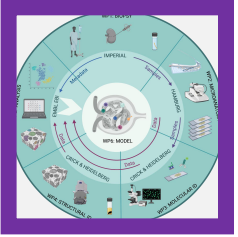
$\sim 100 \mu\text{m}$

Hoechst CD16



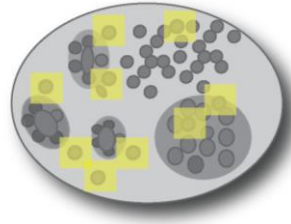
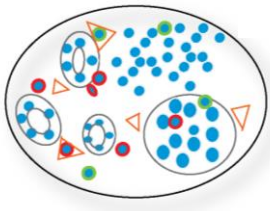
~20 vibratome slices imaged by FM



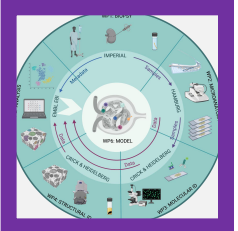


Purpose of molecular labelling

Labelled slices → Resin embedded slices → volume Electron Microscopy
(Re-HiTT)



- To acquire molecular information on cells of interest, to be overlaid with EM images



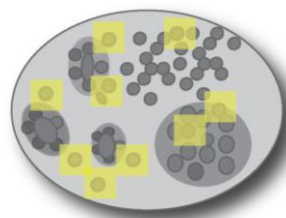
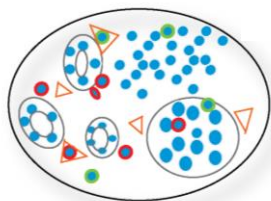
Targeted volume EM acquisition

Resin embedding and targeted Serial Block Face SEM

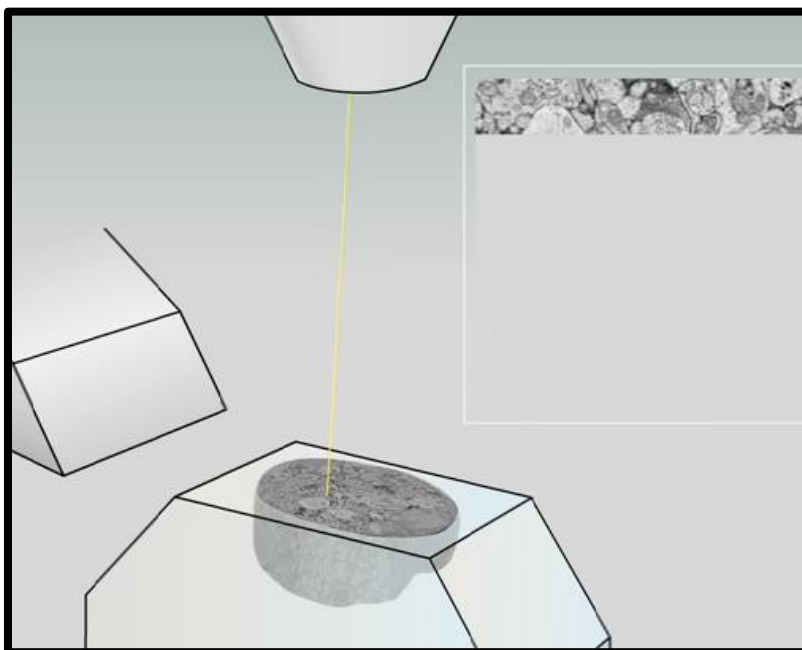


Paolo Ronchi Yannick Schwab

Labelled slices → Resin embedded slices → volume Electron Microscopy

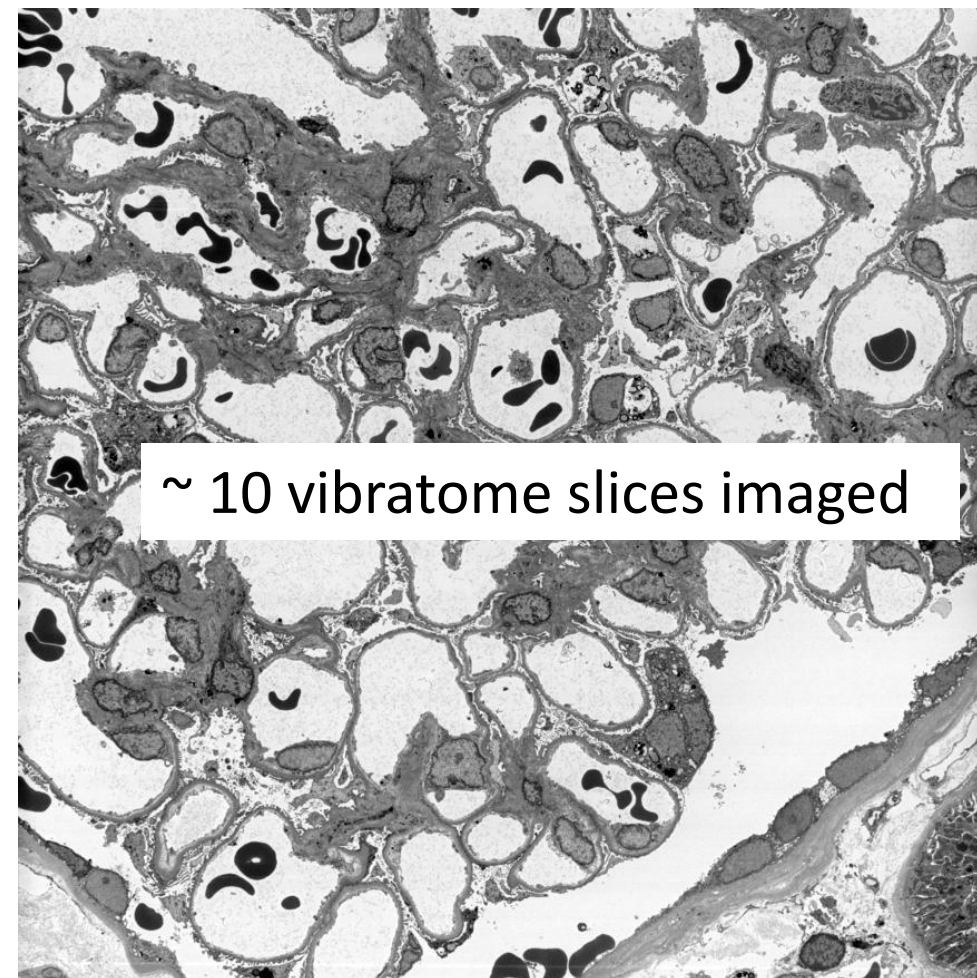


Serial Block Face SEM



<http://www.gatan.com/products/sem-imaging-spectroscopy/3view-system>

Human kidney biopsy



Alana Burrell

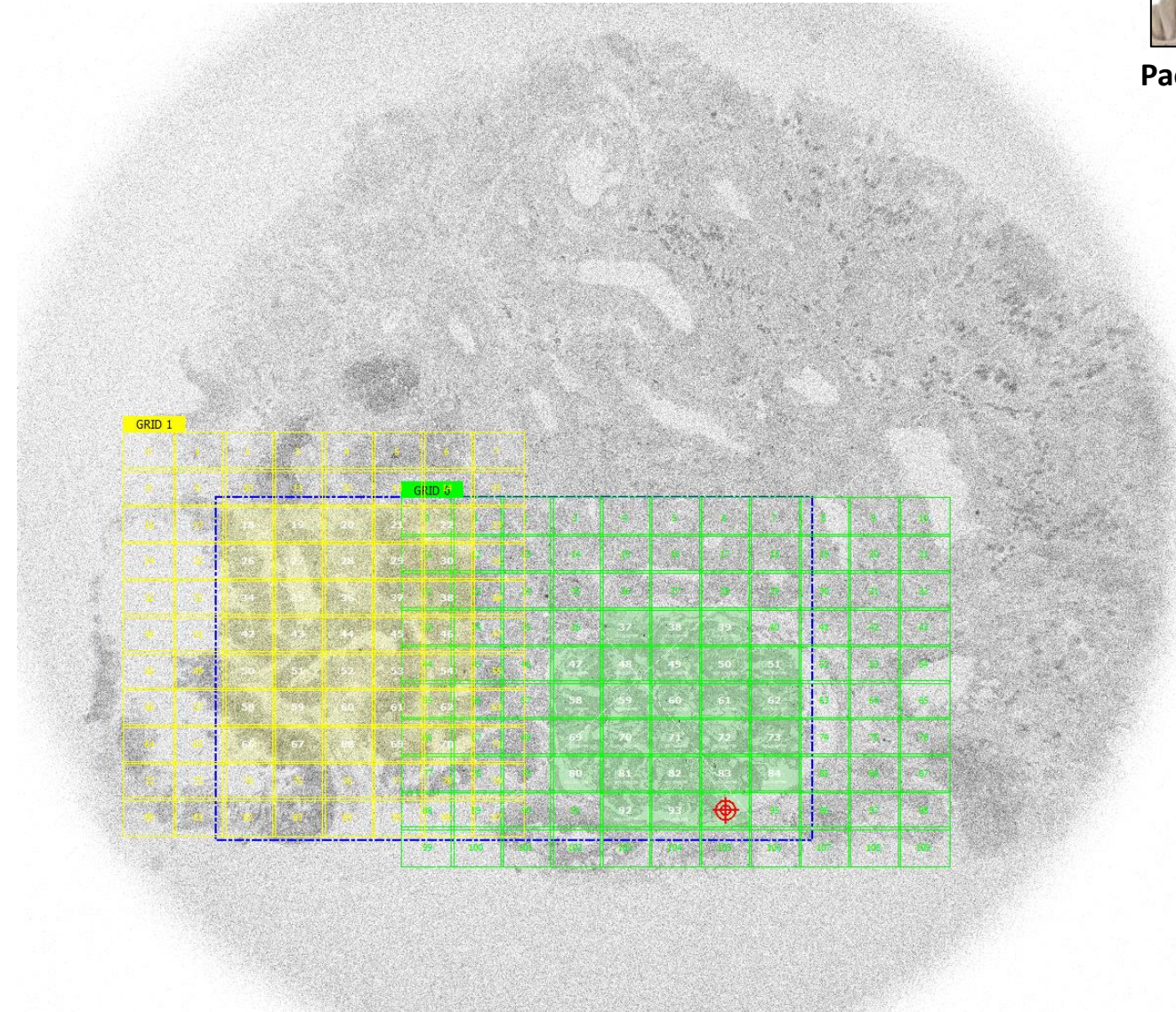


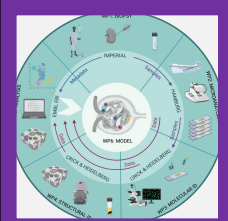


Paolo Ronchi Yannick Schwab

Intelligent SBF-SEM

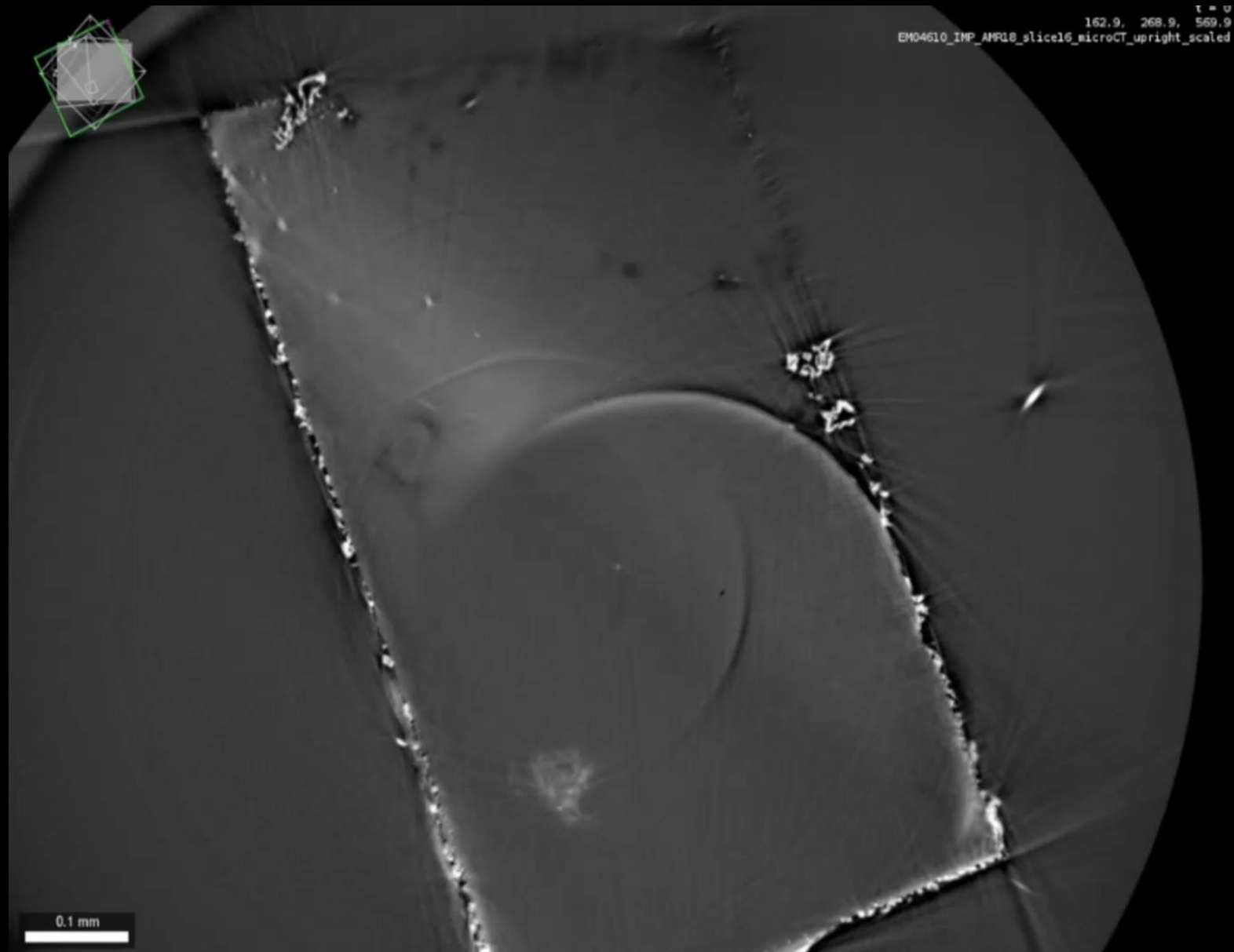
- large ROIs are defined ($200 \times 300 \times 50 \mu\text{m}^3$)
- acquiring at high resolution ($10 \times 10 \times 50 \text{ nm}^3$)
- ~ 3 weeks
- generates 700 GB of data



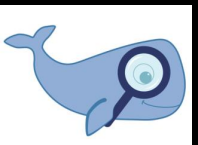


Correlation of multiscale image data

3D overlay of HiTT, FM, EM



MOBIE

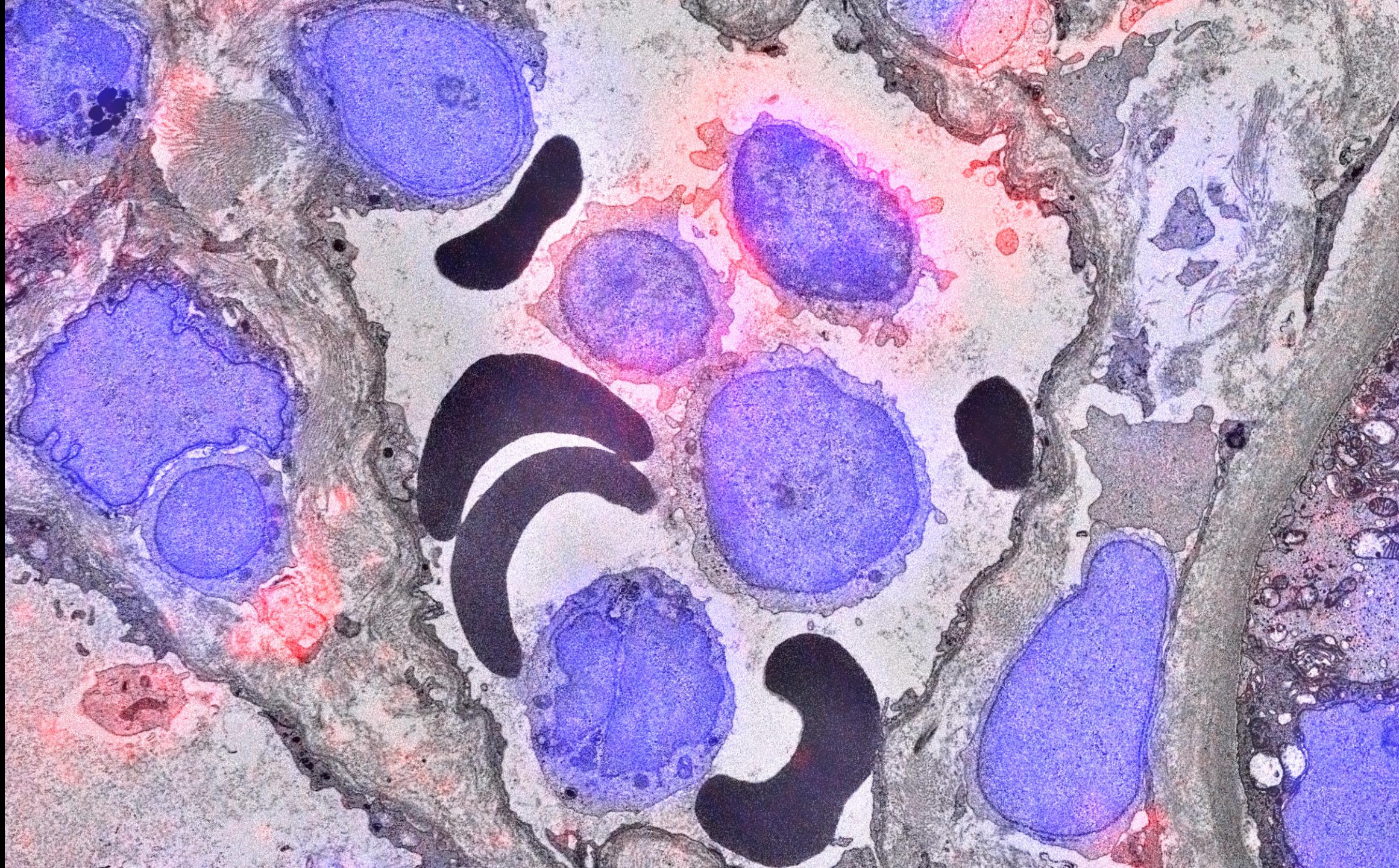


EMBL

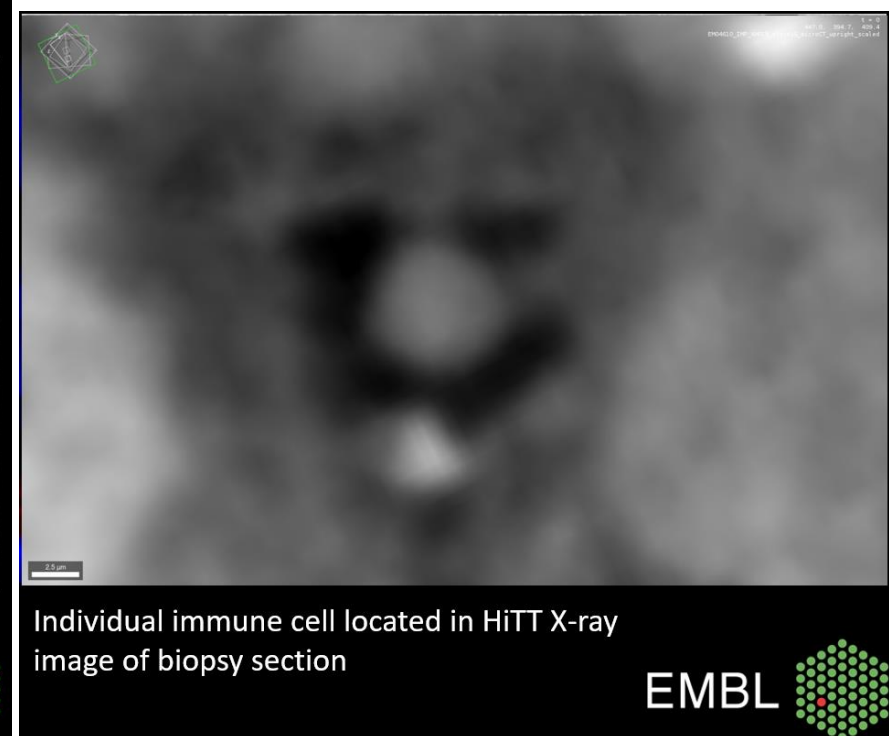
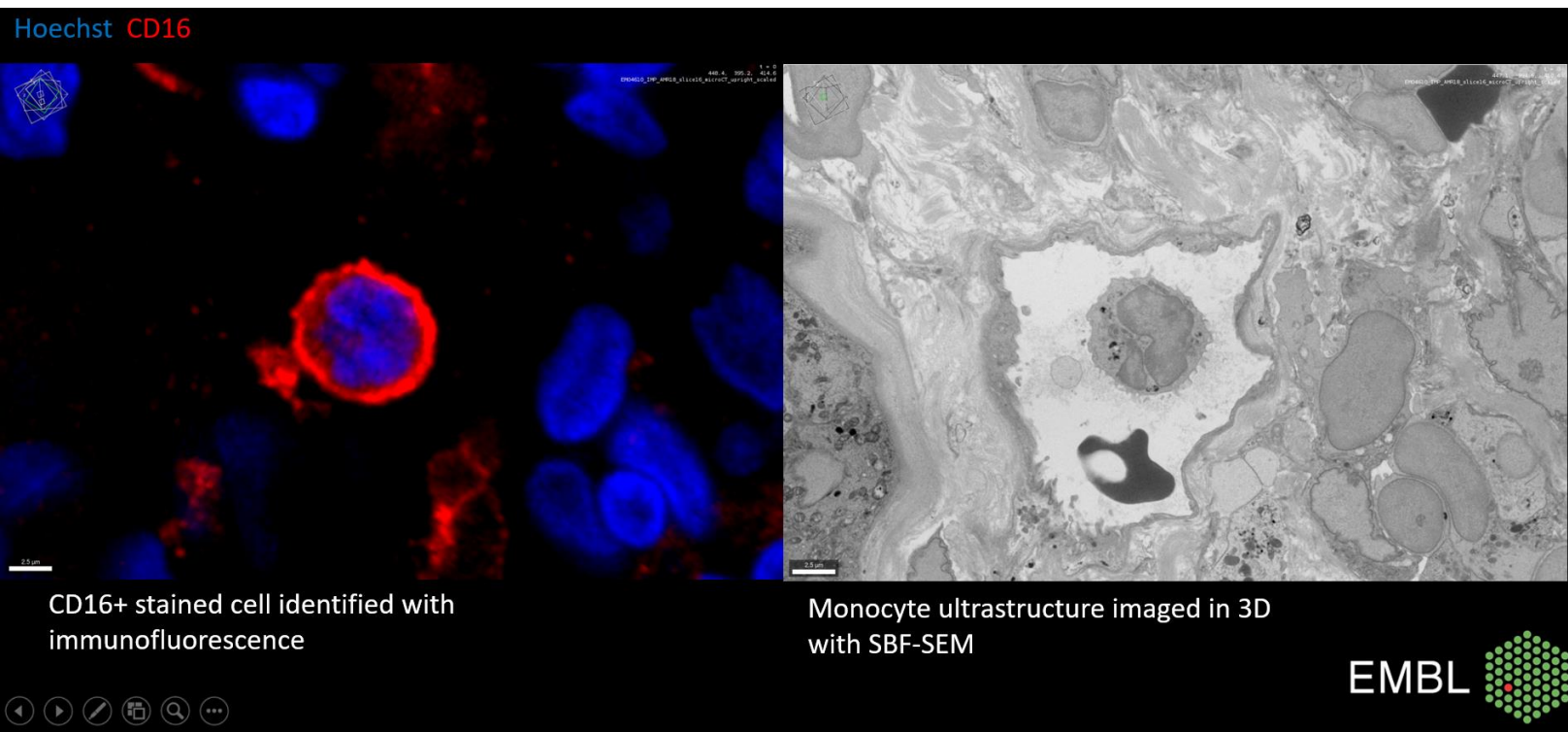


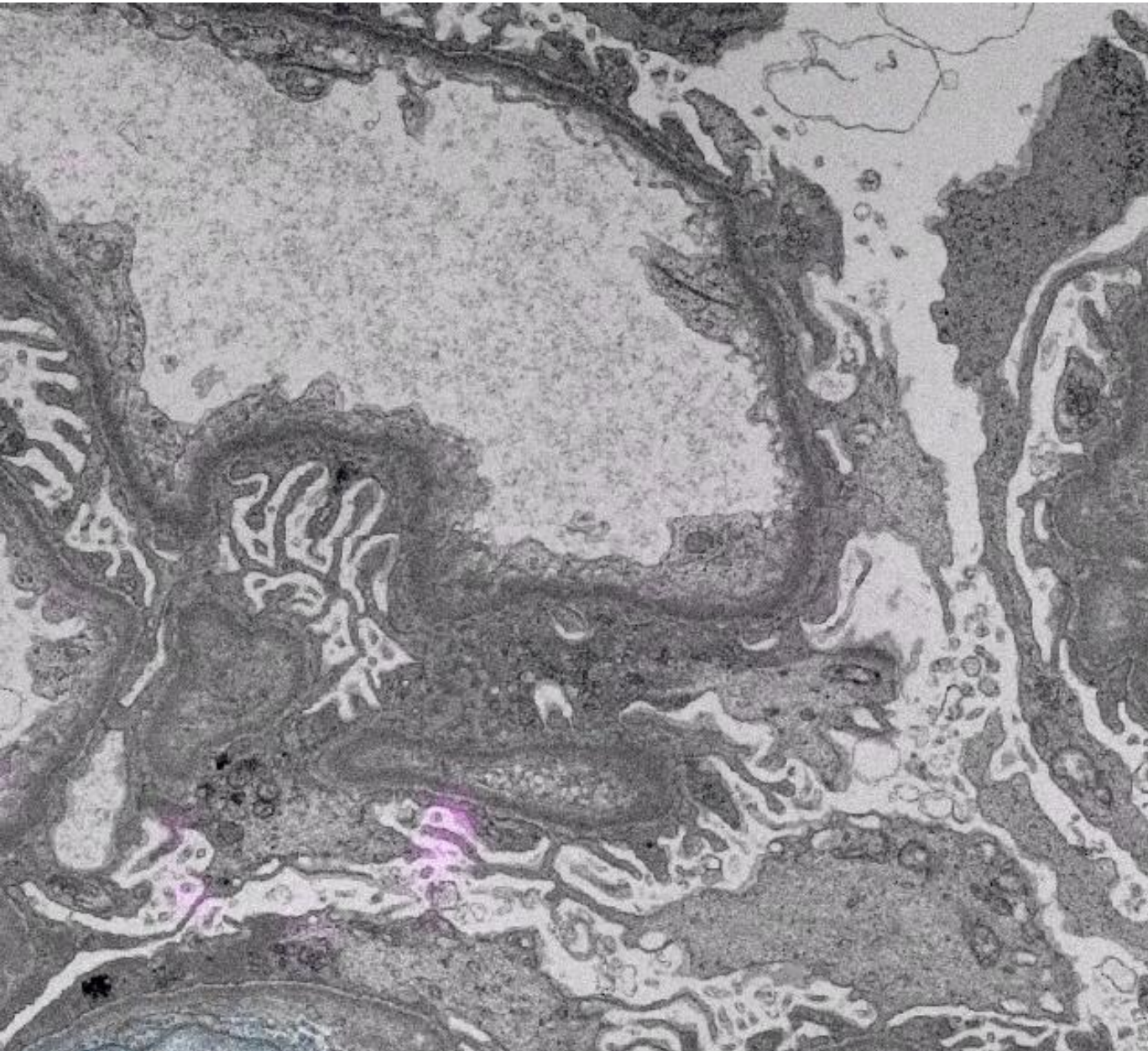
THE
FRANCIS
CRICK
INSTITUTE





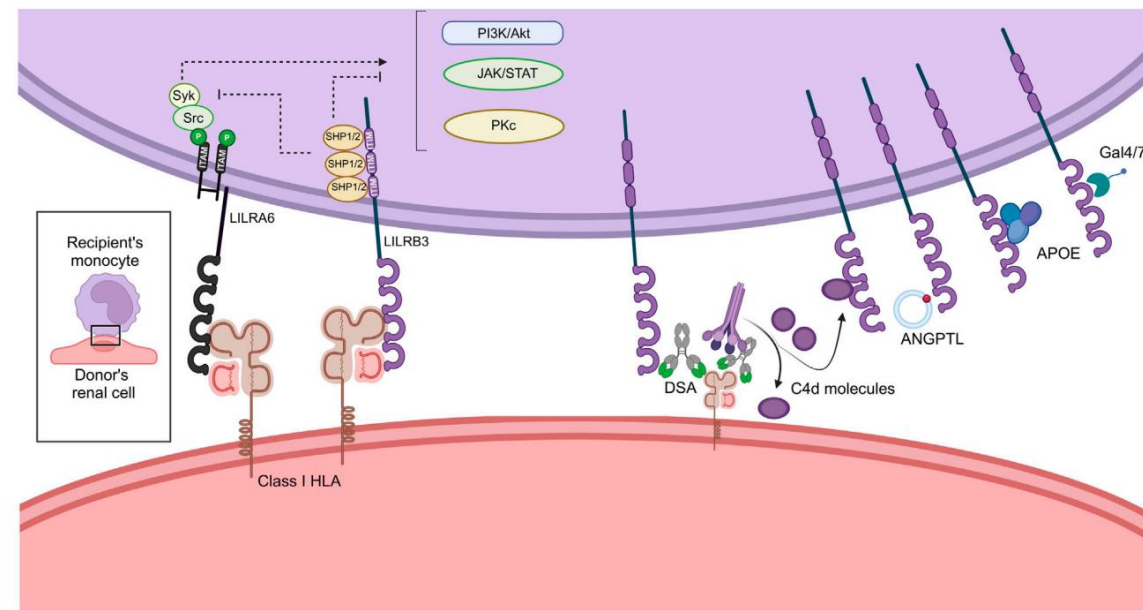
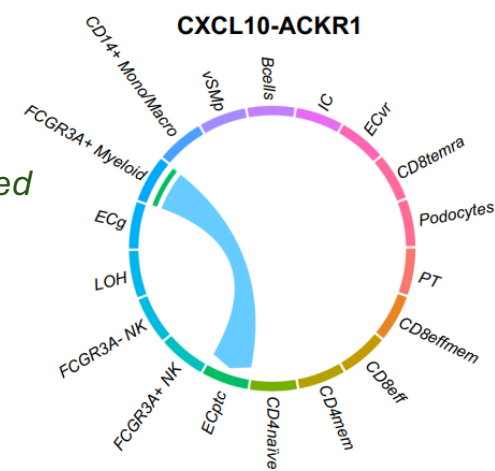
Interesting initial observations...



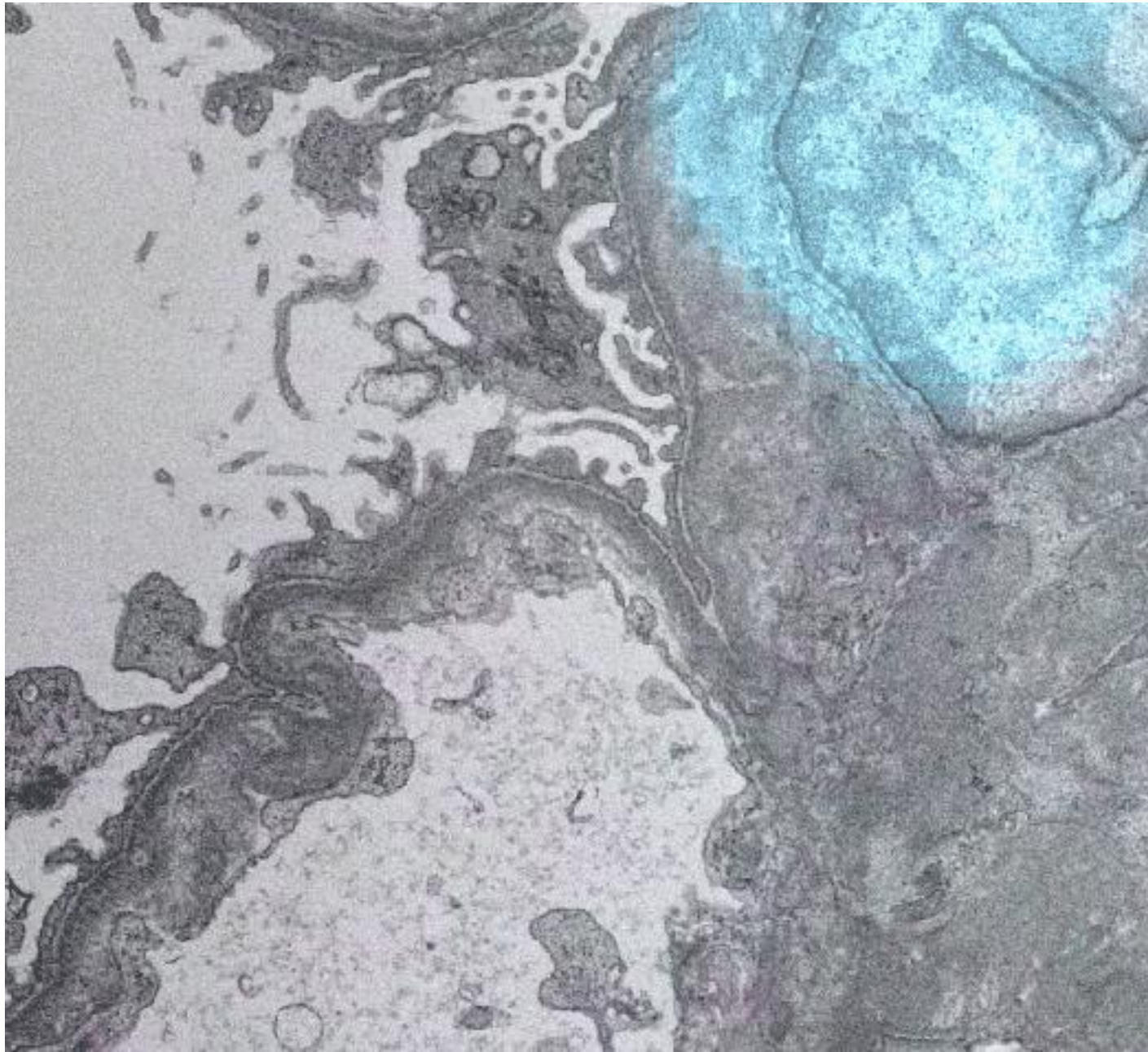


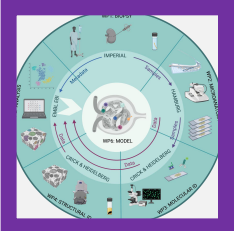
FCGR3A+ monocytes
 - Interact with ptc endothelial cells via CXCL10-ACKR1 (DARC)

Lamarthée et al Nat Med 2023



Palvair et al. Transplant International 2024





Machine learning for nanoscale feature extraction

MorphoFeatures: unsupervised exploration of cell types, tissues and organs in volume electron microscopy

Valentyna Zinchenko^{1,4†}, Johannes Hugger^{2†}, Virginie Uhlmann², Detlev Arendt^{3*}, Anna Kreshuk^{1*}

¹Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; ²European Bioinformatics Institute, European Molecular Biology Laboratory (EMBL), Cambridge, United Kingdom; ³Developmental Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; ⁴Collaboration for joint PhD degree between EMBL and Heidelberg University, Faculty of Biosciences

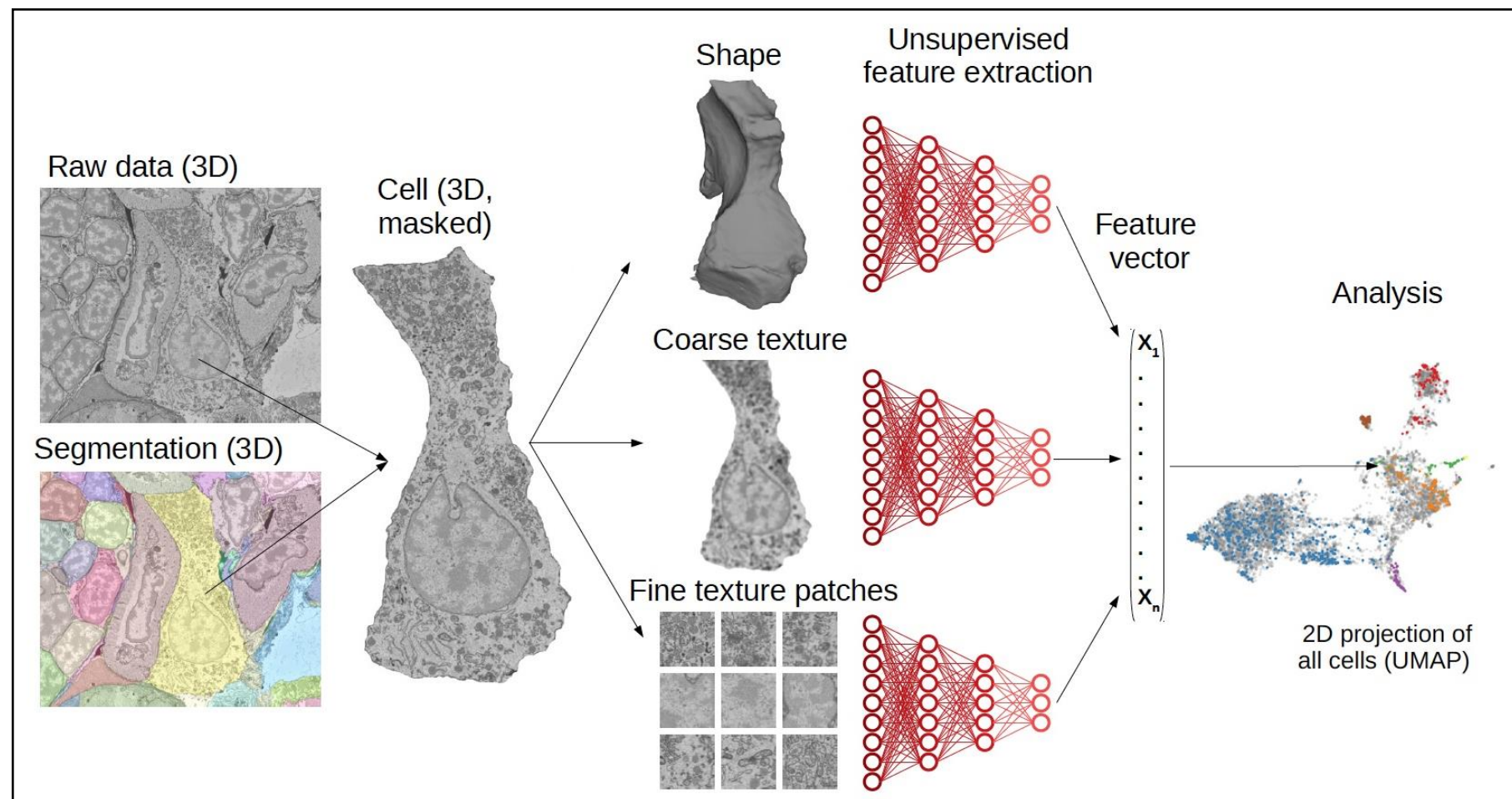
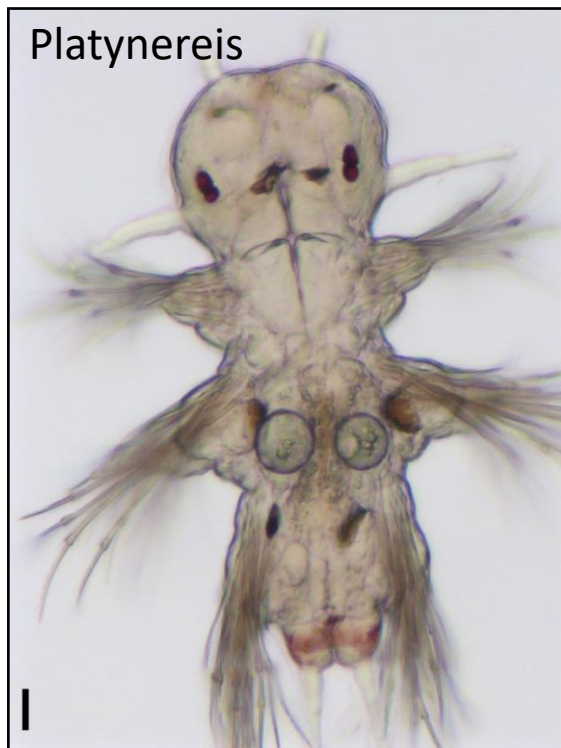


Virginie Uhlmann

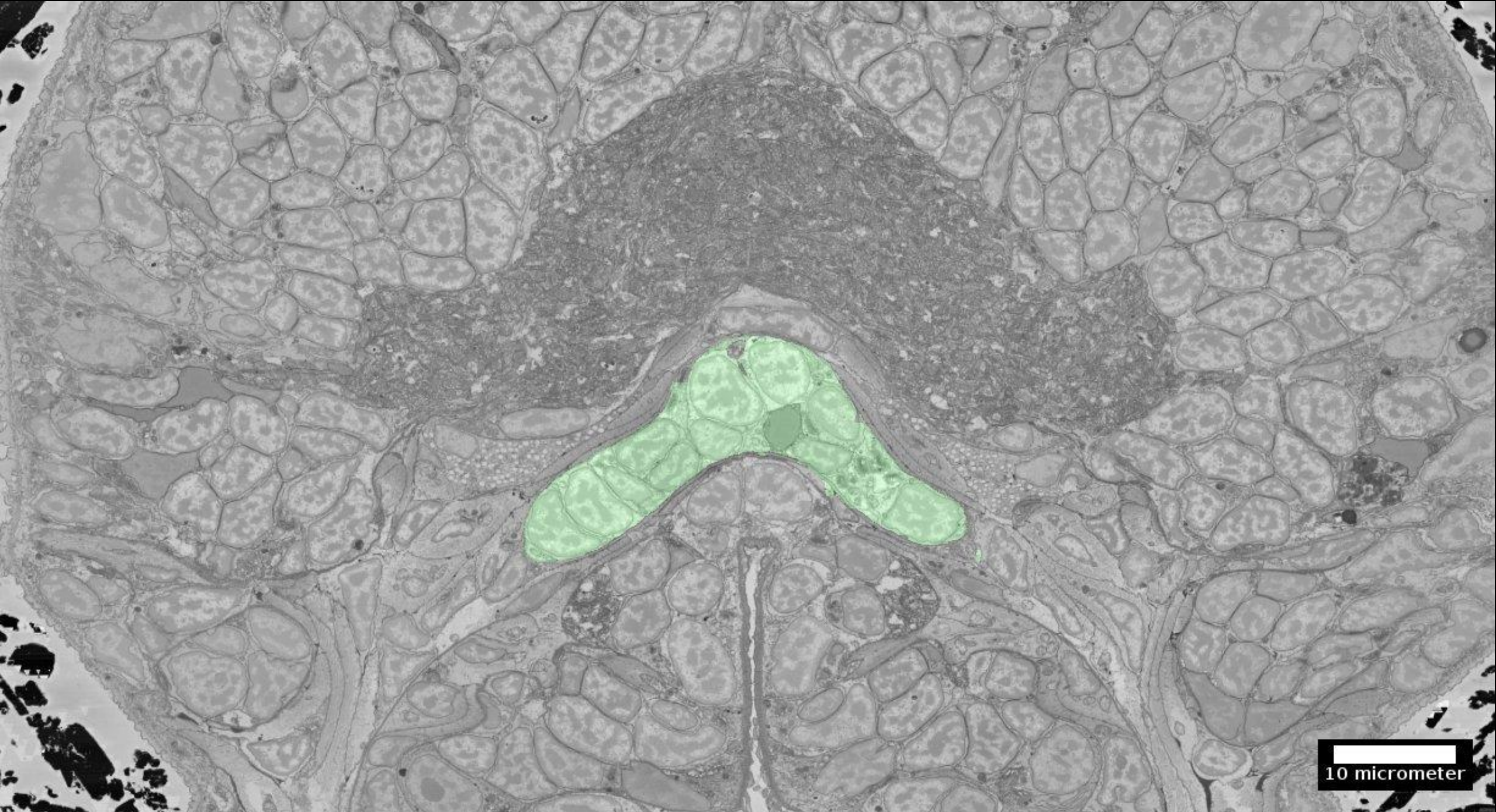


Xiaofan Xiong

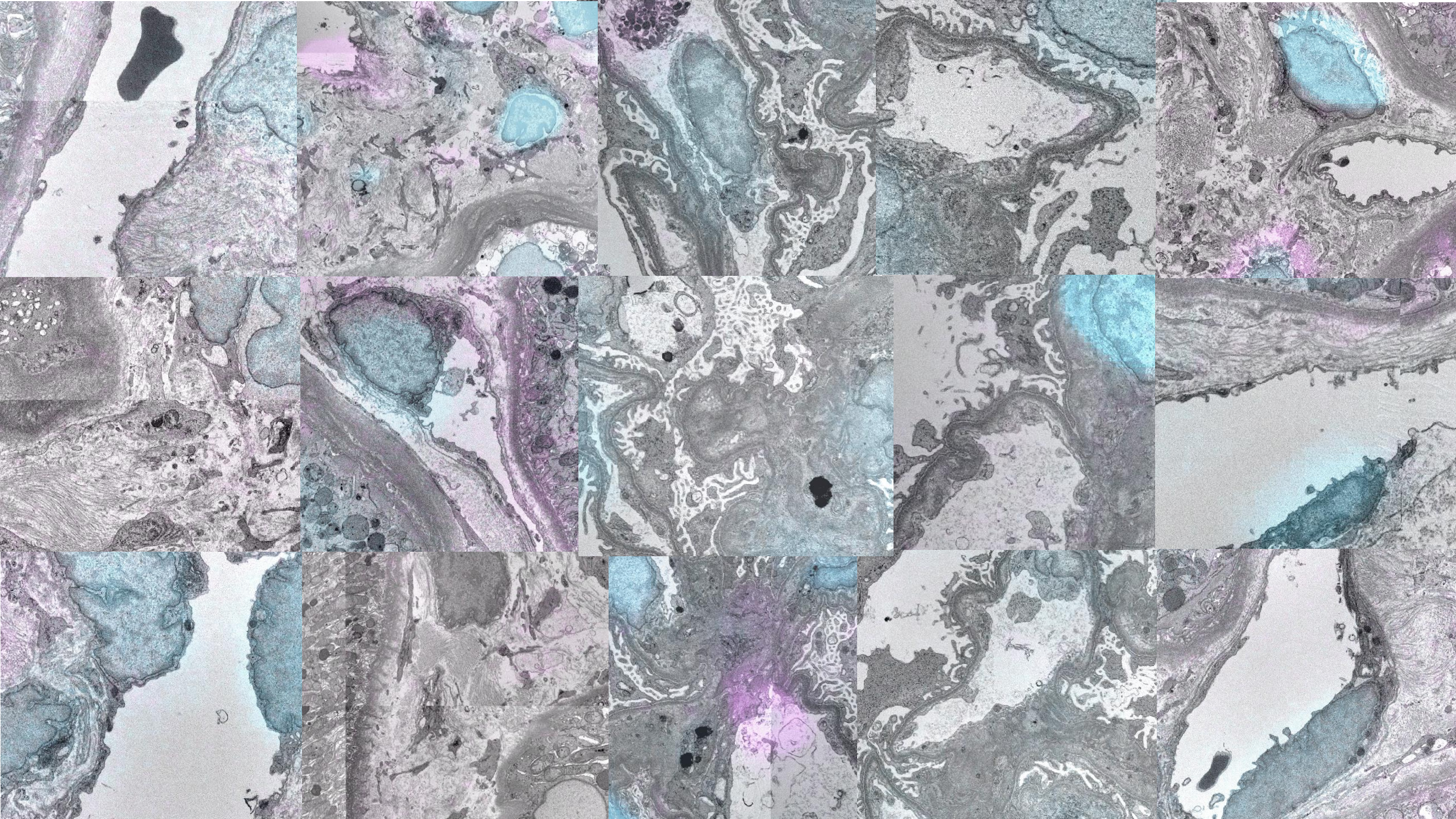
Images to Cells to Shapes & Textures to Classes

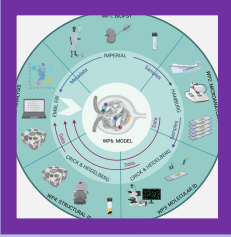


Zinchenko et al, 2022



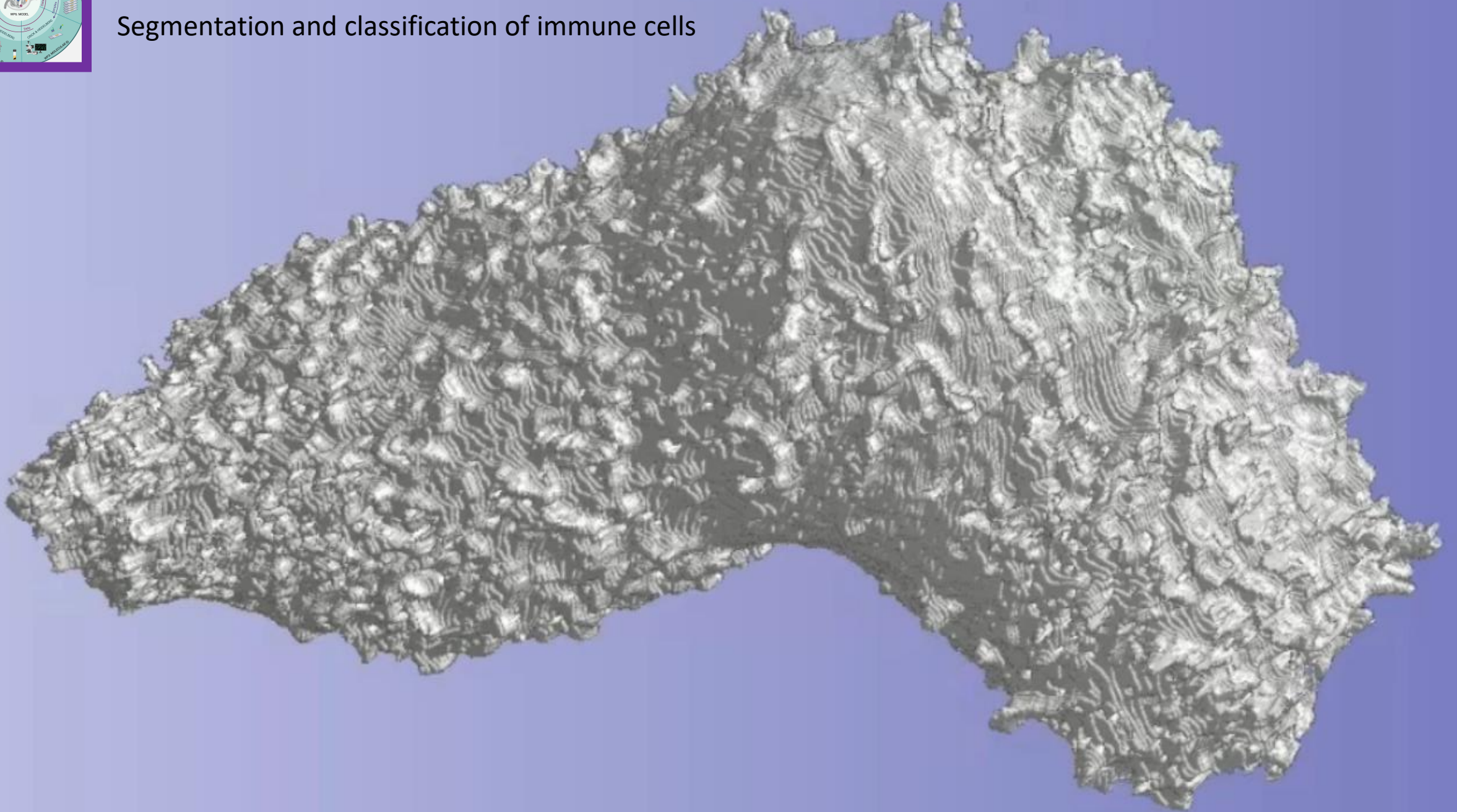
10 micrometer

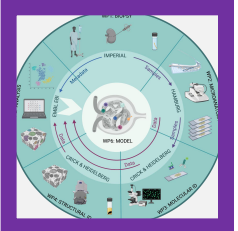




Machine learning for nanoscale feature extraction

Segmentation and classification of immune cells





Machine learning for nanoscale feature extraction

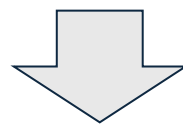
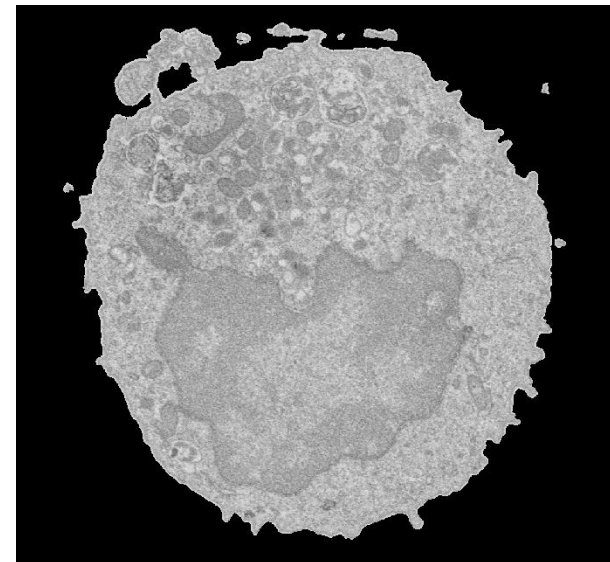
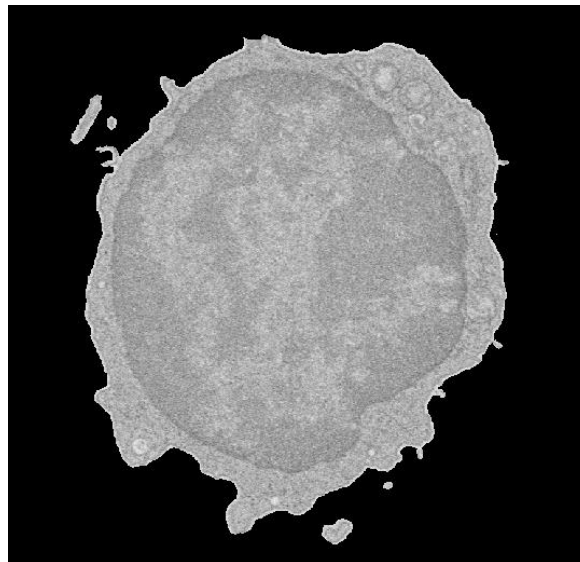
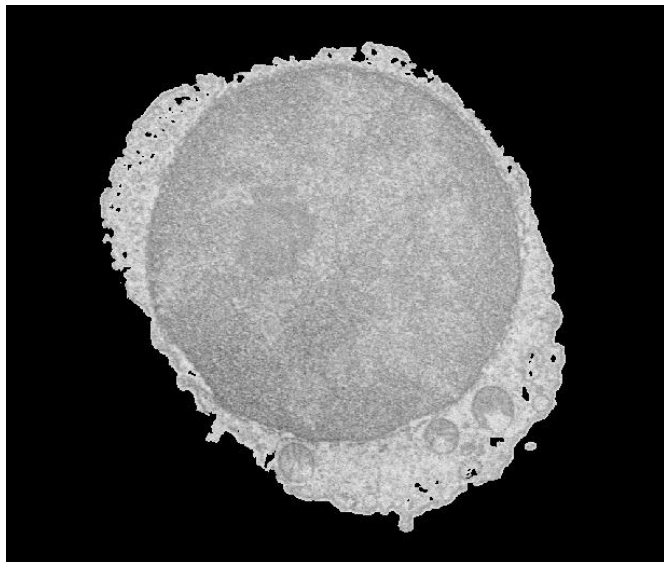
Feature extraction using unsupervised classification



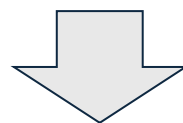
Virginie Uhlmann



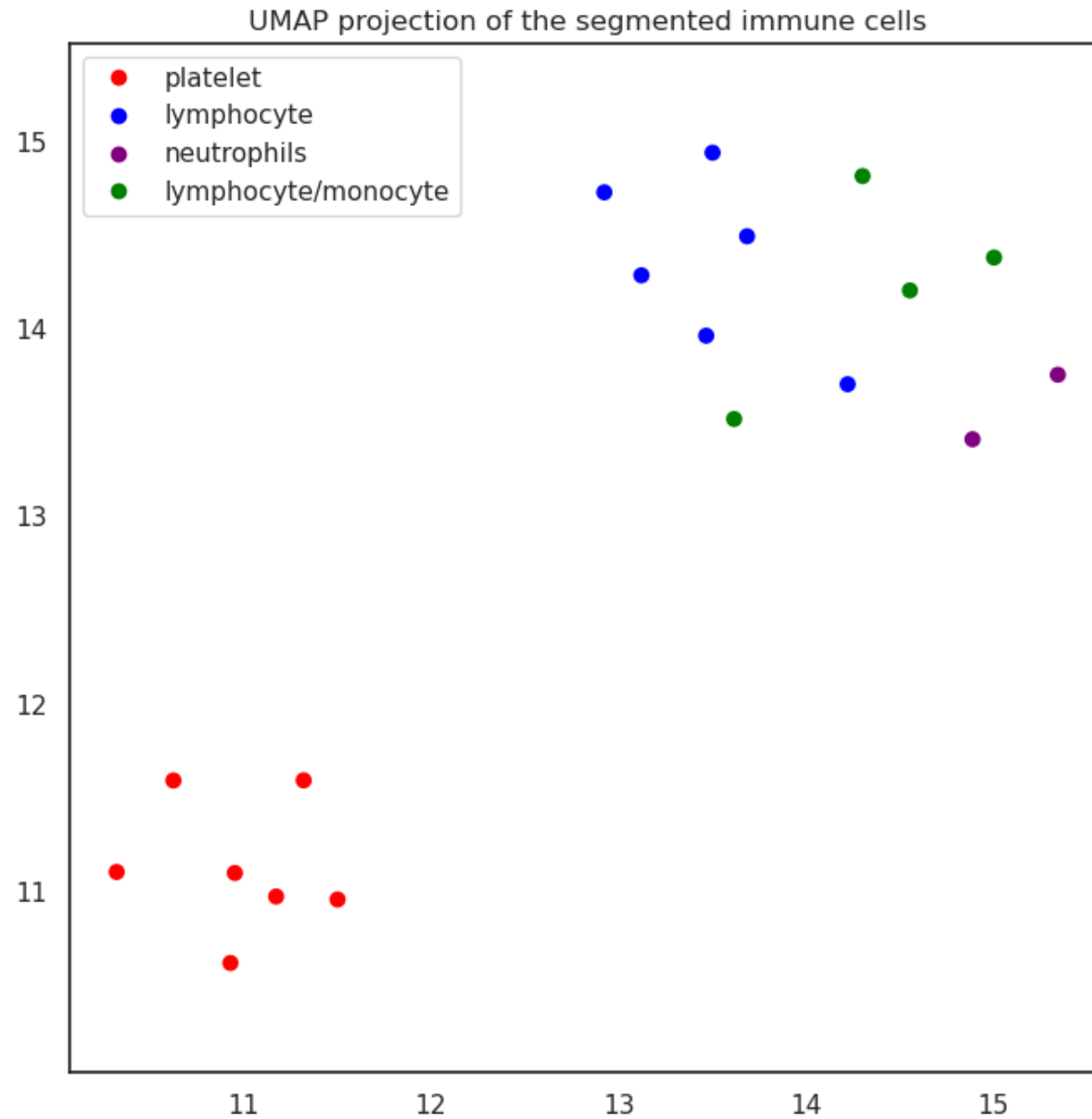
Xiaofan Xiong



Classic image feature extraction: intensity, texture, shape features, etc.



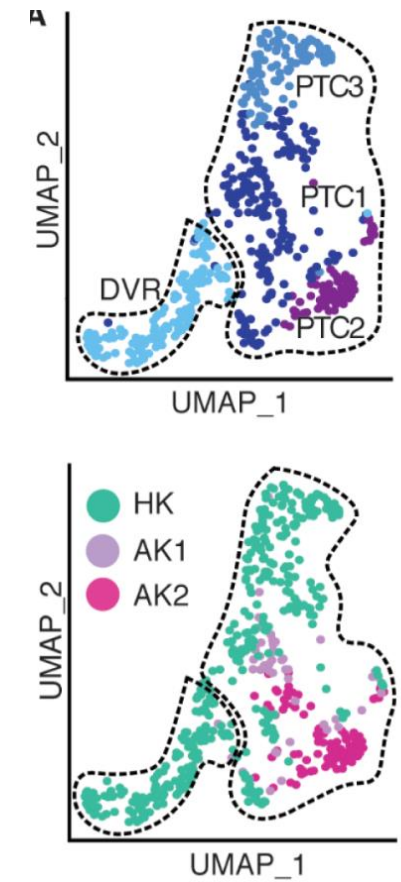
UMAP (dimension reduction)



Virginie Uhlmann



Xiaofan Xiong



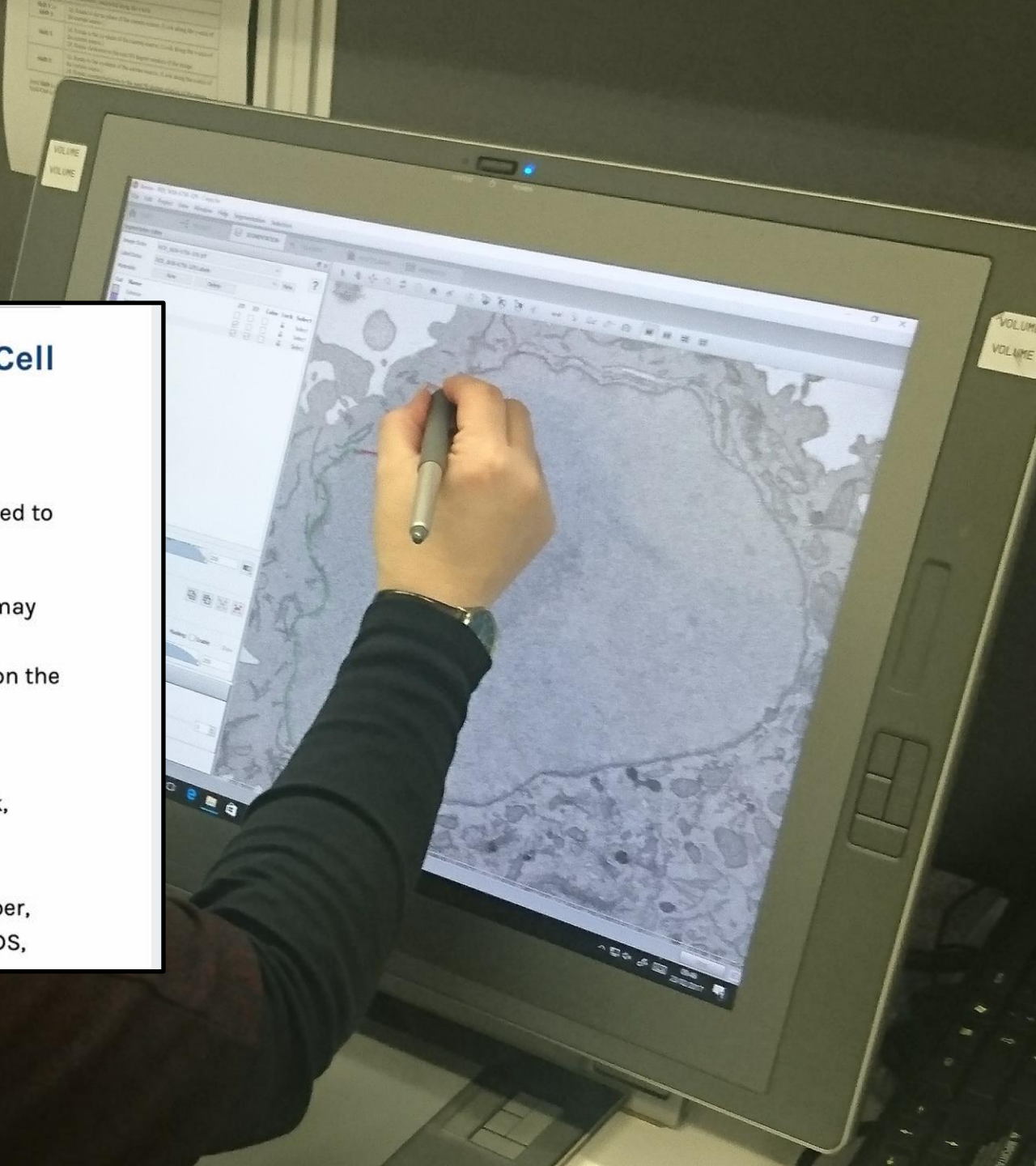
Etch-a-Cell Going Nuclear

Thank you to everyone who has contributed to Etch A Cell so far!

We would like to say an enormous **THANK YOU!** to every single person who has contributed to Etch A Cell. Below is a list of the all the volunteers who have contributed to Etch A Cell so far.

This list was generated on the 23rd July 2020 (so if you have contributed since you may not be listed below) and includes the 'credited name' of all volunteers who have contributed to Etch A Cell. If you aren't on this list but would like to be added, or are on the list and would like to be removed, please get in touch with the Etch A Cell team.

Campbell Allen, Adam McMaster, Kristina Klug, Tanya Oleksuik, John D. Pieper, John Fairweather, x303, Cassie Crowe, Johan Klein Entink, Grant Miller, Coleman Krawczyk, Ingrid Ludeke, Shawn Cruze, Elisabeth Baeten, Jim O'Donnell, Chris Lintott, DrRogg, Christine V. Macmillan, R J Mellor, David BROWNE, Dave Kincla, Sarah Pomfrett, Meg Schwamb, Rebecca Smethurst, David Wardrop, Dennis Brogan, Ercydive, Nancy Godber, Anthony Jakominic, Lukas Hackl, Dave Weilant, Léon McGregor, Philip J. Eversman DDS,





Helen Spiers

TASK

TUTORIAL

Please draw around the cell using the pen

Pen

0 drawn

Done



Multimodal (morphology + molecules) cross-scale data

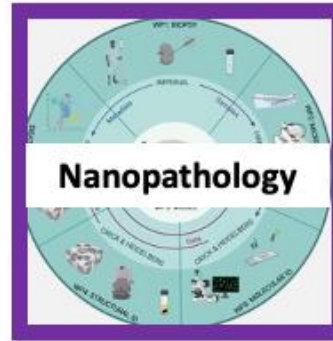
New *insights into disease*

New *pathways to diagnosis*

Pipeline enables Hi-res imaging of a target of interest within the tissue sample; time down from 50 years to ~ 1 month

Further speed up pipeline through targeting down to single cell level (multiplex fluorescence)

- > Roll out nanopathology platform to cancer, neuropathology, human embryology, infection...



Lucy Collinson



Candice Roufosse Alana Burrell

Liz Duke

Jonas Albers

Mat Lawson

Yannick Schwab

Paolo Ronchi



Virginie Uhlmann

Xiaofan Xiong

Amy Strange

Joost de Folter

Graham Ross

Matthew Hartley

Martin Jones

Helen Spiers



Imperial College Healthcare
NHS Trust

Adam McLean
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Kidney transplant patients in our clinics at Imperial College Healthcare NHS Trust

IMPERIAL

Imperial BRC funding
Spatial transcriptomic data

Hannah Lomax-Browne
Karolina Kwiatkowska
Lottie Bottomley

