

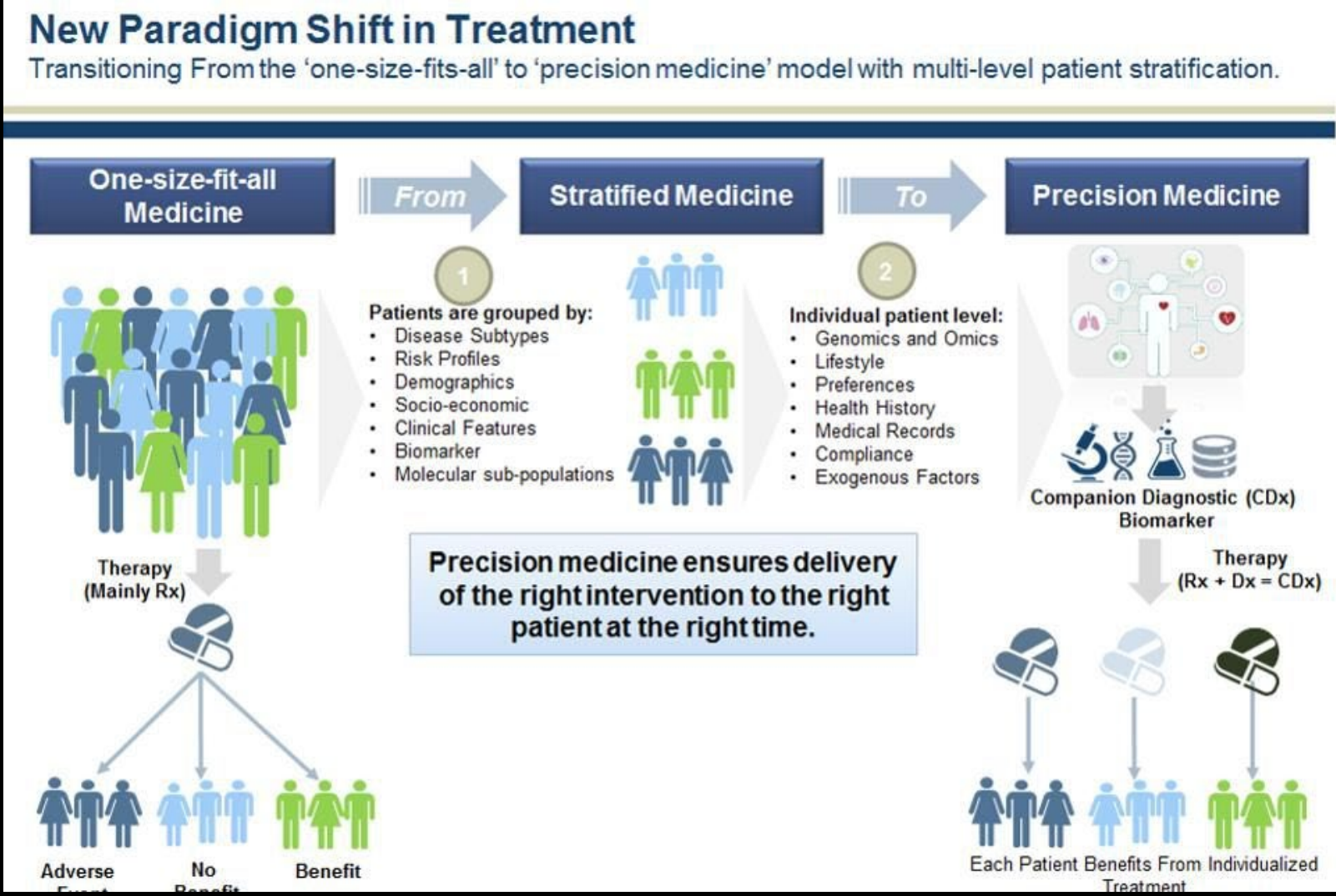
# Bi-directional Interrogation of Image Volumes and Segmented Cells With 3D Tissue Cytometry

**Seth Winfree and Tarek Ashkar**

Indiana University School of Medicine, Medicine, Division of Nephrology

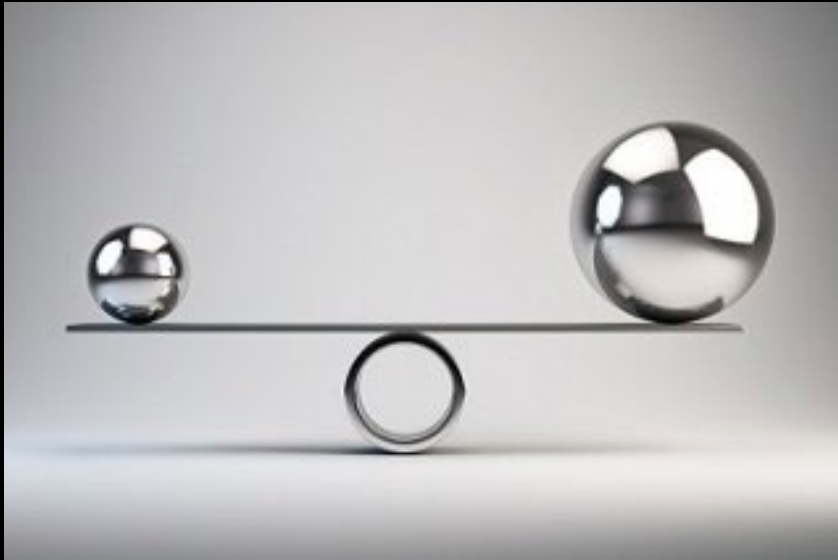
IU/OSU Tissue Interrogation Site, KPMP

# Era of precision medicine



# “Small” changes can have “big” effect

“Small” changes can cause “big” effects

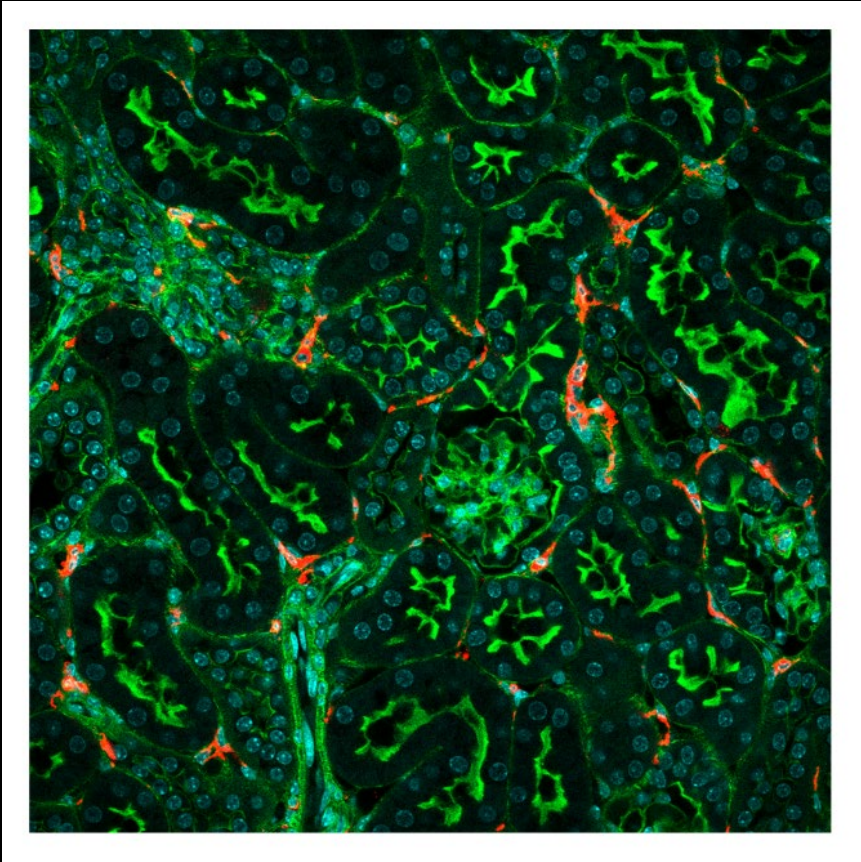


Changes can be a needle in a haystack

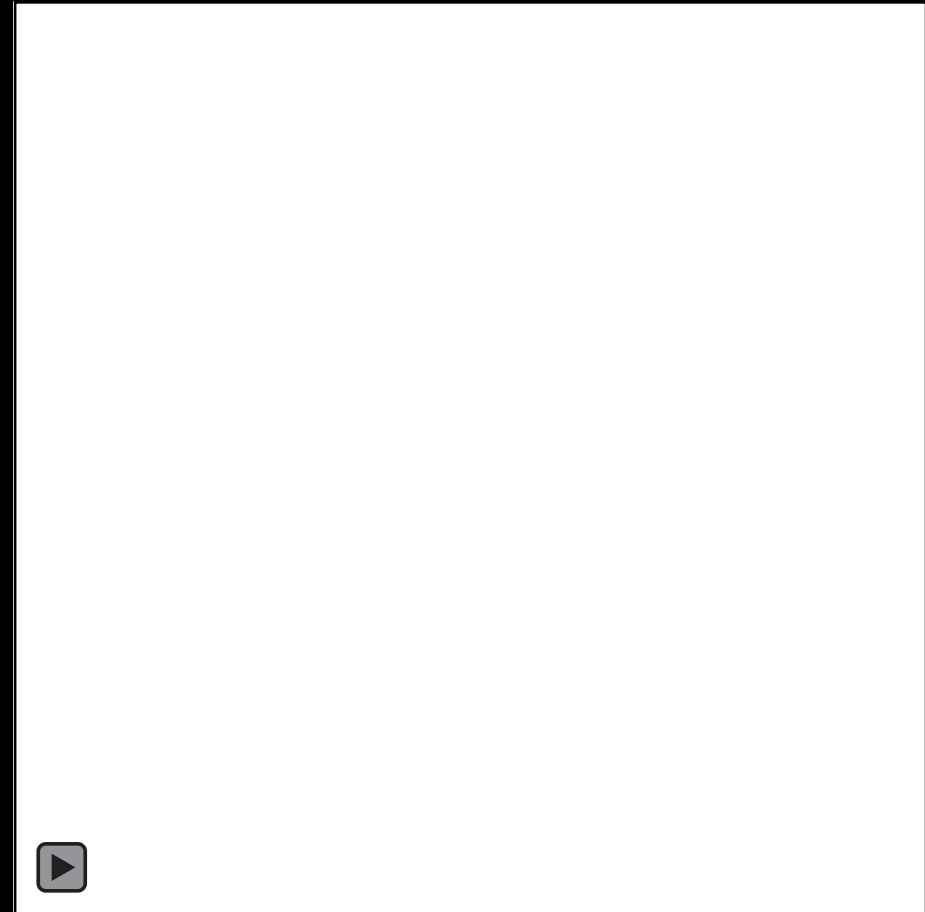


- High resolution methods with high sensitivity
- Ability to analyze multiparametric datasets
- Look for small changes unique for patient and for disease

# Why 3D imaging? Tissue complexity



DAPI MHCII F-Actin

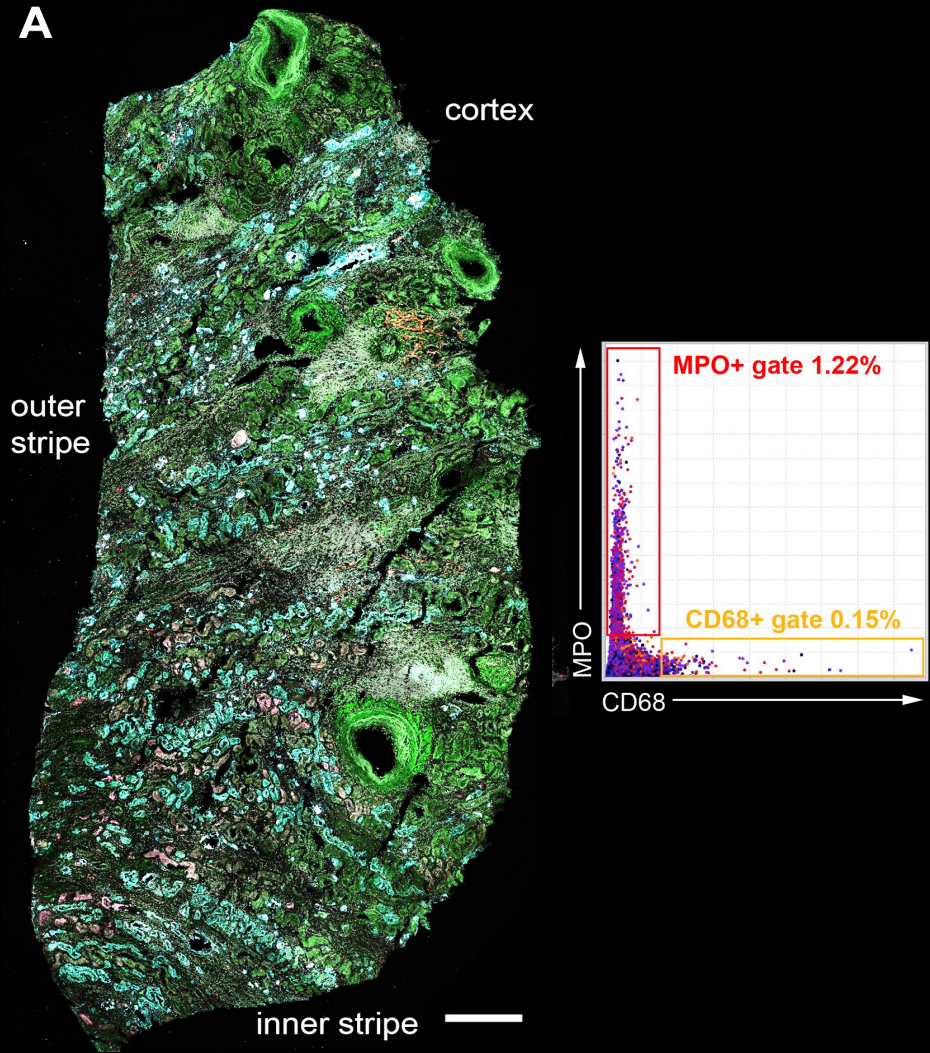
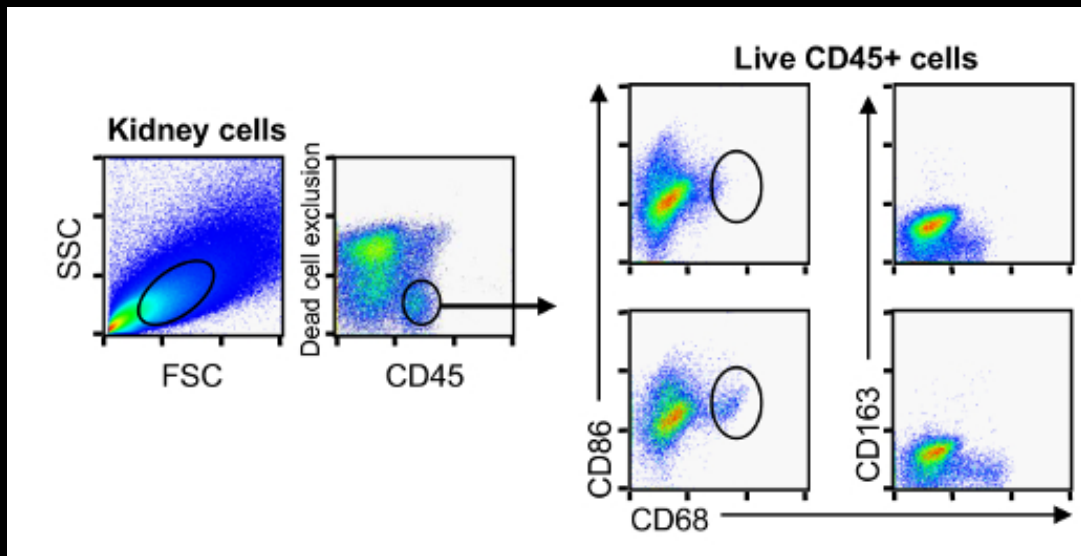
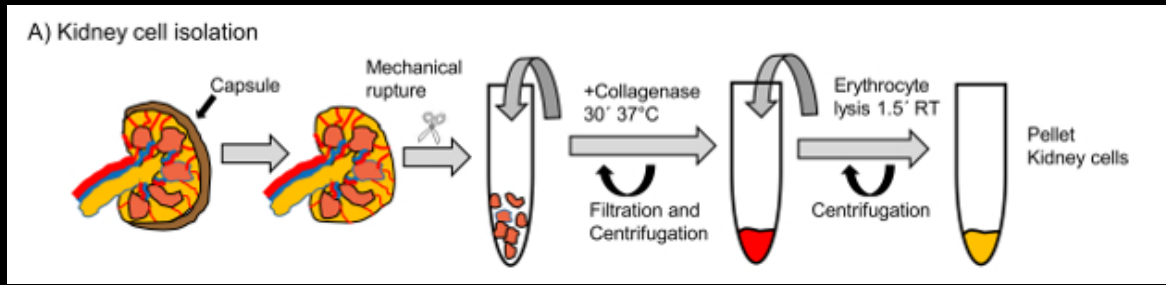


MHCII CD11C F-Actin

# Tissue complexity, 3D and large scale



# Flow Cytometry approach is robust...Can we do something similar in intact kidney tissue?



# 3D cytometry-segmenting individual cells

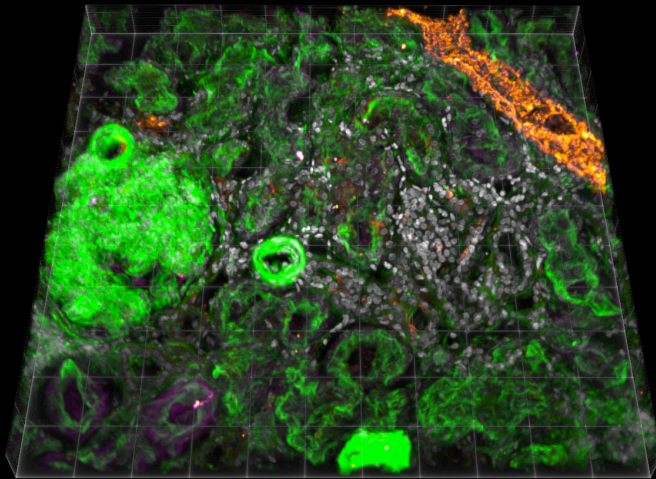
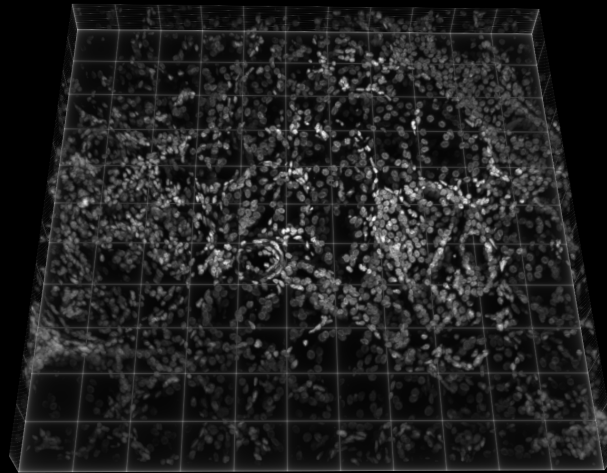


Image data in 3D



Nuclei

Segment



Single cells segmented in 3D

# 3D cytometry-single cell analysis in 3D



Single cells segmented in 3D

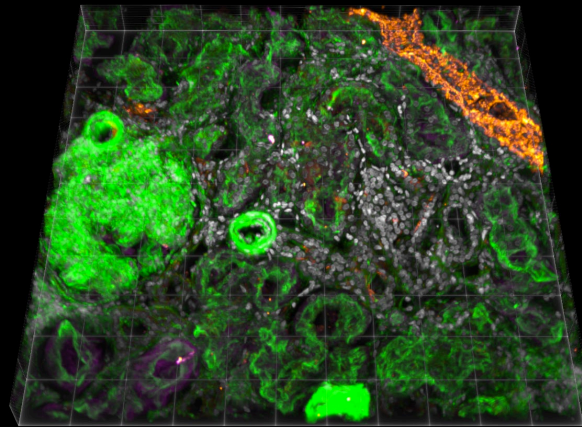
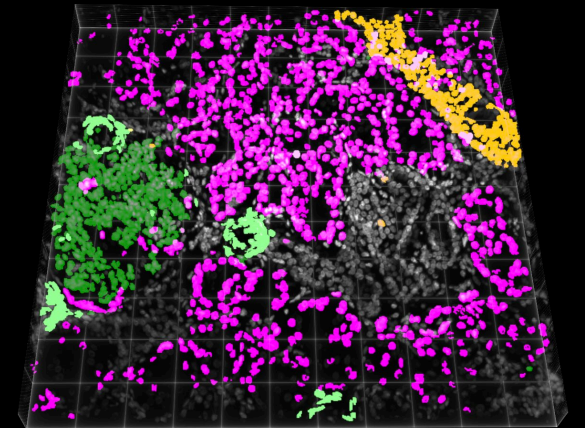


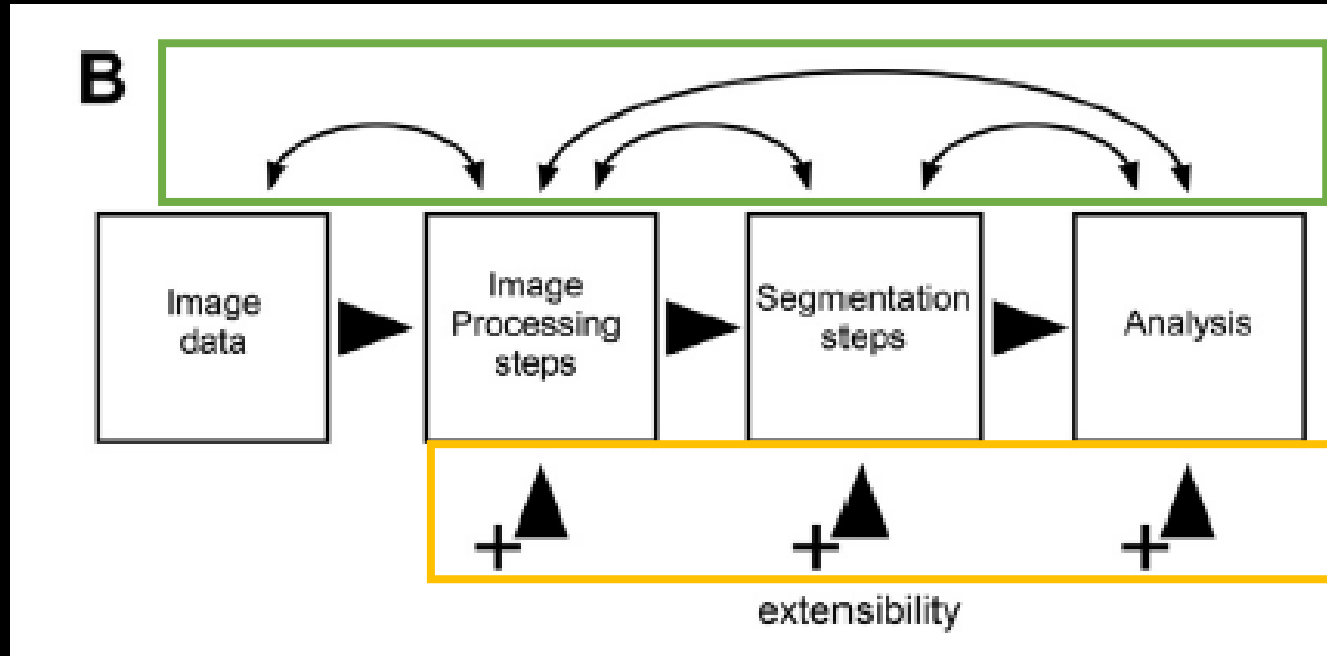
Image data in 3D



Classify and count



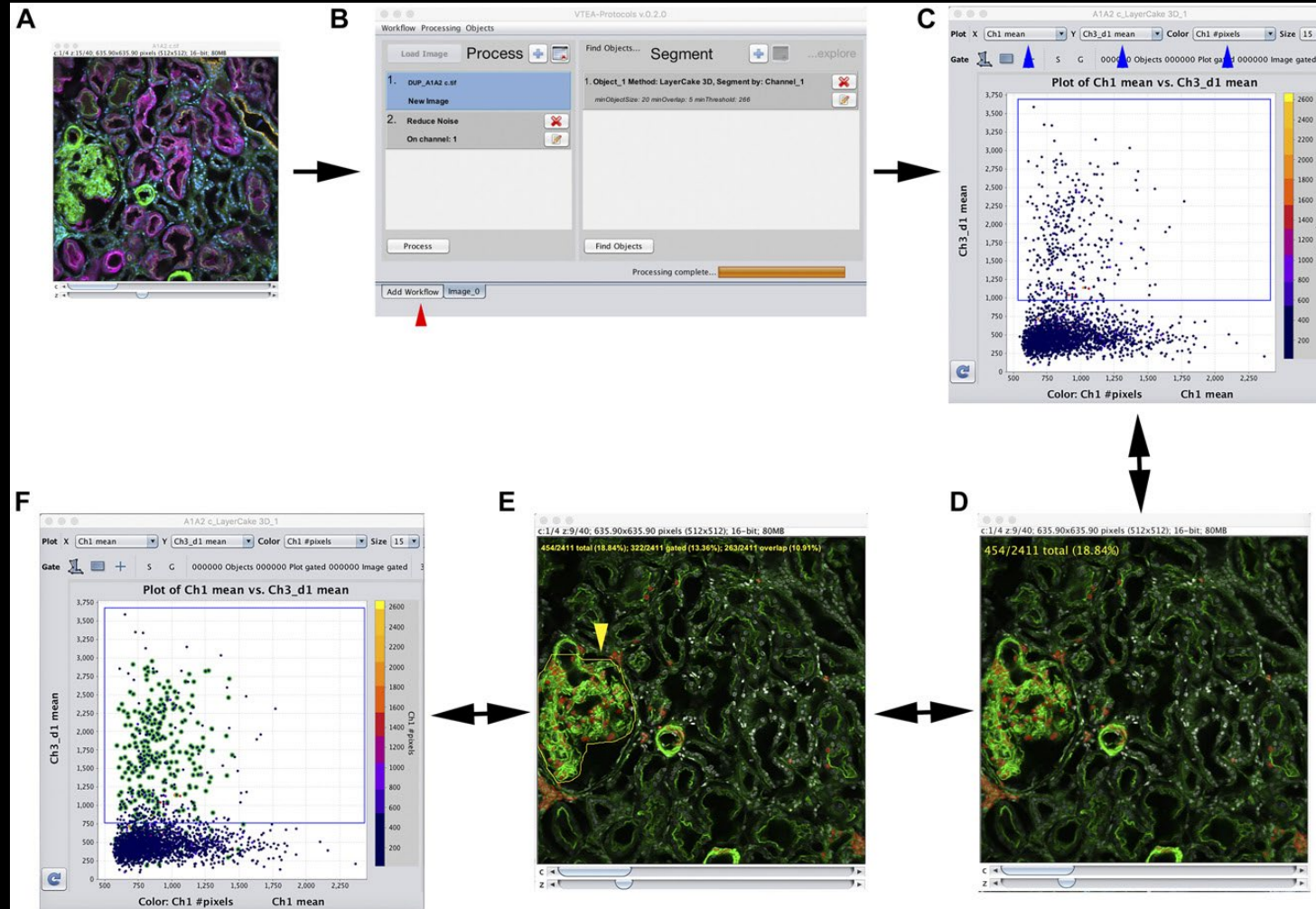
# Designing an approach for tissue cytometry



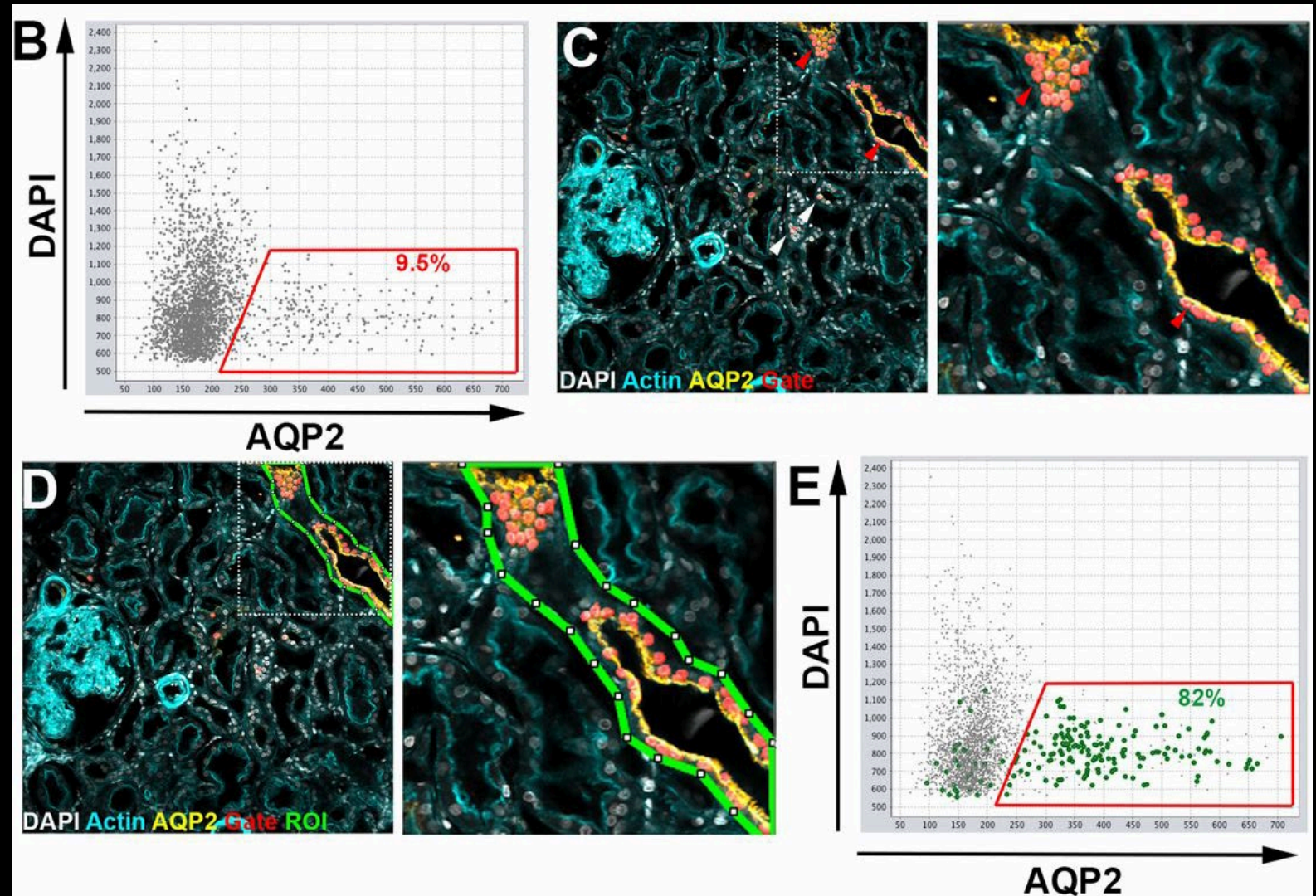
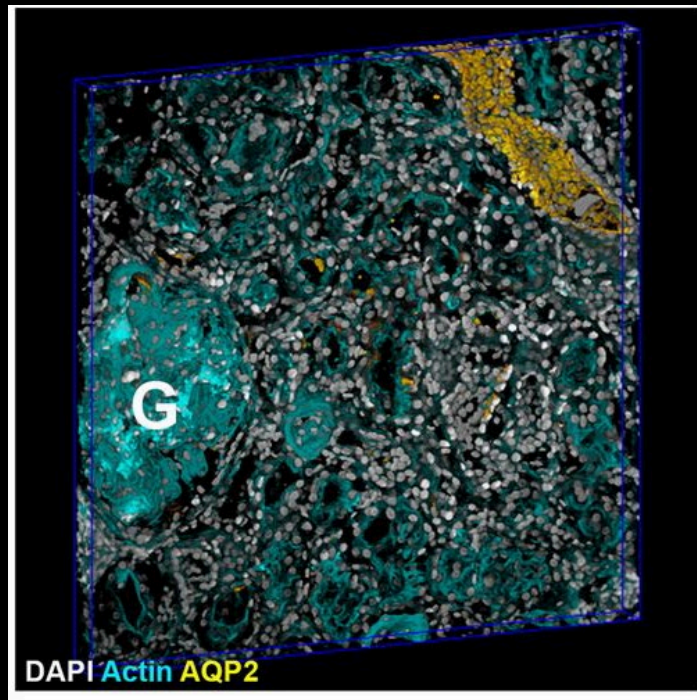
Tool use

Development and growth

# Volumetric Tissue Exploration and Analysis: VTEA

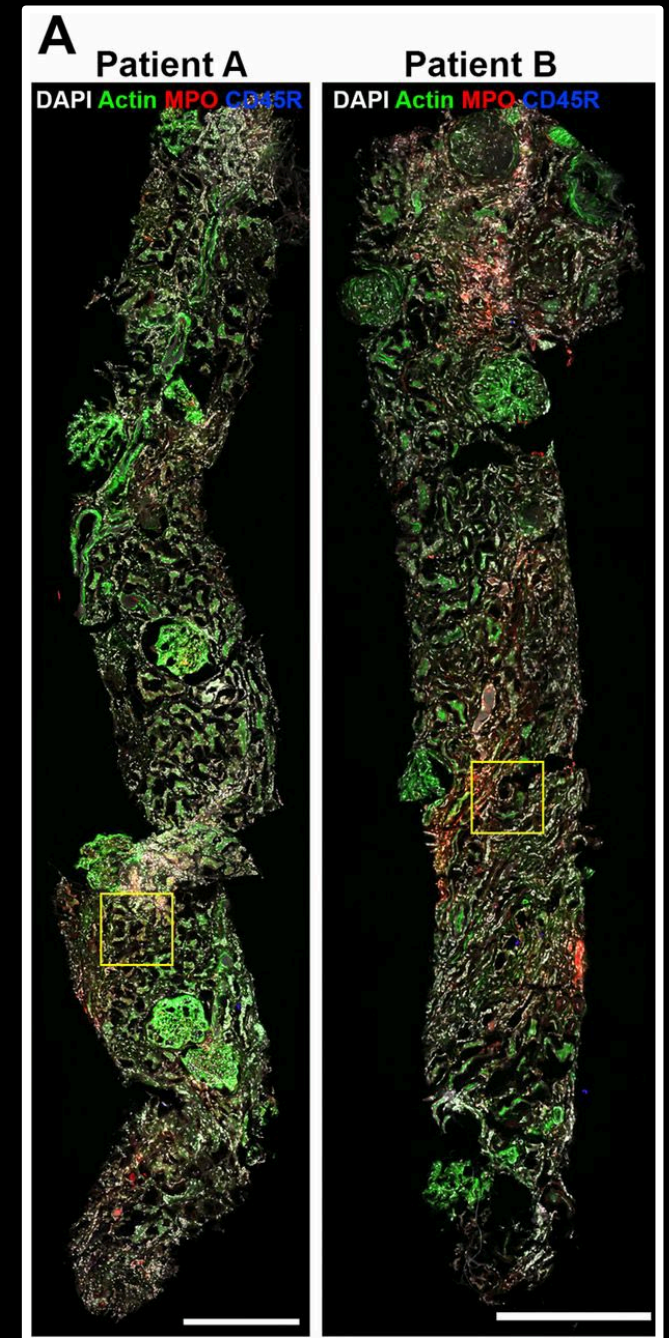
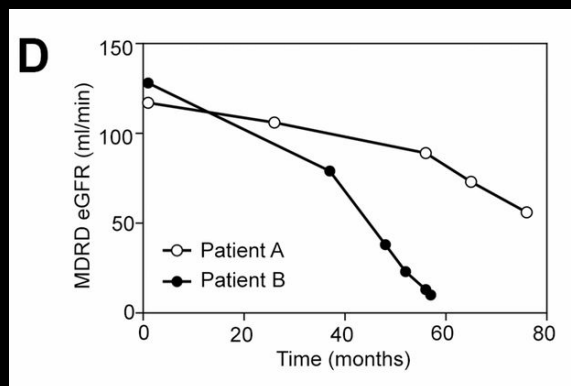
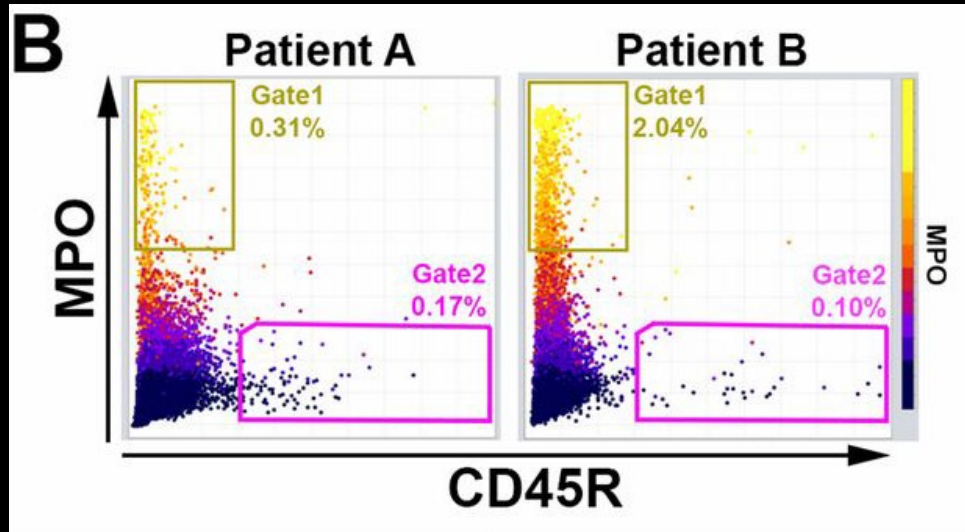


# 3D tissue cytometry using VTEA

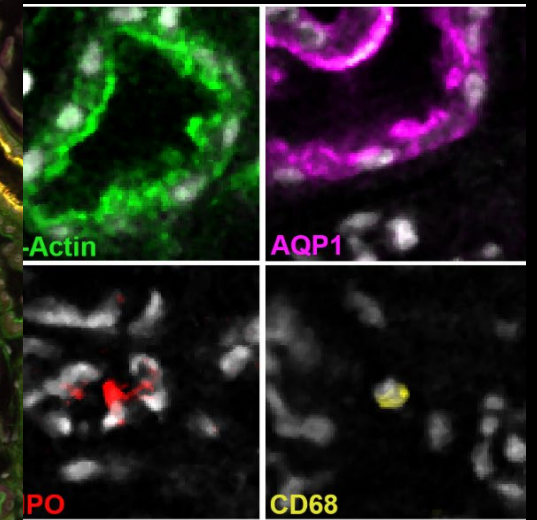
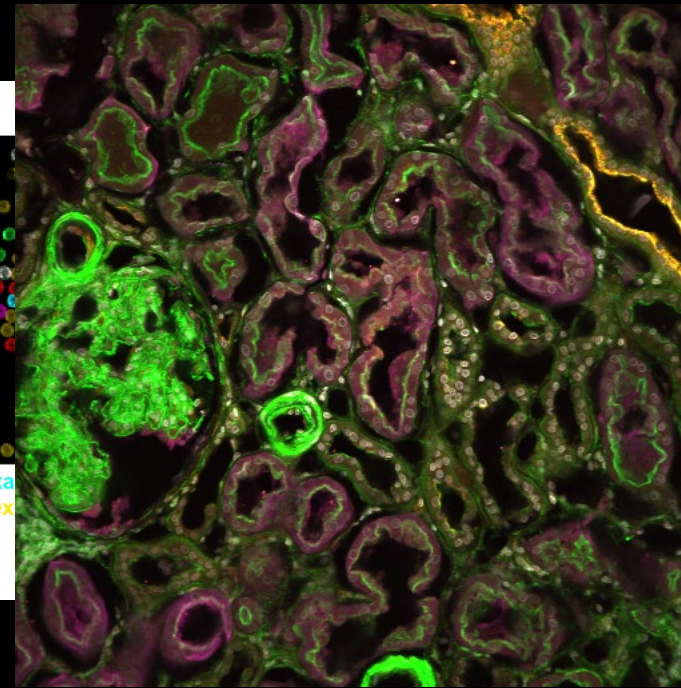
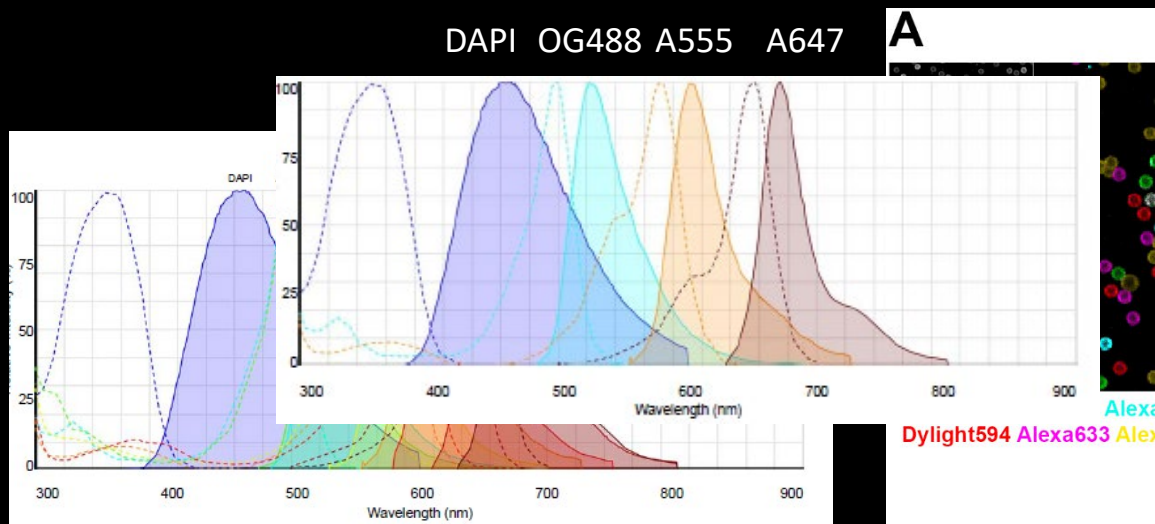


# Application to human biopsies in a clinical setting

VTEA

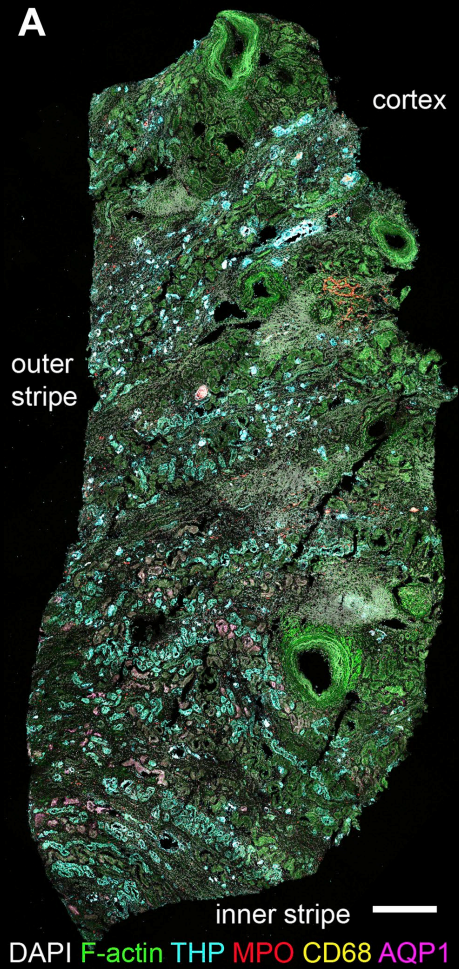


# Marker complexity structures and cells of interest

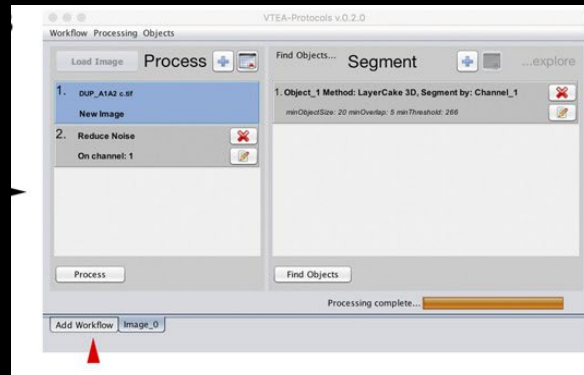




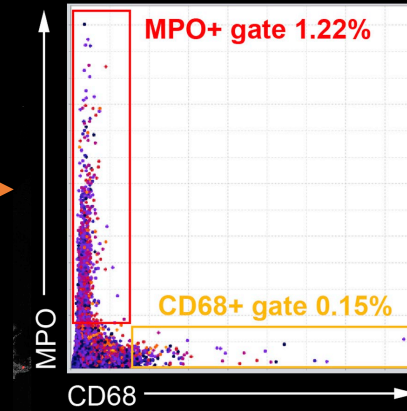
# Tissue "Big" data exploration, analysis and interpretation in one workflow



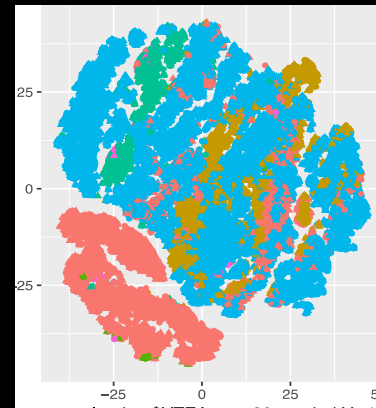
VTEA



Supervised analysis

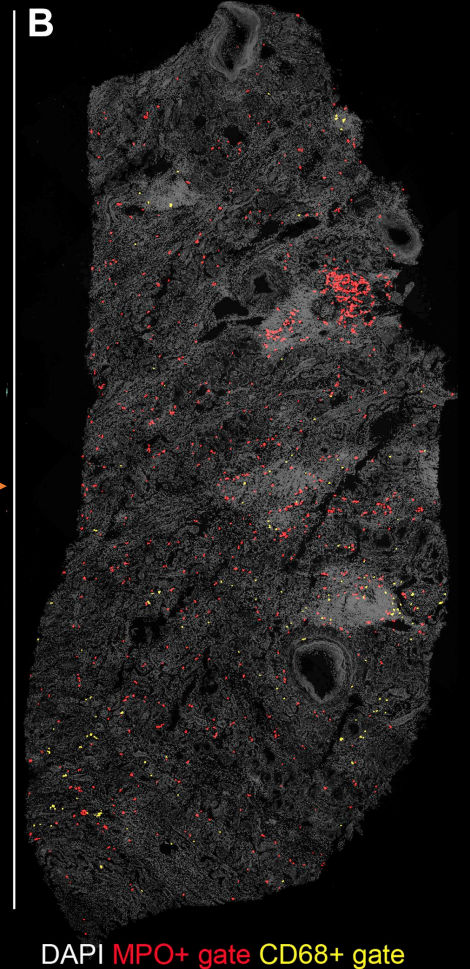


>10<sup>5</sup> segmented nuclei  
>100 attributes



Unsupervised analysis

Link to biology *in situ*



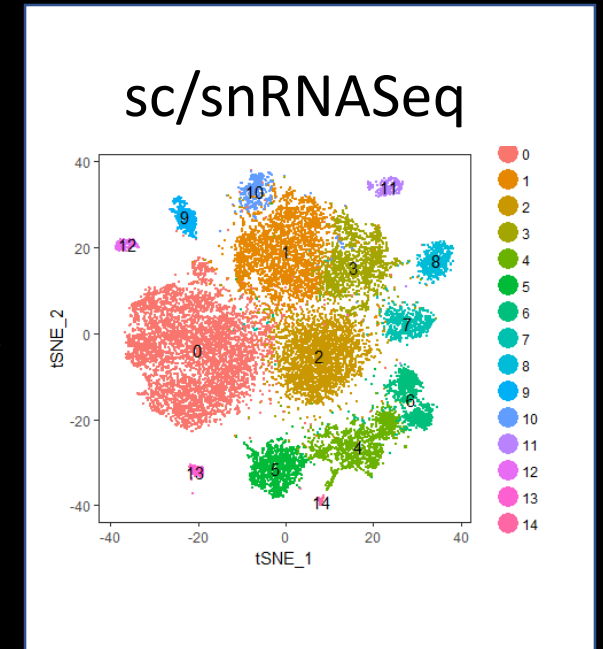
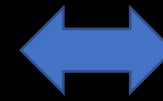
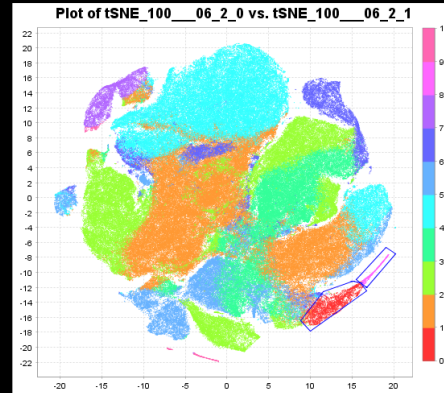
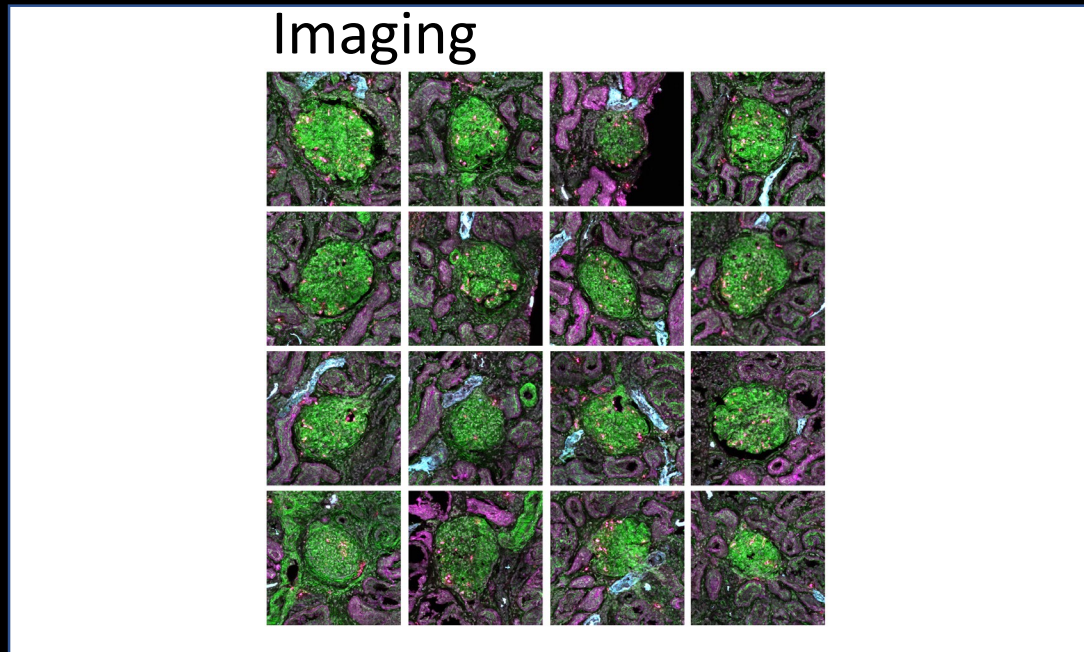
# VTEA in use

The image displays a complex software interface for VTEA (Virtual Tissue Environment Analysis) in use. The interface is composed of several overlapping windows and panels:

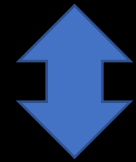
- Main Image Window:** Shows a multi-channel microscopy image of biological tissue with various colors (green, magenta, cyan).
- Process Panel:** A workflow editor with steps: 1. A1A2.c.tif, 2. Denoise, 3. Enhance Contrast, 4. Background Subtraction. Each step has a 'Process' button and a 'Find Objects' button.
- Segment Panel:** Shows 'Object\_1: Connect 3D, by channel 1' with a 'Find Objects' button.
- Ward Hierarchical Clustering Dendrogram:** A tree diagram showing the hierarchical clustering of objects, with 'Height' on the y-axis (0 to 390).
- Feature Panel:** A 'Feature' window showing a list of features for analysis, including 'X-means Clustering', 't-SNE', and 'Ward Hierarchical Clustering'. It includes a 'Find Features' button and a progress indicator 'Completed 5%'.
- Plot of tSNE:** A scatter plot titled 'Plot of tSNE\_100\_15\_58\_0 vs. tSNE\_100\_15\_58\_1' showing data points clustered into several groups, color-coded by cluster.
- Processing Steps:** Three 'Processing' windows showing intermediate steps: 'Processing, Step 2' (Denoise), 'Processing, Step 3' (Enhance Contrast), and 'Processing, Step 4' (Background Subtraction).
- Object\_1 Window:** A window showing 'Object\_1 (50%)' with a 'Segment on Channel' dropdown and 'Object building method' set to 'Connect 3D'. It includes a 'Dark background' and 'Stack histogram' checkbox, and a 'Watershed' checkbox.
- Clustering and Reduction Panels:** Three panels showing 'Type of feature' (Cluster or Reduction) and 'Clustering Method' (Ward Hierarchical Clustering, X-means Clustering, or Reduction Method t-SNE). They include checkboxes for 'Select All Data' and 'Z-scale all data', and a 'Maximum number of clusters' slider.



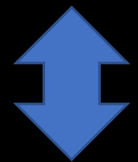
# Signatures across approaches and modalities



Proteomics

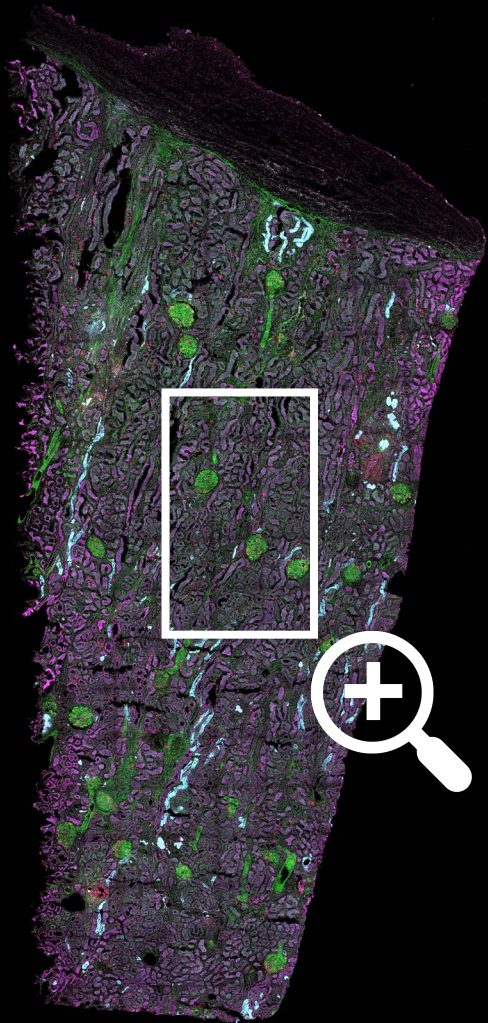


LMD (omics)



Metabolomics

# Popup Query From 3D image volume to omics



## DE Analysis

GeneX

GeneY

...

## Metabolomics

MetaboliteX

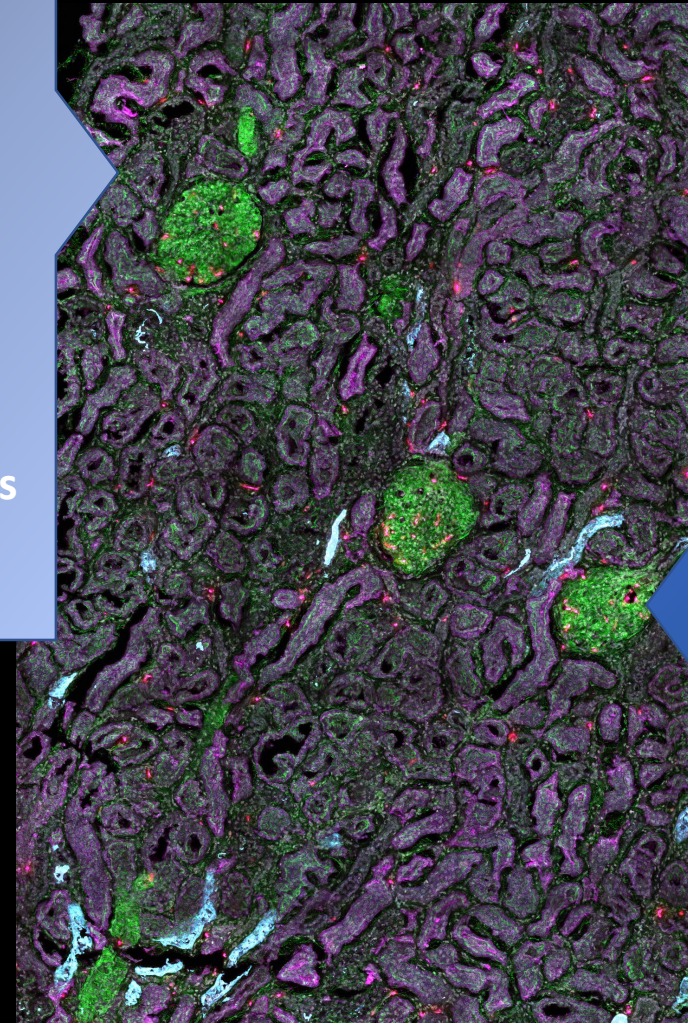
MetaboliteY

...

## LMD transcriptomics

GeneX

GeneY



## DE Analysis

GeneX

GeneY

...

## Metabolomics

MetaboliteX

MetaboliteY

...

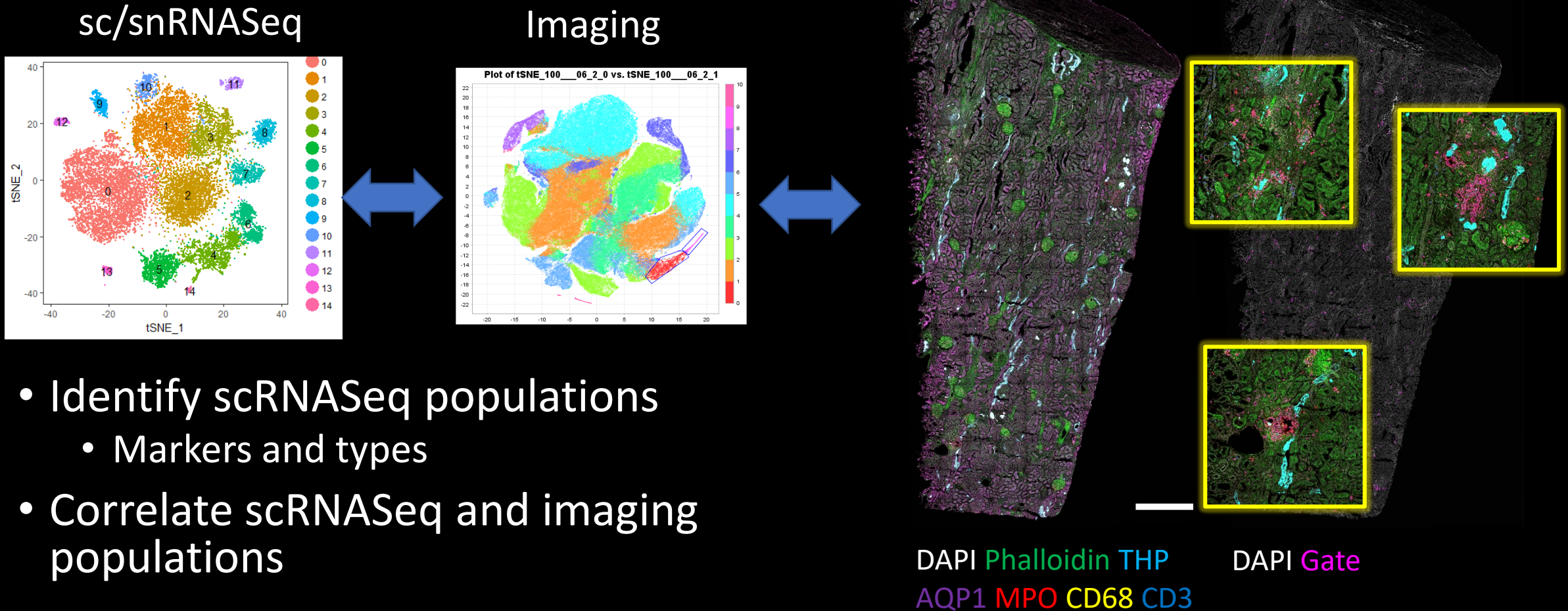
## LMD transcriptomics

GeneX

GeneY

# Localizing -omics

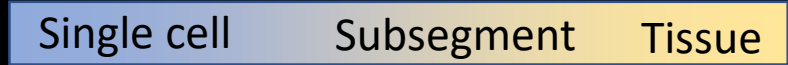
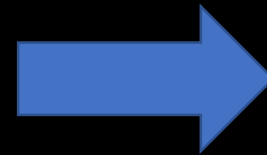
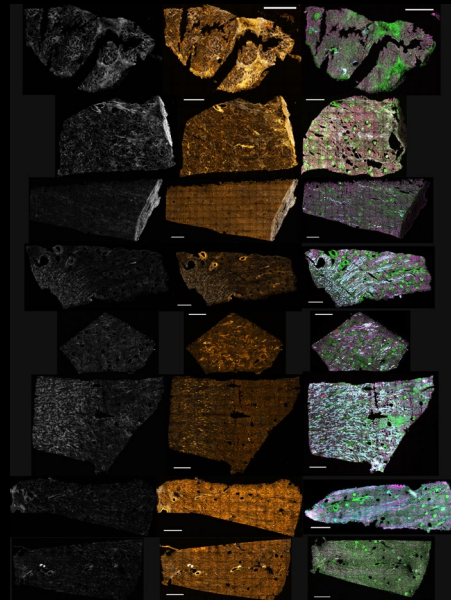
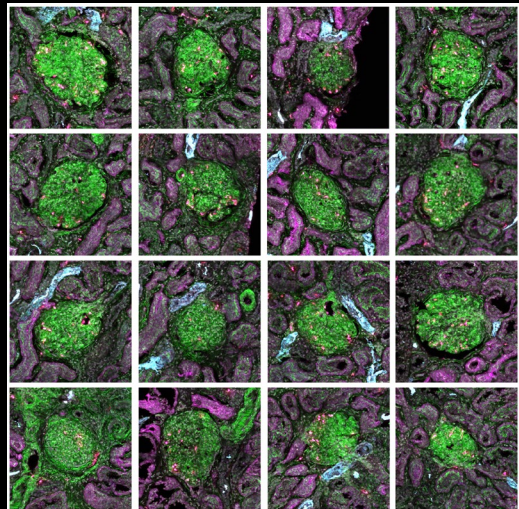
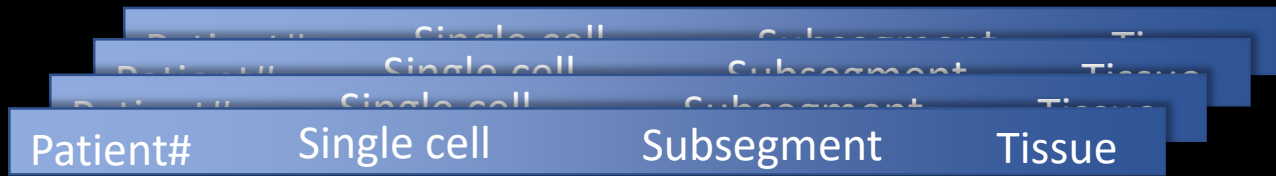
From transcript to 3D image volume



# Making a model...across scale

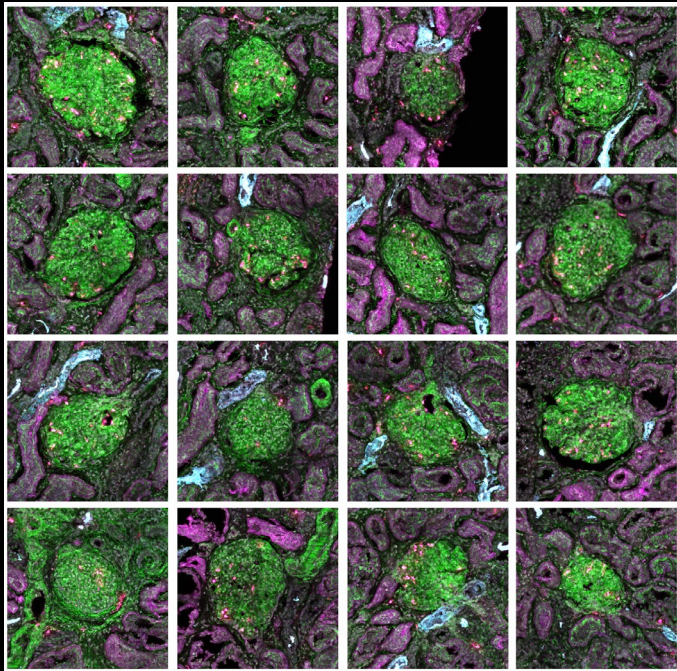
Individual (patients)

Model

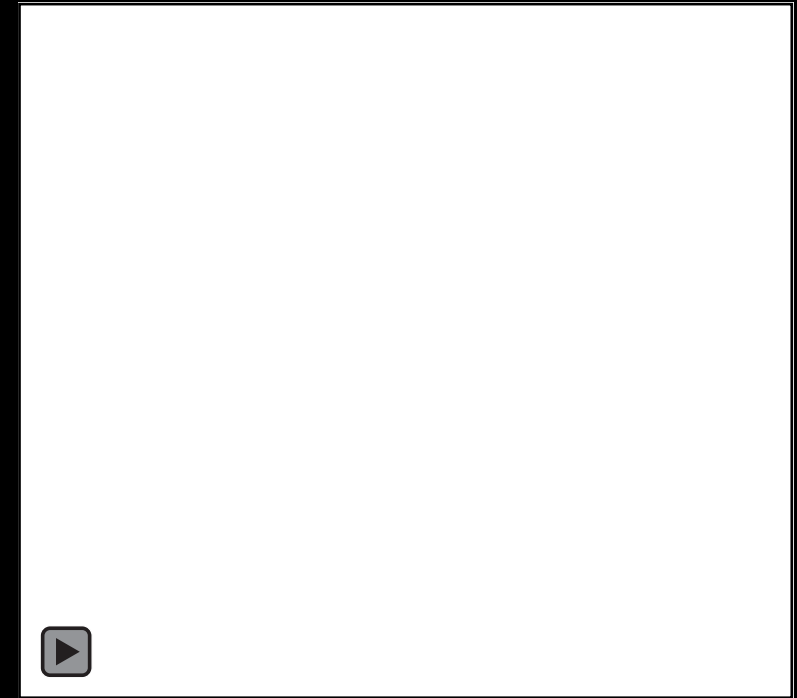


# Modeling the glomerulus

Sections-sparse data

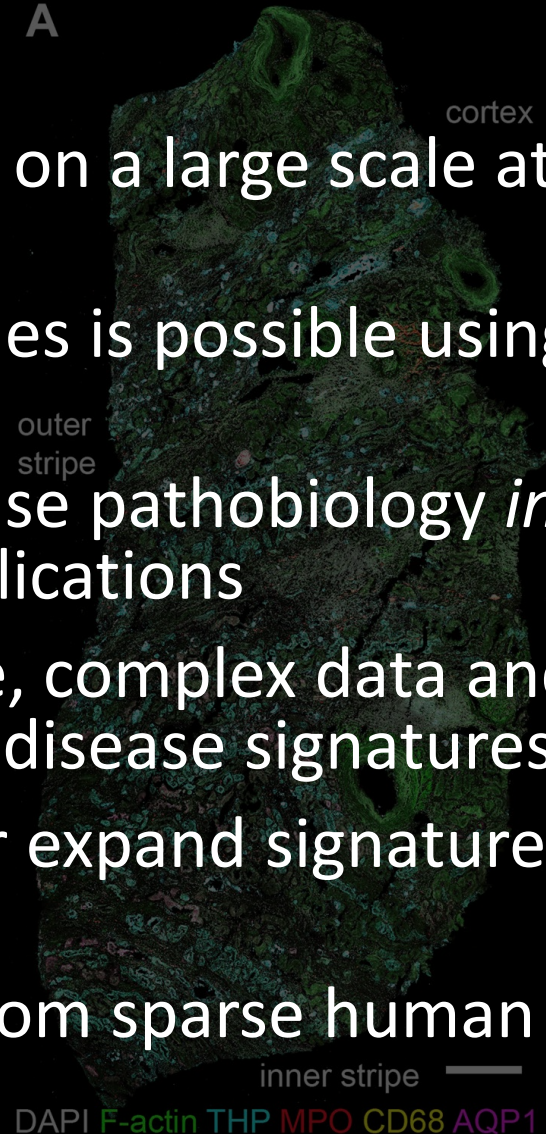


Deep Learning



# Conclusions

- Human kidney biopsies can now be interrogated on a large scale at high resolution using 3D fluorescence imaging
- Efficient quantitative analyses on large 3D volumes is possible using tools such as VTEA
- VTEA analysis can link 3D image analysis to disease pathobiology *in situ*, and can be used for precision medicine applications
- Multivariate analyses has potential to mine large, complex data and provide an unbiased approach to discover novel disease signatures.
- Integrating imaging data with –omics will further expand signature of disease state
- A model “average” kidney may be determined from sparse human biopsy data



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