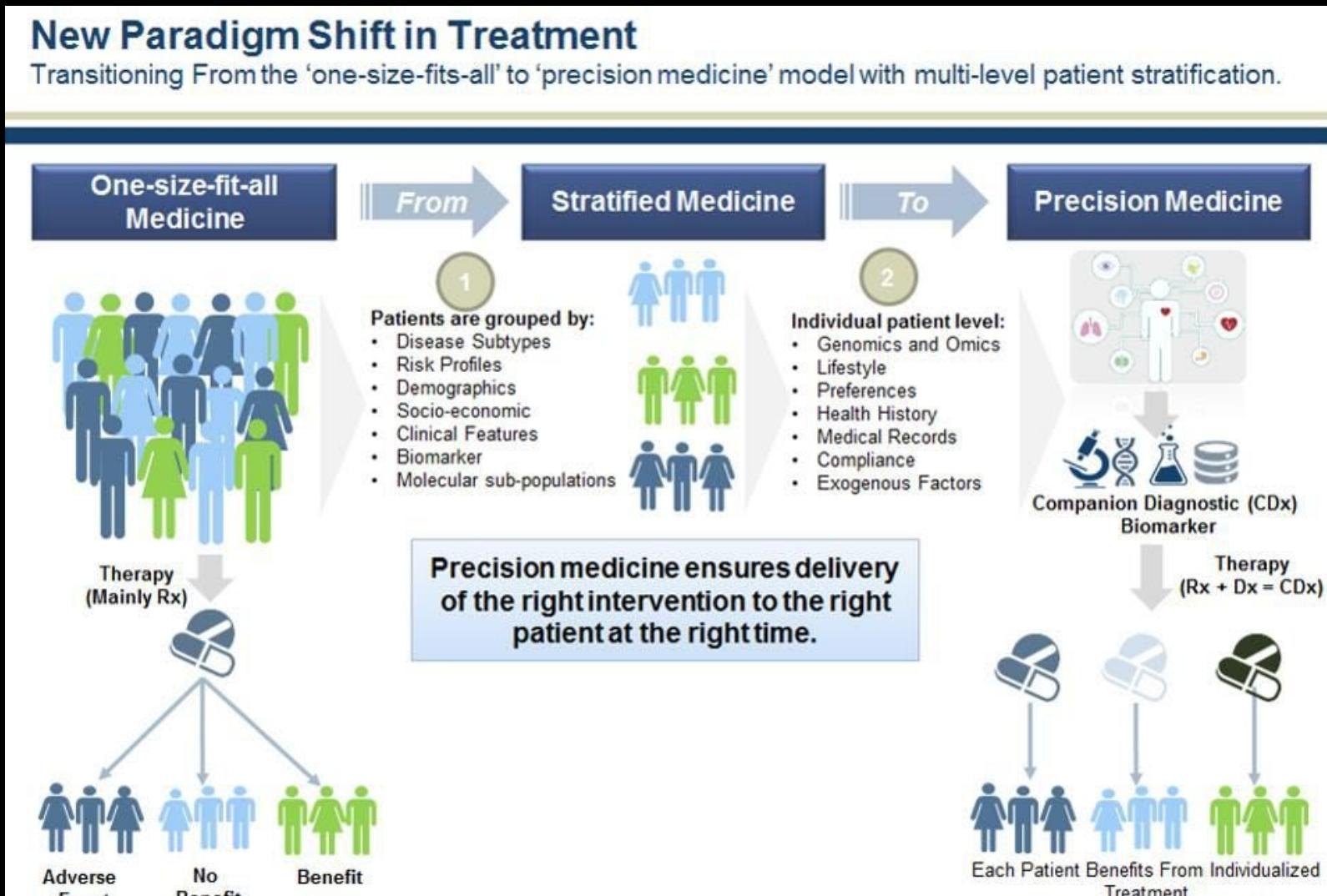


Bi-directional Interrogation of Image Volumes and Segmented Cells With 3D Tissue Cytometry

Seth Winfree and Tarek Ashkar

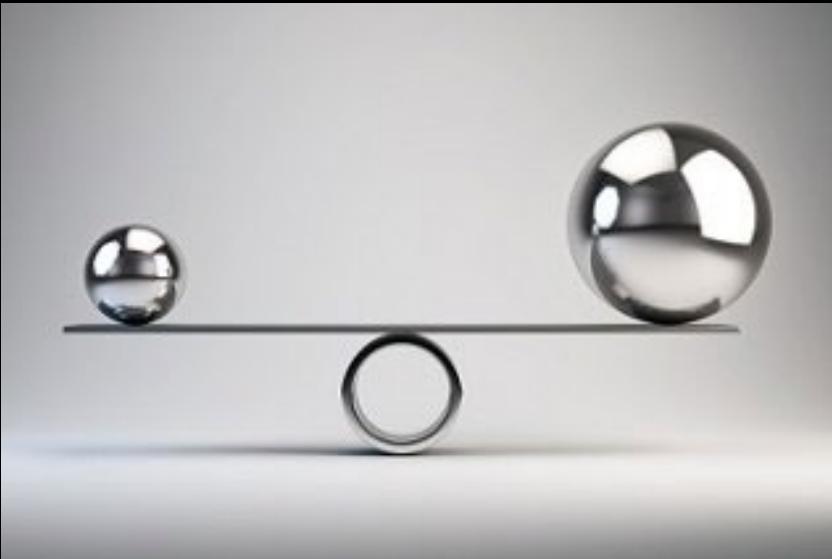
Indiana University School of Medicine, Medicine, Division of Nephrology
IU/OSU Tissue Interrogation Site, KPMP

Era of precision medicine



“Small” changes can have “big” effect

“Small” changes can cause “big” effects

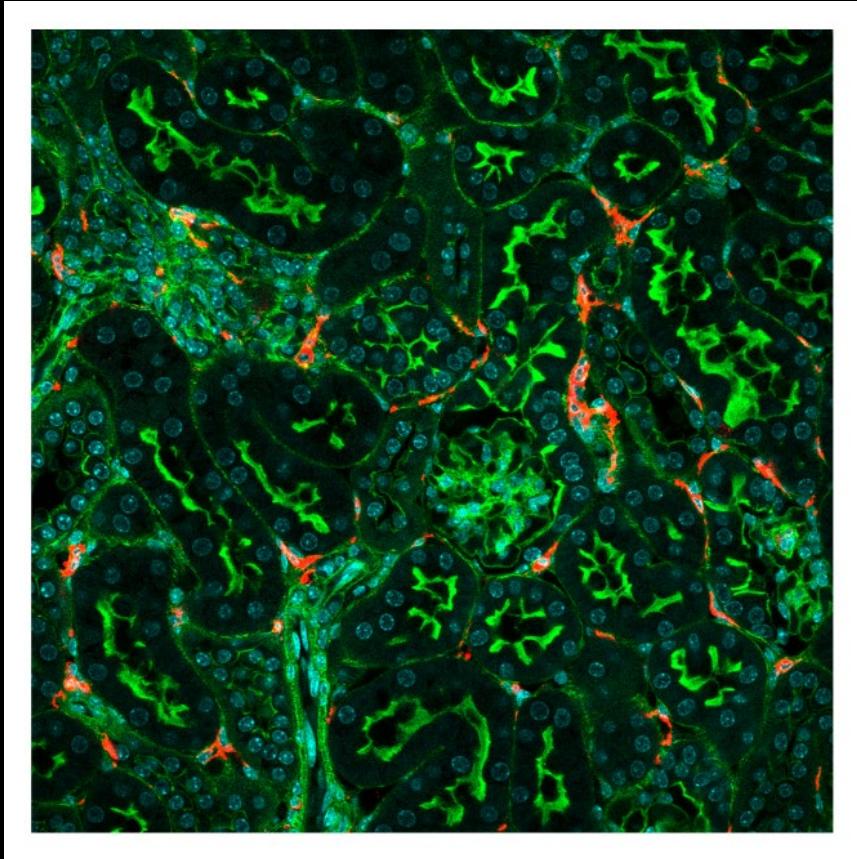


Changes can be a needle in a haystack

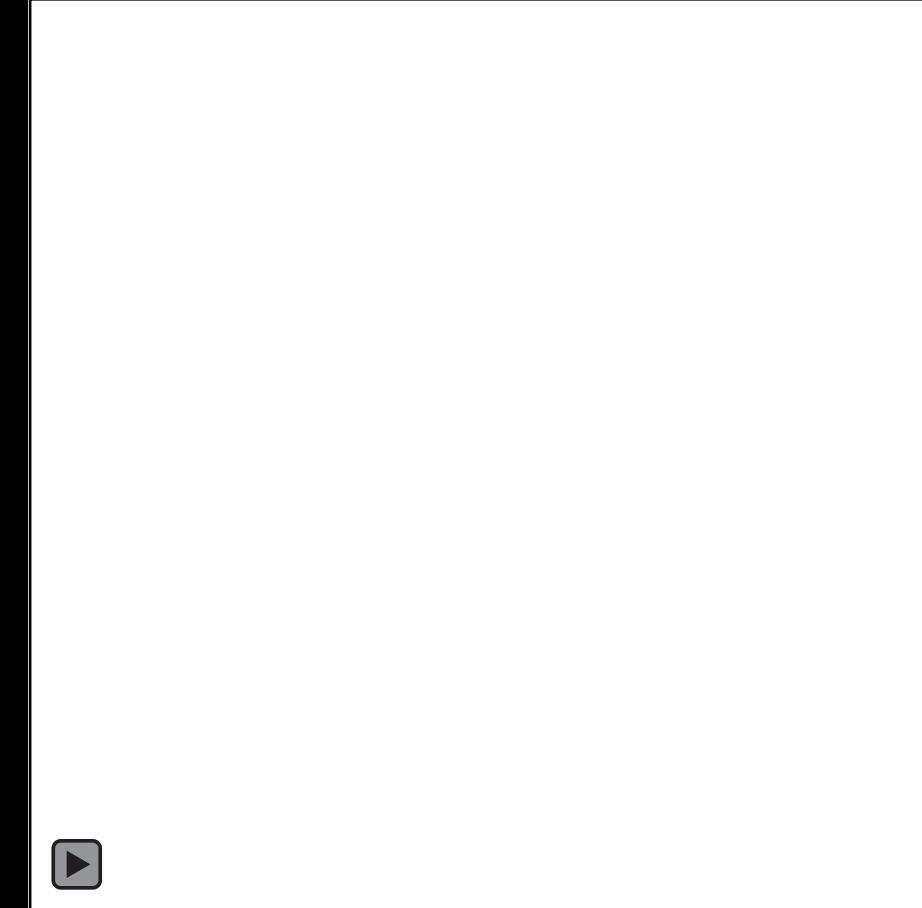


- High resolution methods with high sensitivity
- Ability to analyze multiparametric datasets
- Look for small changes unique for patient and for disease

Why 3D imaging? Tissue complexity



DAPI MHCII F-Actin

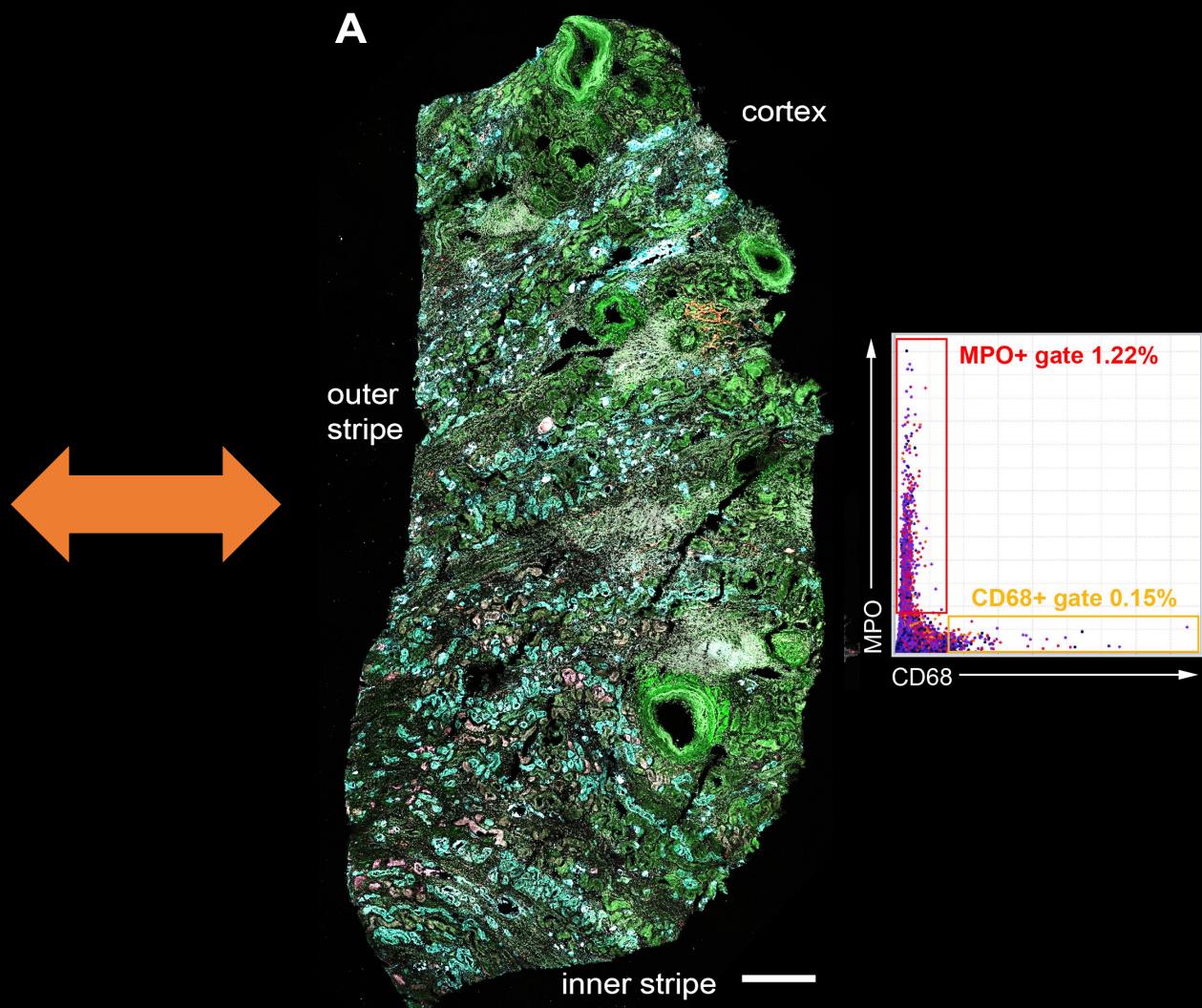
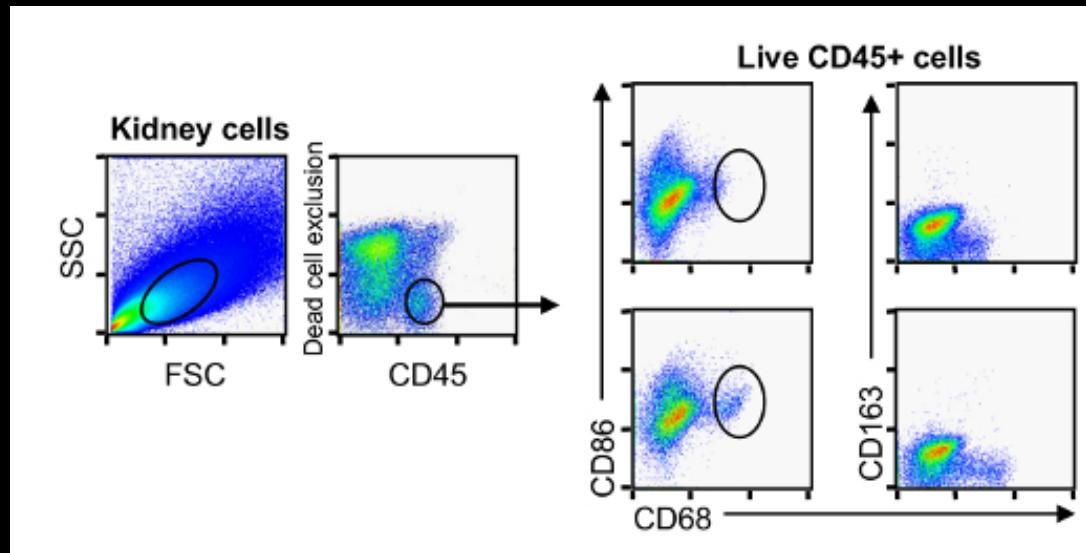
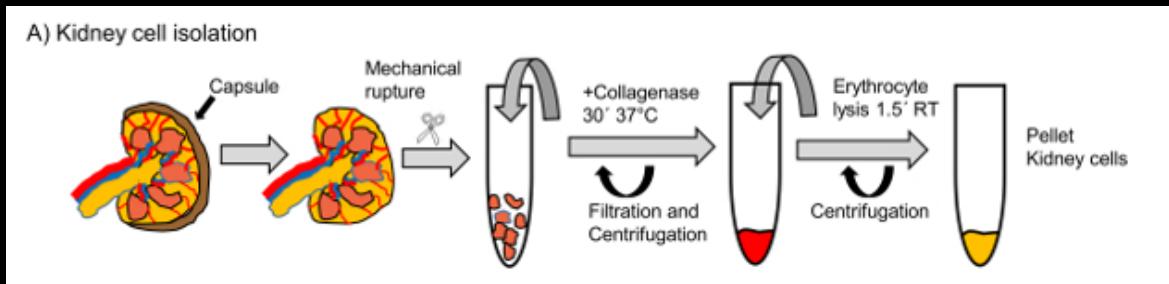


MHCII CD11C F-Actin

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

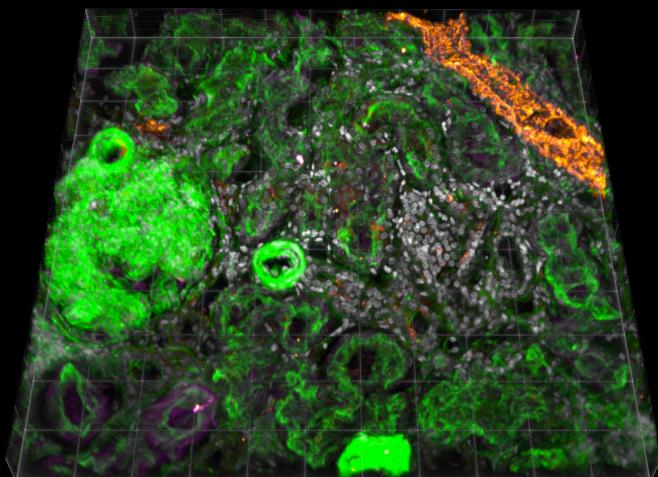
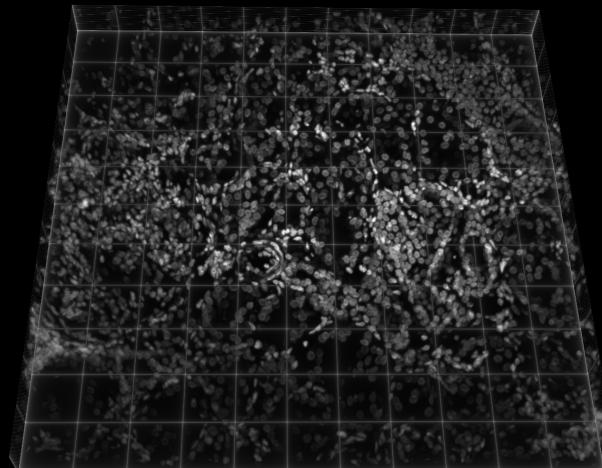
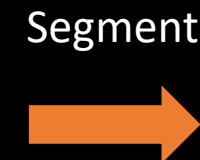


Image data in 3D



Nuclei



Single cells segmented in 3D

3D cytometry-single cell analysis in 3D



Single cells segmented in 3D

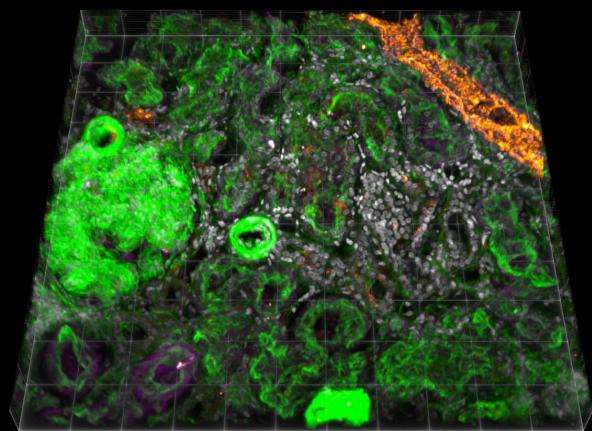
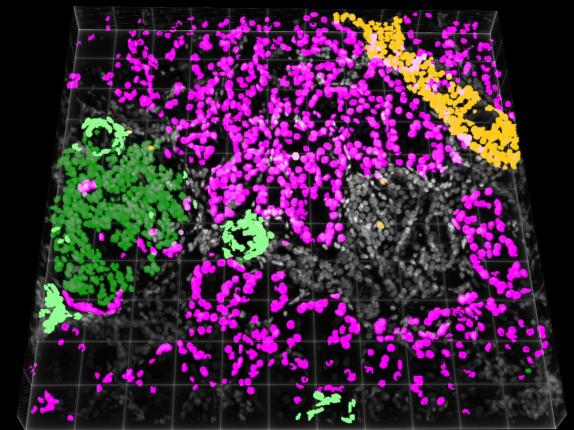
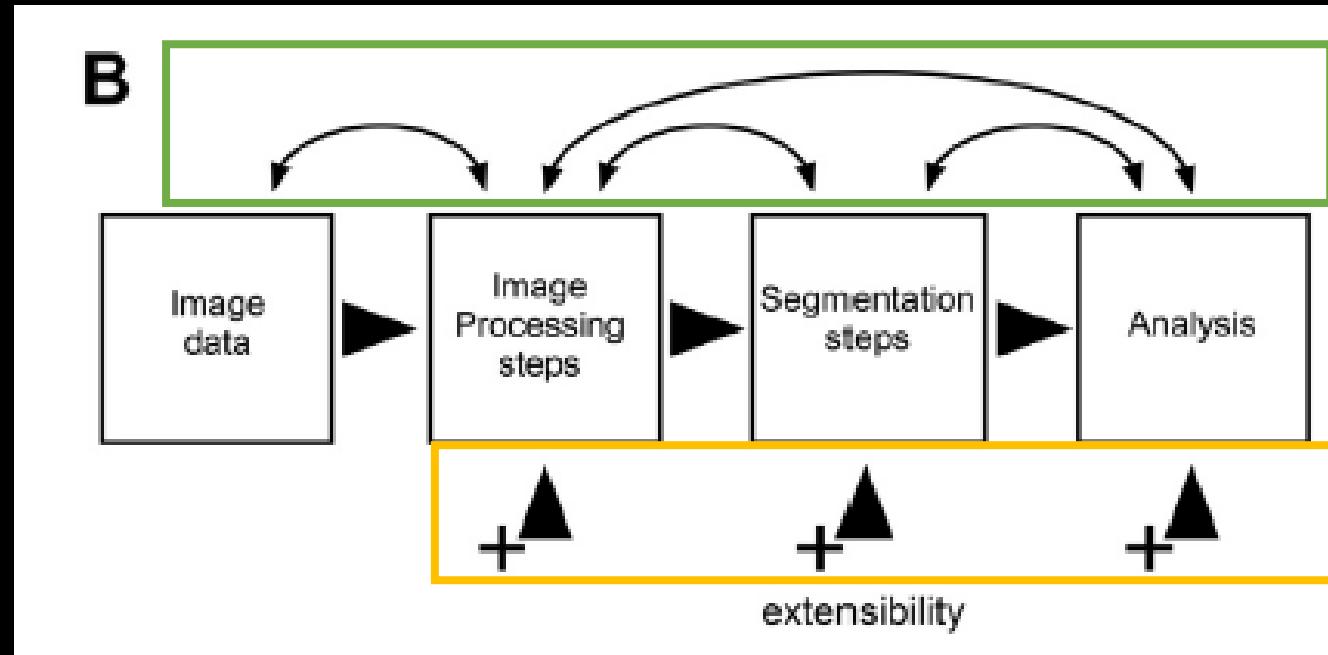


Image data in 3D



Classify and count

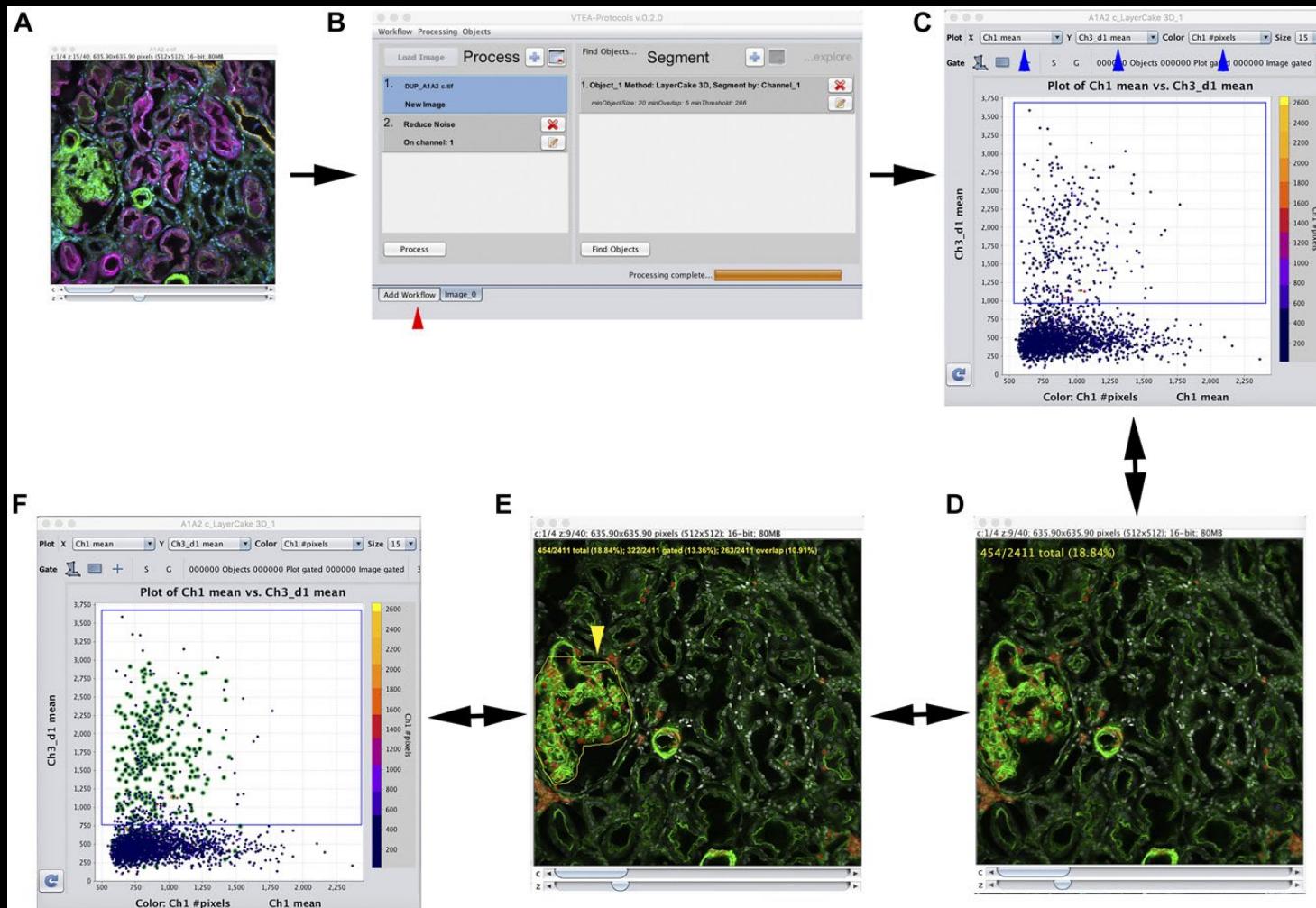
Designing an approach for tissue cytometry



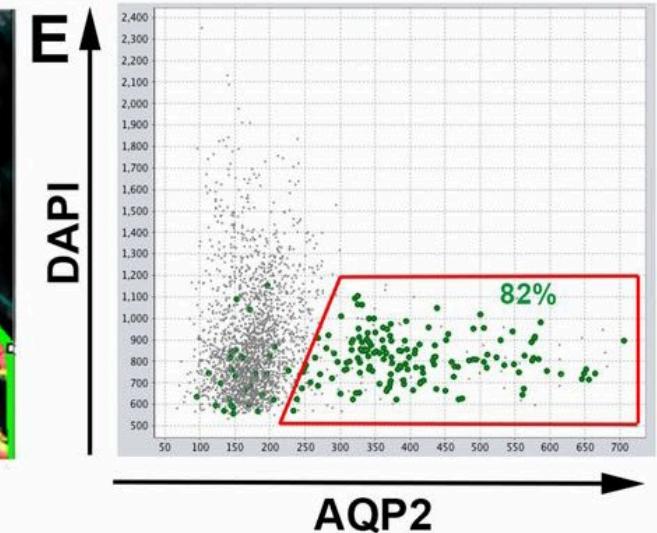
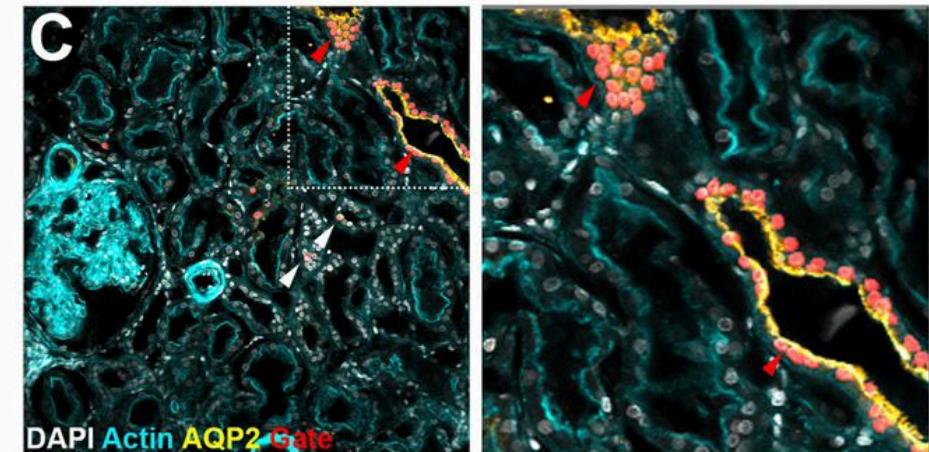
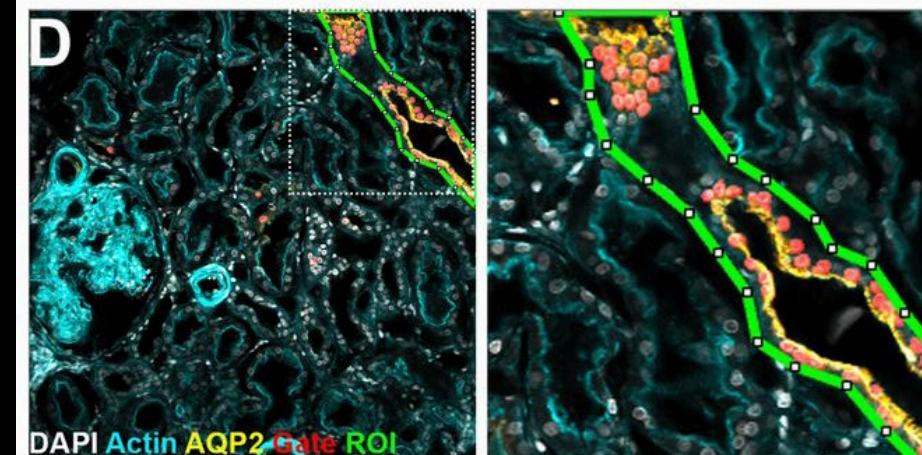
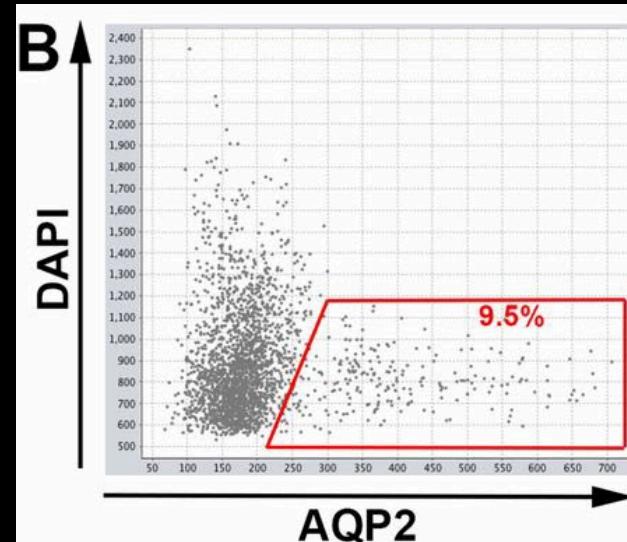
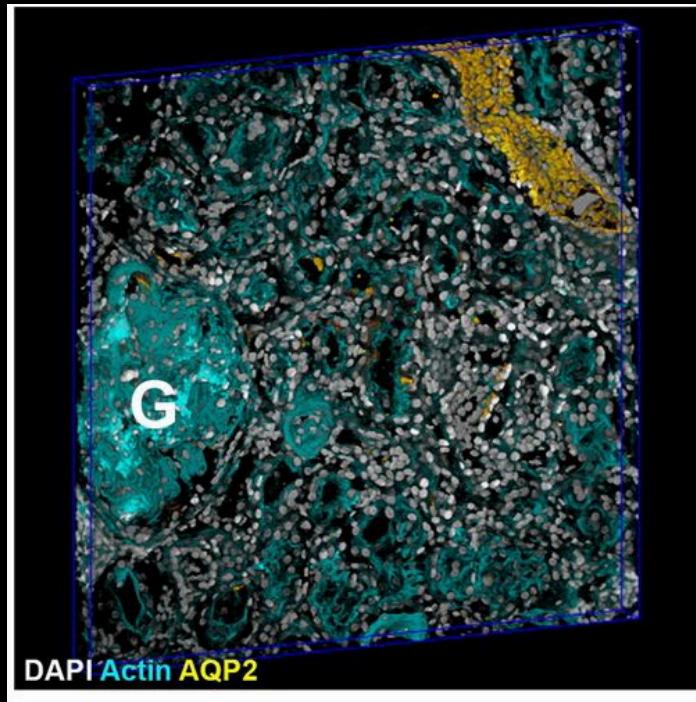
Tool use

Development and growth

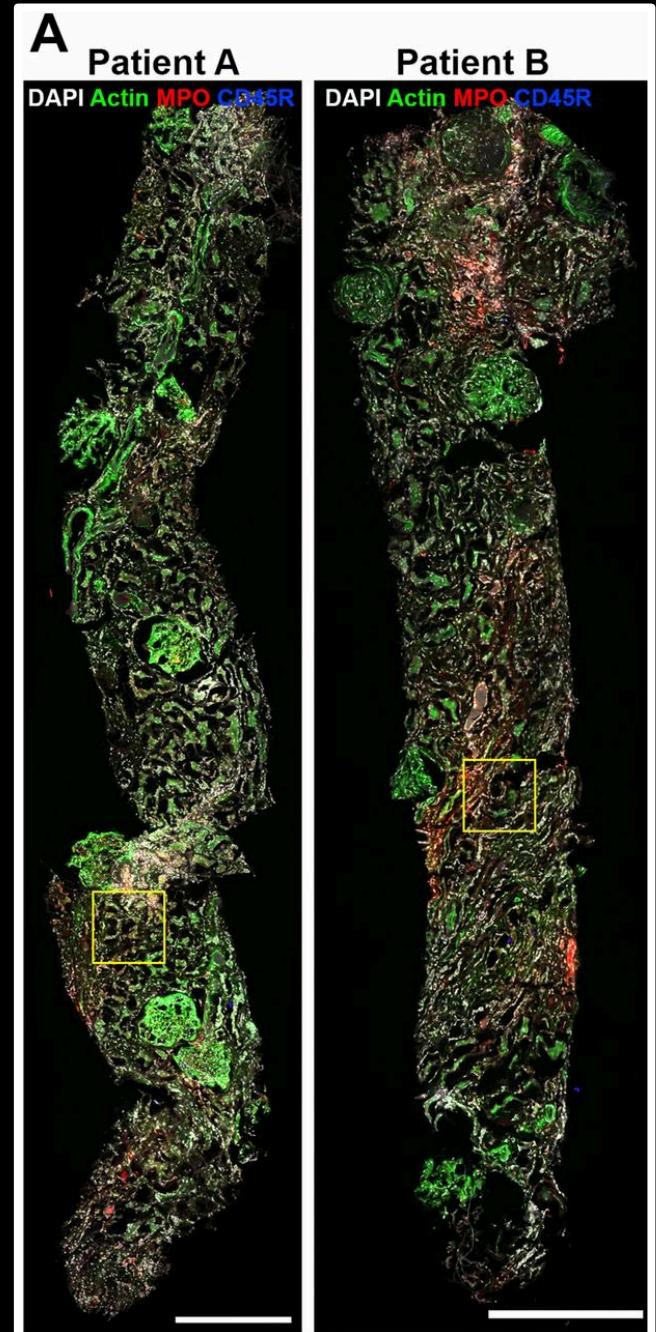
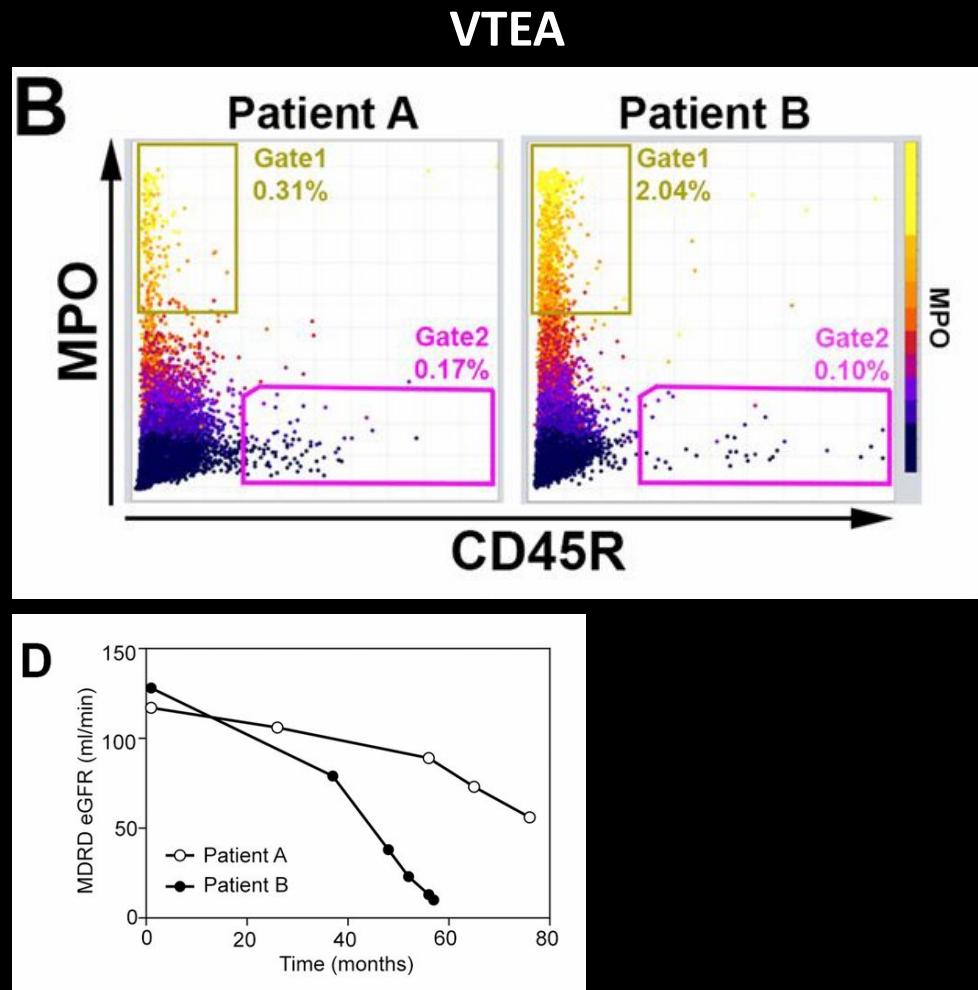
Volumetric Tissue Exploration and Analysis: VTEA



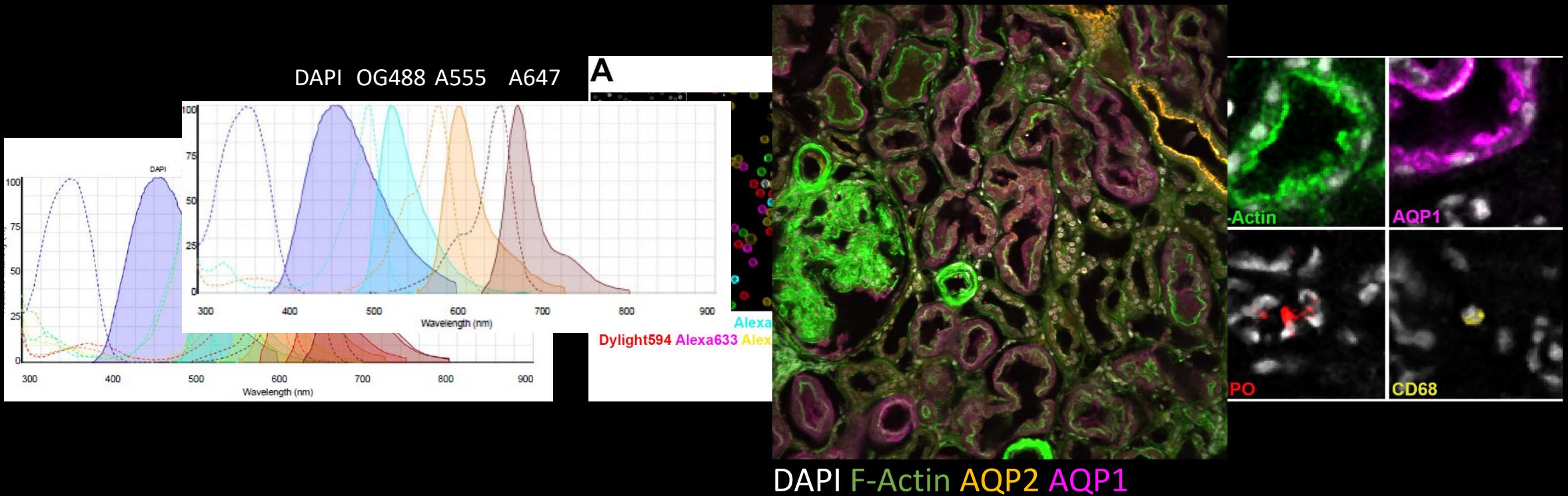
3D tissue cytometry using VTEA



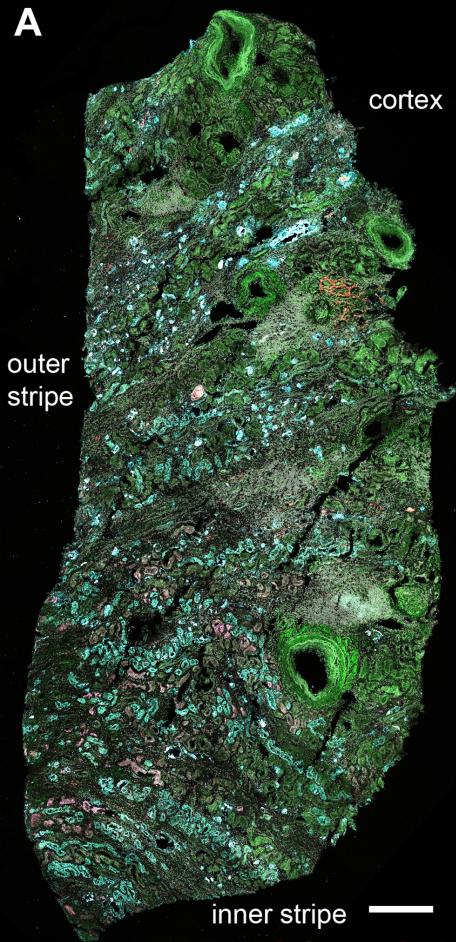
Application to human biopsies in a clinical setting



Marker complexity structures and cells of interest



Multivariate results

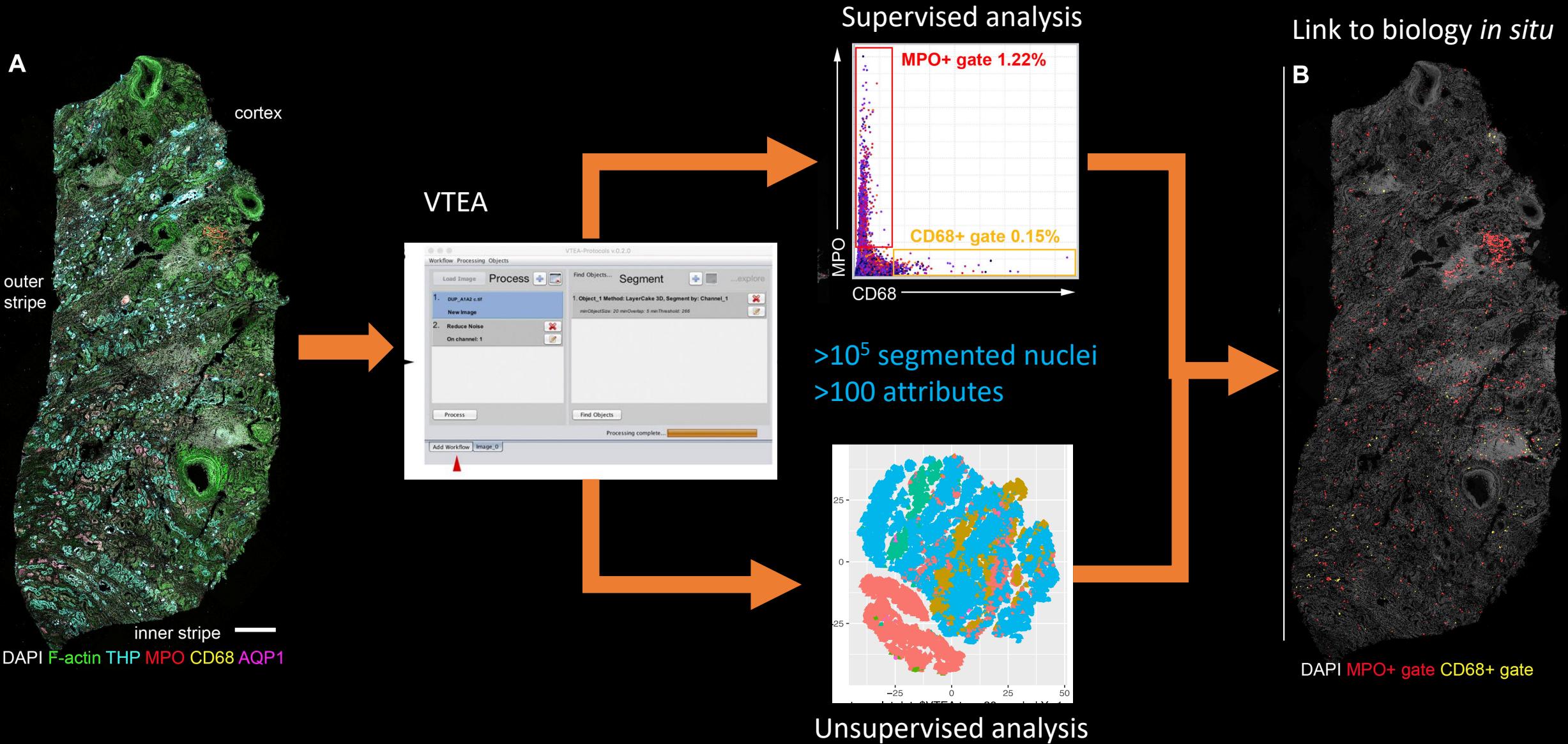


DAPI F-actin THP MPO CD68 AQP1

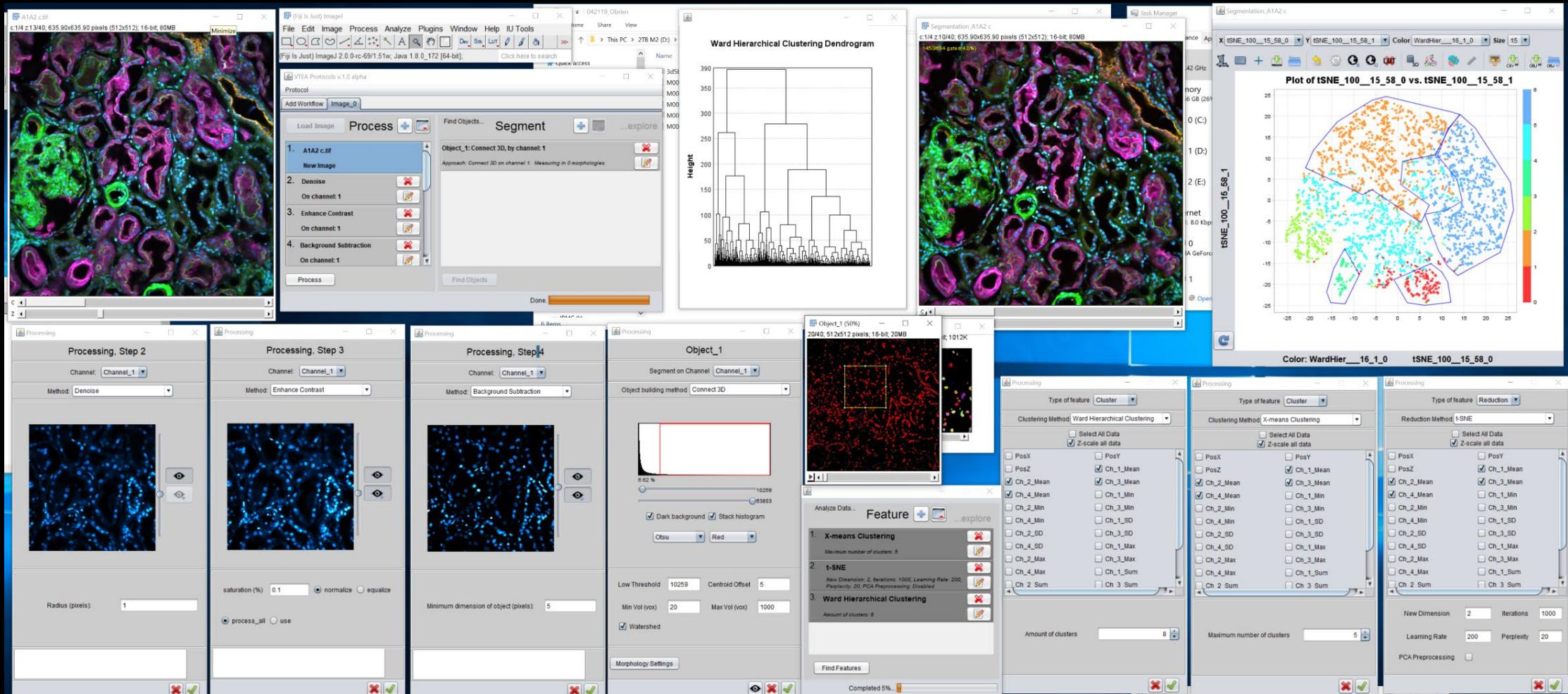
Human nephrectomy section

Quantitative Three-Dimensional Tissue Cytometry to Study Kidney Tissue and Resident Immune Cells. Winfree S et al. J Am Soc Nephrol. 2017 Jul;28(7):2108-2118.

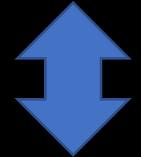
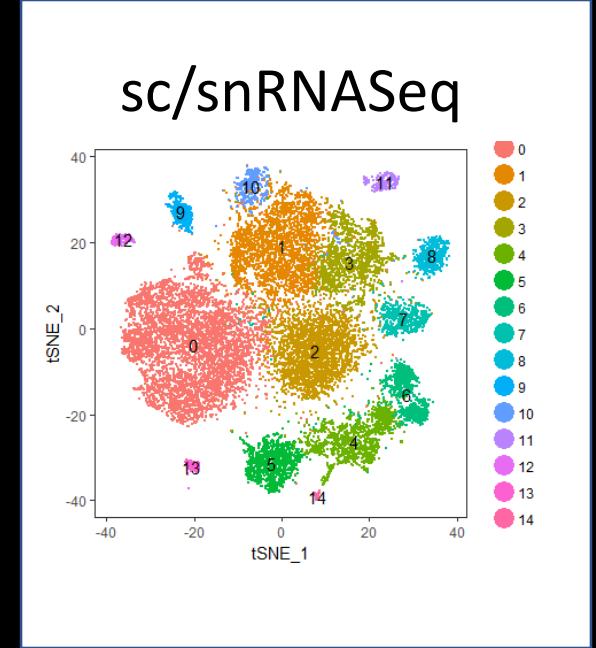
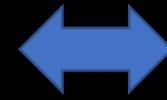
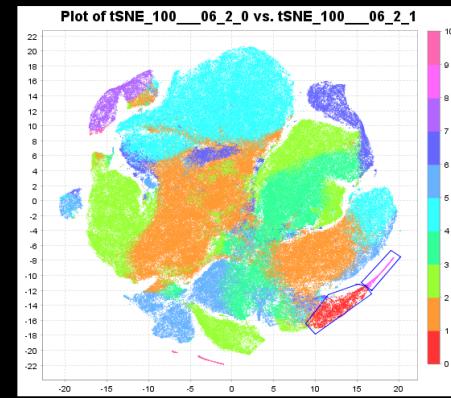
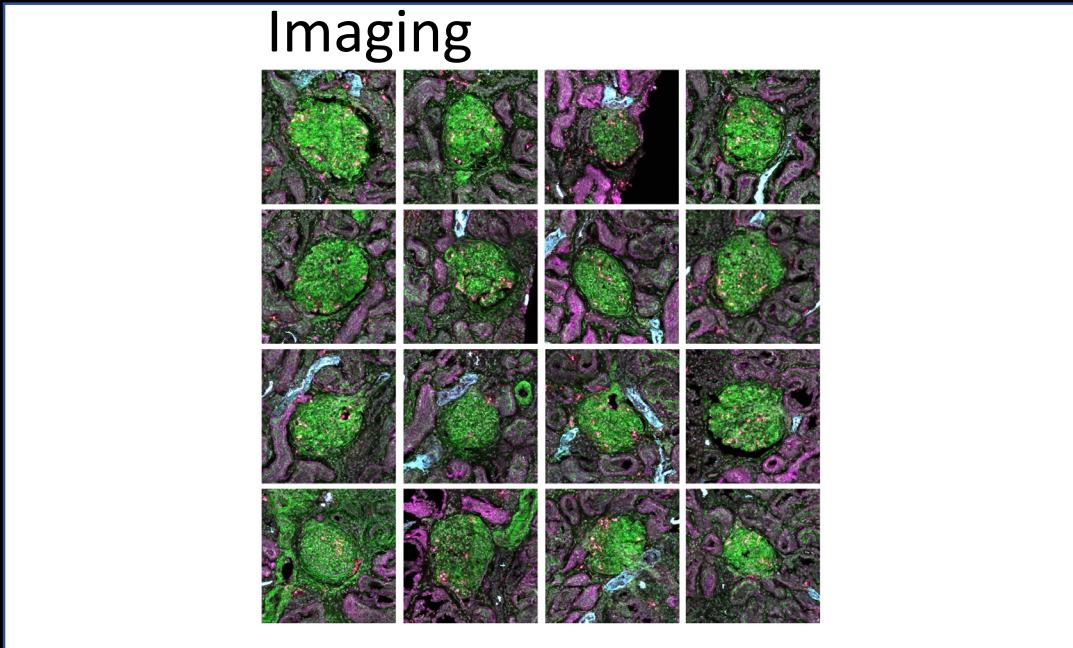
Tissue “Big” data exploration, analysis and interpretation in one workflow



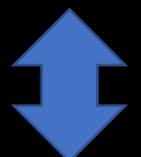
VTEA in use



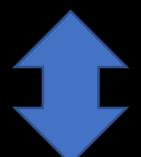
Signatures across approaches and modalities



Proteomics

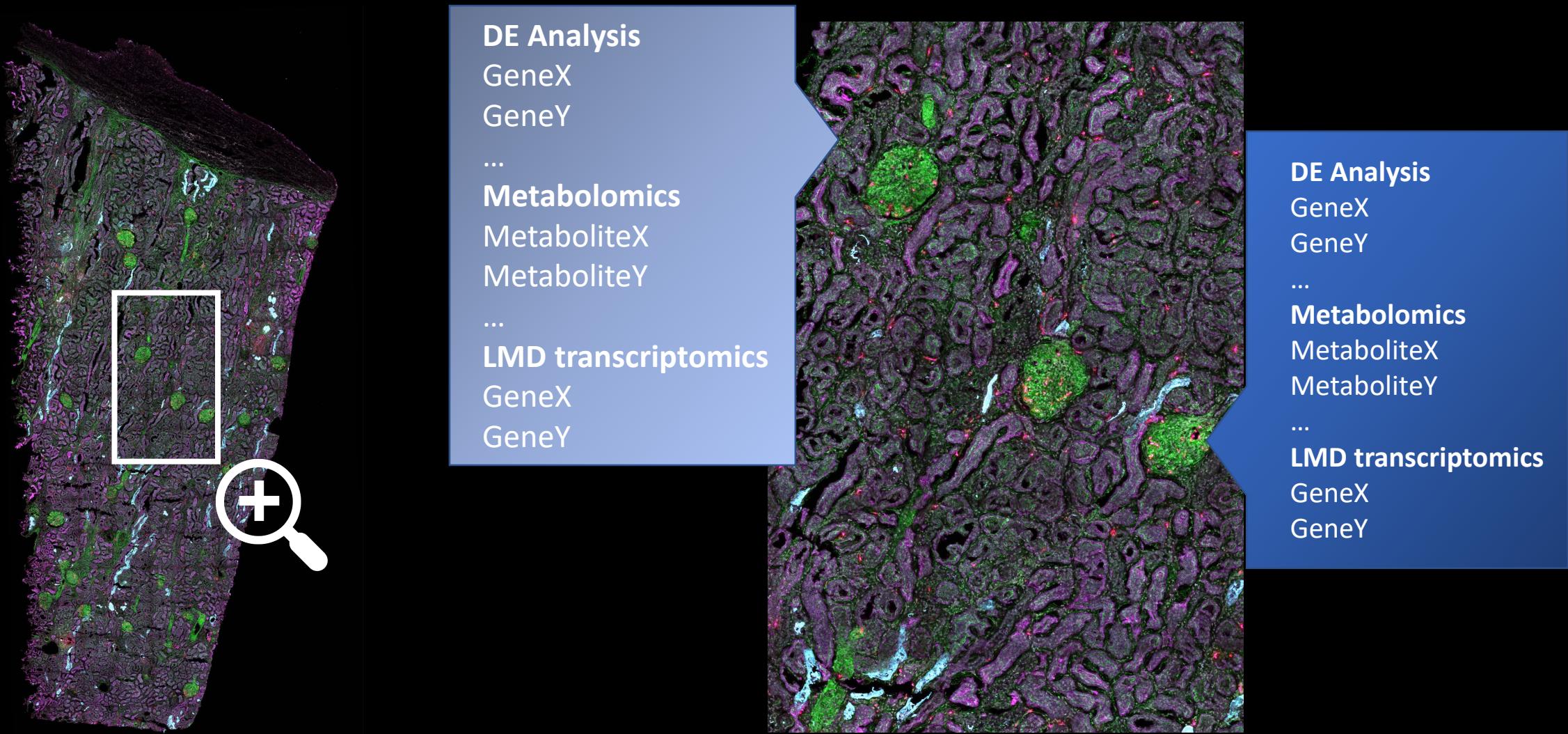


LMD (omics)



Metabolomics

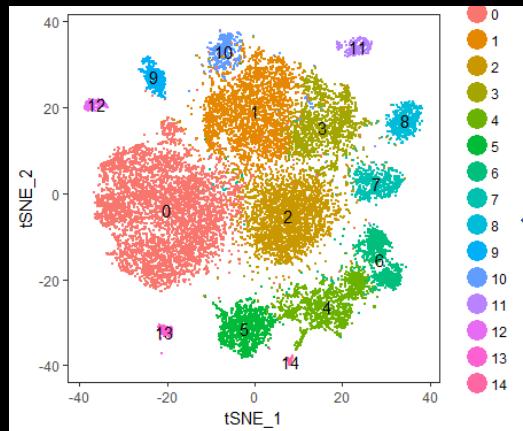
Popup Query From 3D image volume to omics



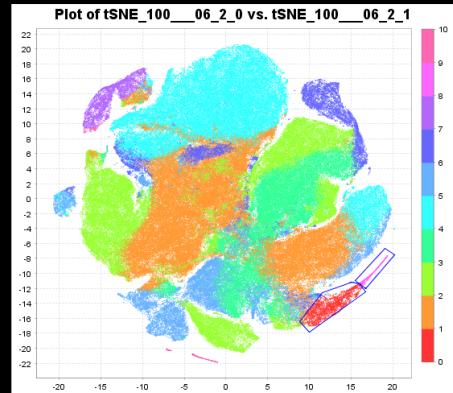
Localizing -omics

From transcript to 3D image volume

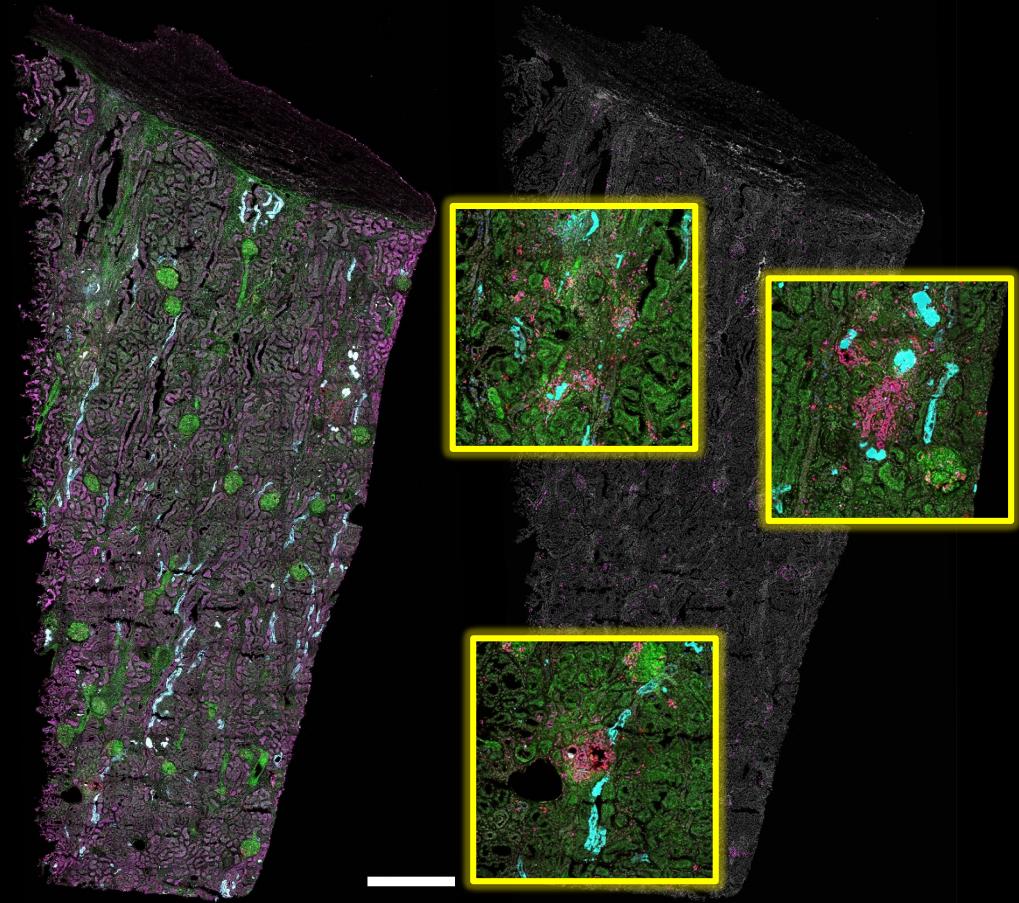
sc/snRNASeq



Imaging



- Identify scRNASeq populations
 - Markers and types
- Correlate scRNASeq and imaging populations



DAPI Phalloidin THP
AQP1 MPO CD68 CD3

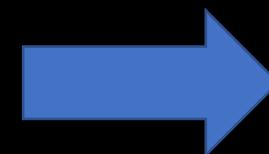
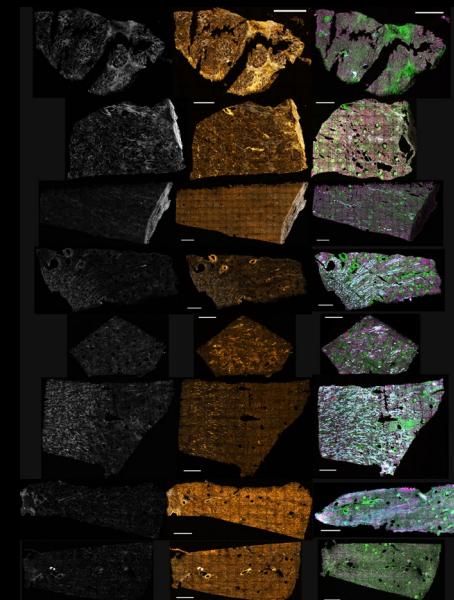
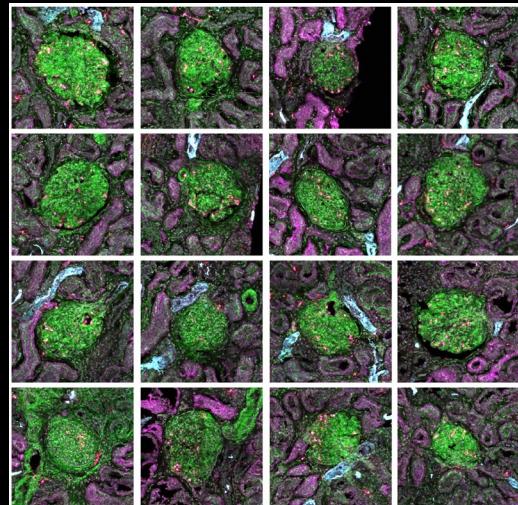
DAPI Gate

Making a model...across scale

Individual (patients)

Model

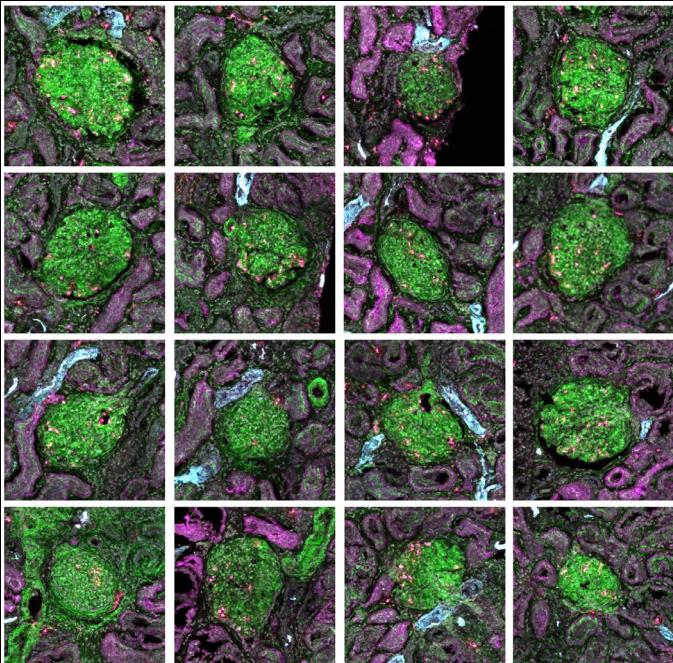
| Patient# | Single cell | Subsegment | Tissue |
|-----------|-------------|------------|--------|
| Patient 1 | Single cell | Subsegment | Tissue |
| Patient 2 | Single cell | Subsegment | Tissue |
| Patient 3 | Single cell | Subsegment | Tissue |



Single cell Subsegment Tissue

Modeling the glomerulus

Sections-sparse data

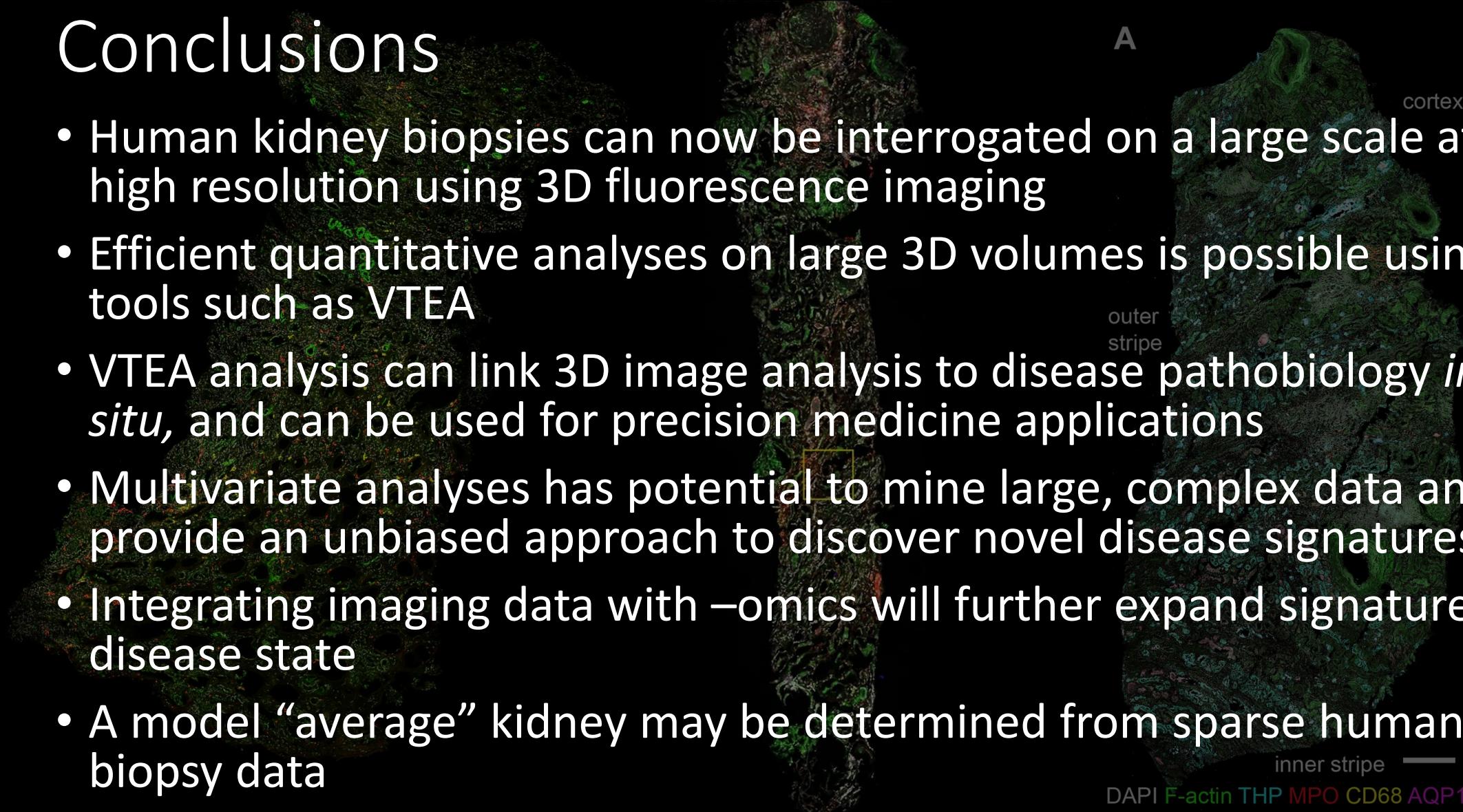


Deep Learning



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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