Workshop Goals and Results: Immuno-LCM

Amanda Pellowe
Gonzalez Lab
Yale University, Biomedical Engineering

Goals

- Stain mouse kidney sections for microvascular pericytes
  - NG2 primary antibody
  - αSMA primary antibody (Alexa488 direct conjugate)
- Immunofluorescent guided microdissection
- Nuclear Staining

NG2 Primary Antibody
Goal: Achieve Fluorescent Signal

- Experimental Set-Up
  - No blocking
  - 30 minute incubation
  - 1:50 dilution, 25 µl per tissue section
  - 30 minute incubation with secondary antibody
    - Alexa488 and Alexa555
- Results
  - No signal
  - Non-specific staining

αSMA Primary Antibody
Goal: Achieve Fluorescent Signal

- Experimental Set-Up
  - No blocking
  - 30 minute incubation, 25 µl per tissue section
  - 1:50 dilution

- Results
  - Signal!
  - Clear vascular structures under fluorescent microscope
  - Some background, but signal is clear

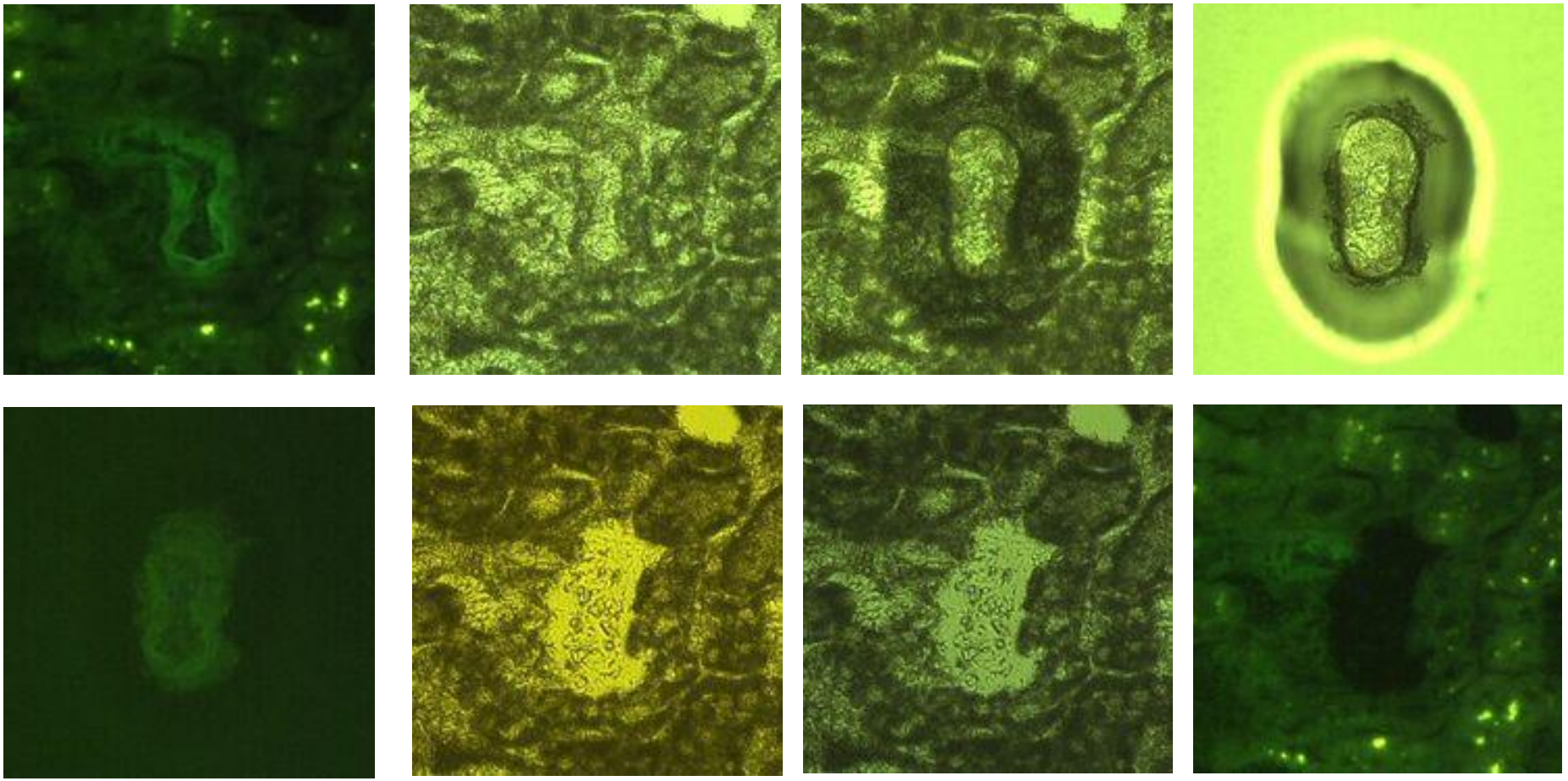
αSMA Primary Antibody Goal: Reduce Incubation Time

- Experimental Set-Up
  - No blocking
  - 1:50 dilution, 25 µl per tissue section
  - 15 minute, 5 minute, 30 second incubations

- Results
  - Signal!

αSMA Primary Antibody
Goal: LCM

1:50 dilution (25 µl, 30 sec)



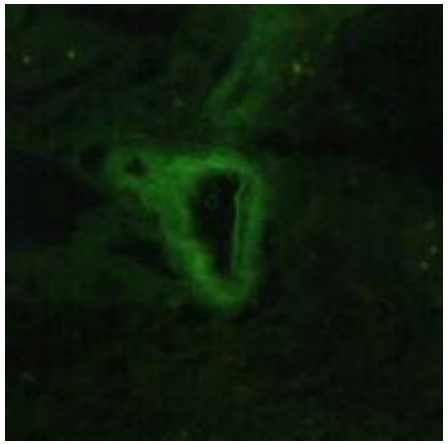
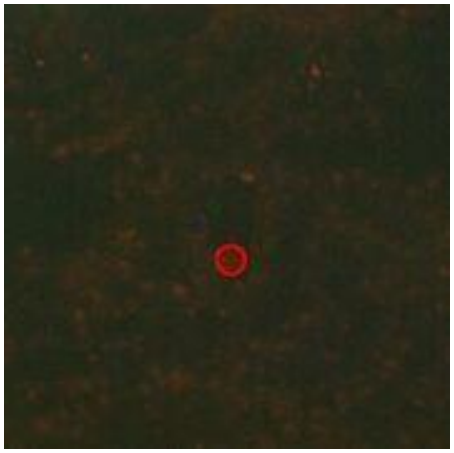
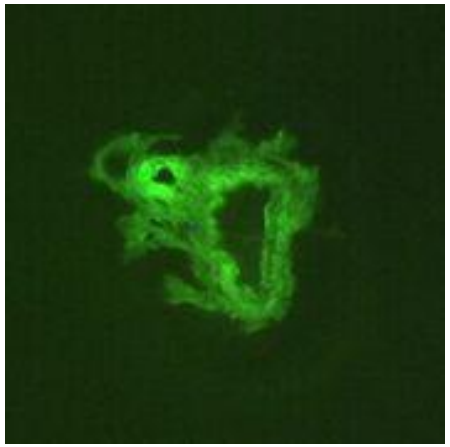
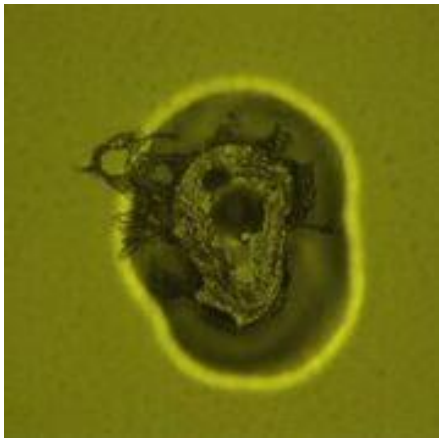
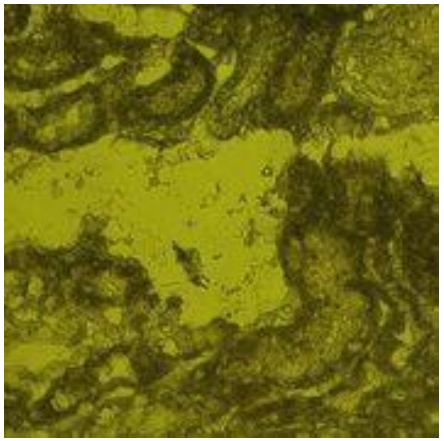
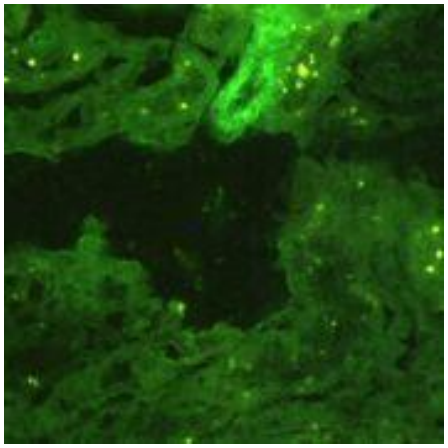
αSMA Primary Antibody
Goal: Reduce Staining Volume

- Experimental Set-Up
  - No blocking
  - 1:25 dilution, 12.5 µl per tissue section
  - 30 second incubation

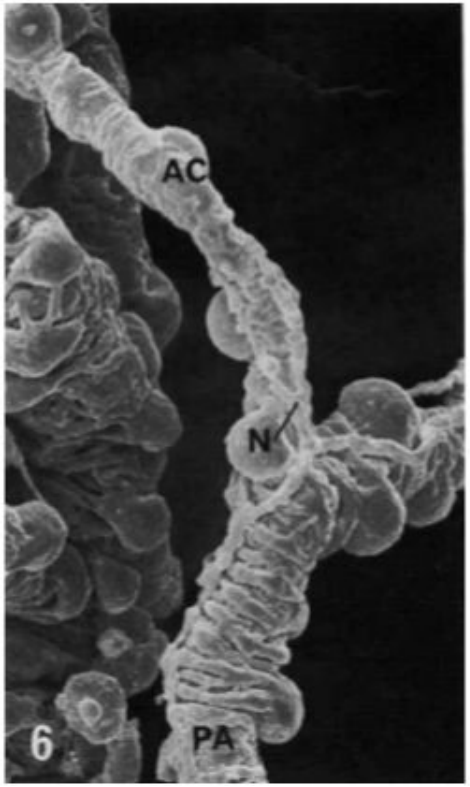
- Results
  - Signal!

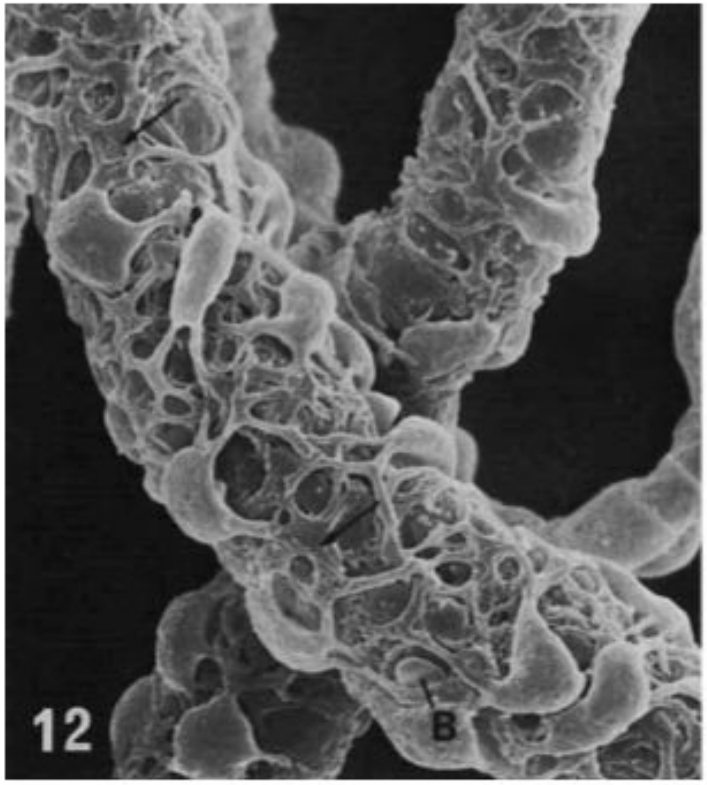
αSMA Primary Antibody Goal: LCM

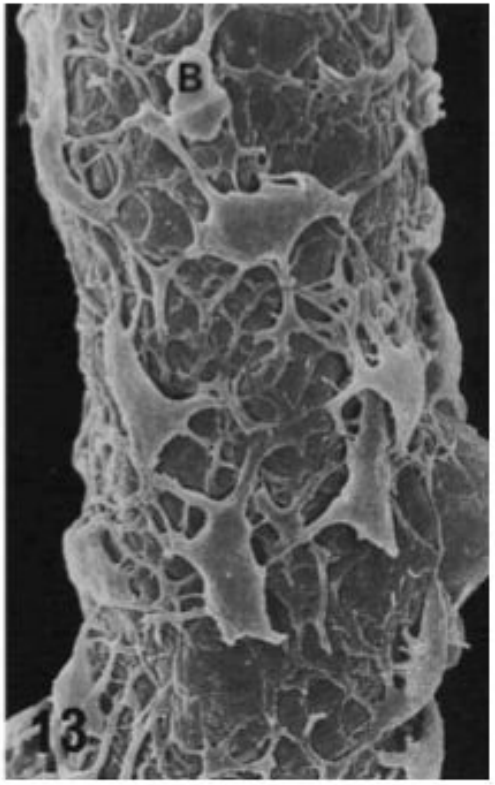
1:25 dilution (12.5 µl, 30 sec)

Nuclear Staining
Goal: Counterstain αSMA to Identify Single Cells







Nuclear Staining
Goal: Counterstain αSMA to Identify Single Cells

	NUCLEAR FAST RED	DAPI	DRAQ5	SYTO 16
PROS	• Fast • Strong nuclear dye	• Strong • Little bleed through anticipated	• Compatible with GFP/488/FITC (far red)	• Low concentrations stain DNA only
CONS	• High concentration stains cytoplasm	• UV	• High concentration stains cytoplasm	• High concentration stains RNA • Not compatible with GFP/488 (green)
RESULTS	• n/a	• Stained in “blobs” at 30 s and 1 min incubations • No clear nuclei image	• Nuclei stained at 1:10,000 and 1:1,000 • Bleed through • Nuclei do not overlap with stained region	• “Empty Nuclei” staining pattern • Extremely bright

Conclusions

- Secondary antibodies are not compatible with this method
- High affinity antibodies with direct fluorescent conjugates can stain cells in as little as 30 seconds
- Of those tested here, low concentrations (1:10,000) of DRAQ5 work best for nuclear staining