Correcting for Systematics in Multiplex Cancer Imaging in the AstroPath Project

Maggie Eminizer, PhD, for the AstroPath team
Institute for Data Intensive Engineering and Science (IDIES)
Johns Hopkins University, Baltimore, MD, USA

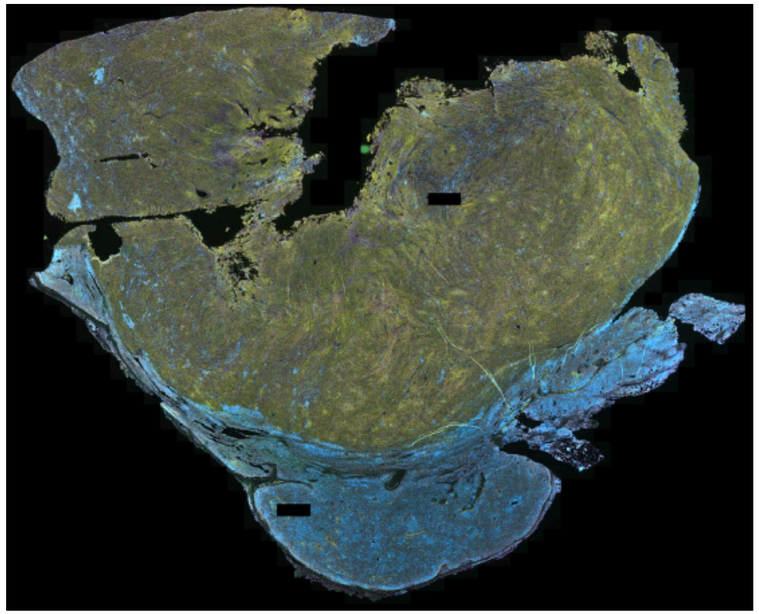
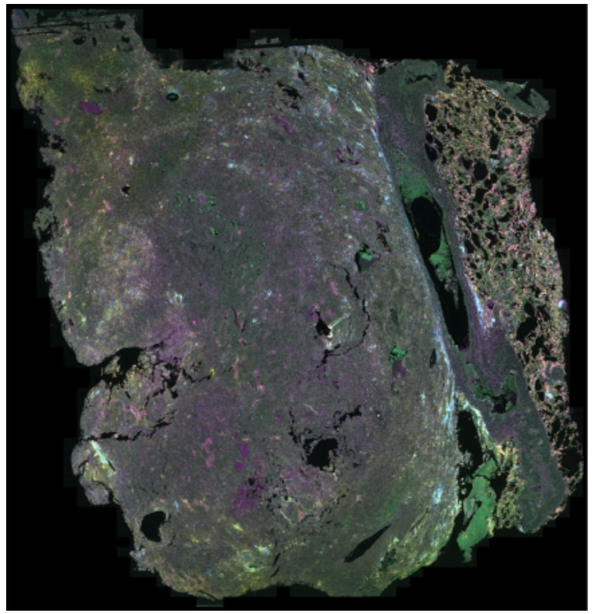
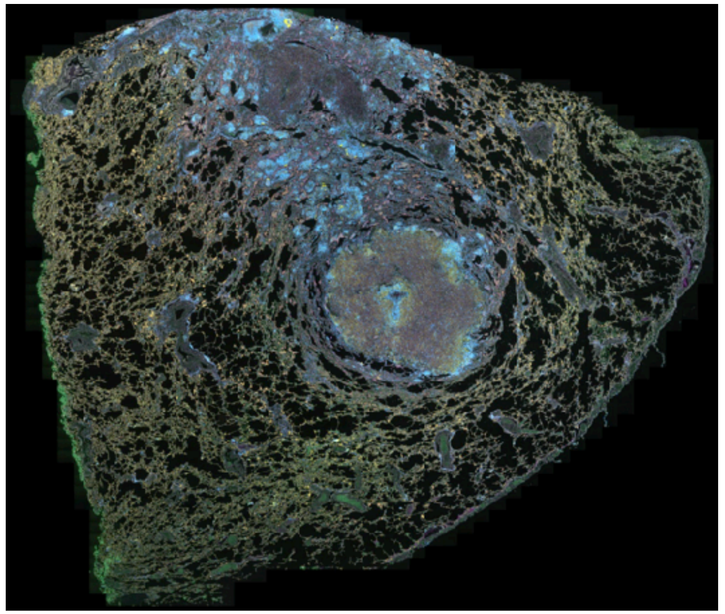
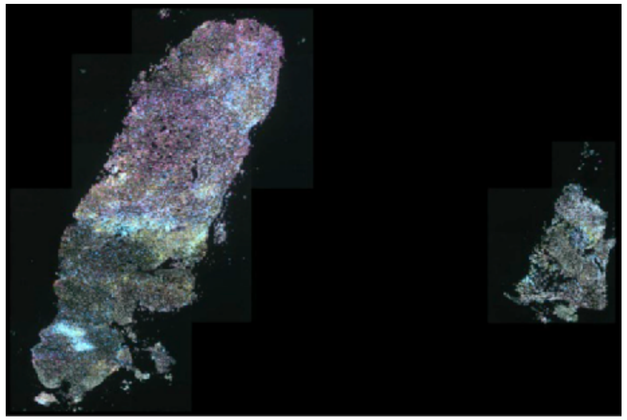
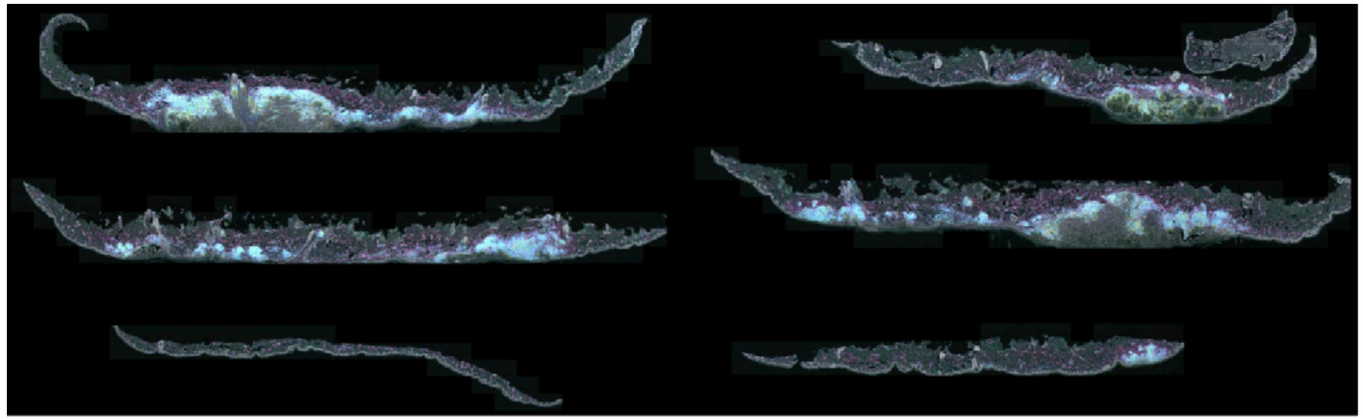
Where the Earth Meets The Sky
Copenhagen, Denmark
May 27th, 2021

Other AstroPath talks from:
- Ben Green (poster earlier today)
- Heshy Roskes (poster earlier today)
- Alex Szalay (talk immediately preceding)
- Joshua Doyle (poster tomorrow)

idies
The Institute for Data Intensive Engineering and Science

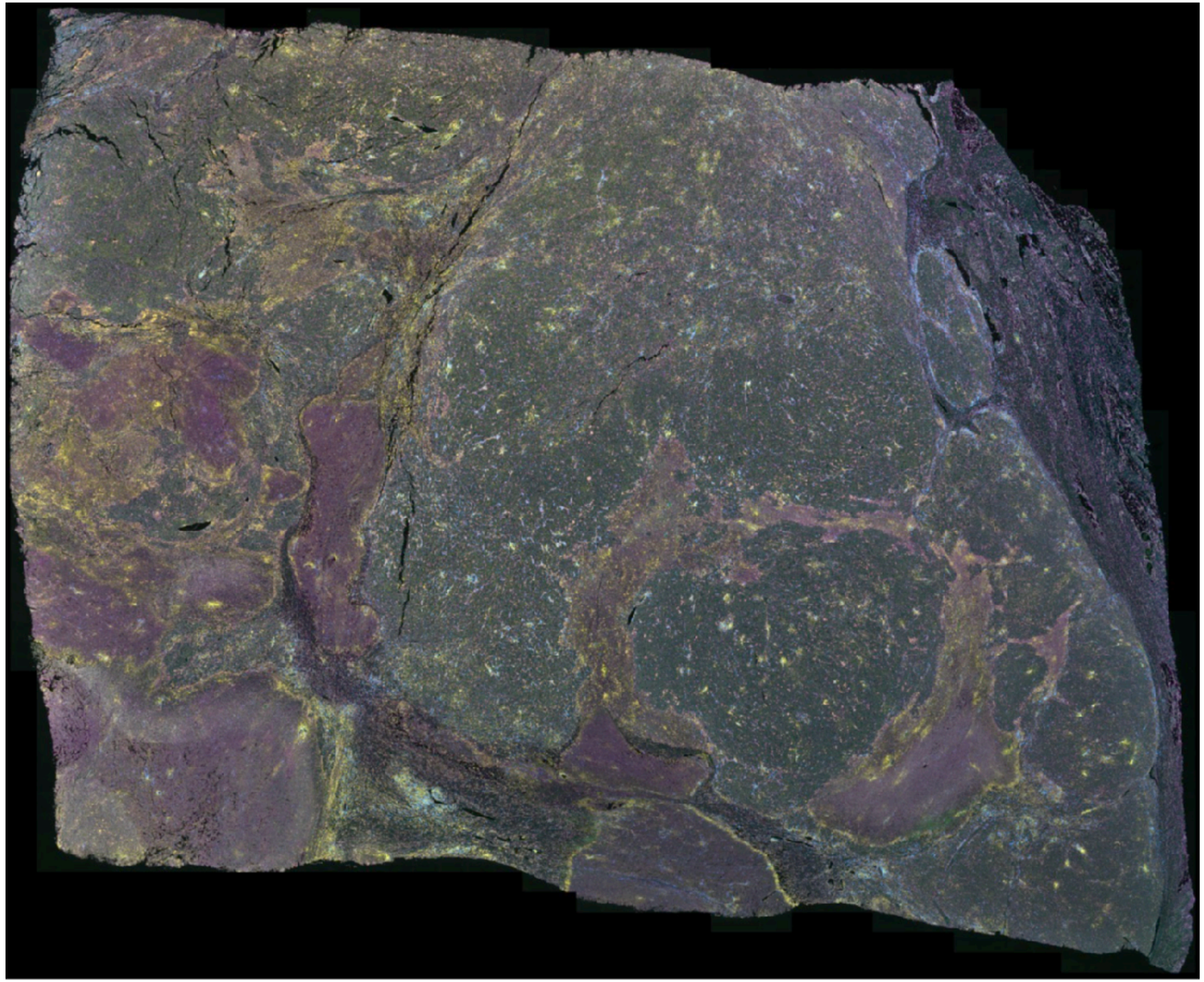
AstroPath Goals and Data

- Curation & analysis of large sets of multispectral immunofluorescence (mIF) microscopy image data
- Apply astronomy & “big data” techniques to cancer pathology

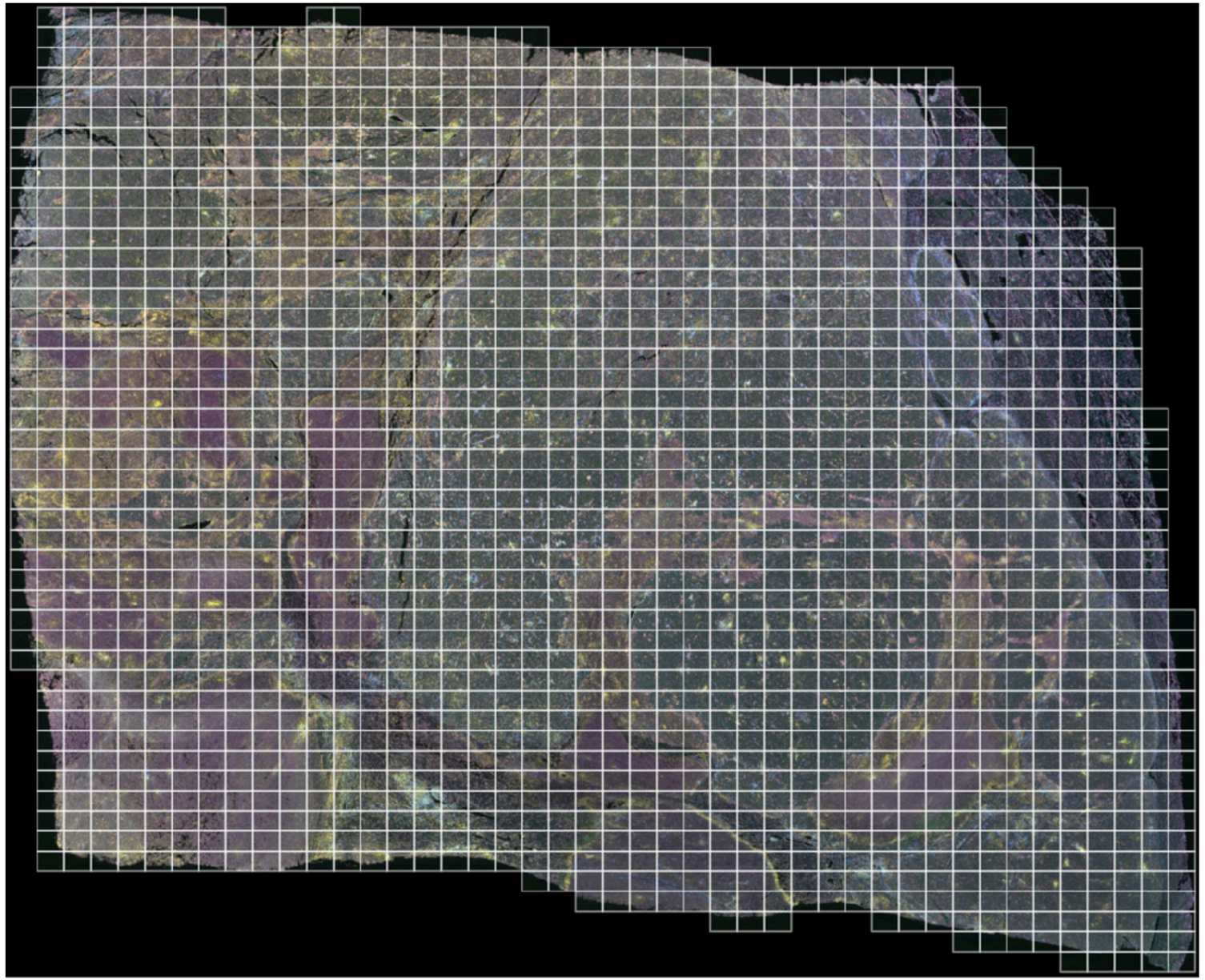
AstroPath Goals and Data

- Tissue specimens imaged w/ Vectra 3 microscope



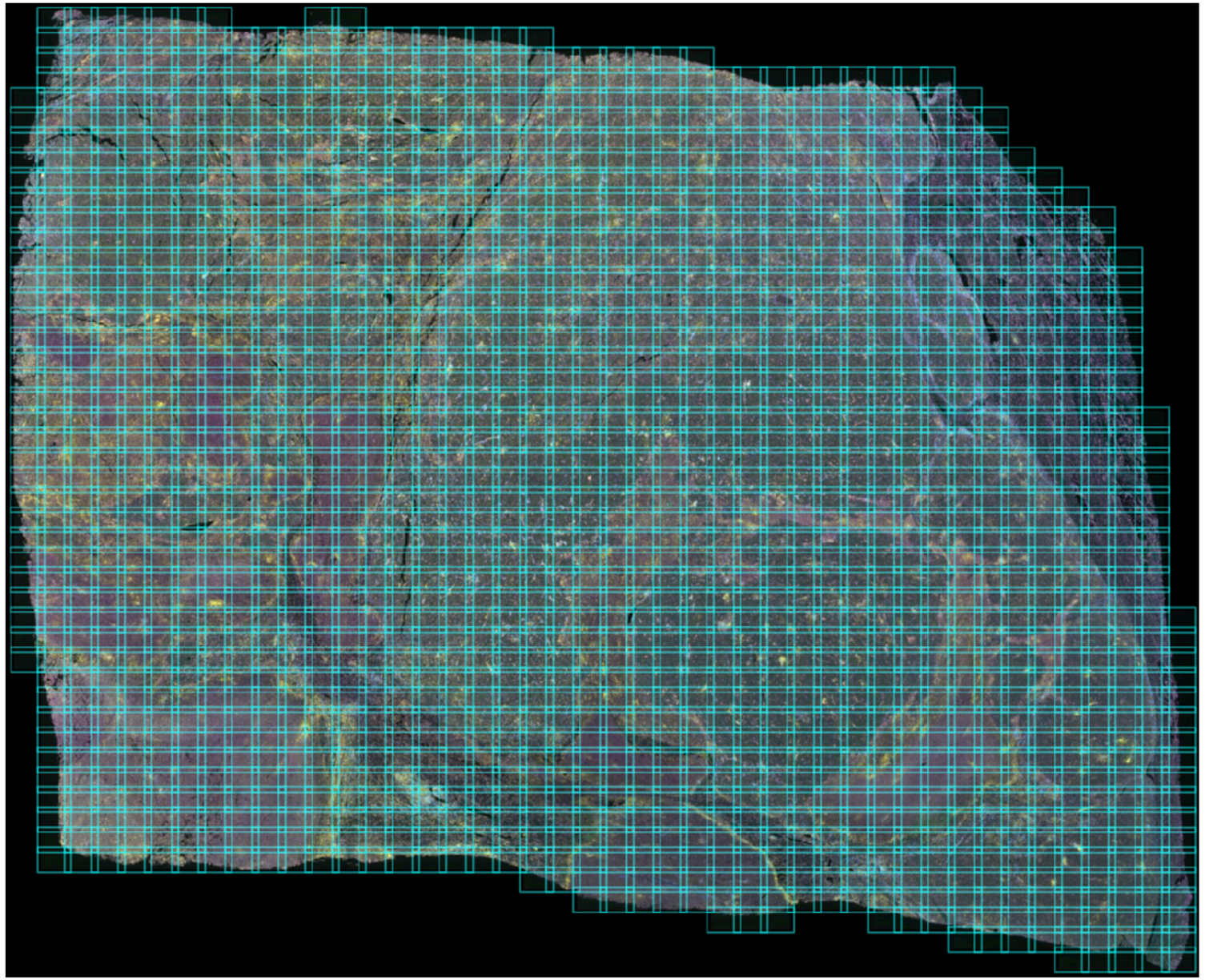
AstroPath Goals and Data

- Tissue specimens imaged w/ Vectra 3 microscope
  - High-power field (HPF) tiles



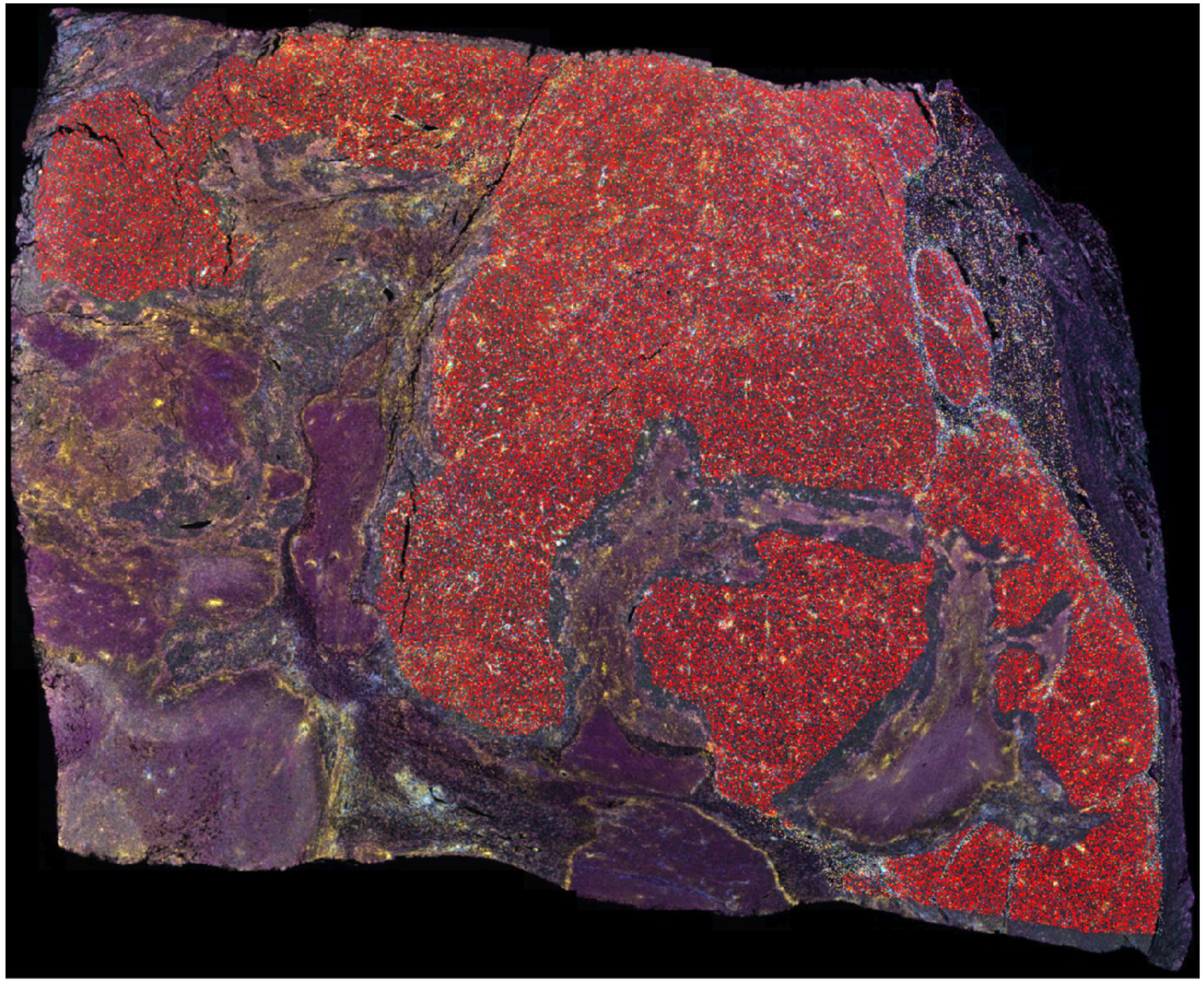
AstroPath Goals and Data

- Tissue specimens imaged w/ Vectra 3 microscope
  - High-power field (HPF) tiles



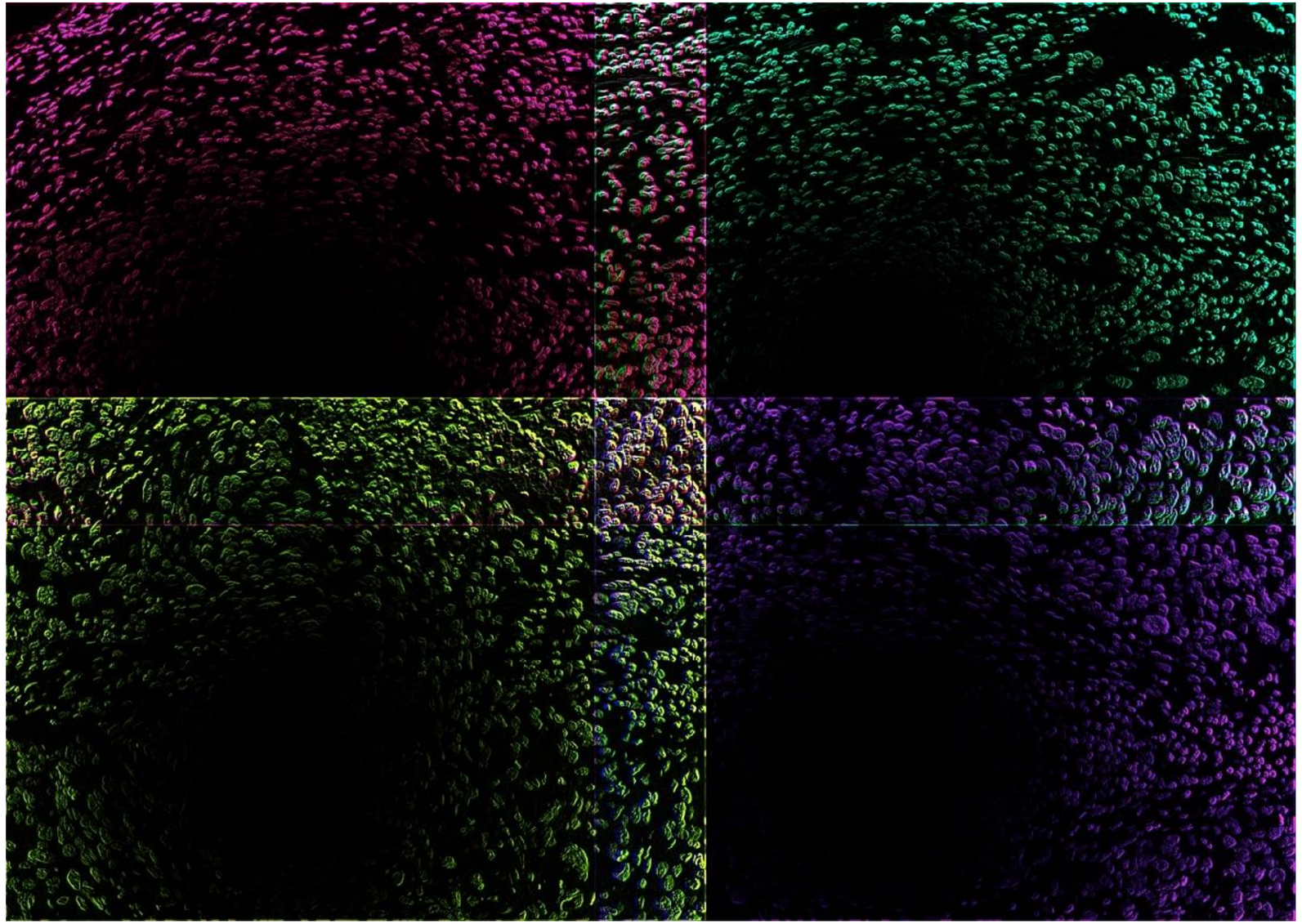
AstroPath Goals and Data

- Tissue specimens imaged w/ Vectra 3 microscope
  - High-power field (HPF) tiles
- Build database for pathology & immunotherapy research
  - Highly automated & quantitative
  - Robust illumination intensity measurements *in situ*



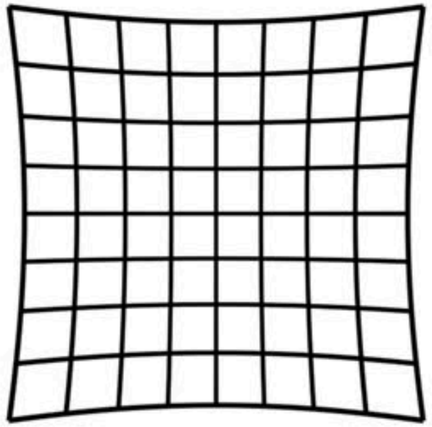
AstroPath Goals and Data

- Correct for systematic effects impacting each HPF
  - Wavelength-dependent warping & illumination variation

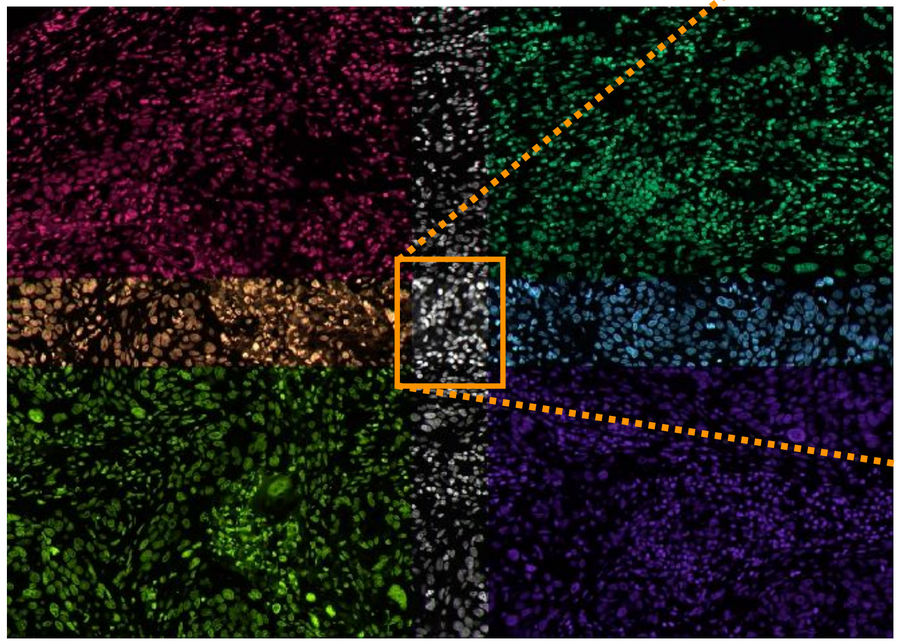


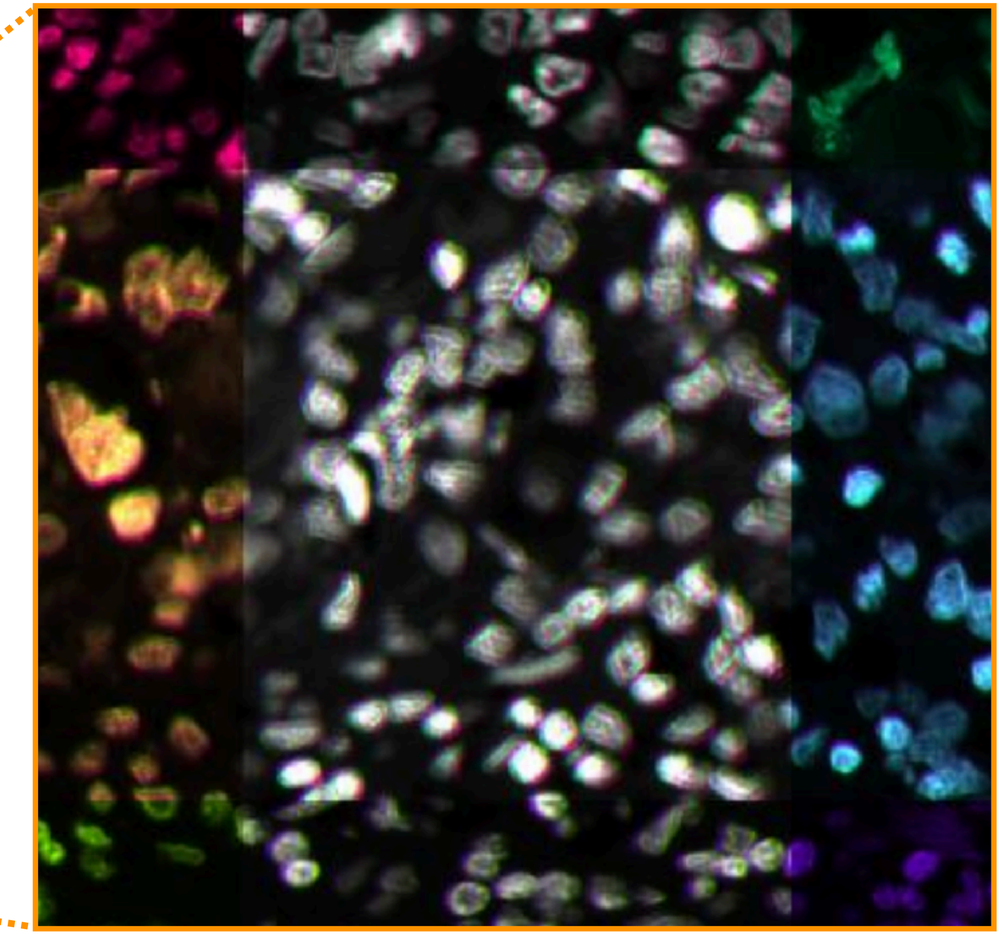
Warping Effects

- Pincushion distortion from objective & camera lenses



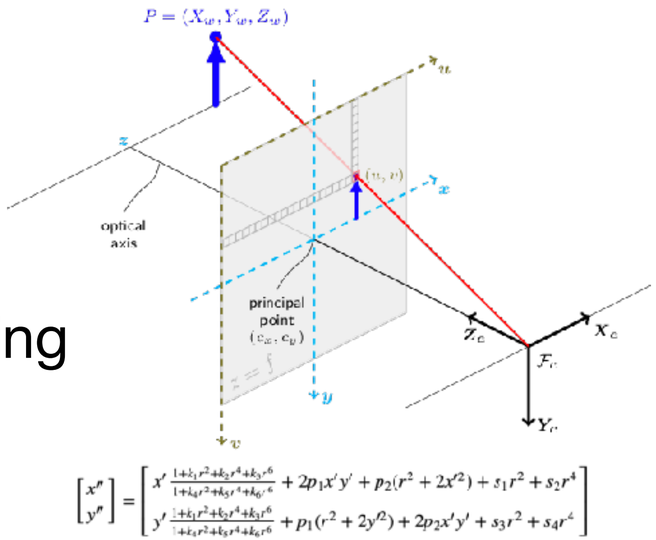
Pincushion Distortion



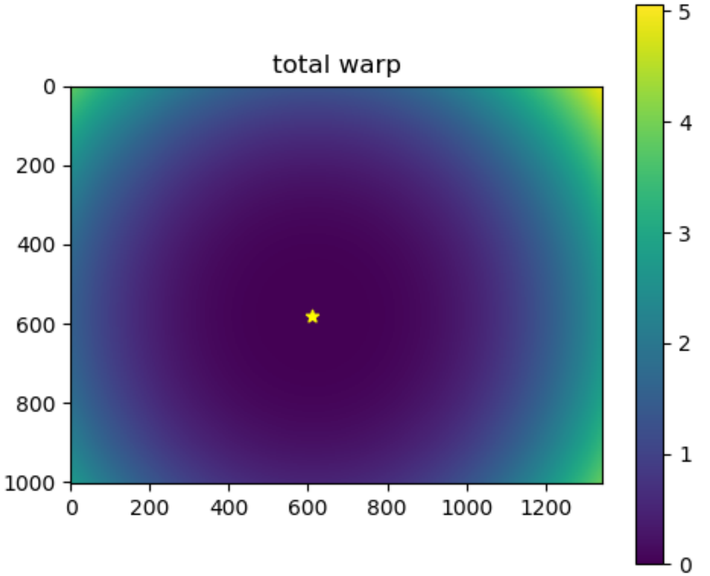


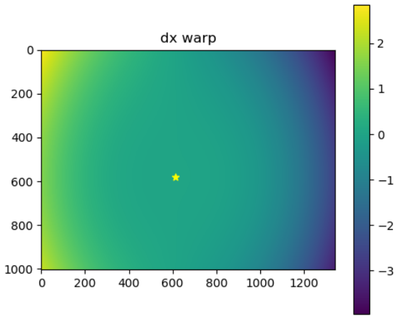
Warping Effects

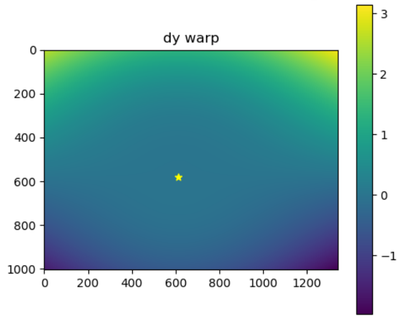
- Pincushion distortion from objective & camera lenses
- Model using OpenCV camera calibration
  - camera matrix + radial distortion parameters
- Minimize MSE between overlapping image regions

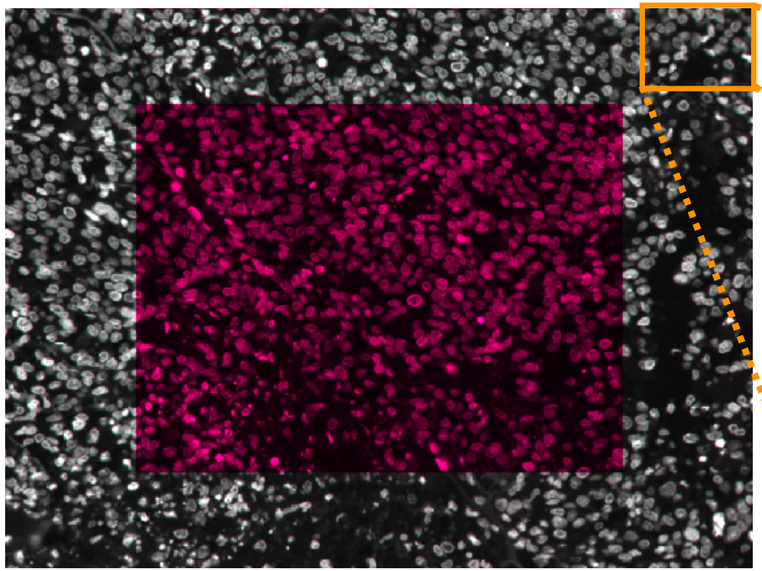


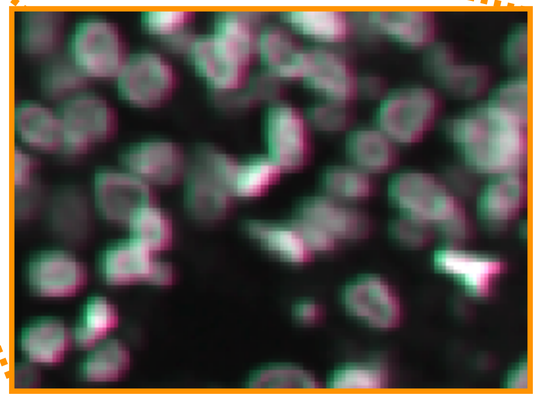
$$\begin{bmatrix} x'' \\ y'' \end{bmatrix} = \begin{bmatrix} x' \frac{1+k_1 r^2+k_2 r^4+k_3 r^6}{1+k_4 r^2+k_5 r^4+k_6 r^6} + 2p_1 x' y' + p_2(r^2+2x'^2) + s_1 r^2 + s_2 r^4 \\ y' \frac{1+k_1 r^2+k_2 r^4+k_3 r^6}{1+k_4 r^2+k_5 r^4+k_6 r^6} + p_1(r^2+2y'^2) + 2p_2 x' y' + s_3 r^2 + s_4 r^4 \end{bmatrix}$$



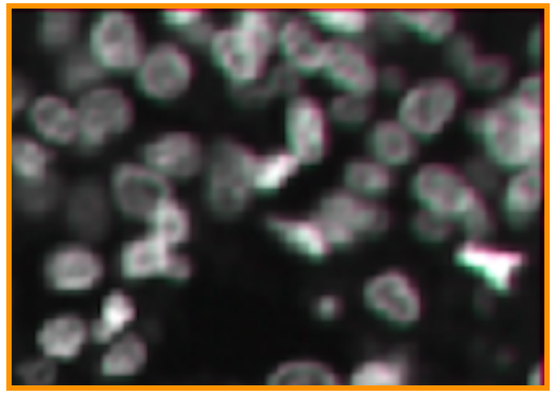




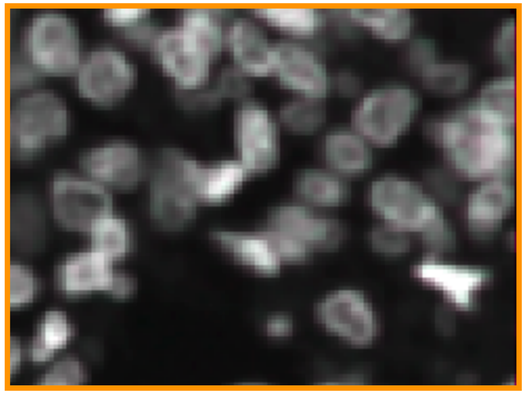




raw, not aligned



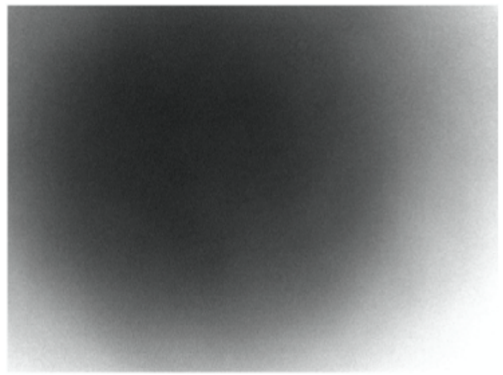
aligned (only) MSE=0.010

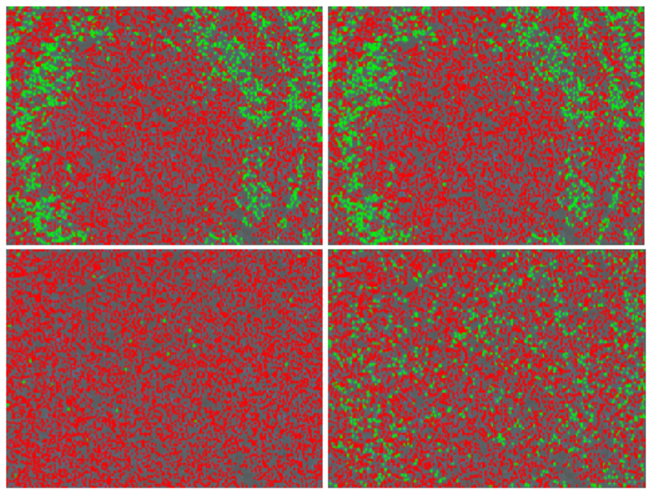


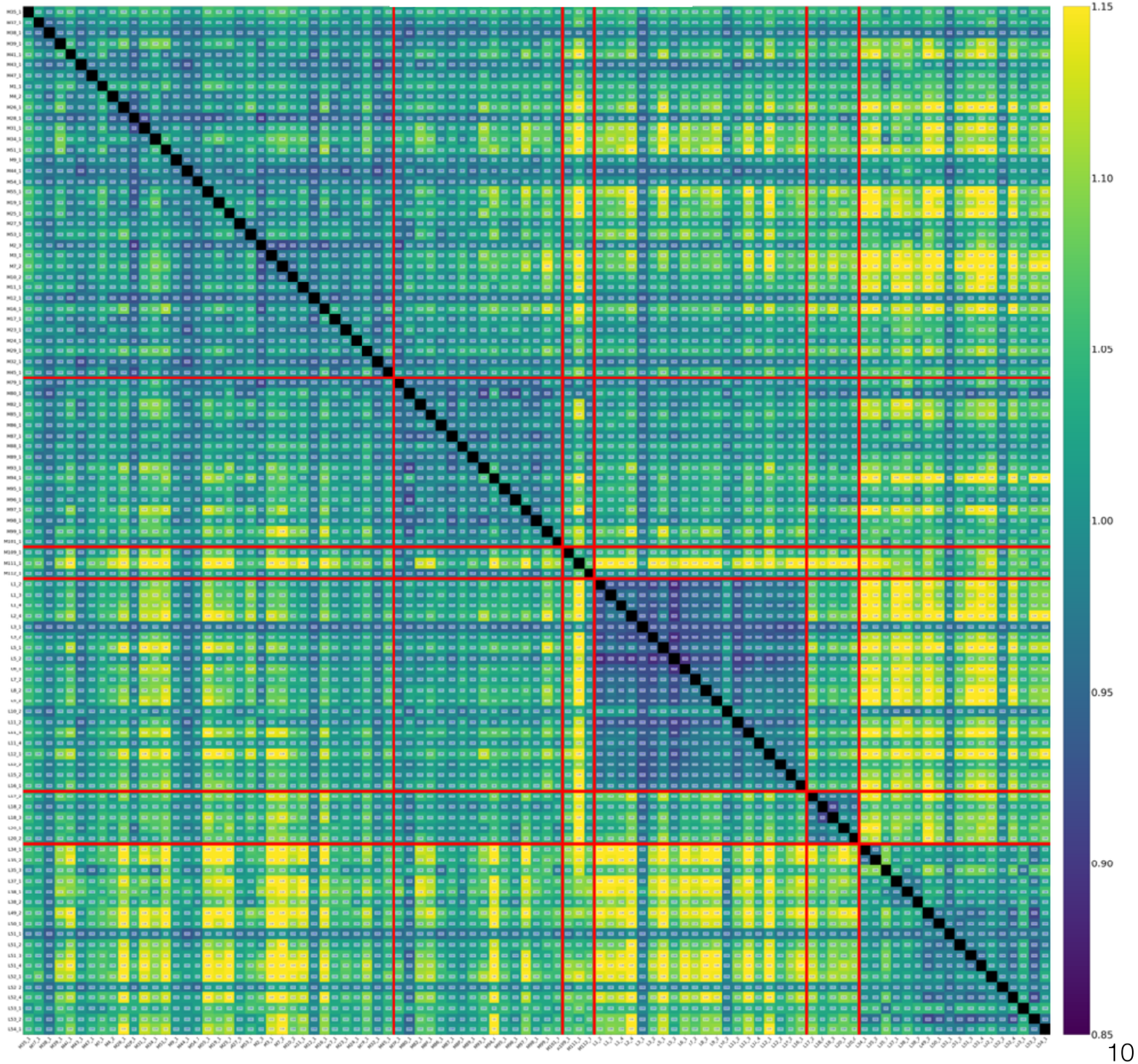
unwarped + aligned MSE=0.003

Flatfielding Effects

- Spatial variation in HPF illumination
  - “vignetting”
  - systematically bright regions (~10%)
  - wavelength-dependent
- Why is it important to measure?
  - Impacts quantitative analyses/illumination
  - Changes over time/per-microscope

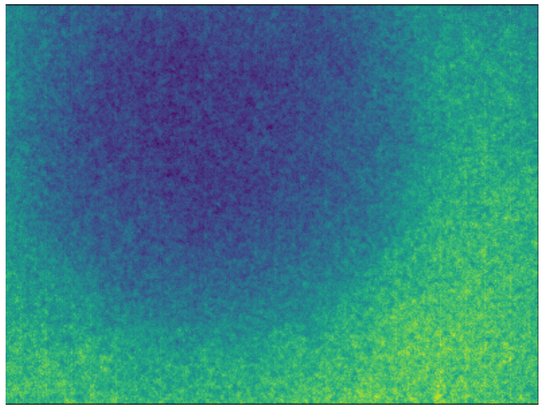




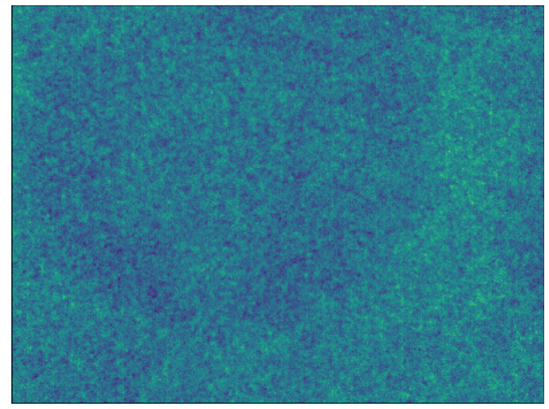


Flatfielding Effects

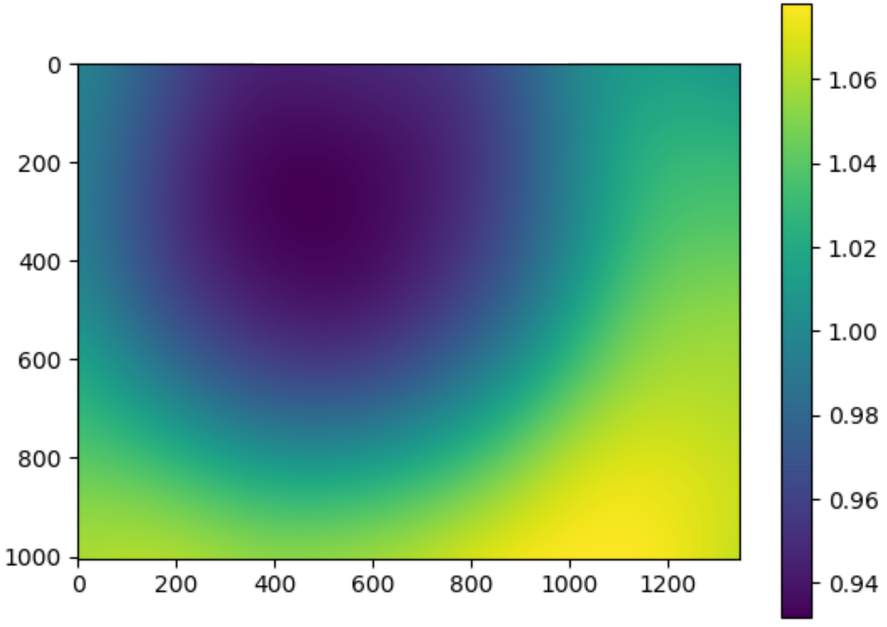
- How do we correct for it?
  - Find mean illumination pattern in each layer
  - Derive/apply correction factors to each HPF



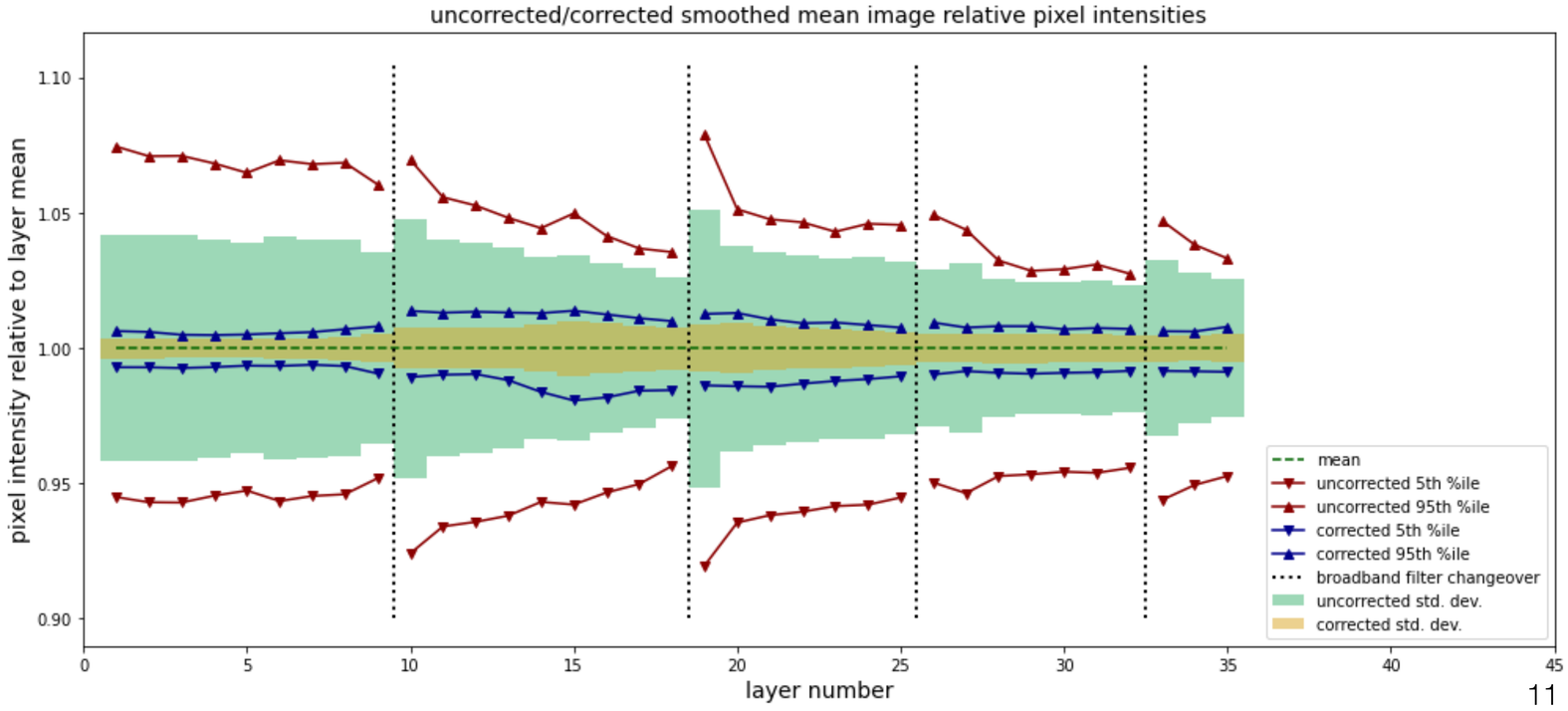
mean of ~21,000 uncorrected images



mean of ~21,000 corrected images

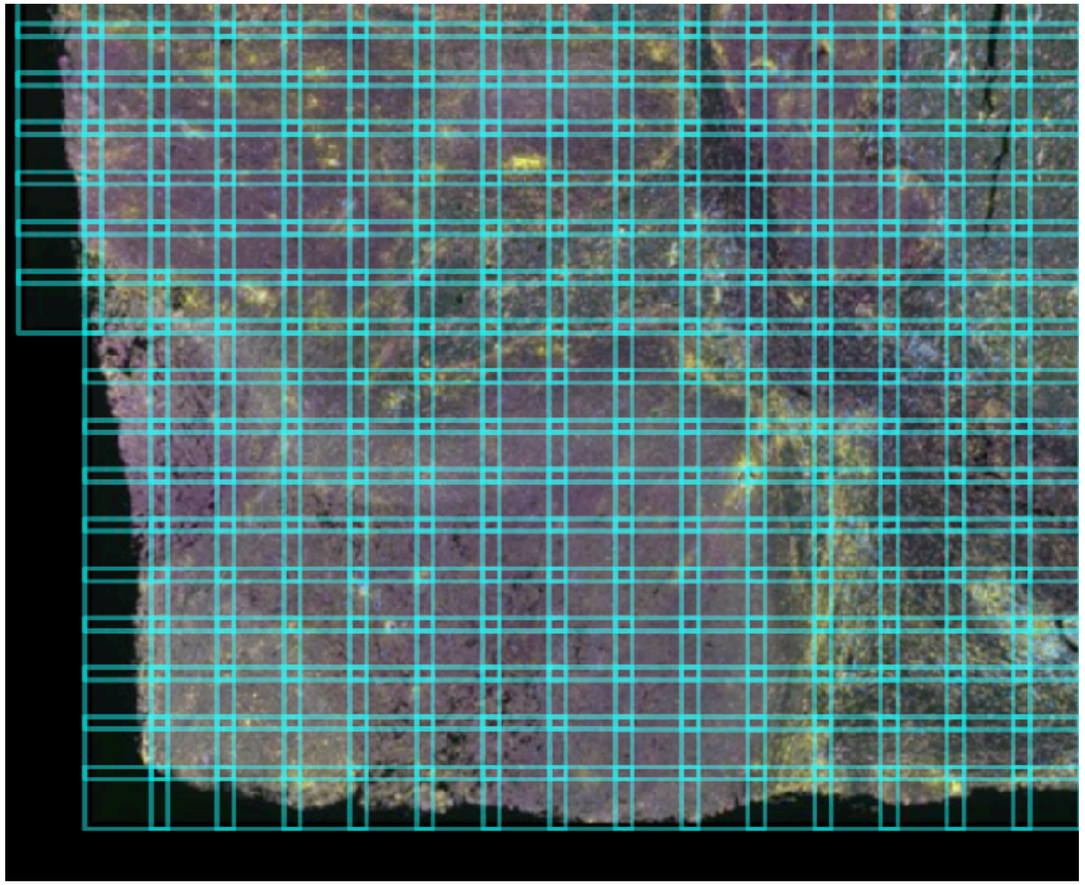


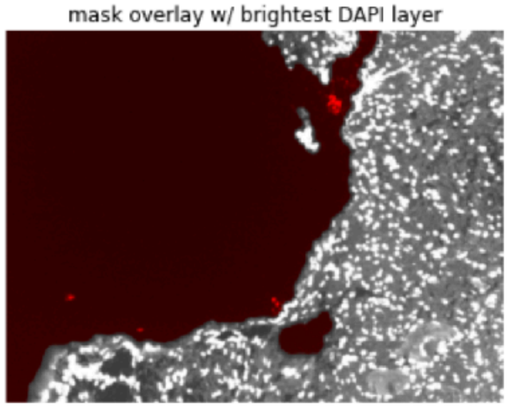
flatfield correction factors

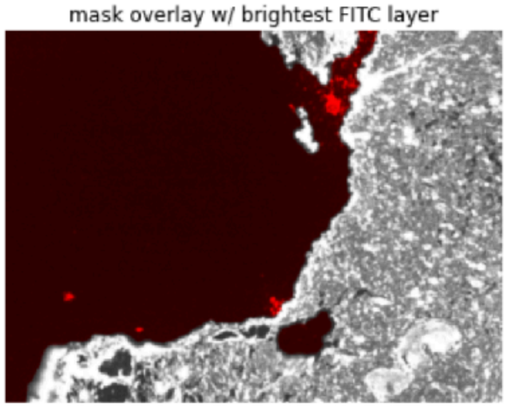


Flatfielding Effects

- Practical considerations
  - Leave out empty background

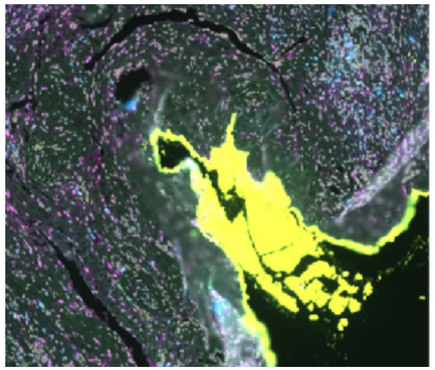
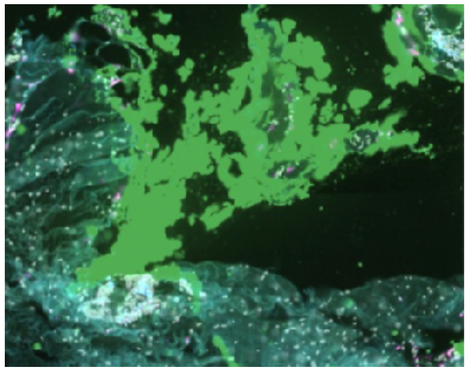
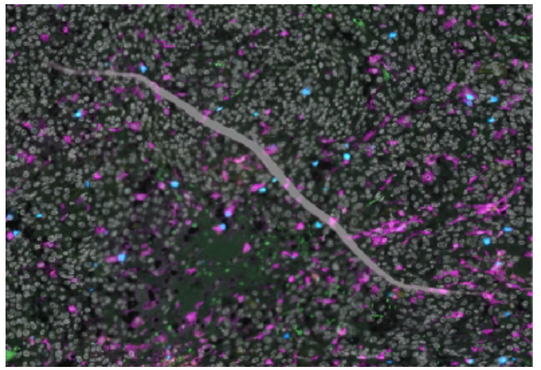
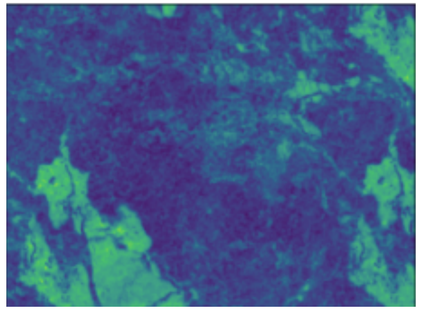
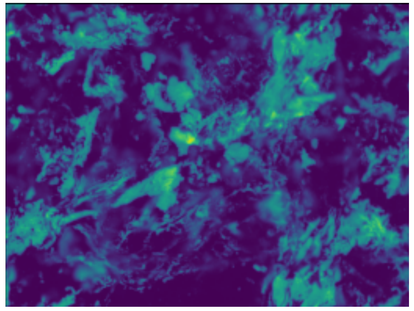
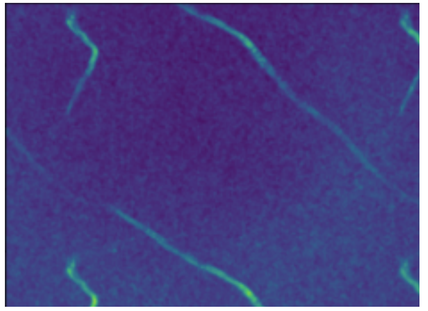
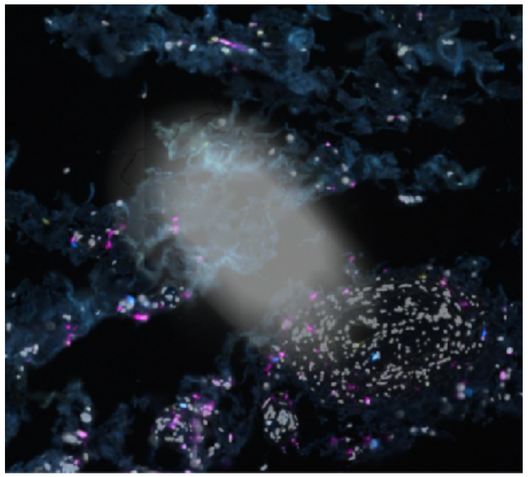
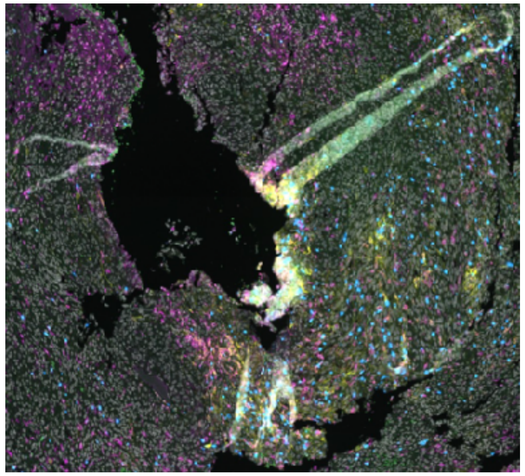






Flatfielding Effects

- Practical considerations
  - Leave out empty background
  - Image artifacts
- Image masks
  - Improve corrections
  - Useful to have in the database

flatfield model	uncorrected illumination variation	corrected illumination variation	reduction in illumination variation
no masking	11.6%	3.20%	**72.4%**
with masking	10.6%	1.95%	**81.6%**

Summary

- Use raw mIF microscopy data to measure and correct for systematic warping and illumination variation effects
- Automatically create masks to remove empty background and other artifacts from individual images
- Bring together large sets of microscopy data for cancer pathology and immunotherapy research in new ways

Acknowledgments

Principal Investigators

Prof. Alexander Szalay & Dr. Janis Taube, MD, MSc

Taube Lab

- Sneha Berry, PhD
- Ben Green
- Liz Engle, MS
- Tricia Cottrell, MD, PhD
- Haiying Xu
- Aleksandra Ogurtsova
- Nicholas Giraldo, MD, PhD

IDIES

- Heshy Roskes, PhD
- Richard Wilton, MD
- Josh Doyle, MD
- Sahil Hamal
- Dmitry Medvedev
- Nate Eisenberg (undergrad)

AI / Computer Vision

- Prof. Alan Yuille
- Seyoun Park, PhD
- Yixiao Zhang (PhD student)

Other Collaborators

- BKI
  - Drew Pardoll, MD, PhD
  - Robert Anders, MD, PhD
  - Suzanne Topalian, MD
  - Evan Lipson, MD
- Akoya
  - Cliff Hoyt, MS
  - Chi Wang
- Bristol Myers Squibb
  - Robin Edwards, MD

JOHNS HOPKINS UNIVERSITY & MEDICINE

idies The Institute for Data Intensive Engineering and Science

The Mark Foundation® for Cancer Research