How I used up Kevin's reagents:

Tissue-Stain LCM
(Matrigel-embedded organoids)

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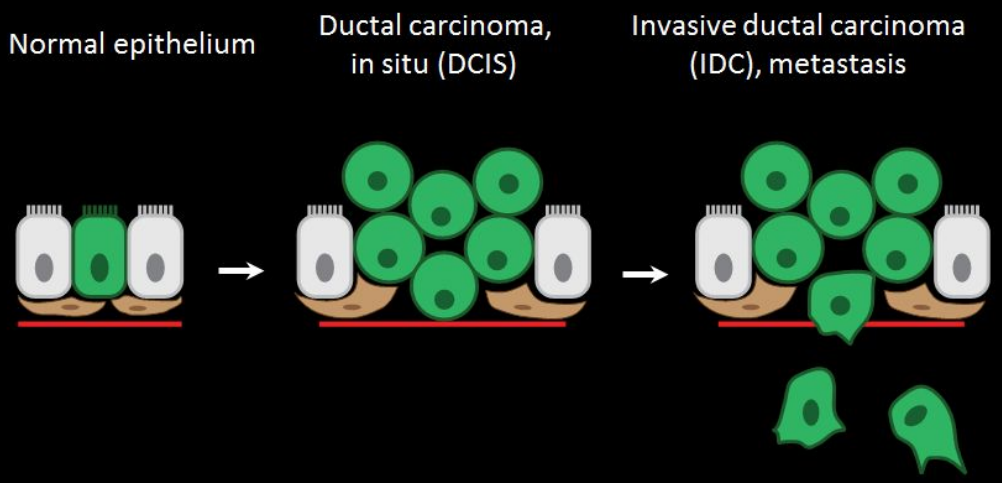
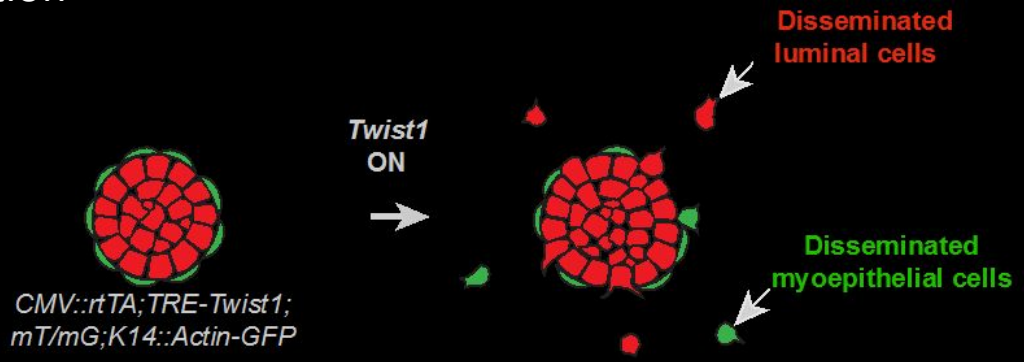
Central Question

- What regulates carcinogenic metastasis?



Preliminary Data

- Twist expression promotes dissemination

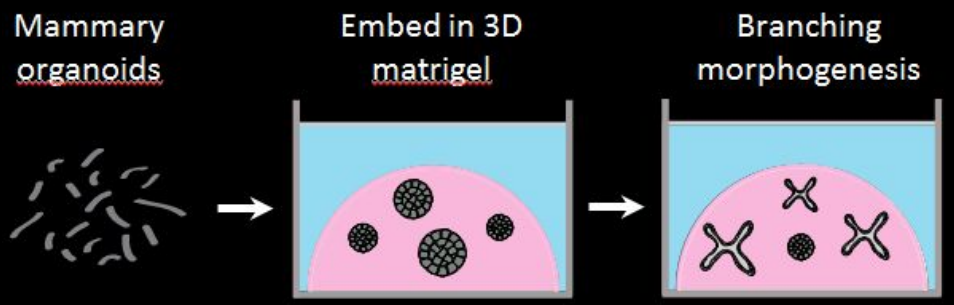


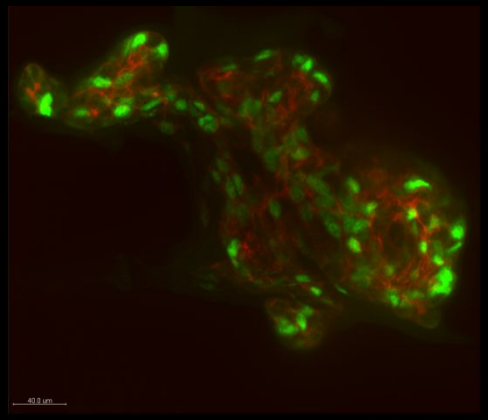
Specific Question

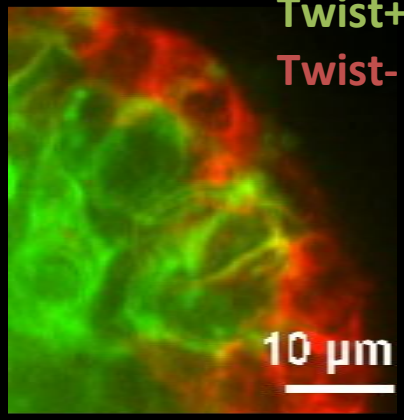
- What is the transcriptomic difference between Twist +/- cells?
- Is there transcriptomic heterogeneity AMONG Twist+ cells

Approach

- Mouse reporter (Green = Twist+) (Red = Twist-) -> Matrigel Culture
- Optimize LCM







Snap-freeze (100% EtOH) → Cryosection → EtOH fixation → Xylene Dehydration → LCM

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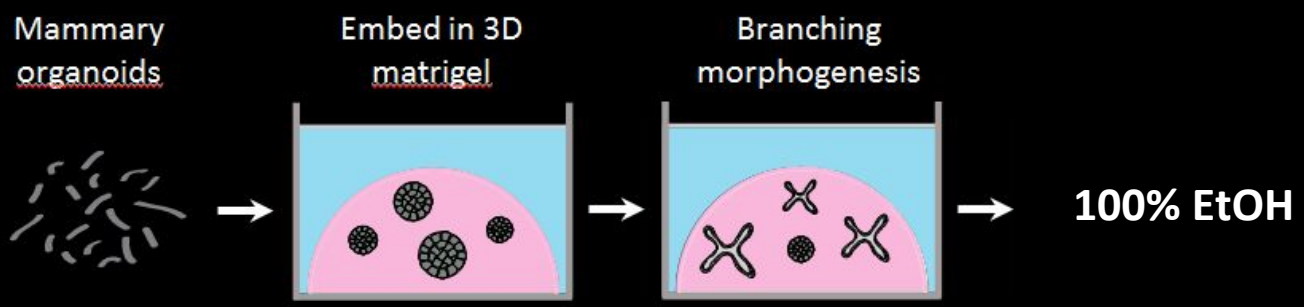
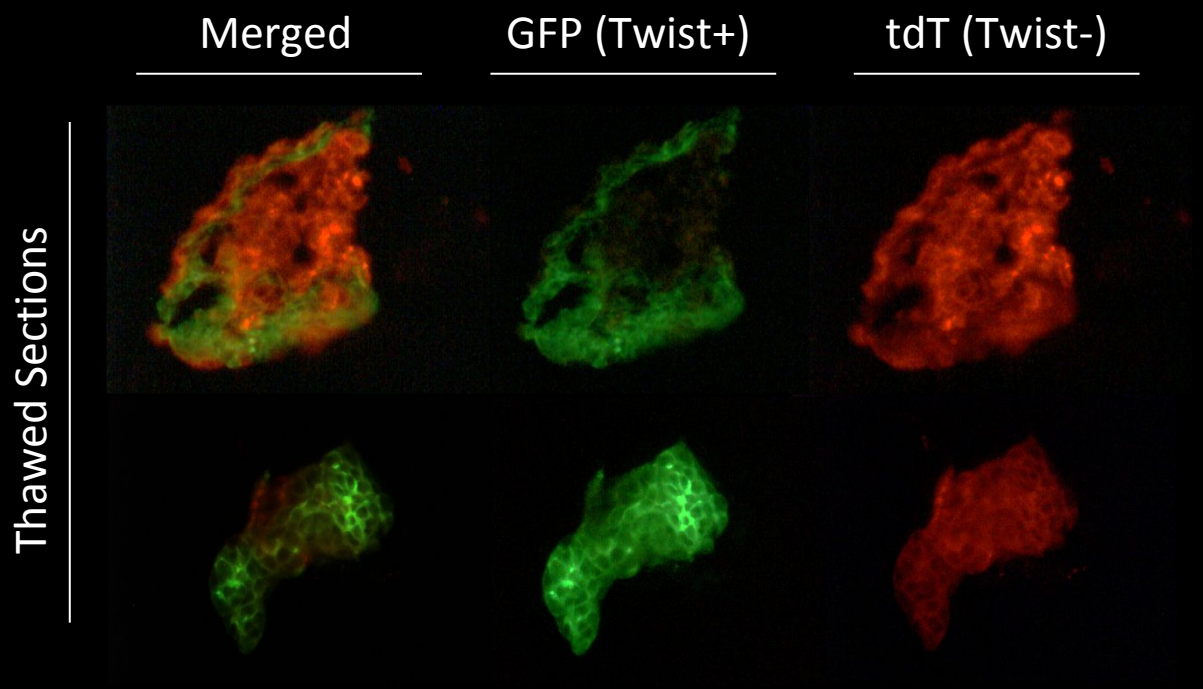
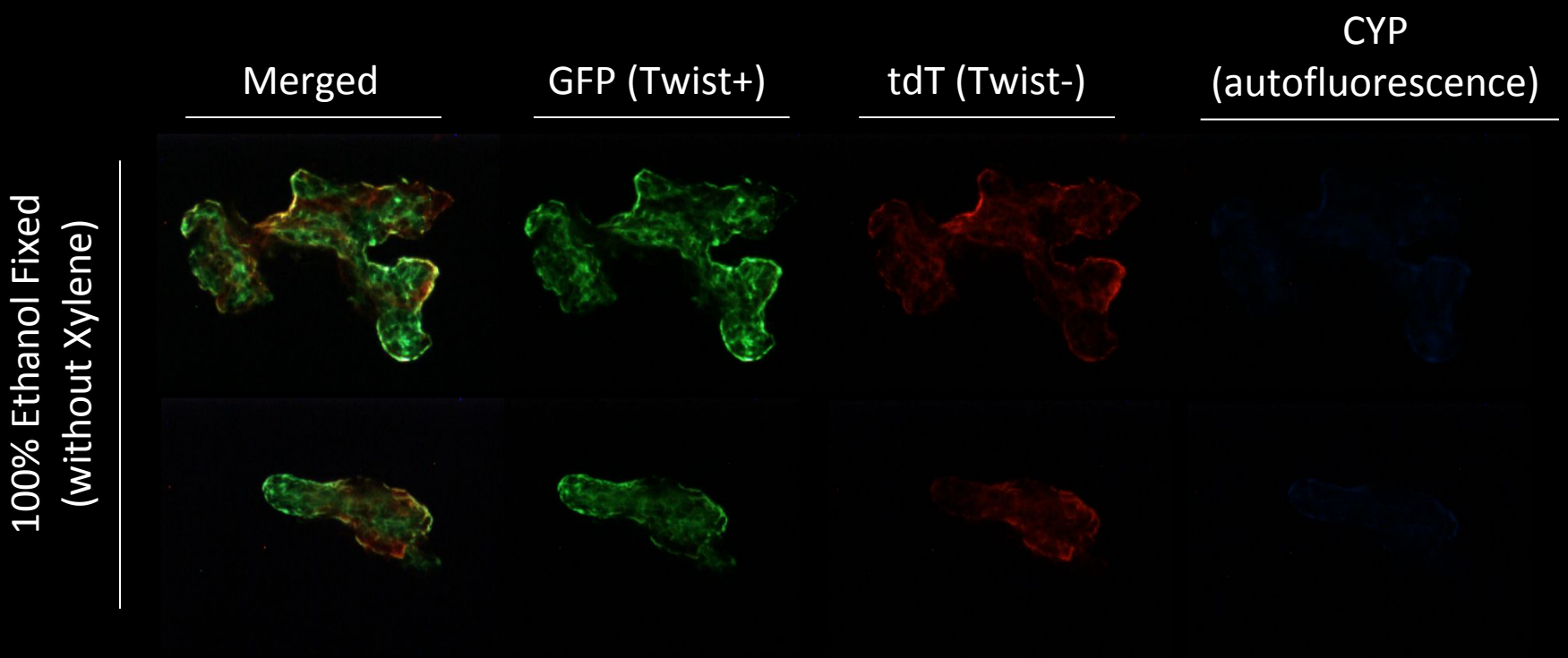


Snap-freeze (100% EtOH) → **Cryosection** → **EtOH fixation** → **Xylene Dehydration** → **LCM**



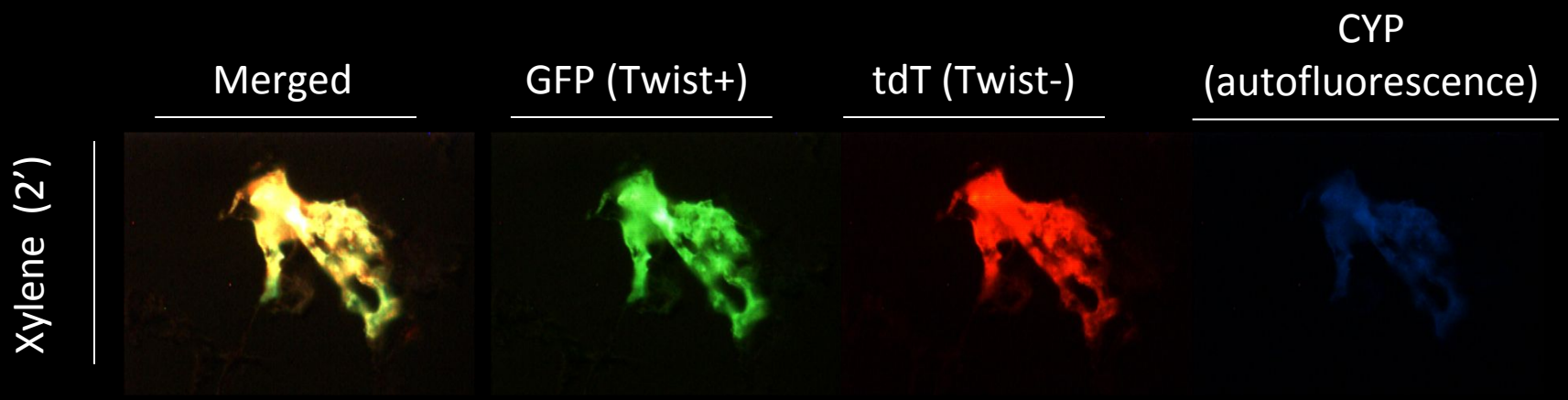
- Snap-Freeze in EtOH + Cryosection preserves fluorescence localization
- Very low cell number and fragmented matrigel upon sectioning

Snap-freeze (100% EtOH) → Cryosection → EtOH fixation → Xylene Dehydration → LCM



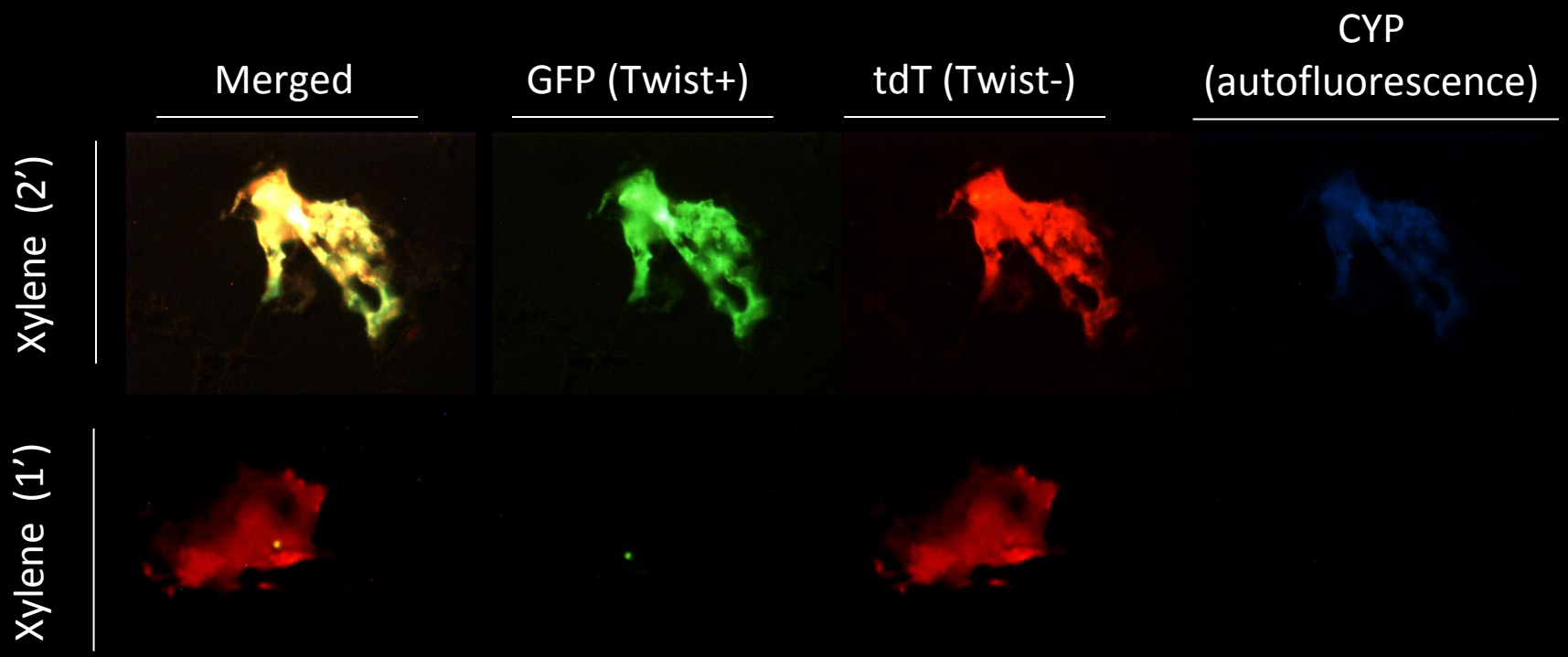
- EtOH fixation protocol preserves fluorescence localization w/ minimal autofluorescence

Snap-freeze (100% EtOH) → Cryosection → EtOH fixation → Xylene Dehydration → LCM



Xylene dehydration compromises fluorescence localization w/ elevated autofluorescence

Snap-freeze (100% EtOH) → Cryosection → EtOH fixation → Xylene Dehydration → LCM

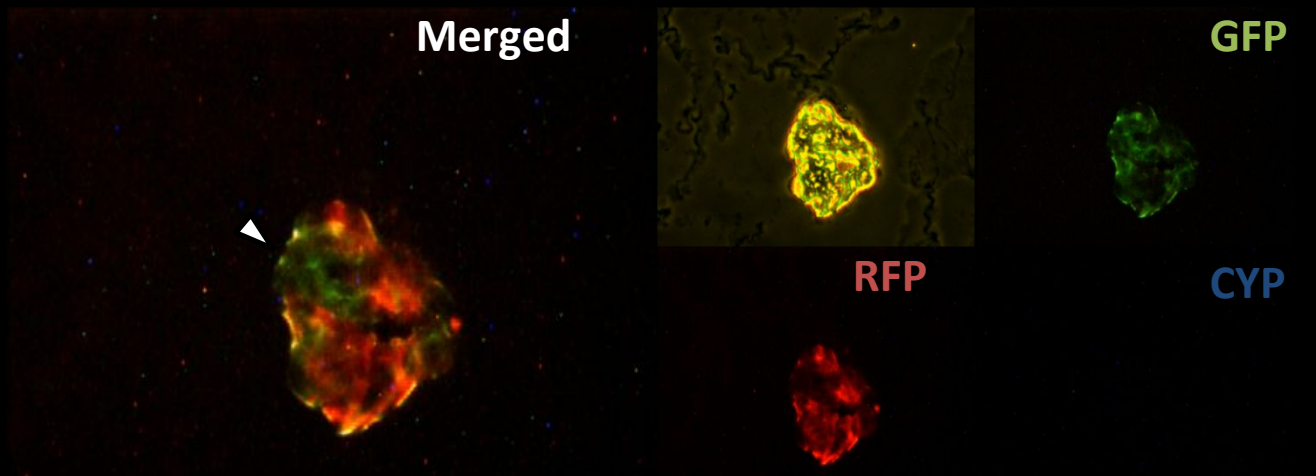


Decreasing Xylene incubation might decrease autofluorescence, but still dampen GFP signal

Can we avoid Xylene dehydration for LCM?

Immunostain to retain cytoplasmic GFP & shorten Xylene incubation

αGFP 30’ (AbCam 50x) w/ xylene (1’)



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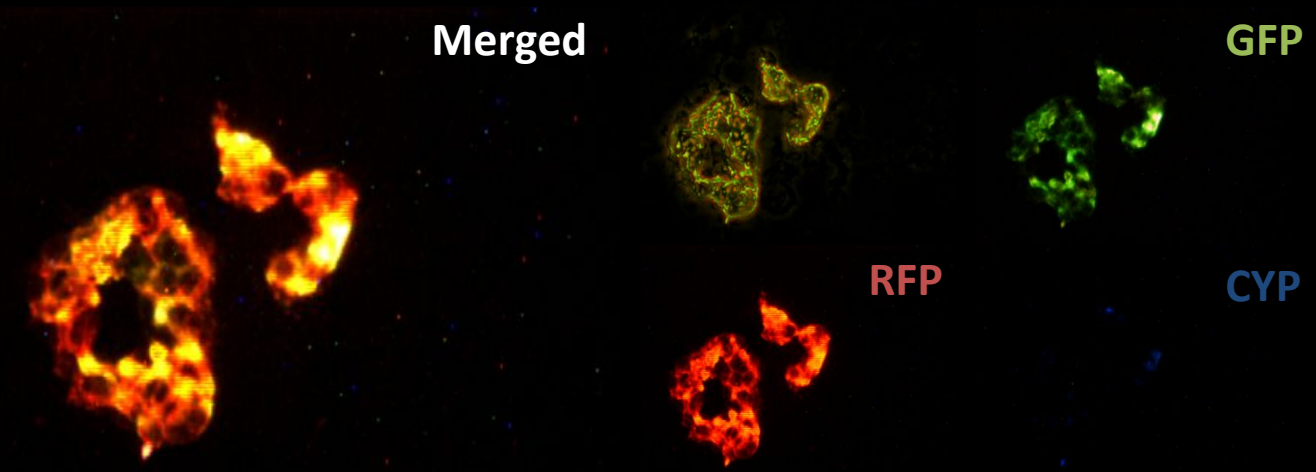


- Immunostain retains GFP localization & shortened Xylene decreases autofluorescence
- Is this dehydrated enough for LCM? (Yet to confirm)

Take-away's from workshop

1. Matrigel plating requires revision
   - culture cells on top of 100% matrigel 'disc' but covered w DMEM + 2% matrigel
   - 'macrodissect' matrigel disc prior to sectioning

2. Cryosectioning protocol was compatible w sample

3. Immunostain with anti-GFP Ab (AbCam 1:10, 10') to preserve GFP signal

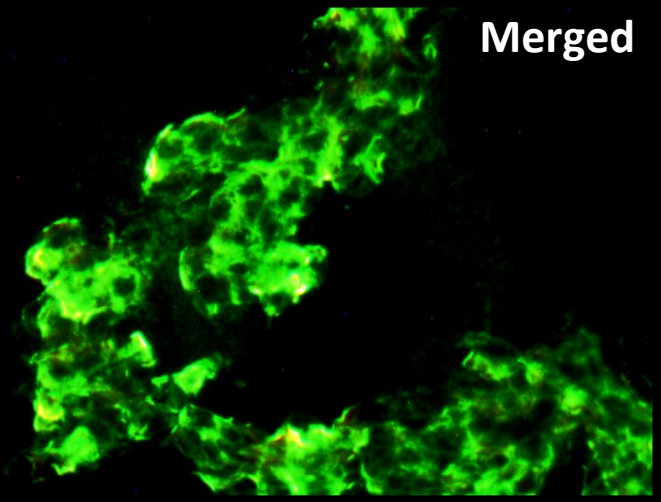
4. EtOH fixation and shortened Xylene dehydration
   - Confirm with LCM (cell count was too low)

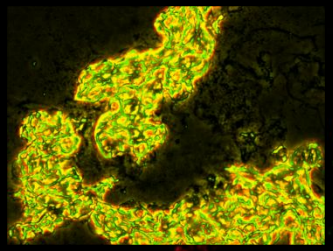
5. Charlottesville > Baltimore

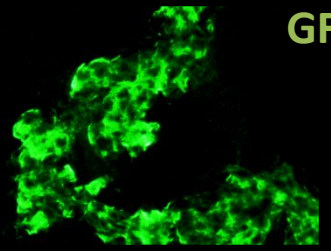
Can we avoid Xylene dehydration for LCM?

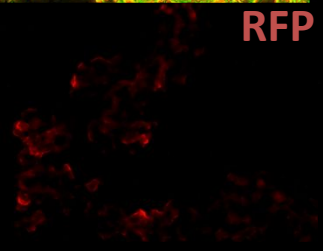
Immunostain to retain cytoplasmic GFP

αGFP 30’ (AbCam 50x)

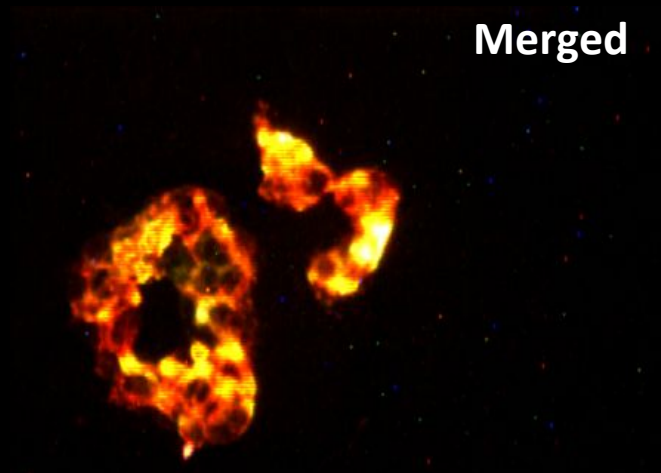


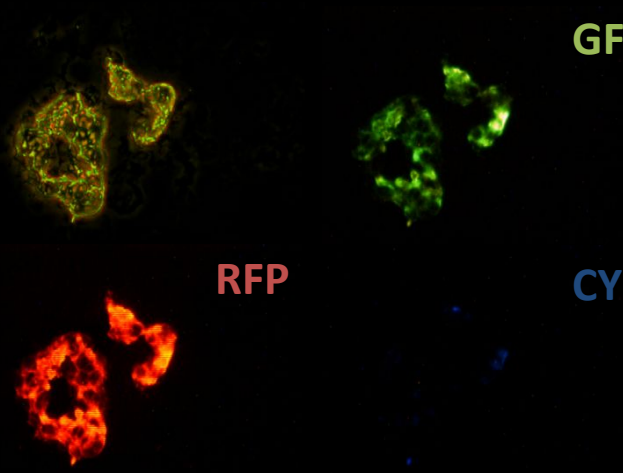




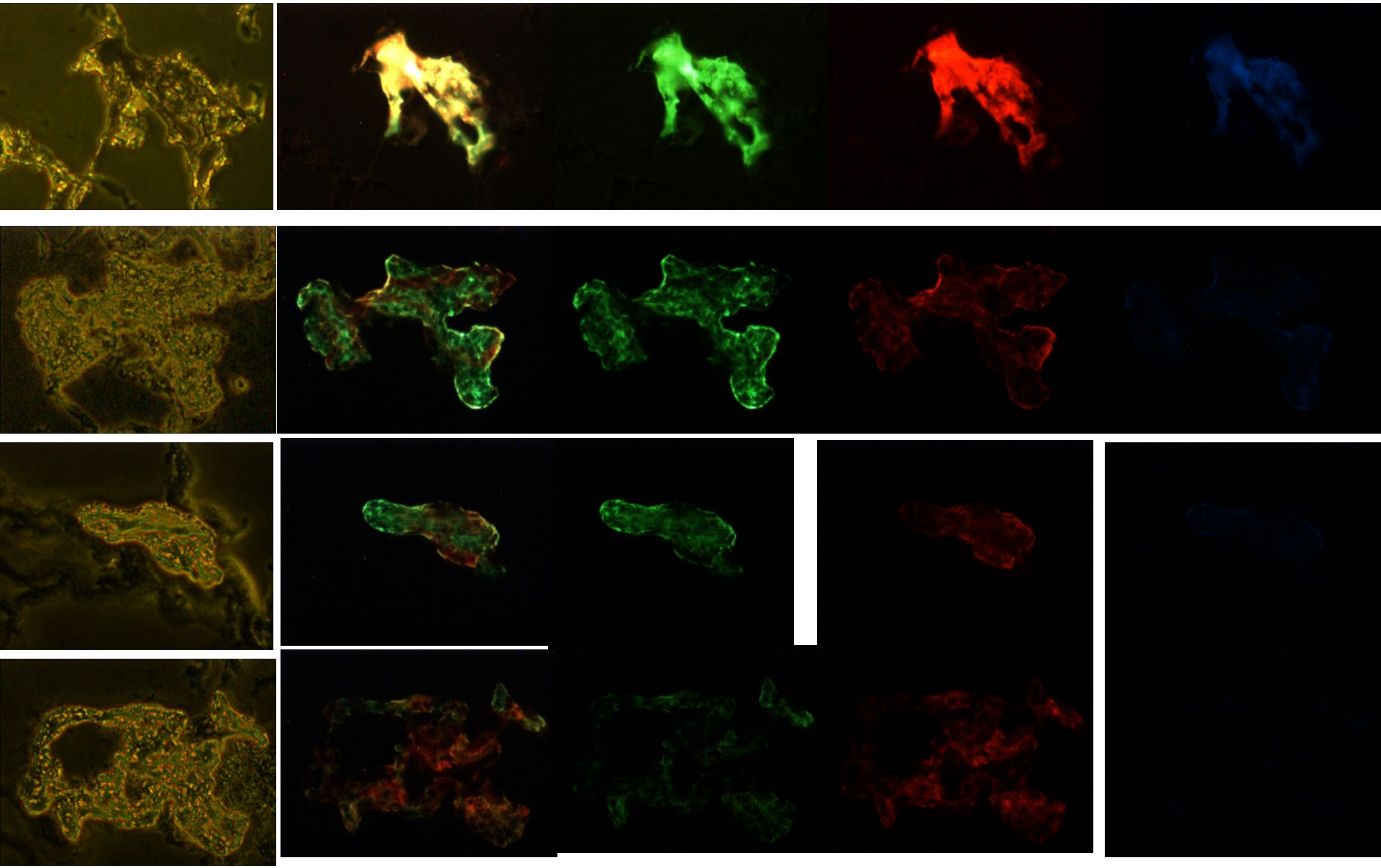


αGFP 30’ (AbCam 50x) w/ xylene



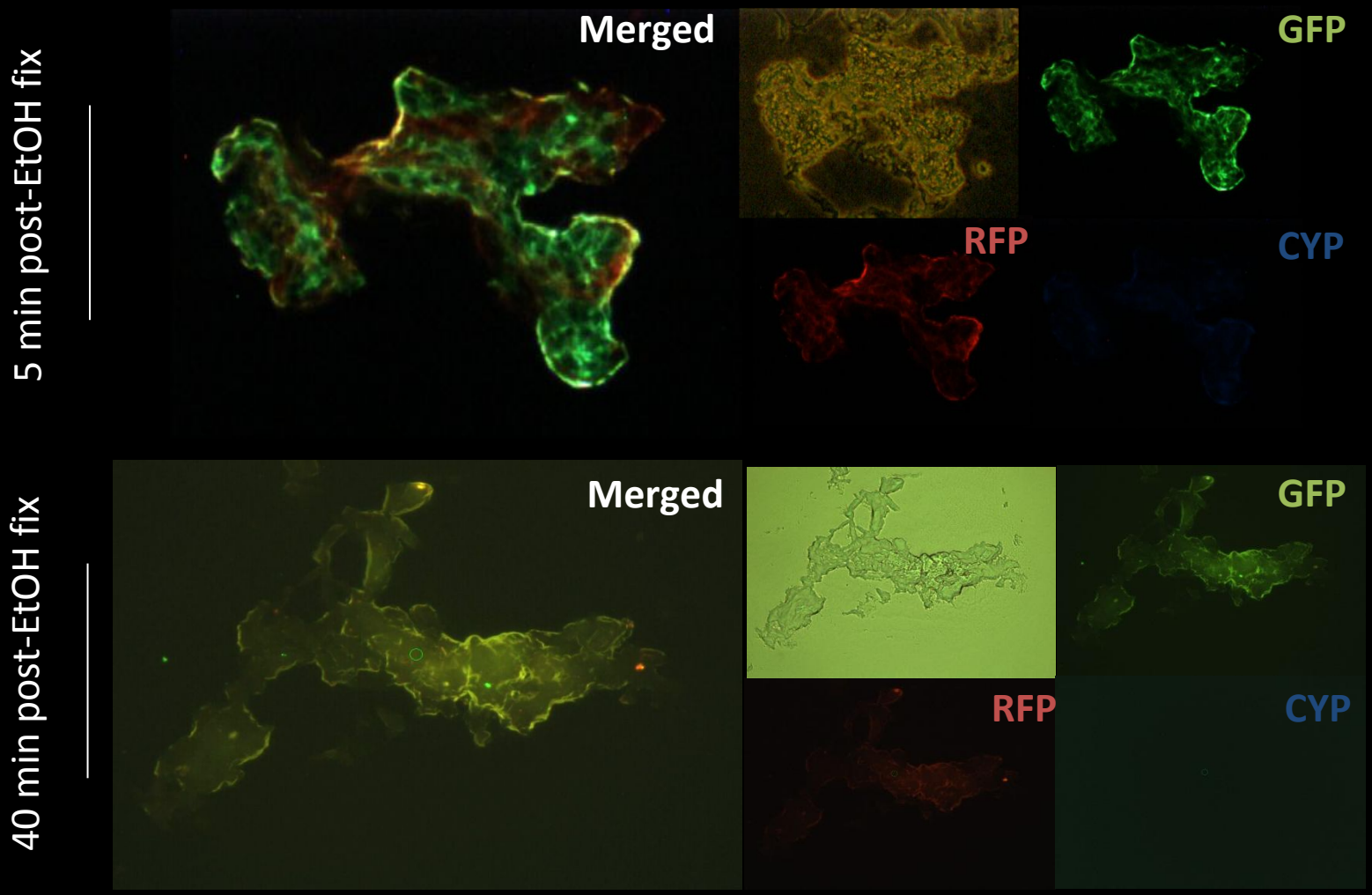


Immunostain retains cytoplasmic GFP & Xylene dampens GFP, but increases autofluorescence



Can we avoid Xylene dehydration for LCM?

Skip Xylene after 100% EtOH fix -> LCM



Possible “pushing” of fluorescent proteins towards periphery secondary to dehydration