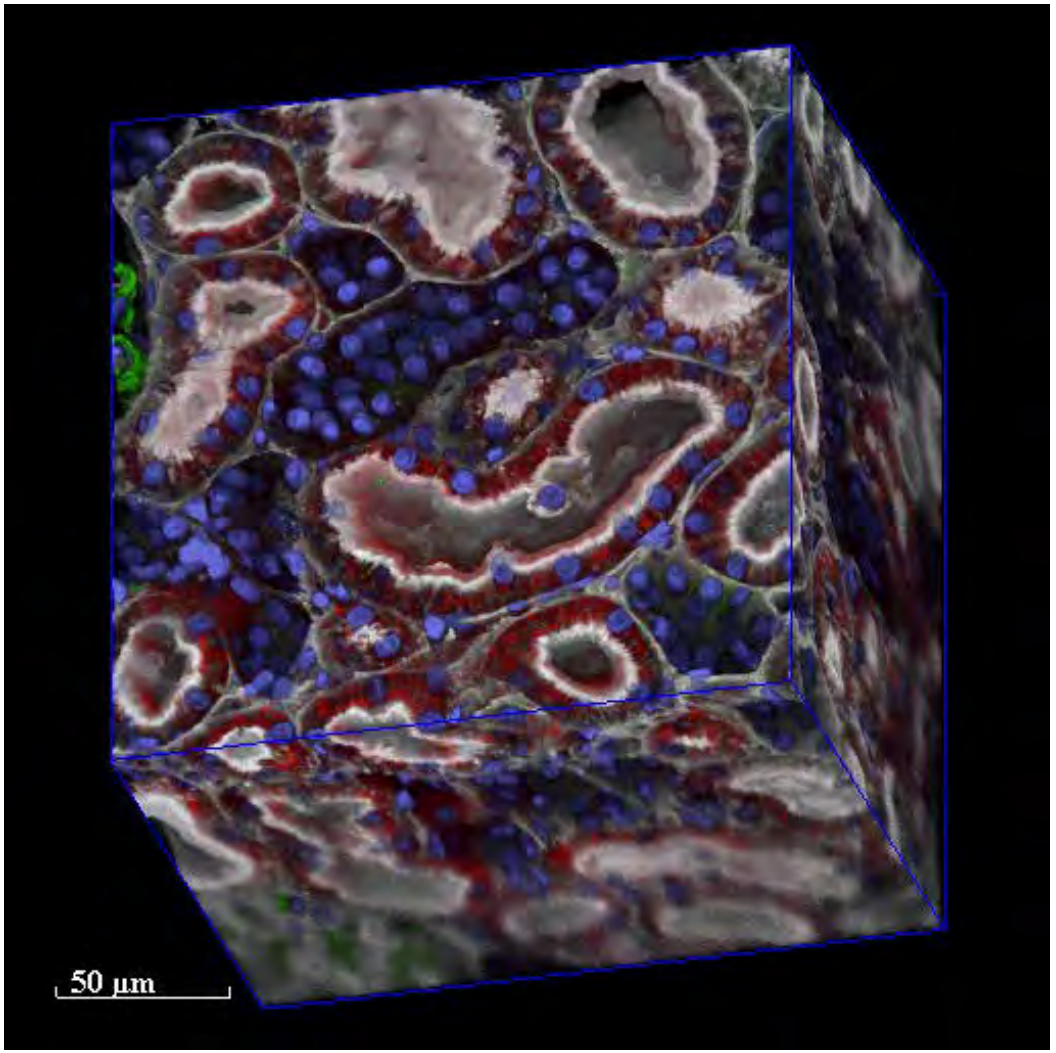
# Large Scale 3D imaging and Analysis

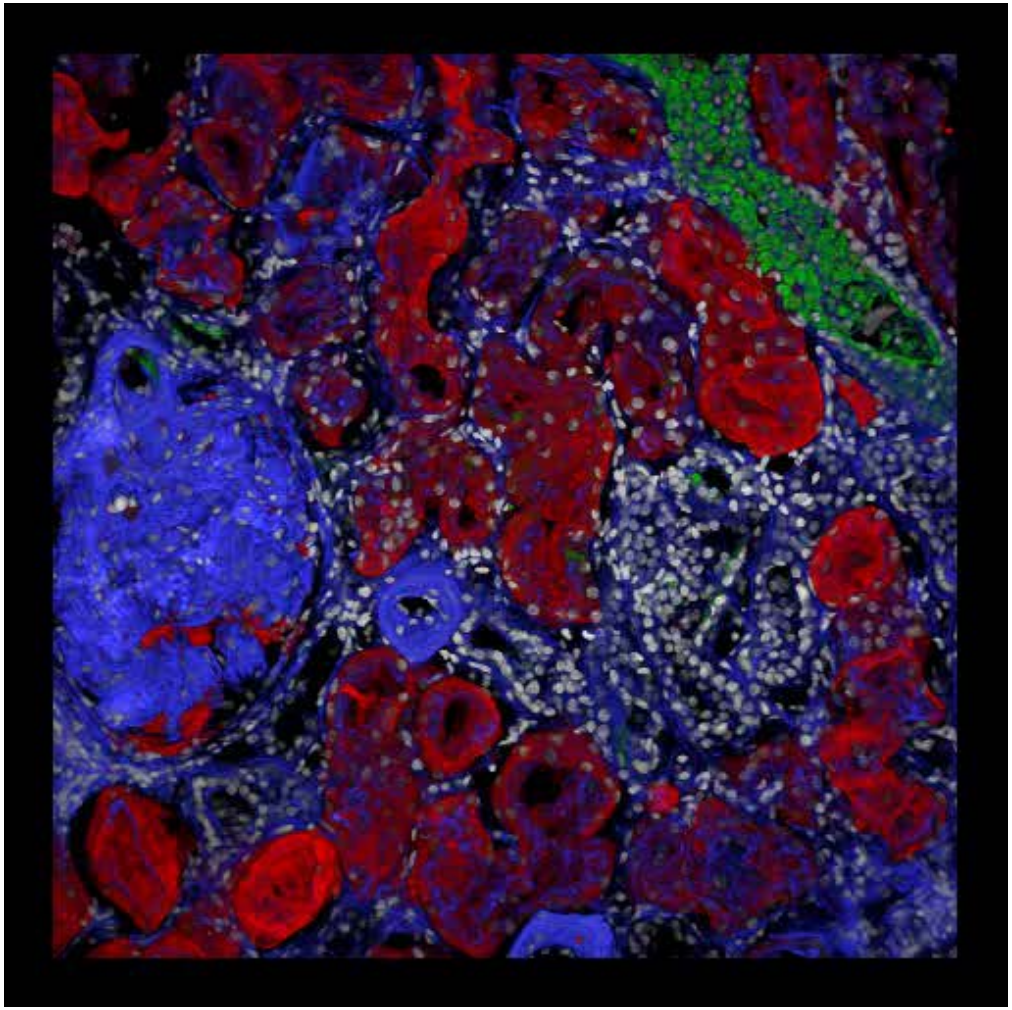
Tarek M. Ashkar (El-Achkar), MD

# 3D imaging captures entire structures

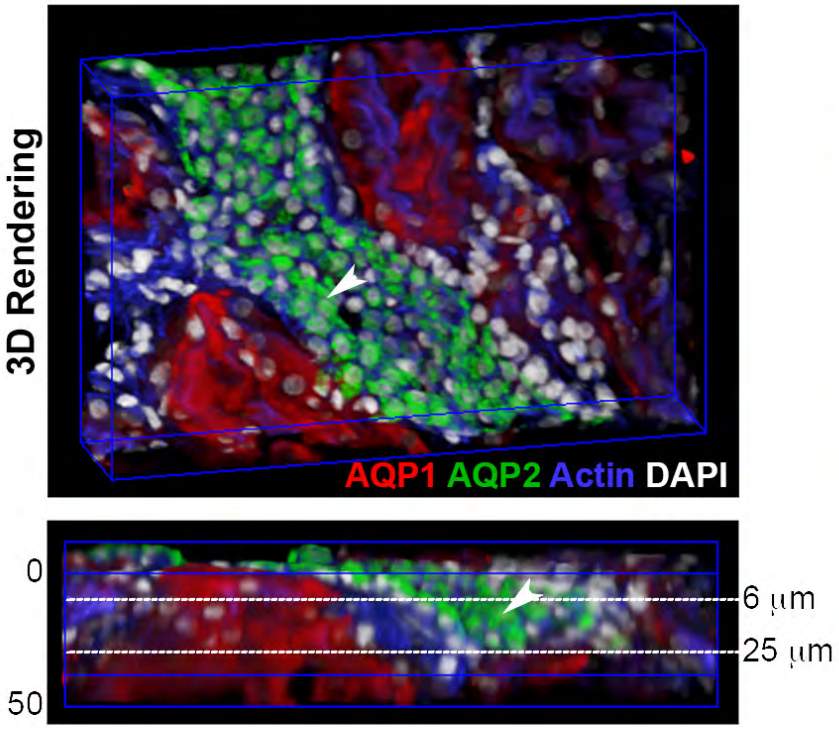


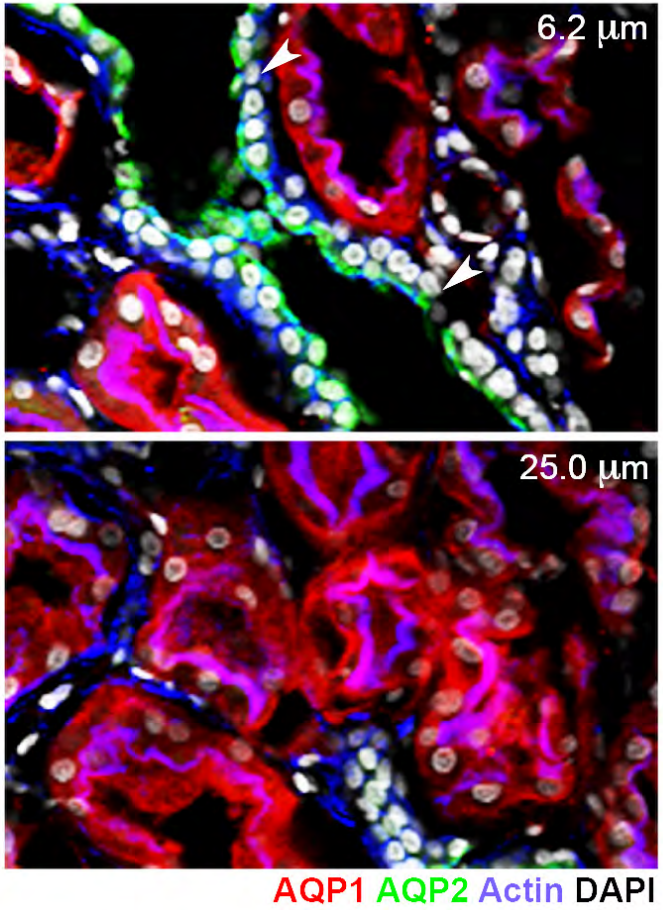

Clendenon, Ferkowicz, Young, and Dunn. 2011. Deep Tissue Fluorescent Imaging in Scattering Specimens Using Confocal Microscopy. Microscopy and Microanalysis.

# 3D imaging captures entire structures

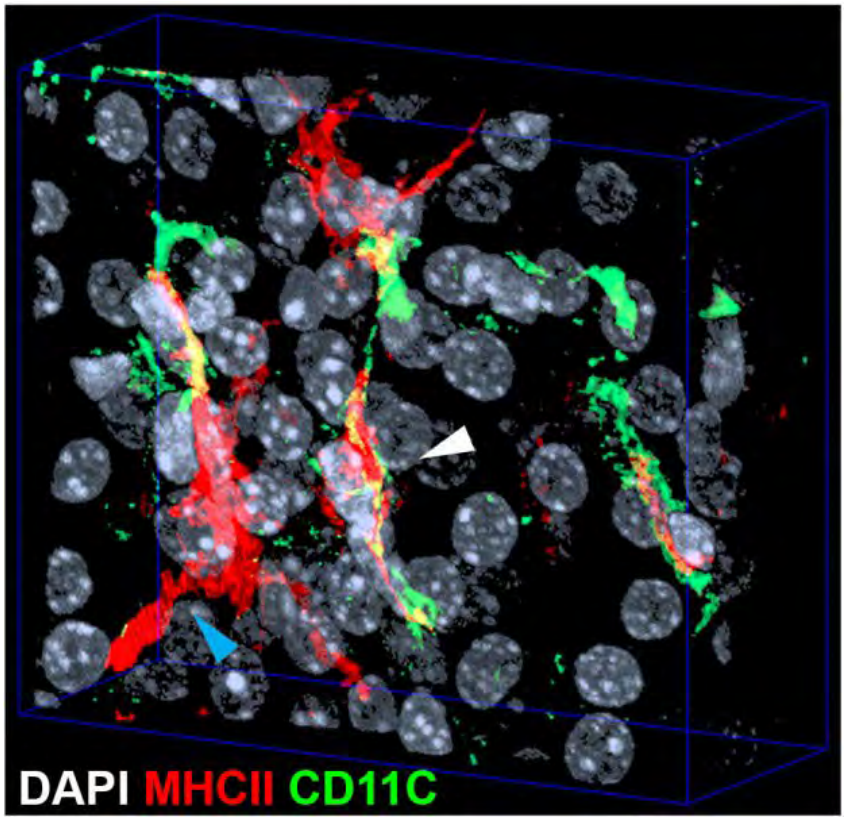


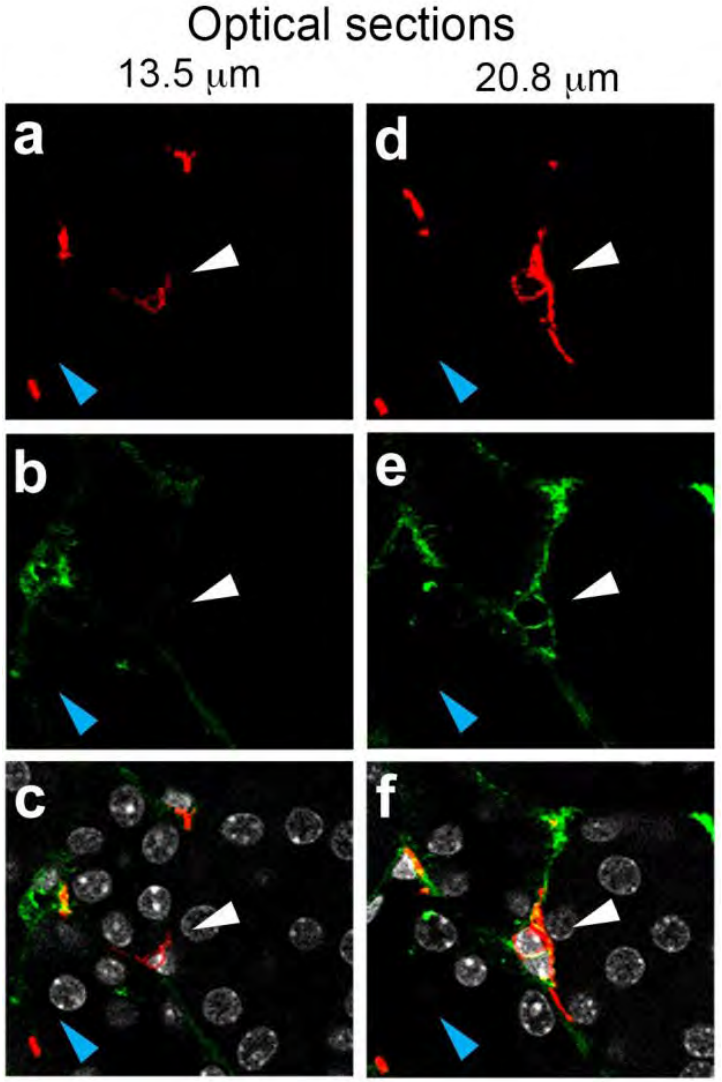
# 3D imaging captures entire structures



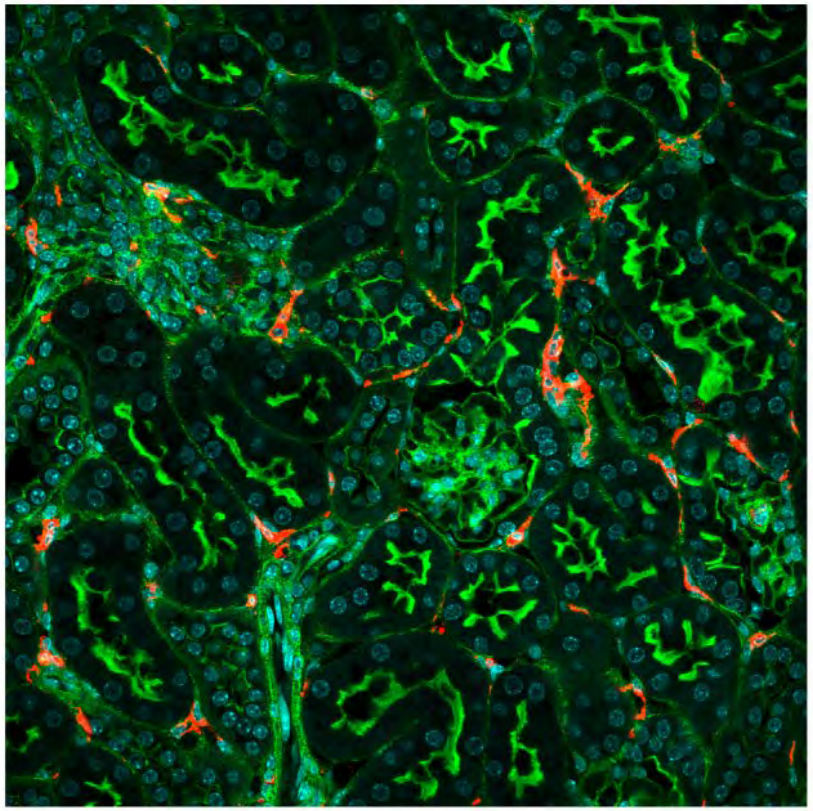



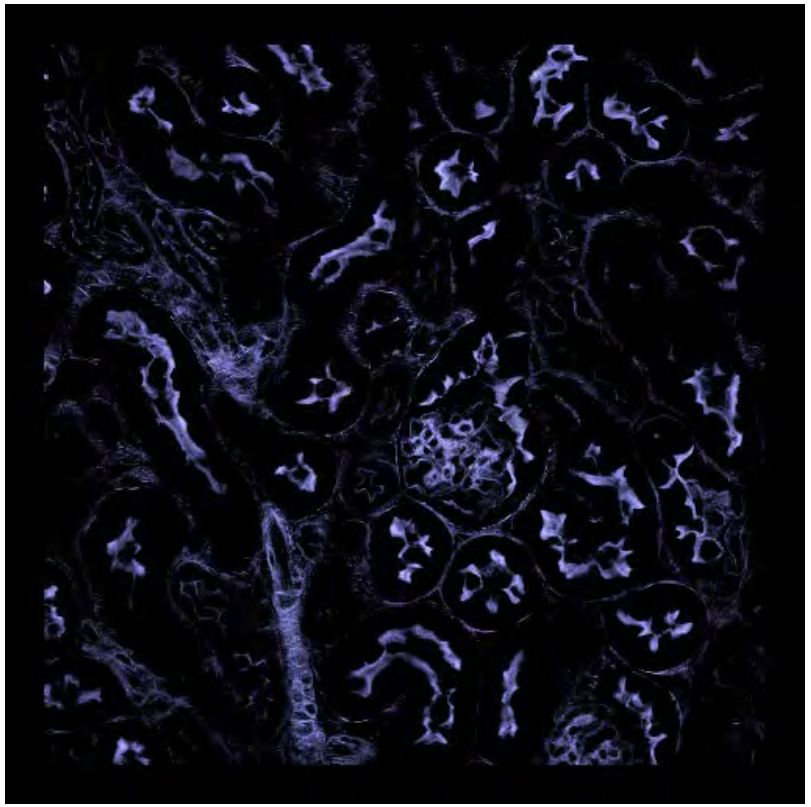
# 3D imaging improves data accuracy



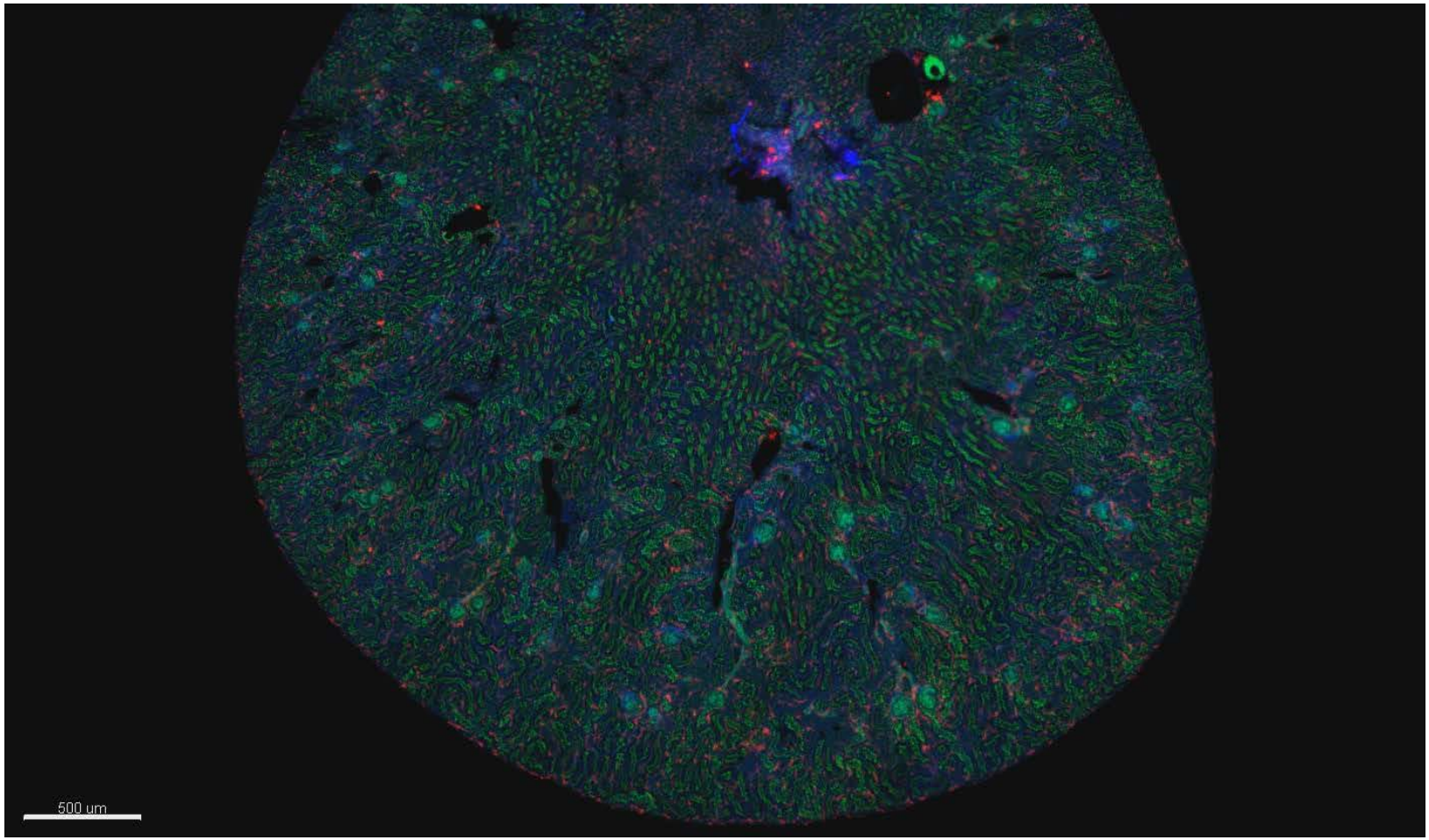



# 3D imaging uncovers interactions between cells

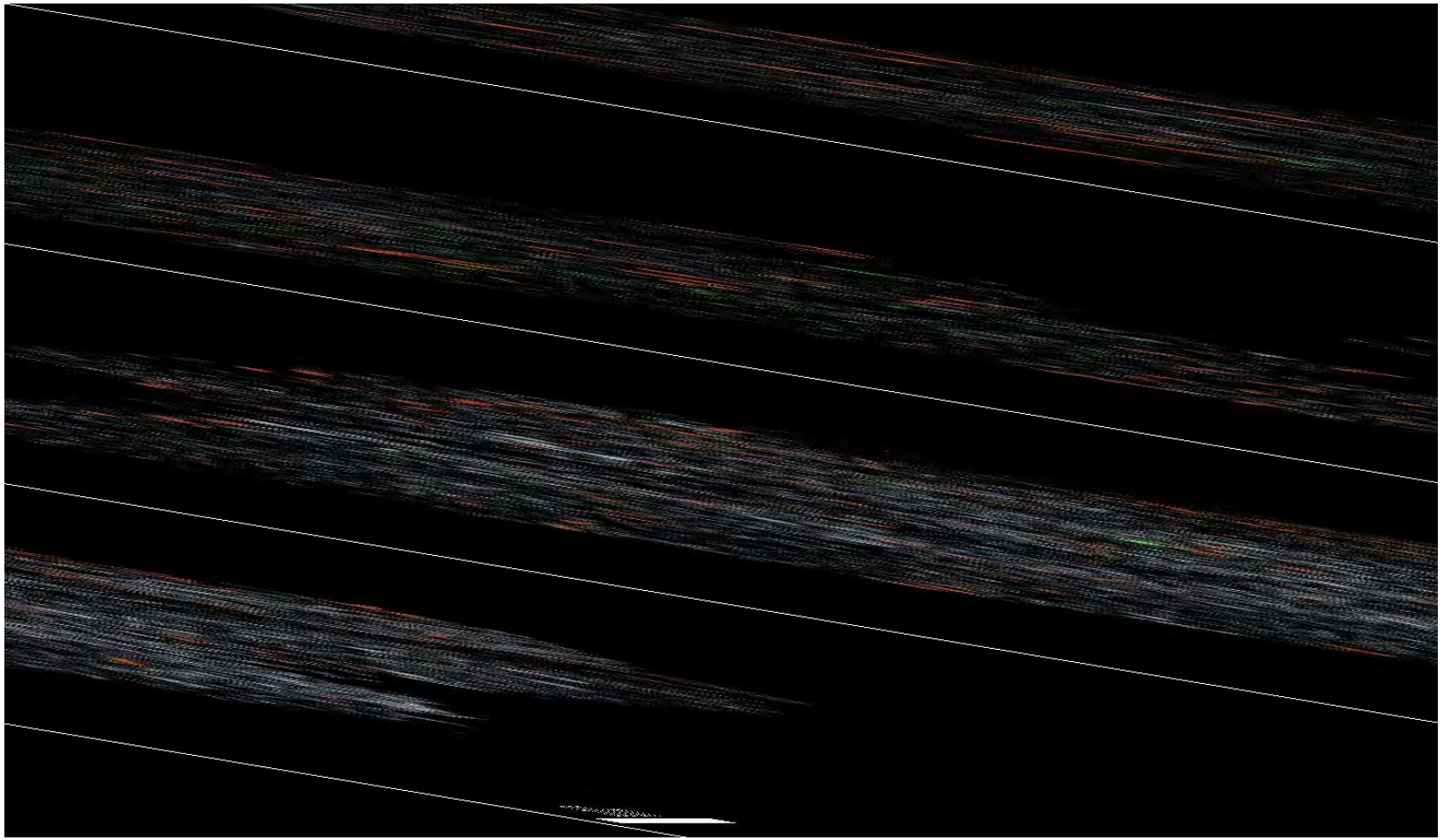




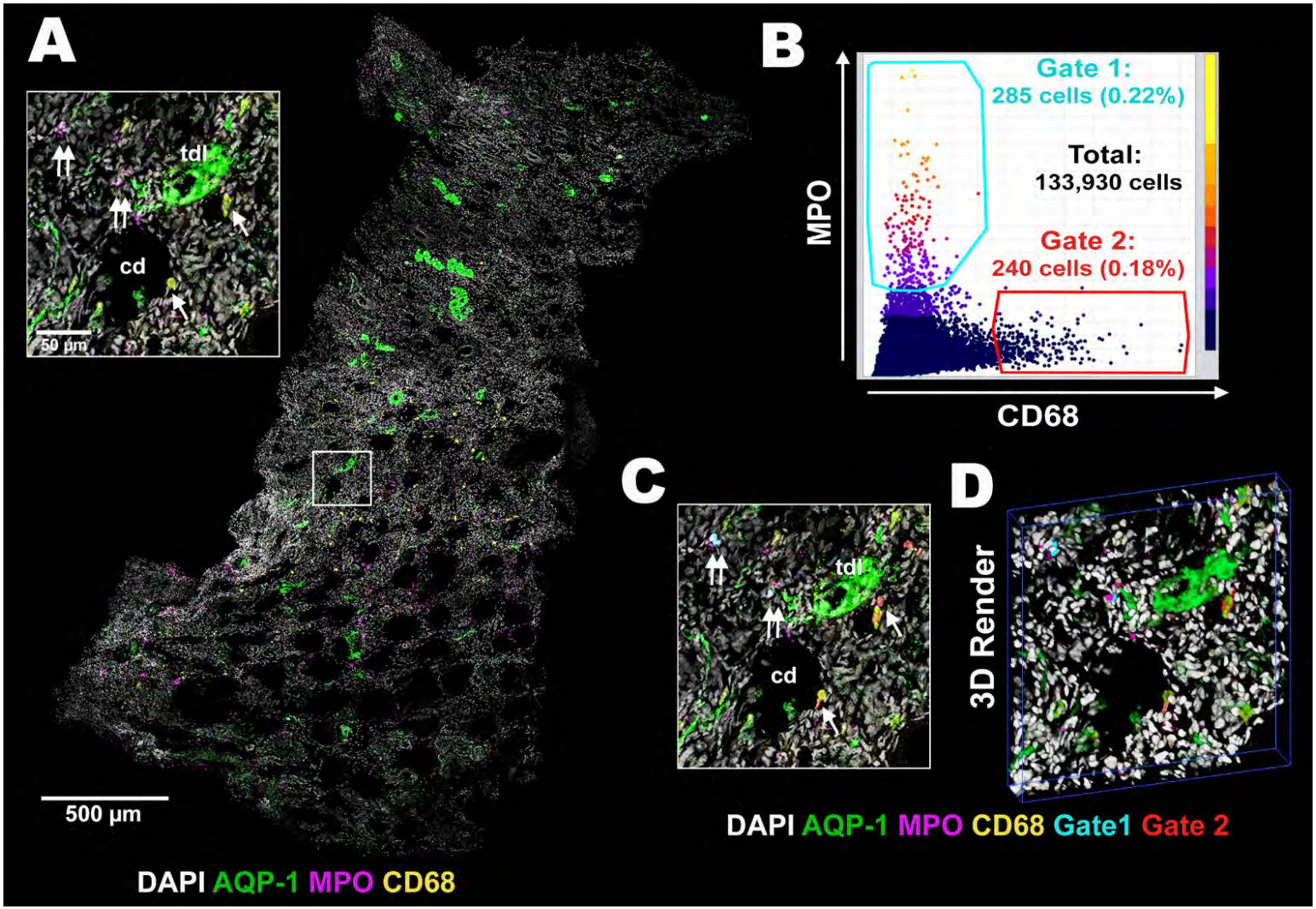
# Large scale imaging: distribution of cells throughout the tissue



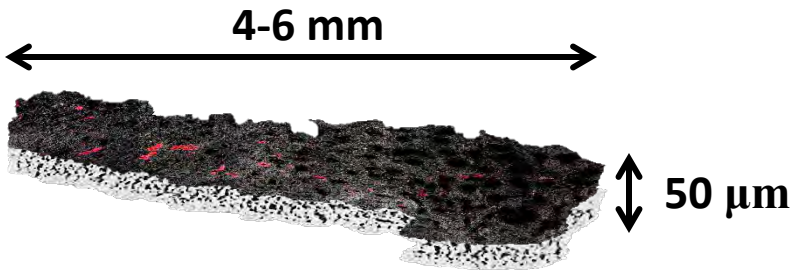
# Large scale imaging: distribution of cells throughout the tissue

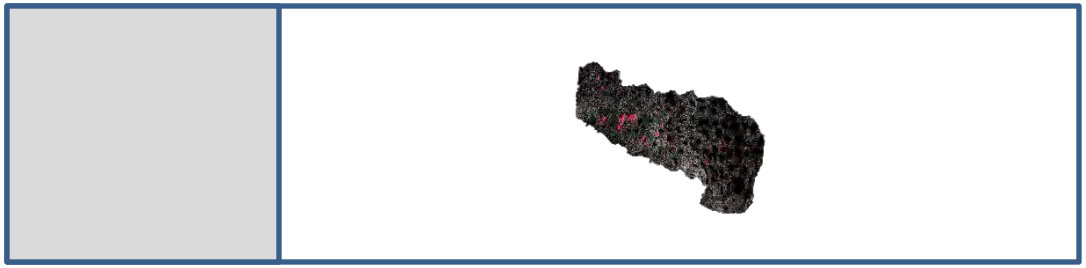


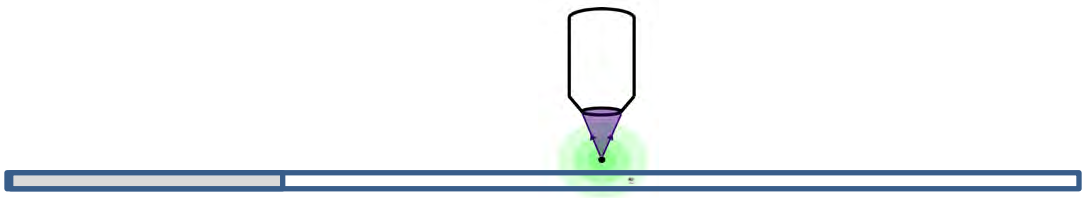
# Large scale imaging: interrogating thousands of cells



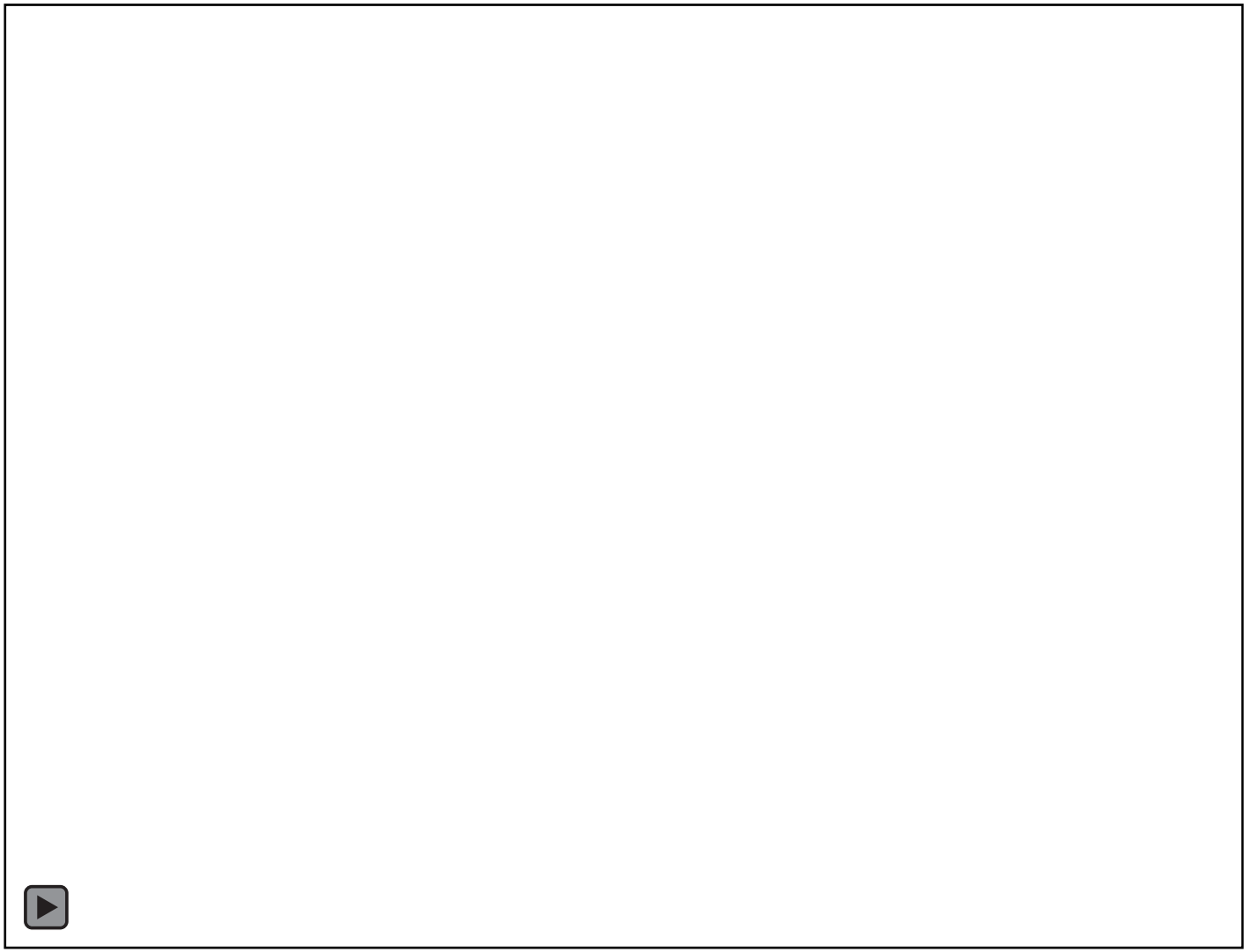
# Tissue Preparation





# Cleared tissue-mouse intestine



Nuclei Myristoylated tdTomato Myeloid cells ~1300 um x 600 um x 275 um

Mike Ferkowicz and Merv Yoder

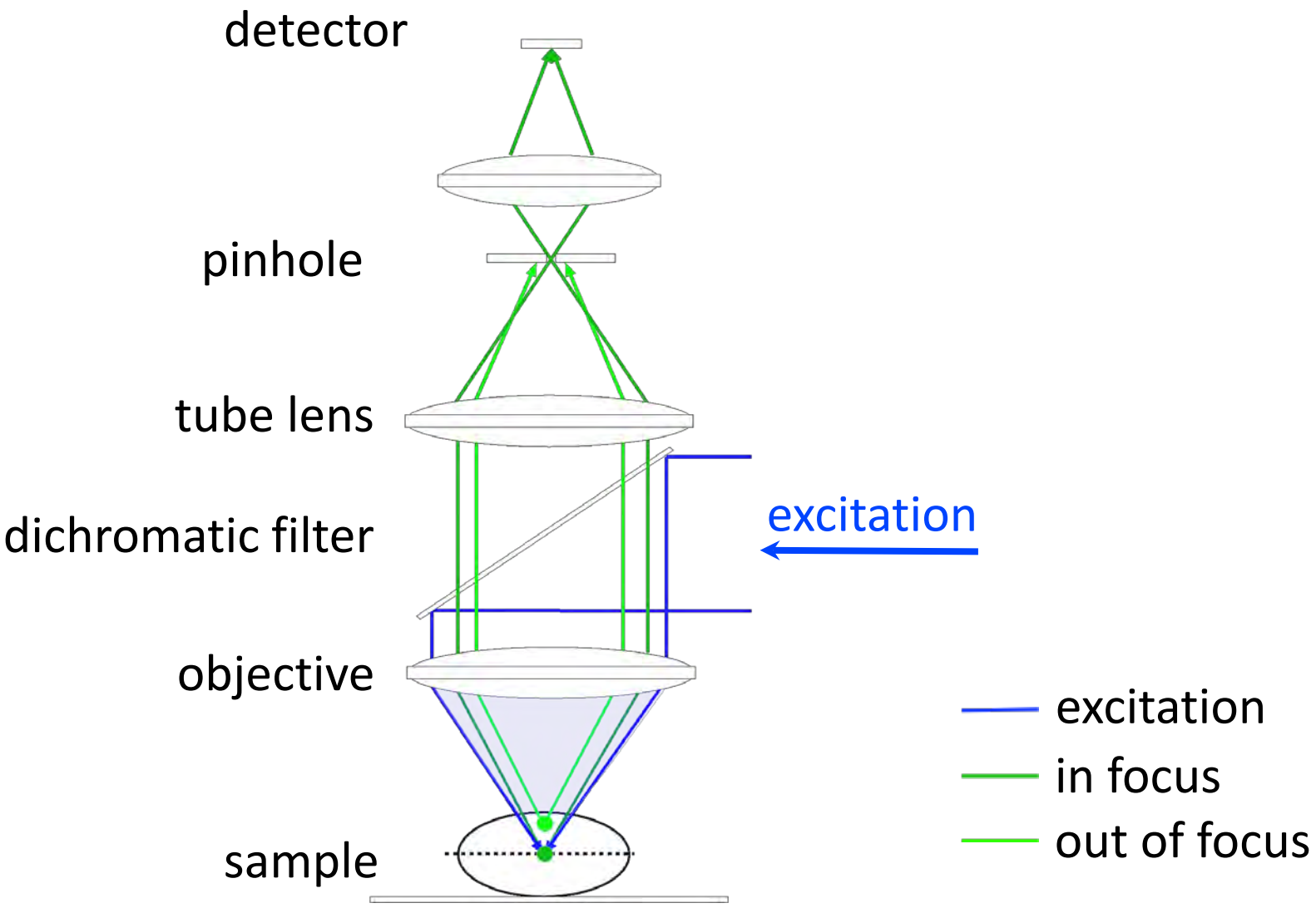
# Optical sectioning modalities

confocal fluorescence microscopy

selective plane illumination microscopy

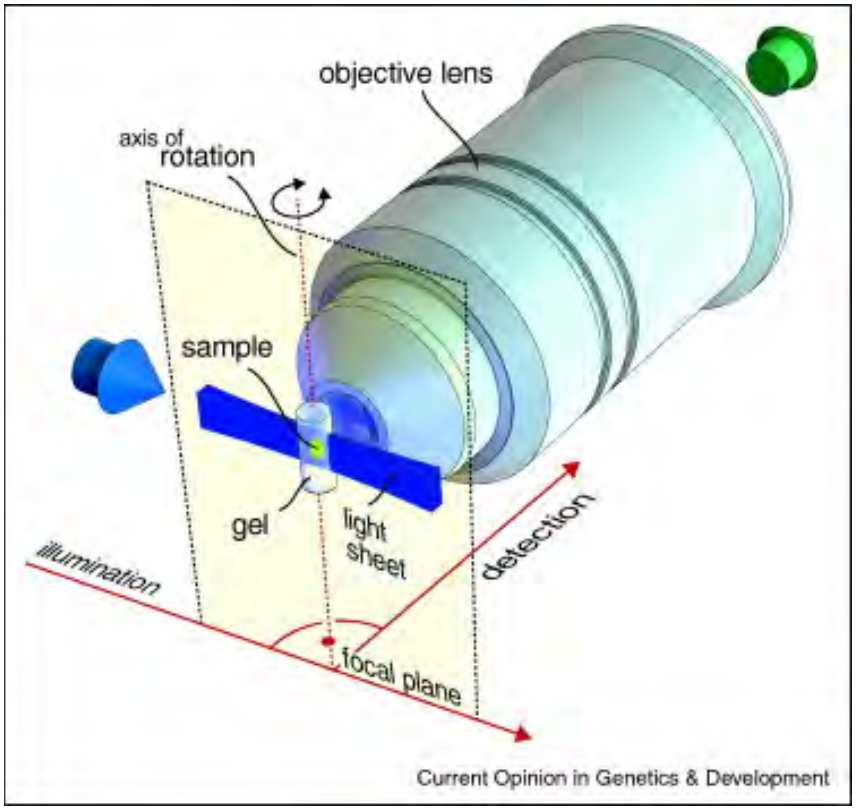
multi-photon excitation fluorescence microscopy

# Confocal fluorescence imaging



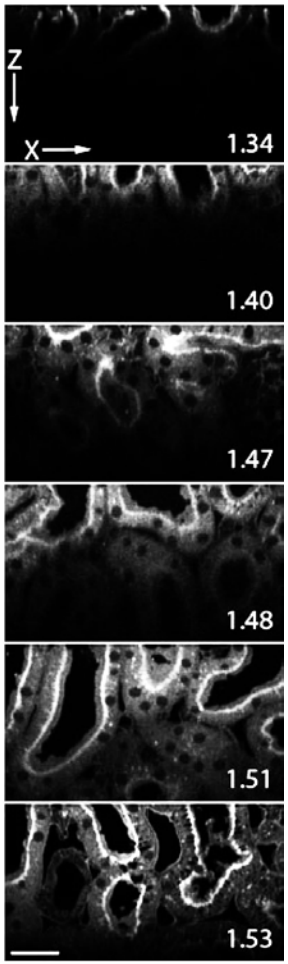
# Light sheet microscopy for image large specimens quickly

- Massive volumes of cleared tissue
- 10-100 times faster than confocal and multi-photon excitation microscopy.
- Samples can be rotated for multiple views.




Curr Opin Genet Dev. 2011 Oct;21(5):566-72. doi: 10.1016/j.gde.2011.09.009. Epub 2011 Sep 30. Light sheet microscopy for real-time developmental biology. Weber M, Huisken J.

# Clearing tissue for *in toto* imaging

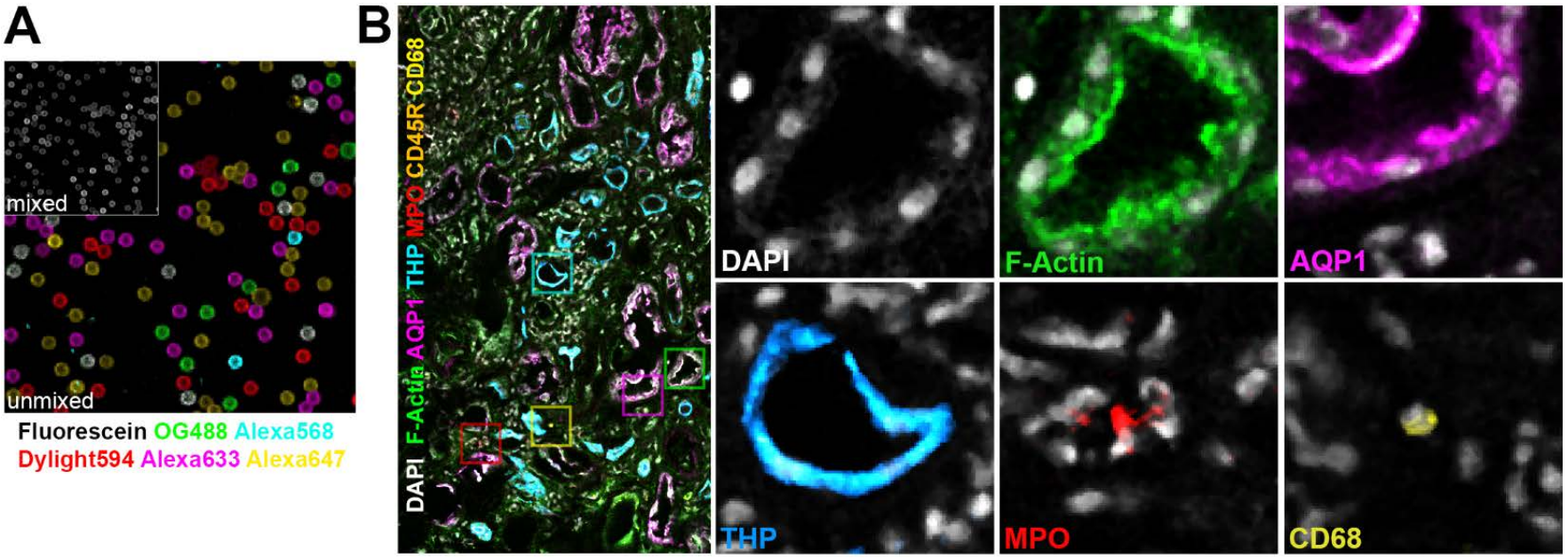



1. remove lipids and partially unfold proteins
2. match tissue and mounting media refractive index

Nat Neurosci. 2013 Aug;16(8):1154-61. doi: 10.1038/nn.3447. Epub 2013 Jun 23. SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. Ke MT(1), Fujimoto S, Imai T.

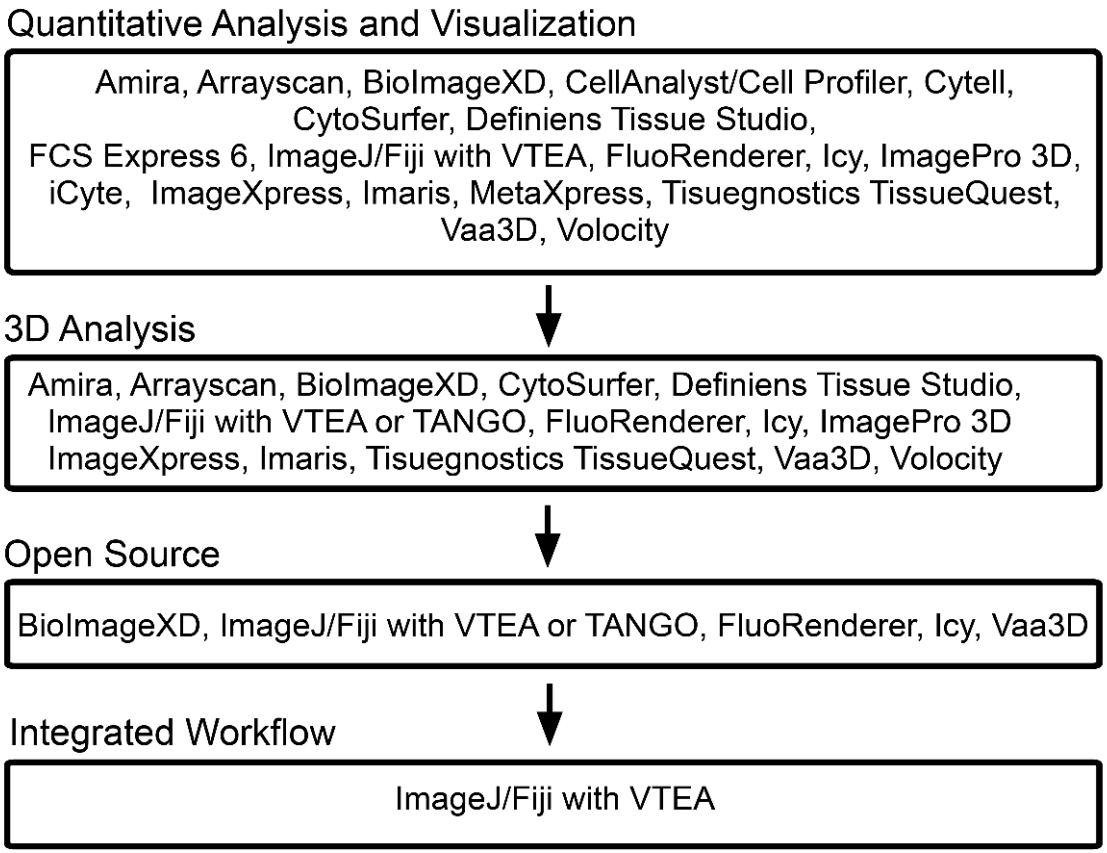
J Microsc. 2011 May;242(2):148-56. doi: 10.1111/j.1365-2818.2010.03448.x. Epub 2010 Sep 27. The effects of refractive index heterogeneity within kidney tissue on multiphoton fluorescence excitation microscopy. Young PA, Clendenon SG, Byars JM, Dunn KW.

# Spectral unmixing of seven labels in human biopsy volumes



# Analysis

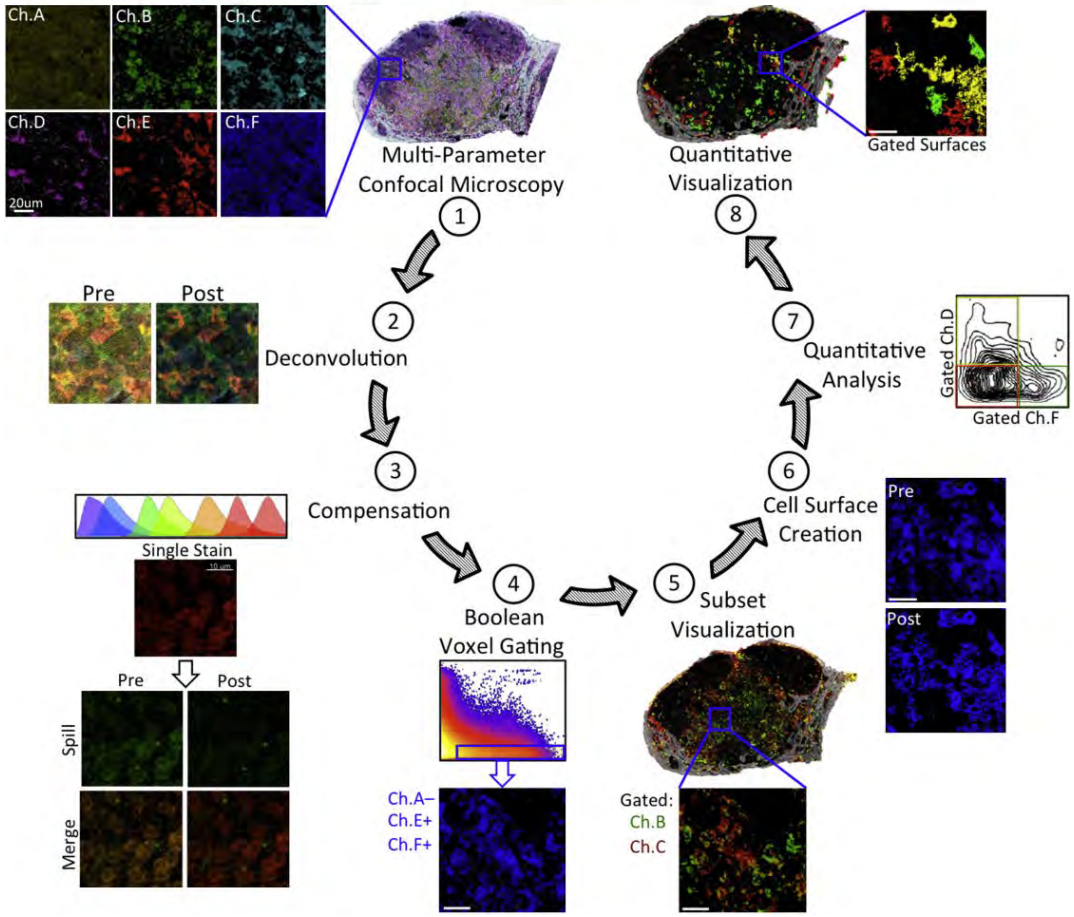
# 3D image analysis software



# Tissue Cytometry

## Histo-Cytometry: A Method for Highly Multiplex Quantitative Tissue Imaging Analysis Applied to Dendritic Cell Subset Microanatomy in Lymph Nodes

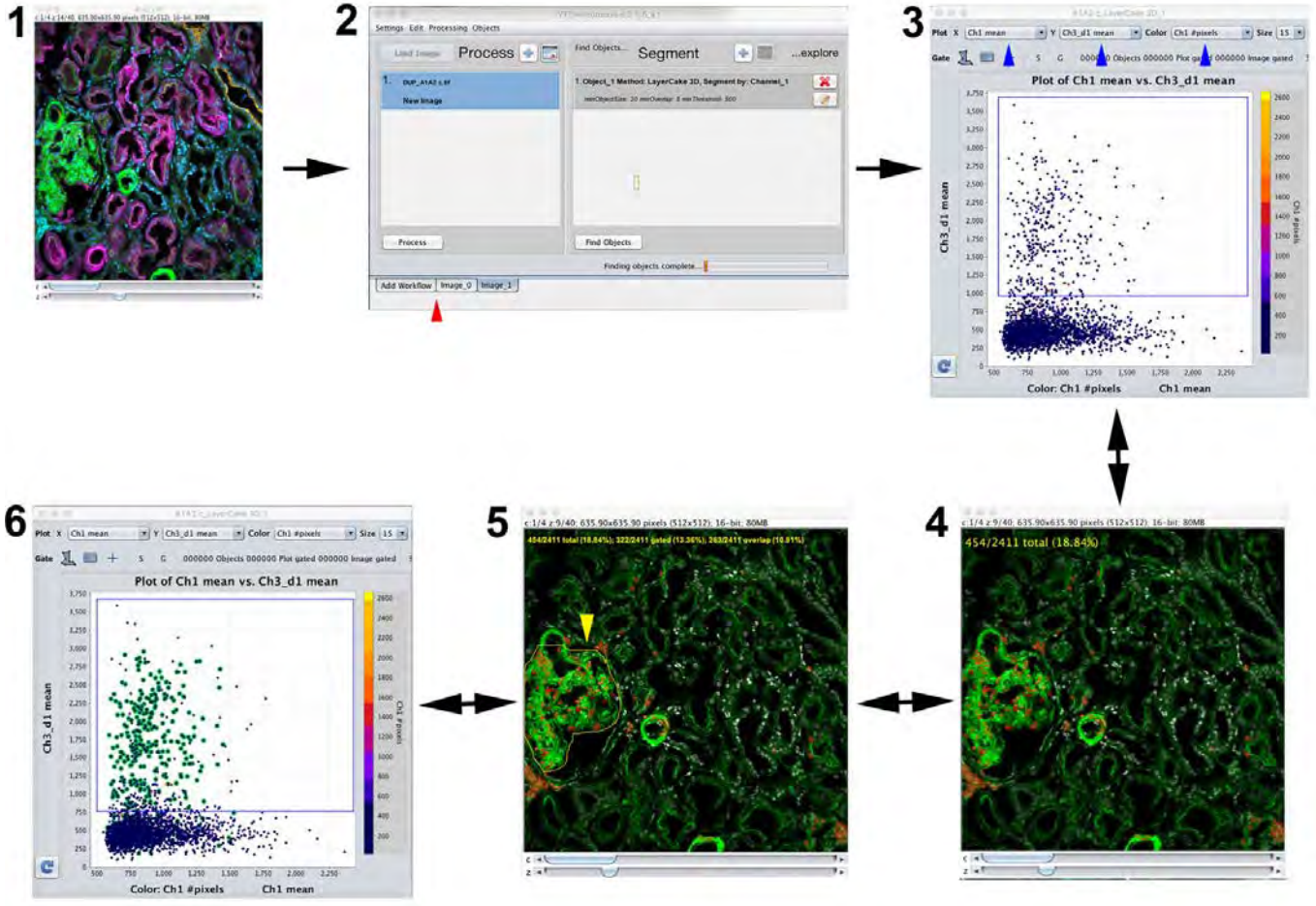
Michael Y. Gerner,[1,*] Wolfgang Kastenmuller,[1] Ina Ifrim,[1] Juraj Kabat,[2] and Ronald N. Germain[1,*]
[1]Lymphocyte Biology Section, Laboratory of Systems Biology
[2]Biological Imaging Section, Research Technology Branch
National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: gernermy@niaid.nih.gov (M.Y.G.), rgermain@nih.gov (R.N.G.)
http://dx.doi.org/10.1016/j.immuni.2012.07.011



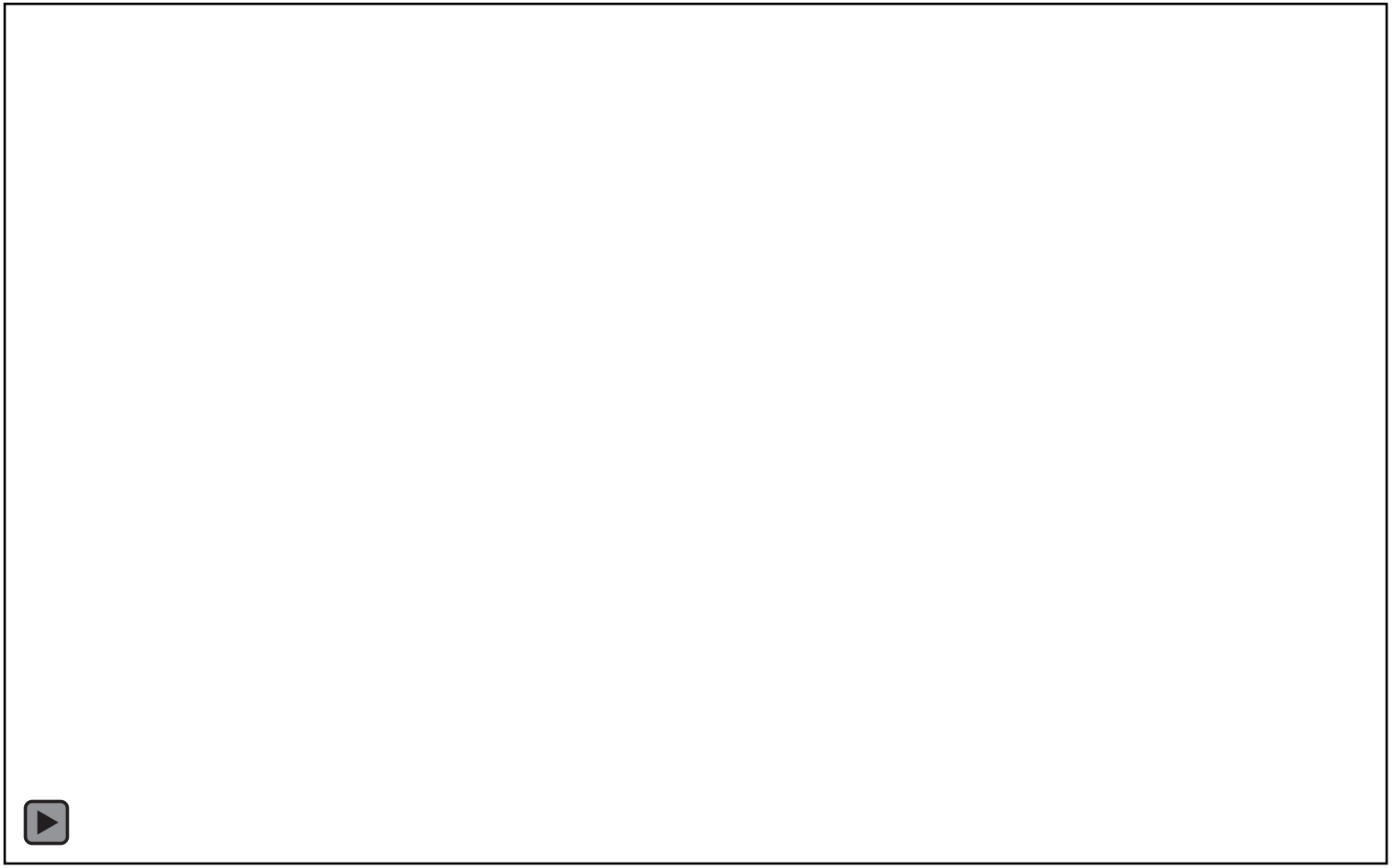

**Software used:**

- Huygen's Essential software
- Imaris
- Excel
- Flojo

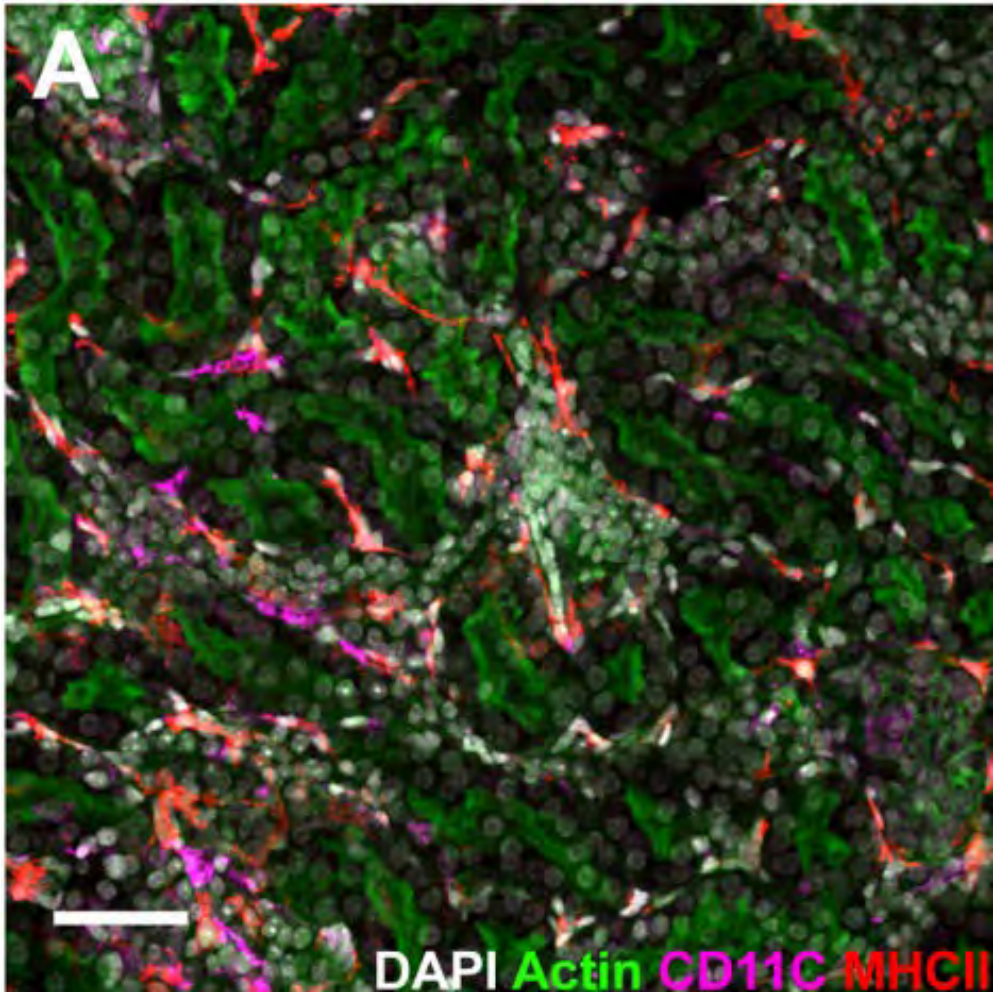
# Volumetric Tissue Exploration and Analysis (VTEA)- ***Seth Winfree***



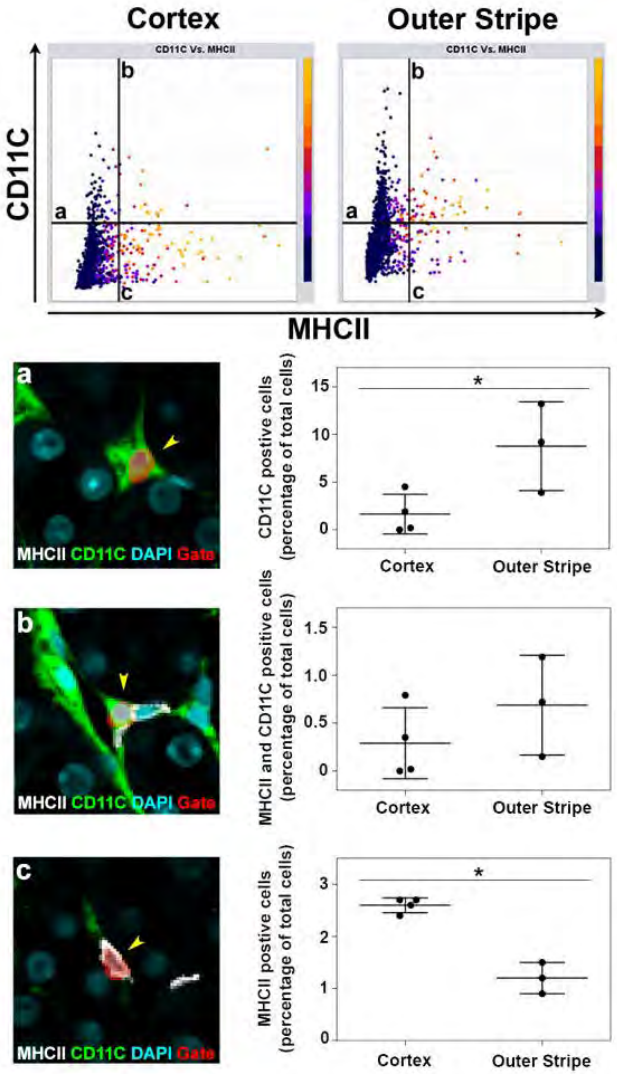
# VTEA in action

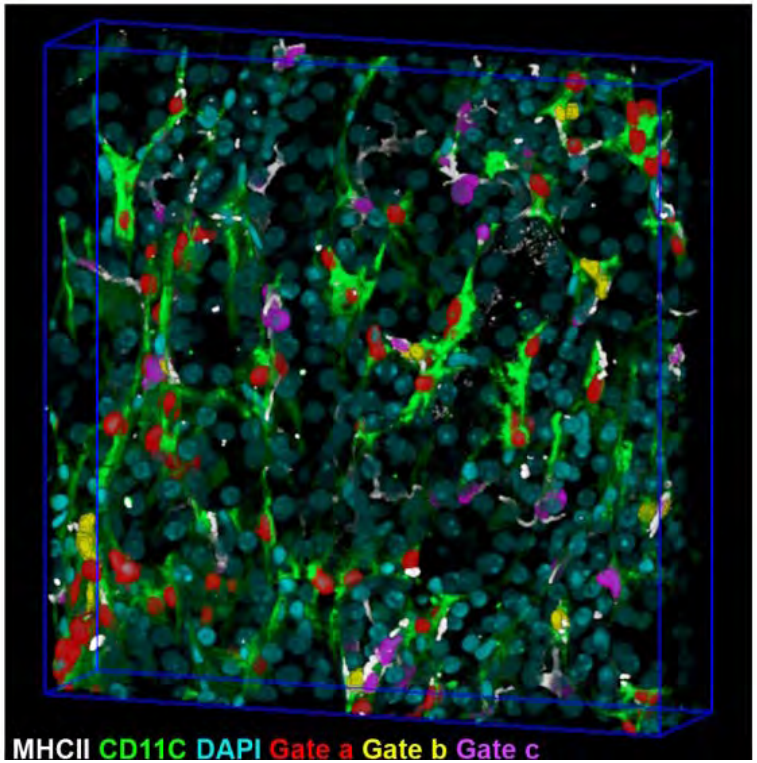


# Applications

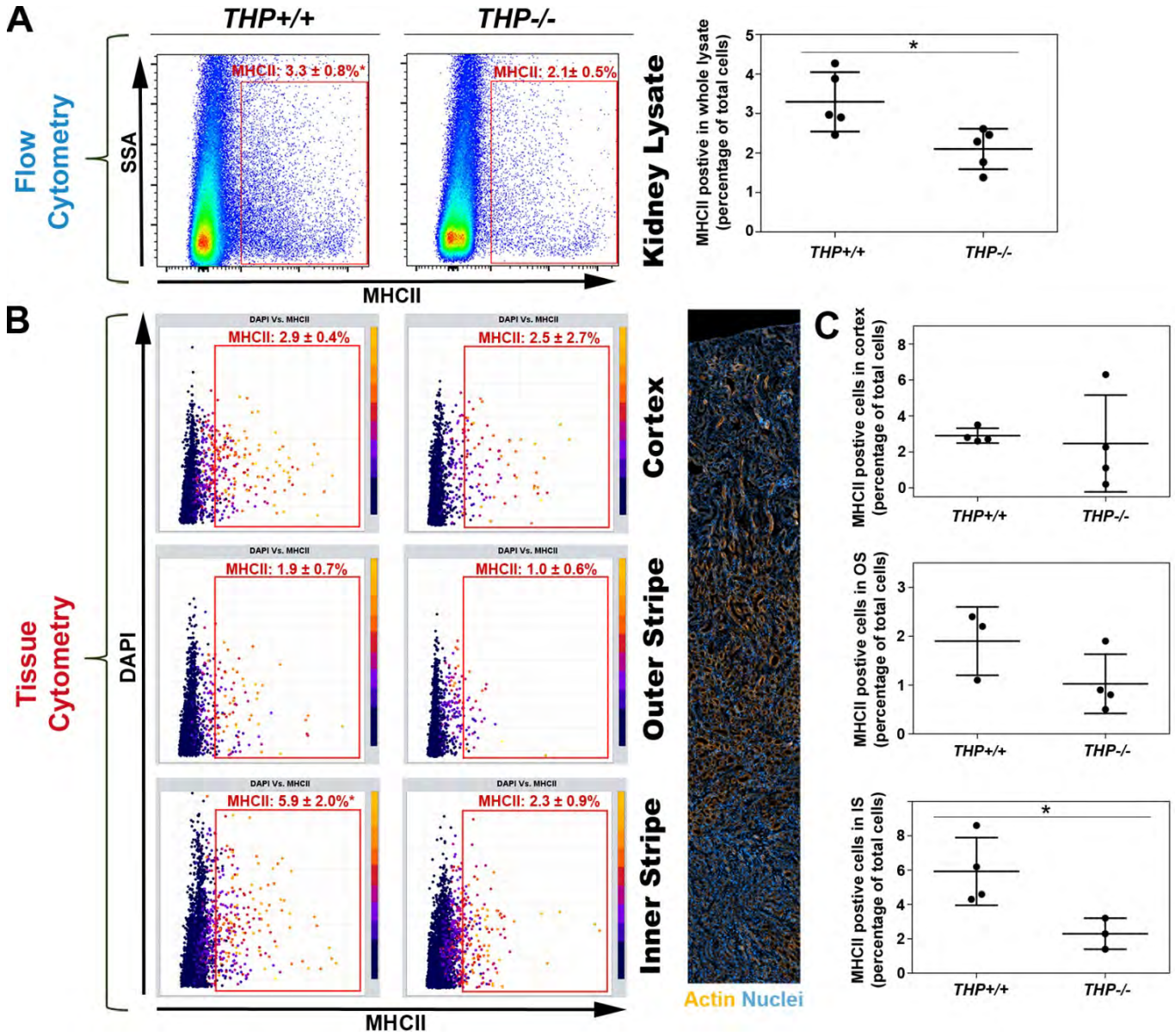


# 3D tissue cytometry of immune cells

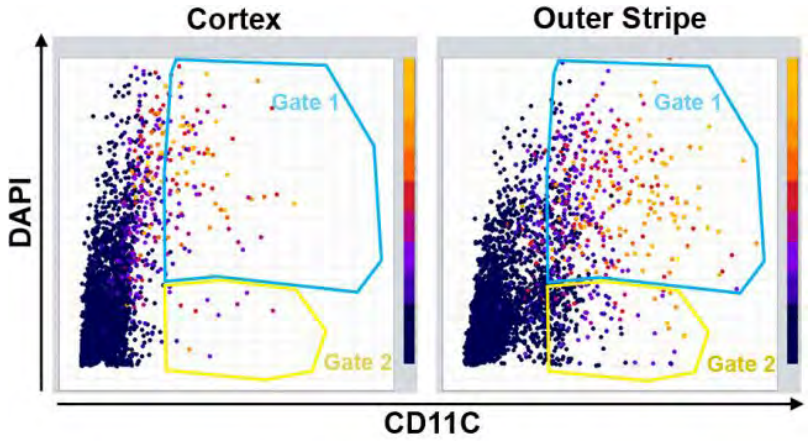


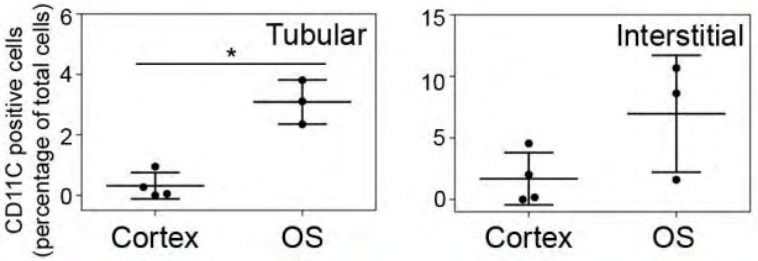



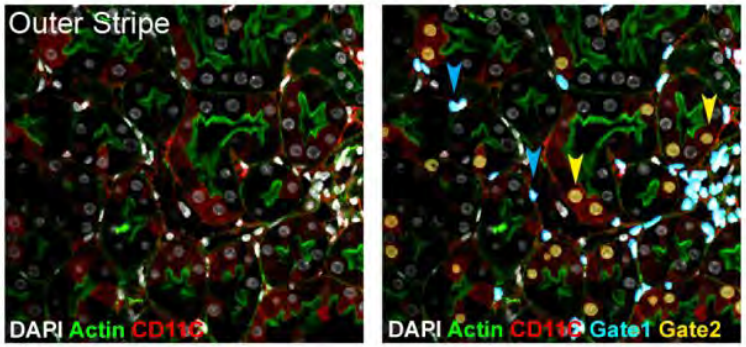
# 3D tissue cytometry of immune cells

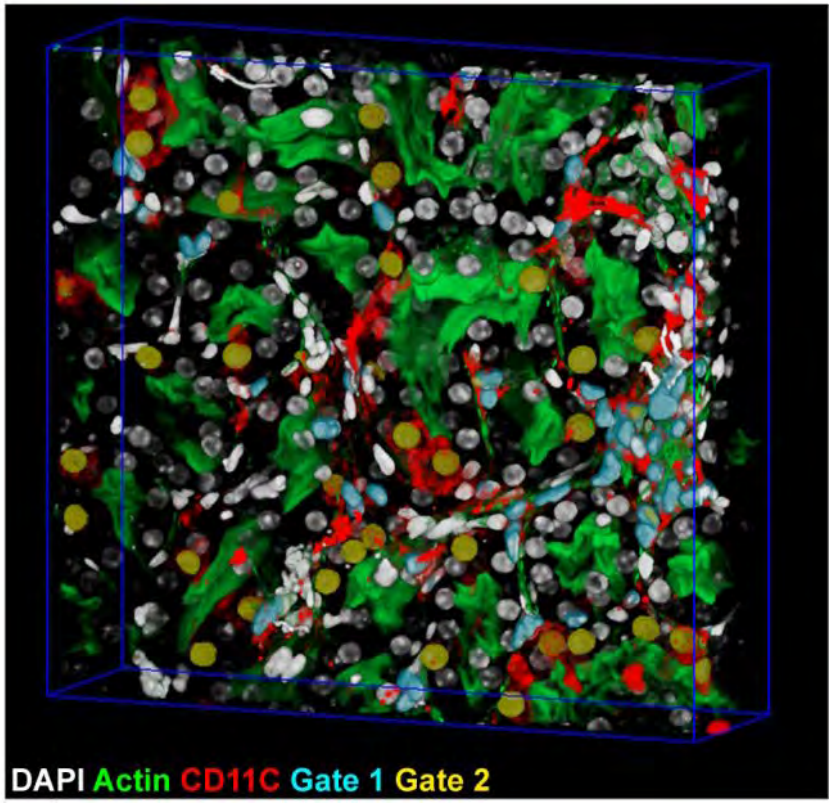


# Using VTEA based analysis to uncover CD11C+ tubular epithelial cells

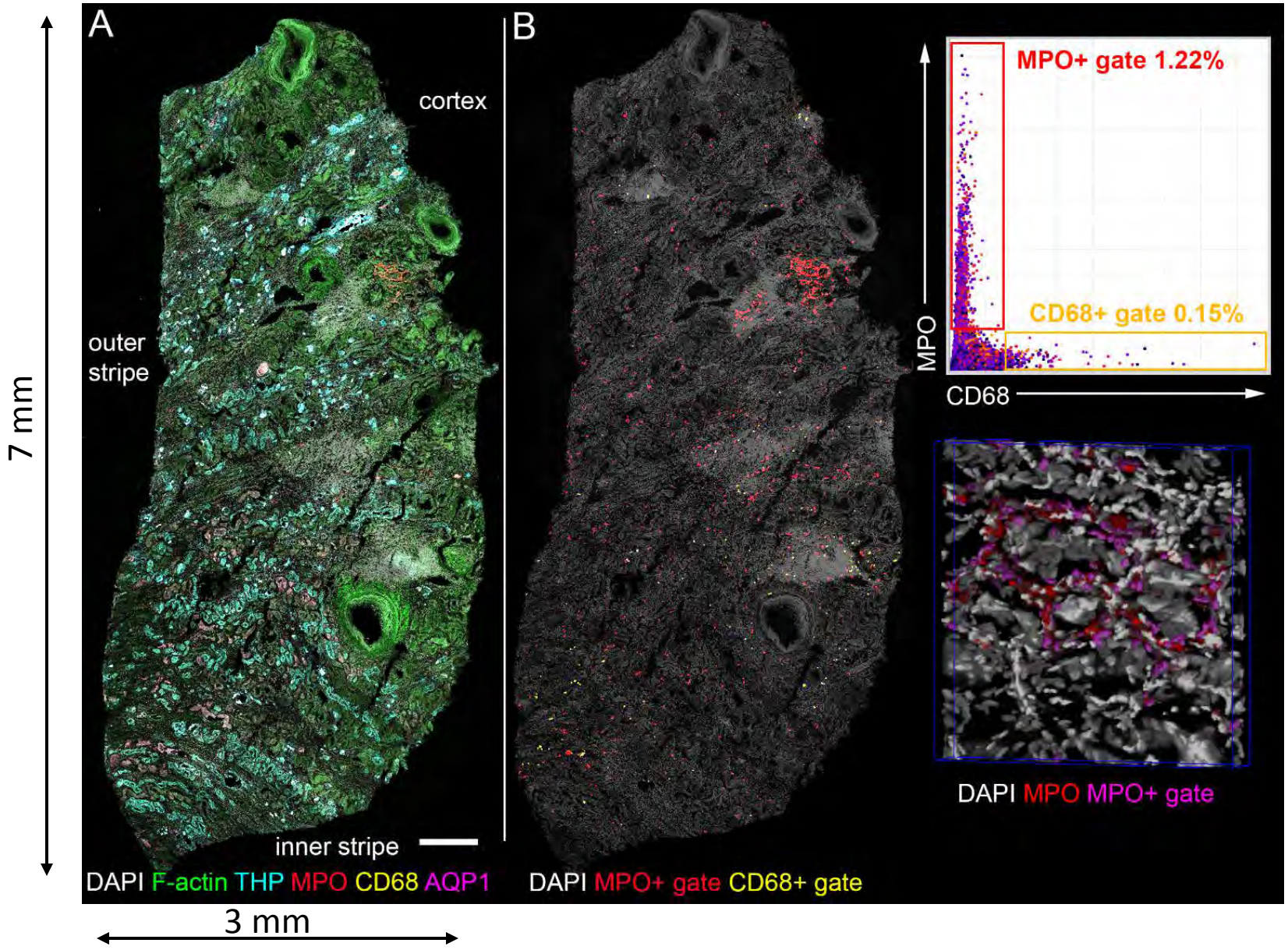


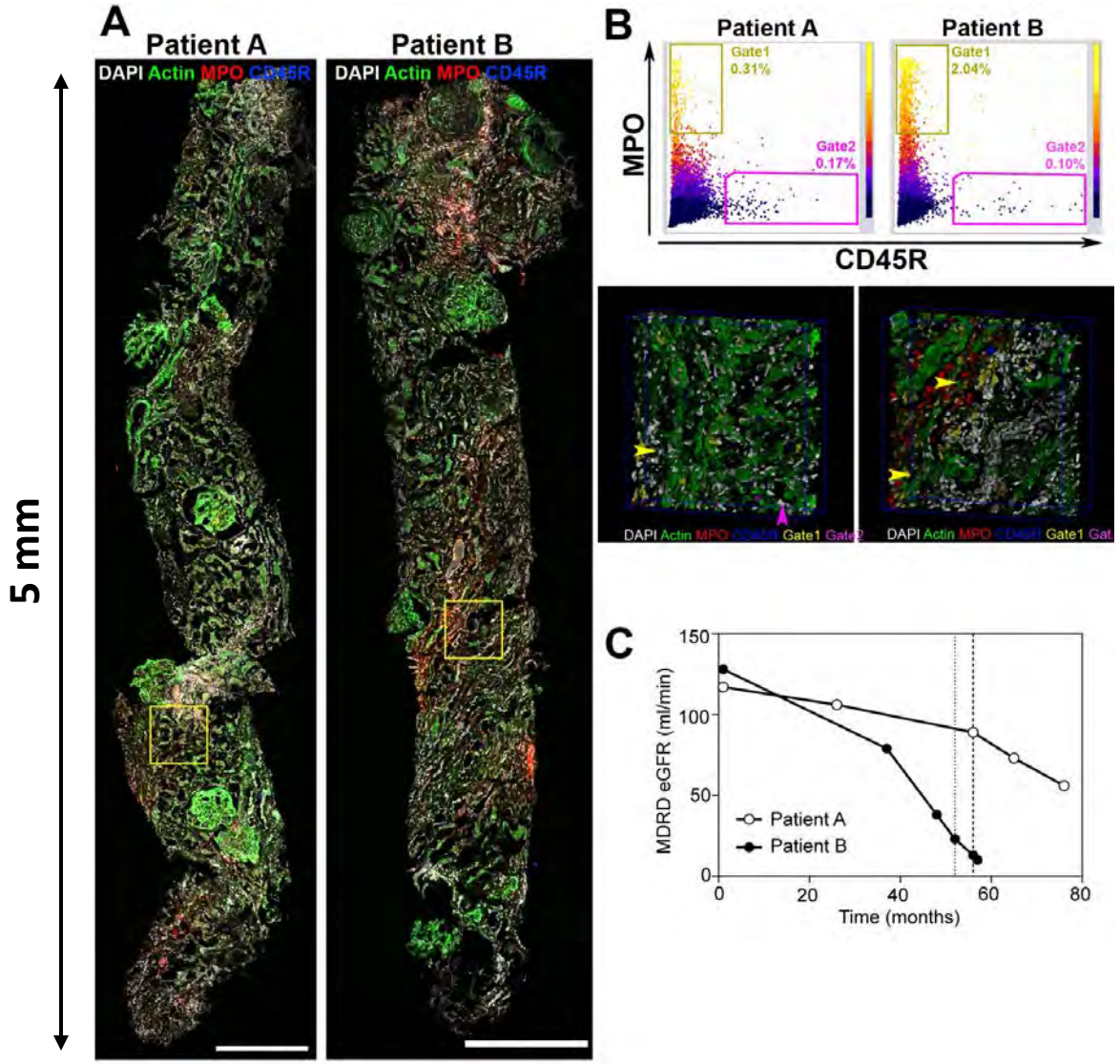



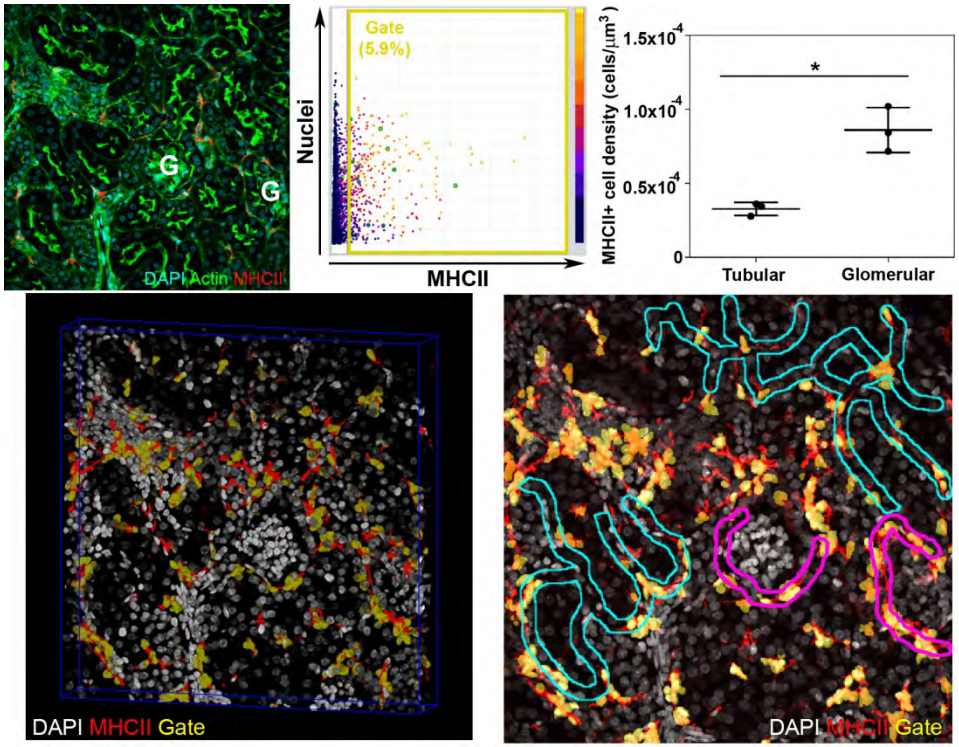
# Six channel 3D mesoscale imaging and analysis of human nephrectomy



# Immune cell analysis of human biopsies and correlation with clinical outcomes

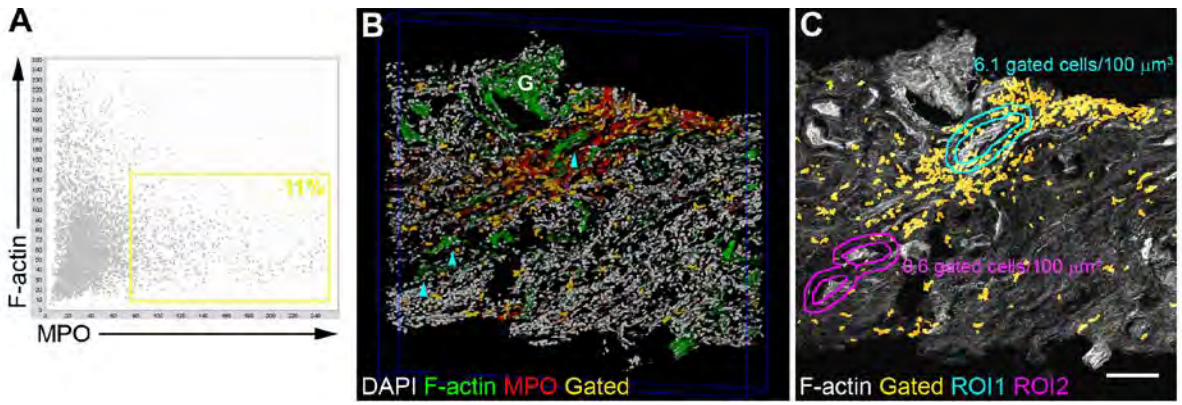


# Quantifying the spatial density of immune cells




Clustering of dendritic cells around glomeruli vs. tubules

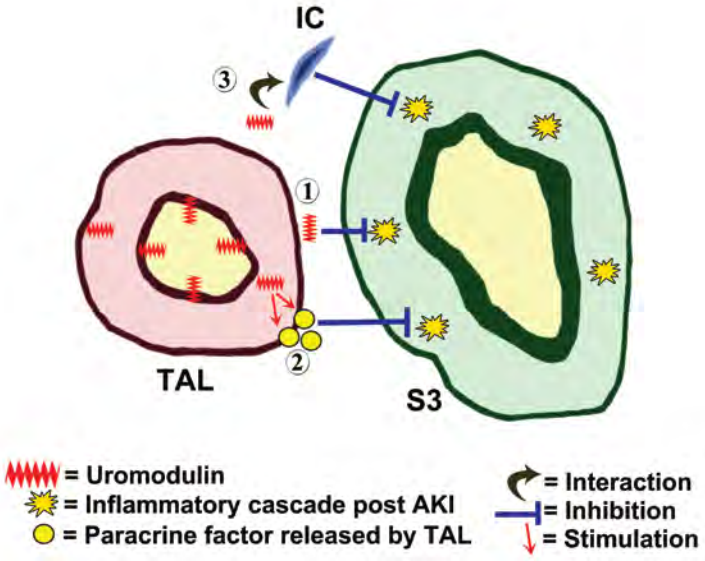
***mouse***



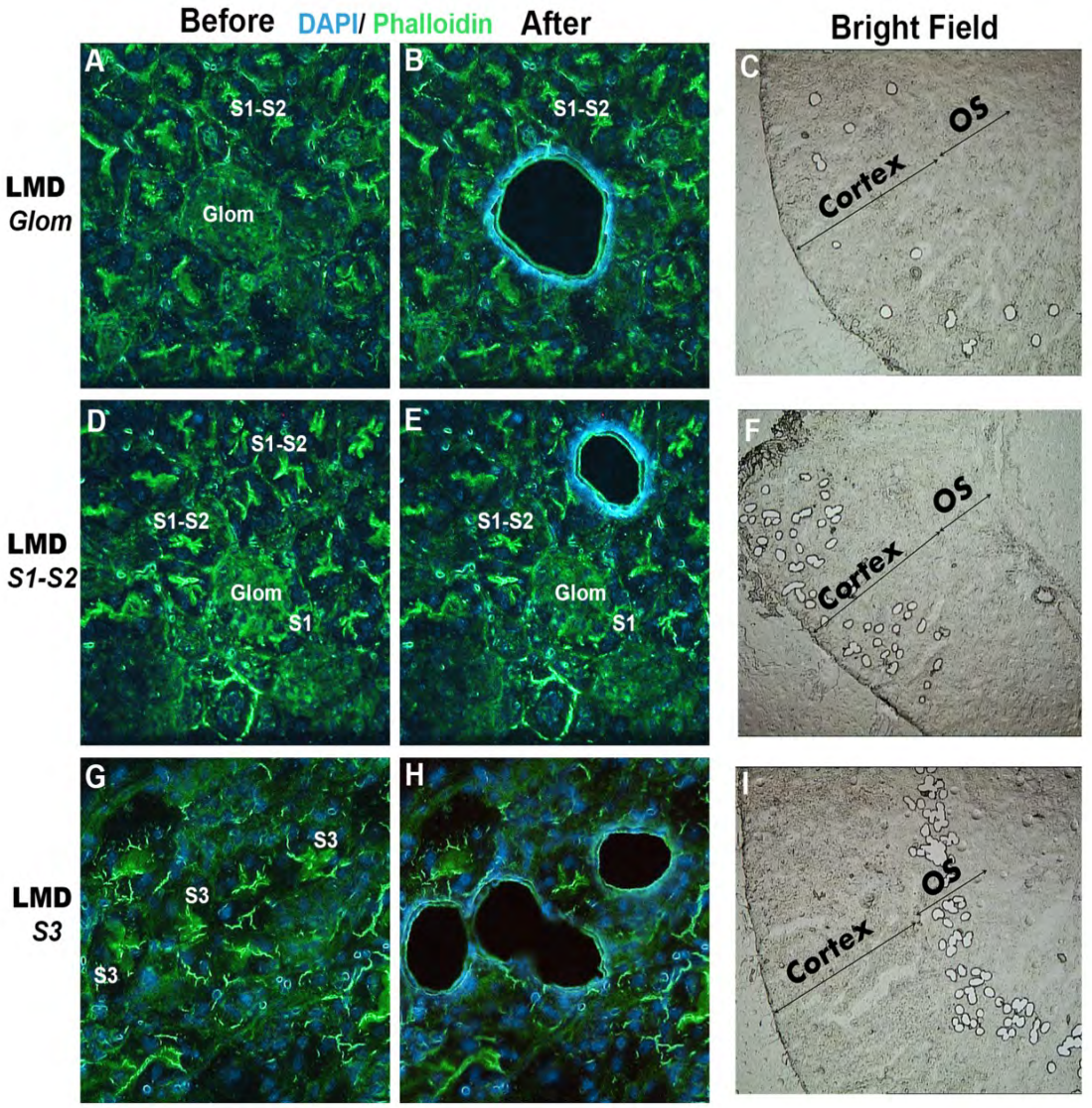

Quantifying the spatial density of infiltrating neutrophils

***human***

# Using VTEA to study signaling pathway activation

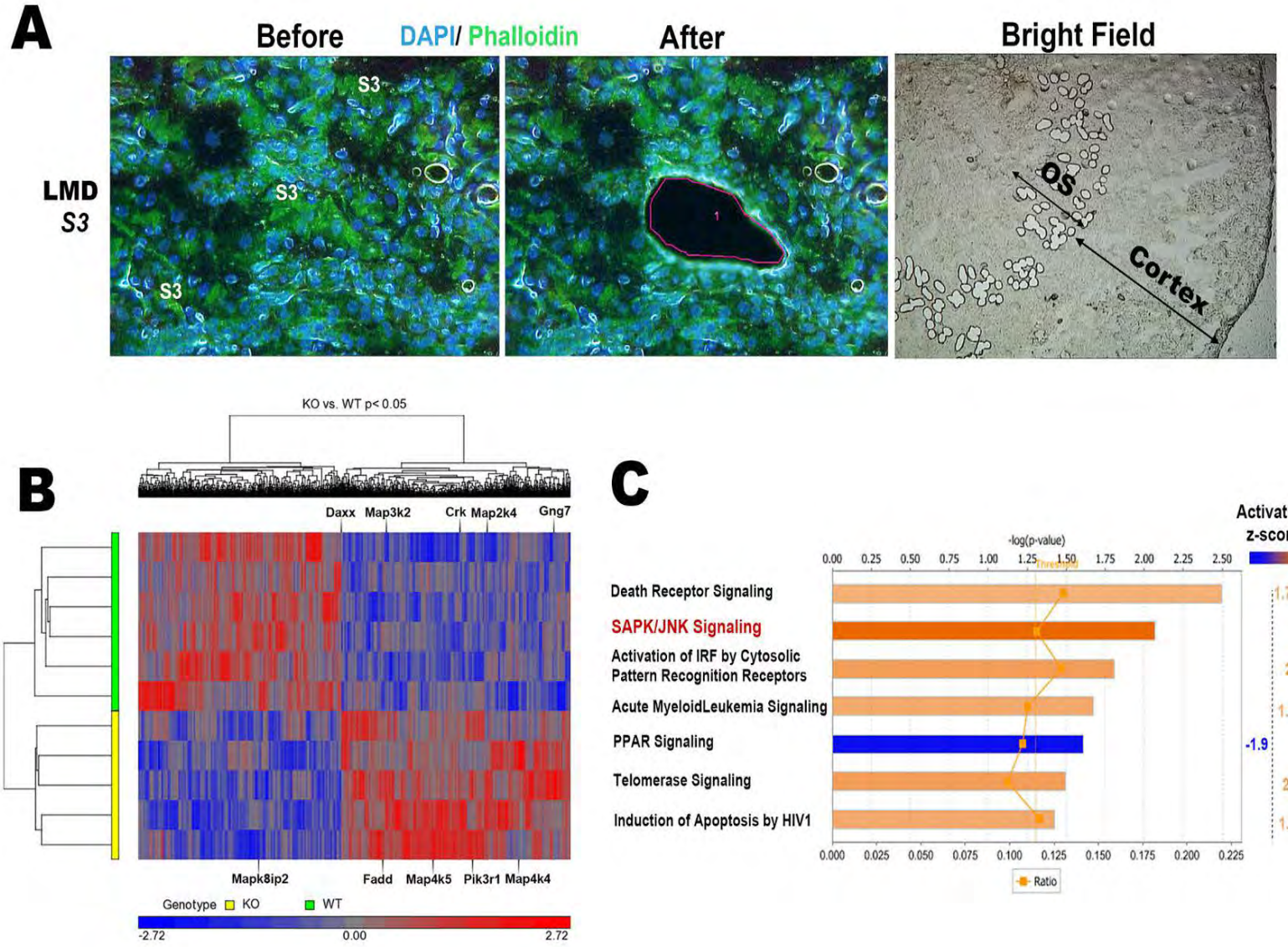



El-Achkar and Wu, AJKD 2012

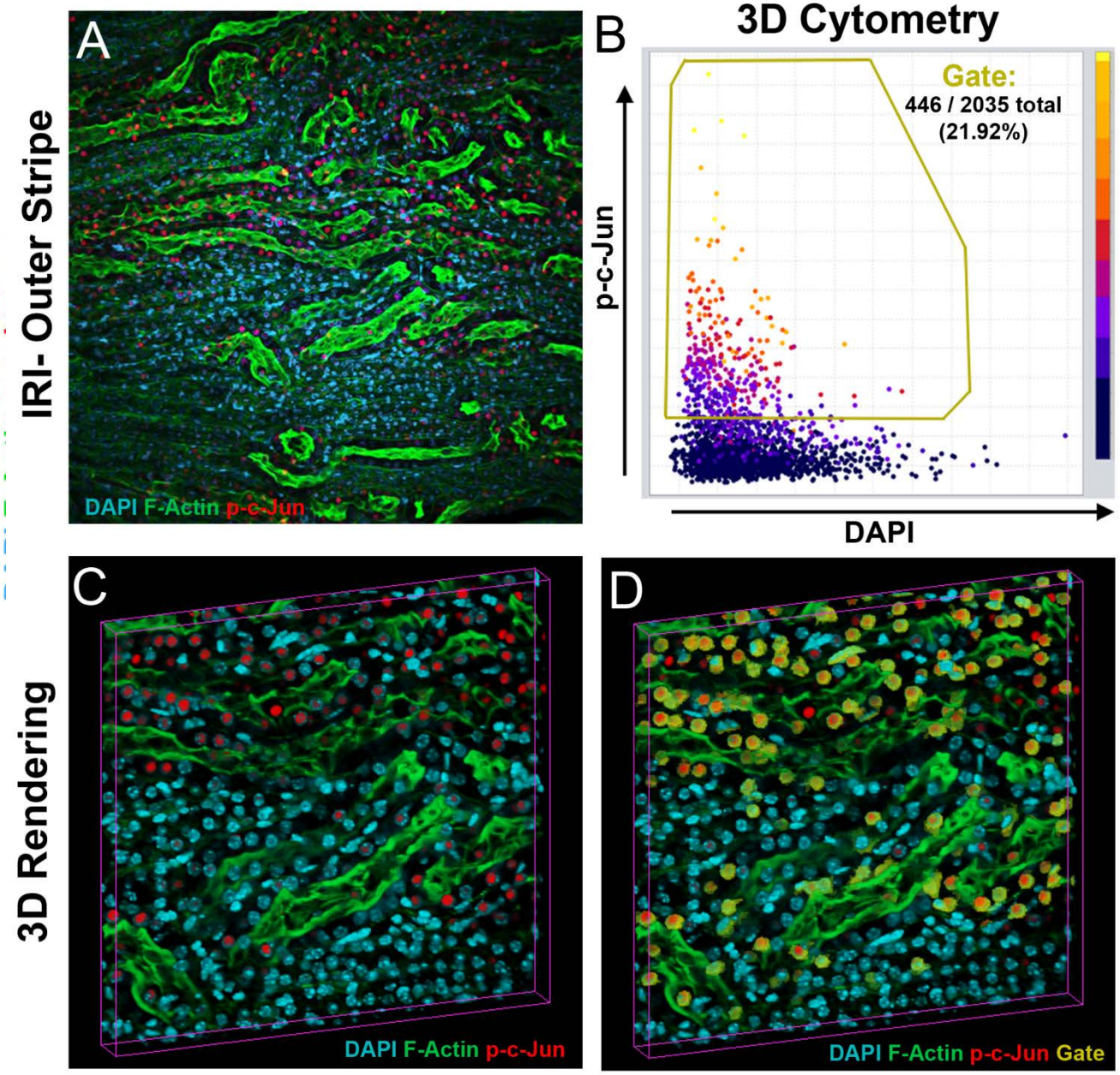



Micanovic R, Khan S, El-Achkar TM, Physio Rep 2015

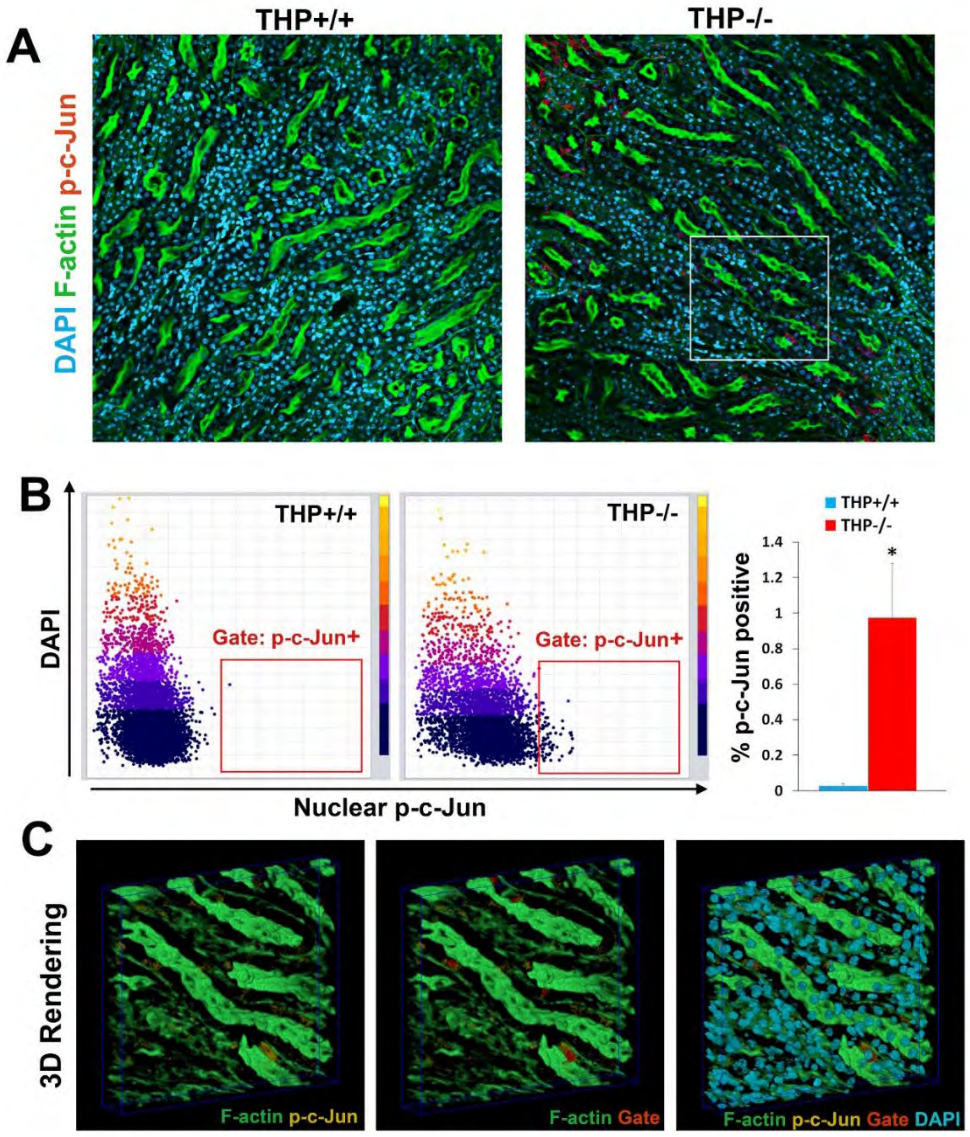
# Transcriptomics of S3 proximal tubules in THP-/- vs. THP+/+



# JNK is activated in kidney injury



# Quantitating c-Jun activation in uninjured S3 proximal tubules using VTEA



# Challenges

- Validated antibodies/probes in tissue
- Improved segmentation in high cell density area
- Capacity of computer hardware, speed, space for large scale analysis

# Exciting potential

- Technical advances:
  - Improved Imaging speed with multichannel SPIM
  - Improved staining and clearing (nanobodies, etc)

- Extensibility of VTEA
  - processing, exploring and analyzing data
  - novel segmentation methods
  - machine learning, shape recognition

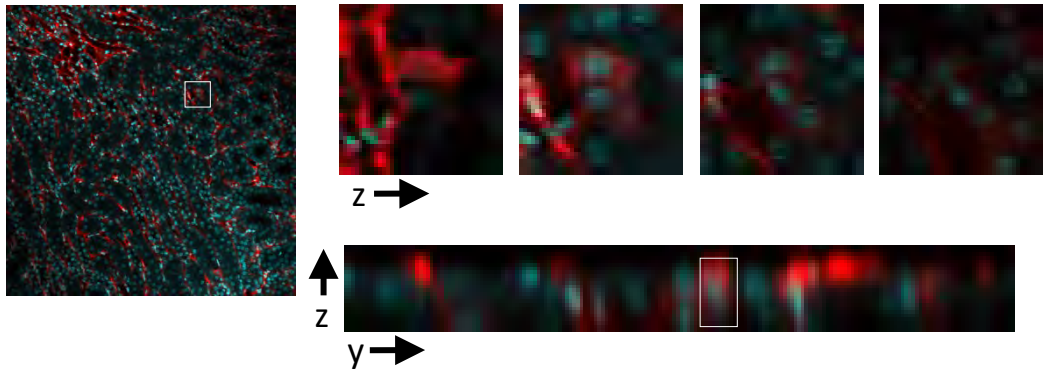
# Acknowledgement


- Seth Winfree, Kenneth Dunn, Gosia Kamocka, Bruce Molitoris
- Pierre Dagher, Katrina Kelly, Michael Eadon, Timothy Sutton
- Michael Ferkowicz, Mervin Yoder, Troy Markel

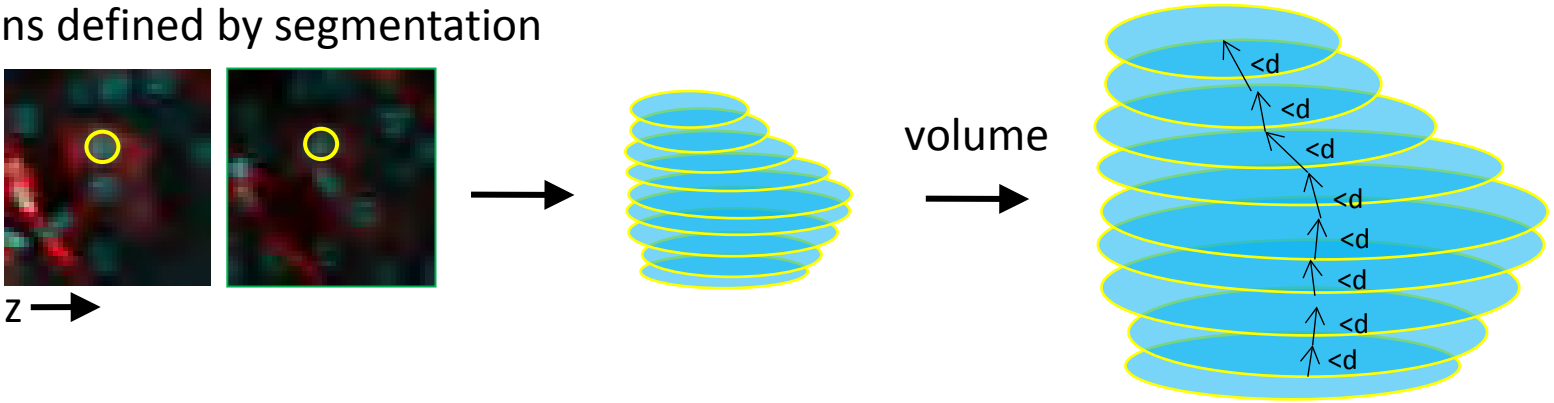
- Funding:
  - VA Merit Award, NIH-NIDDK Program Project Grant (P01DK056788)
  - NIH/NIDDK DK076169 Diacomp
  - Indiana Clinical and Translational Sciences Institute, funded in part by grant #UL1 TR001108 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award.
  - National Institutes of Health O'Brien Center for Advanced Renal Microscopic Analysis (NIH-NIDDK P30 DK079312).

Microscopy studies were conducted at the Indiana Center for Biological Microscopy.

# Volume formation-finding nuclei

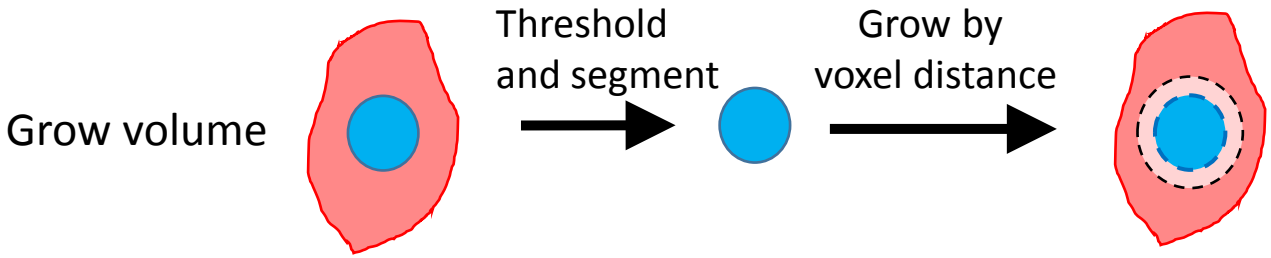


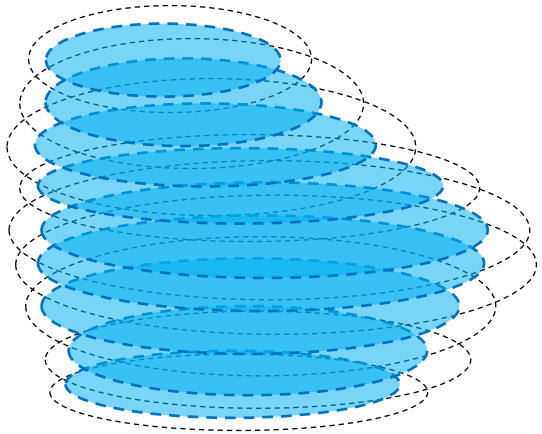

Regions defined by segmentation

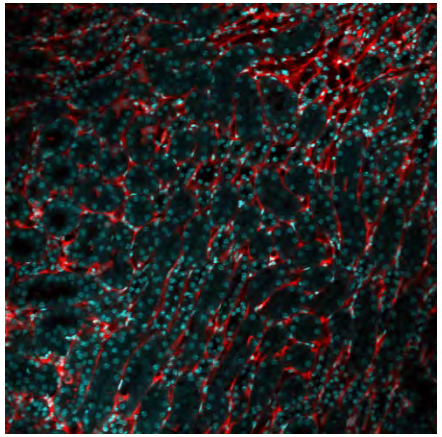



- Use a maximum distance between region centers to build volumes
- Extract means, Feret diameters, aspect ratios, etc.

# Volume formation-finding “cytosol”





…and repeat a couple thousand times…