Correcting for Systematics in Multiplex Cancer Imaging in the AstroPath Project

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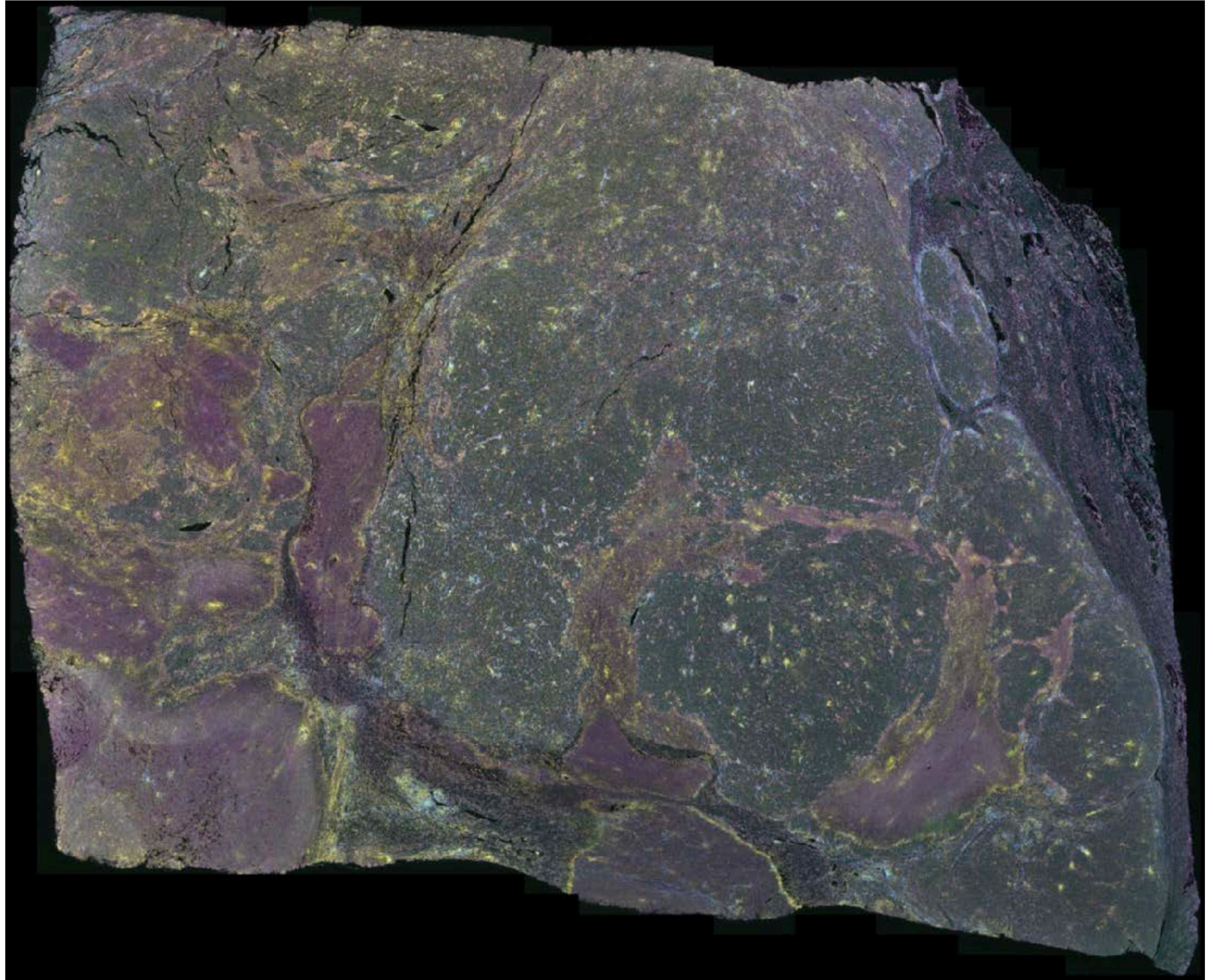- Joshua Doyle (poster tomorrow)

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

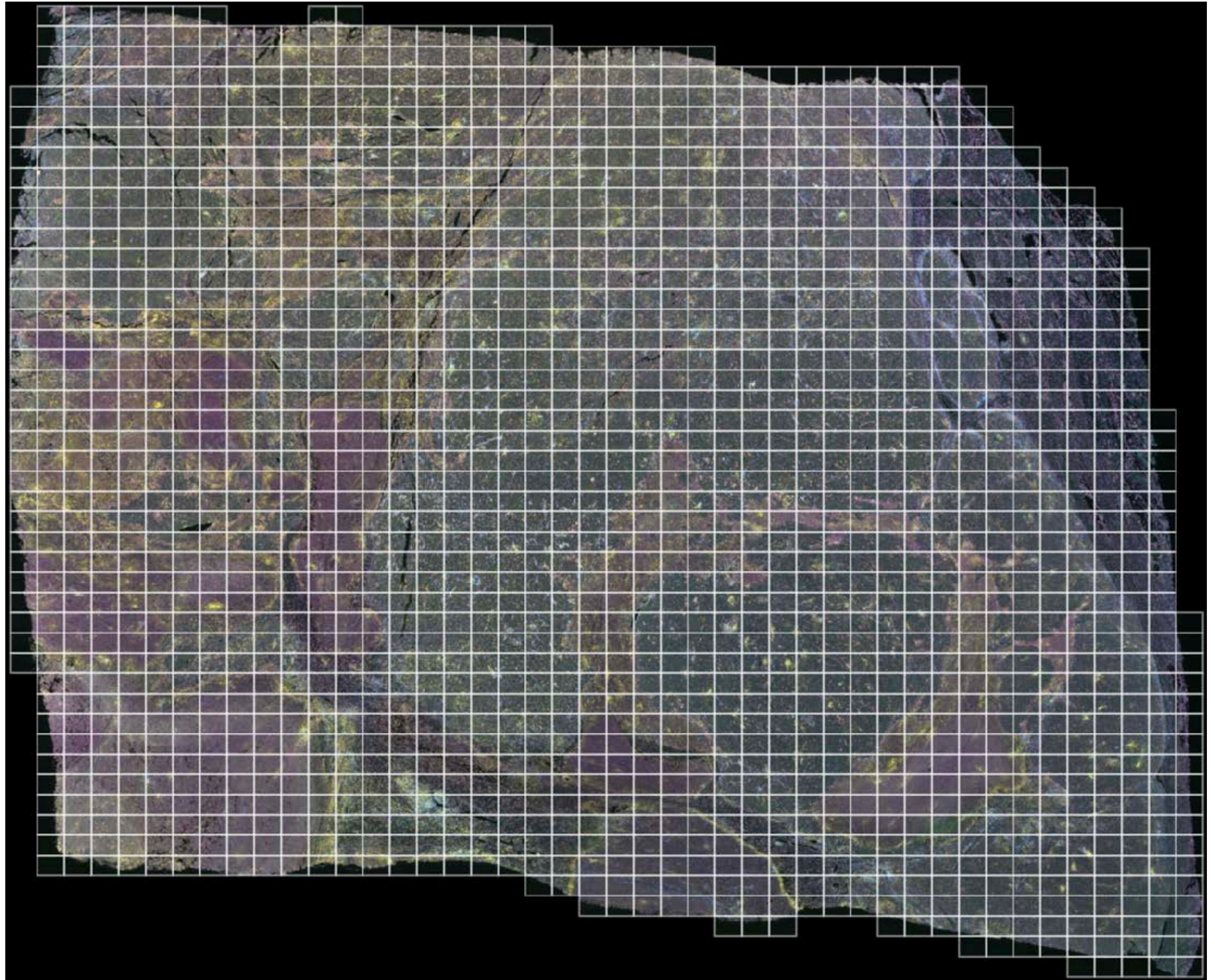- Apply astronomy & "big data" techniques to cancer pathology
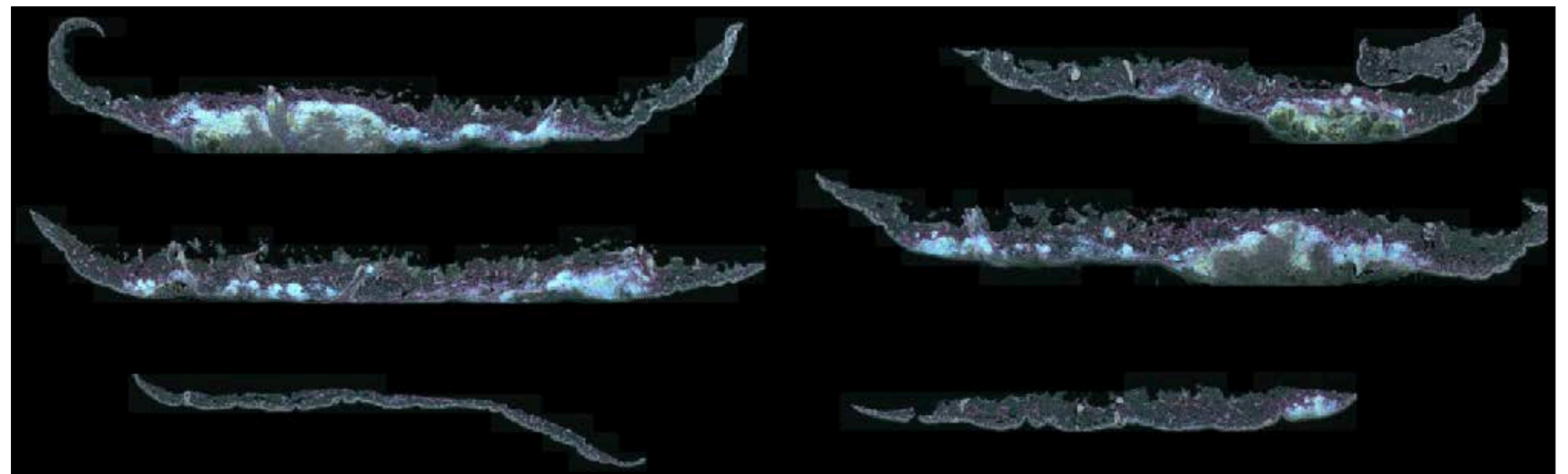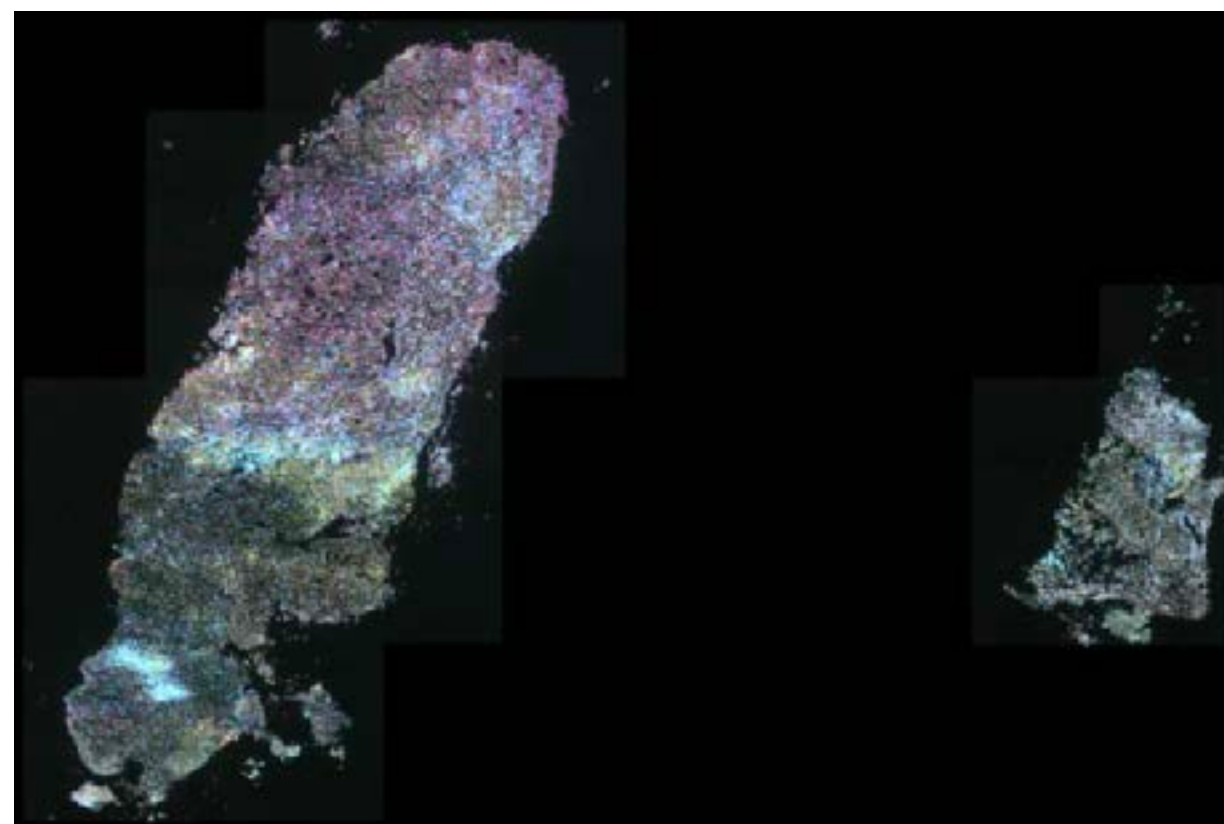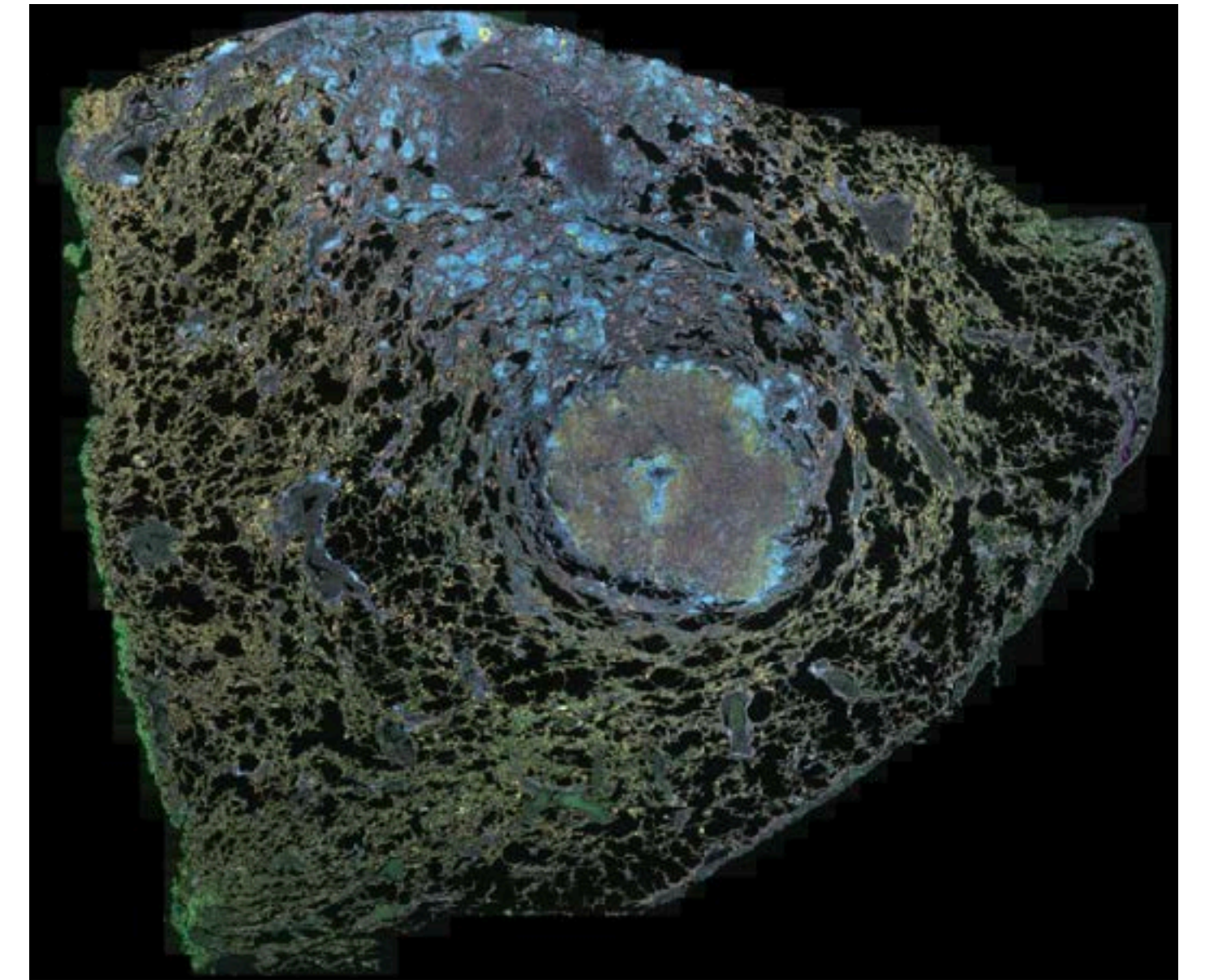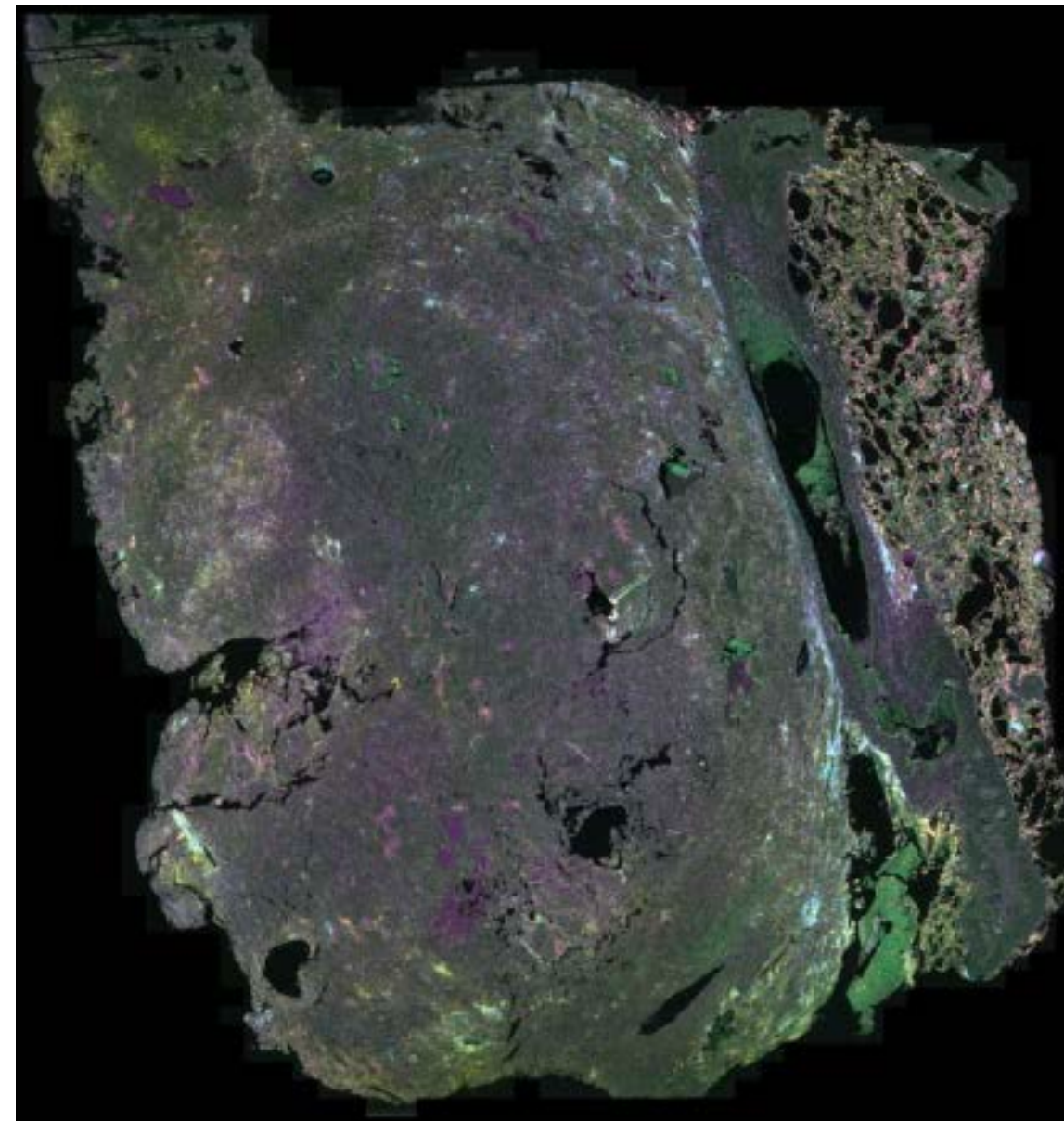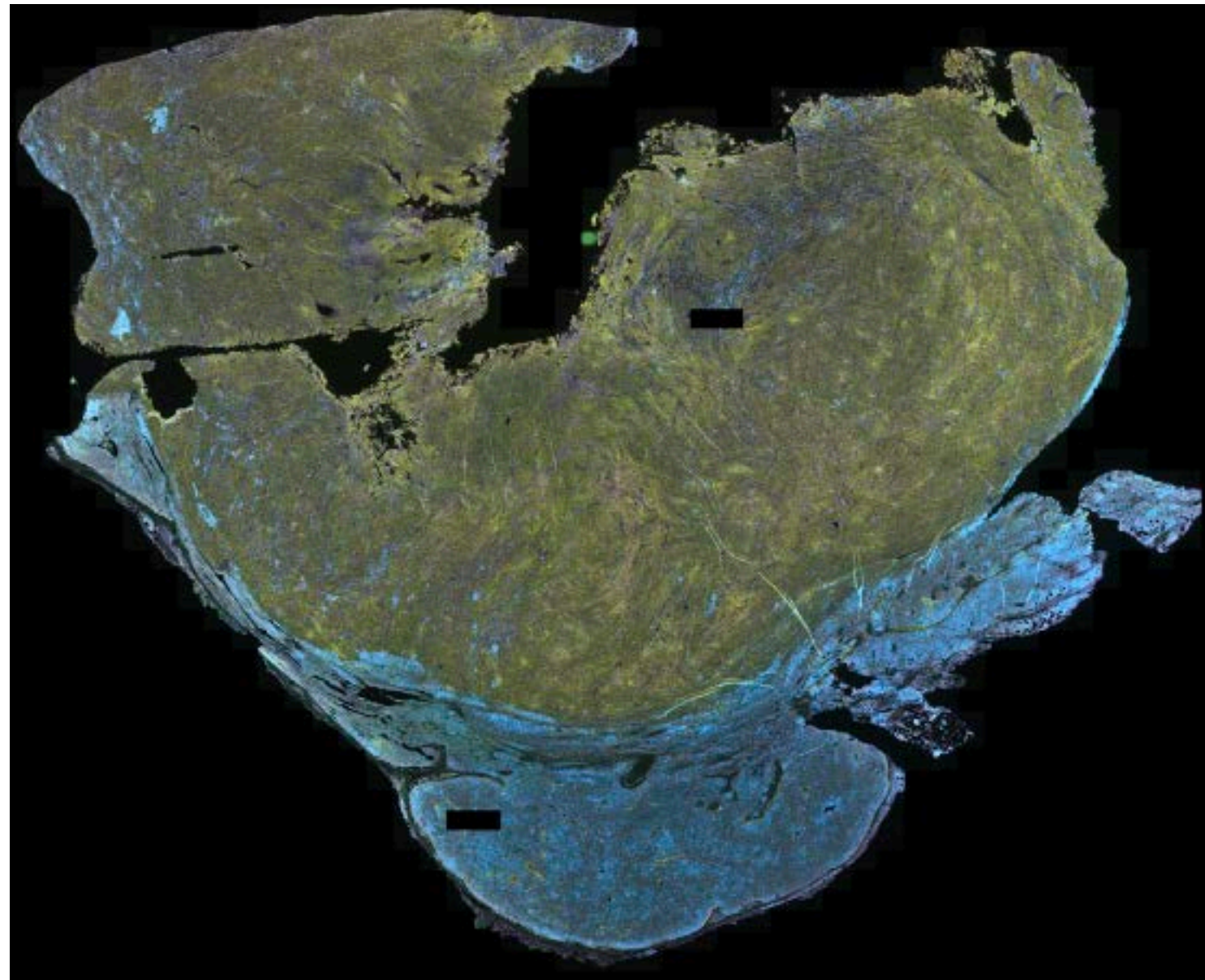
AstroPath Goals and Data

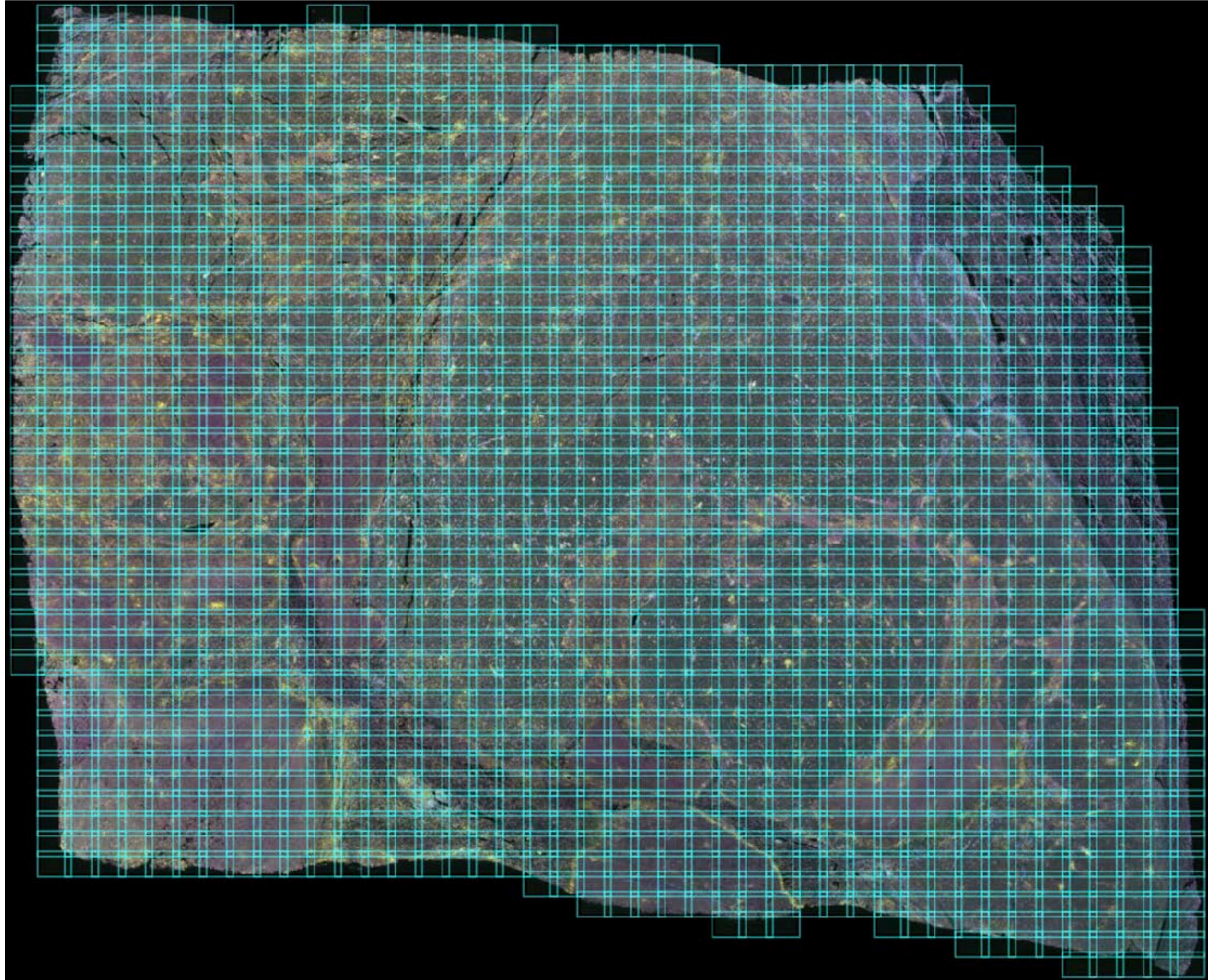- Tissue specimens imaged w/ Vectra 3 microscope

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
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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

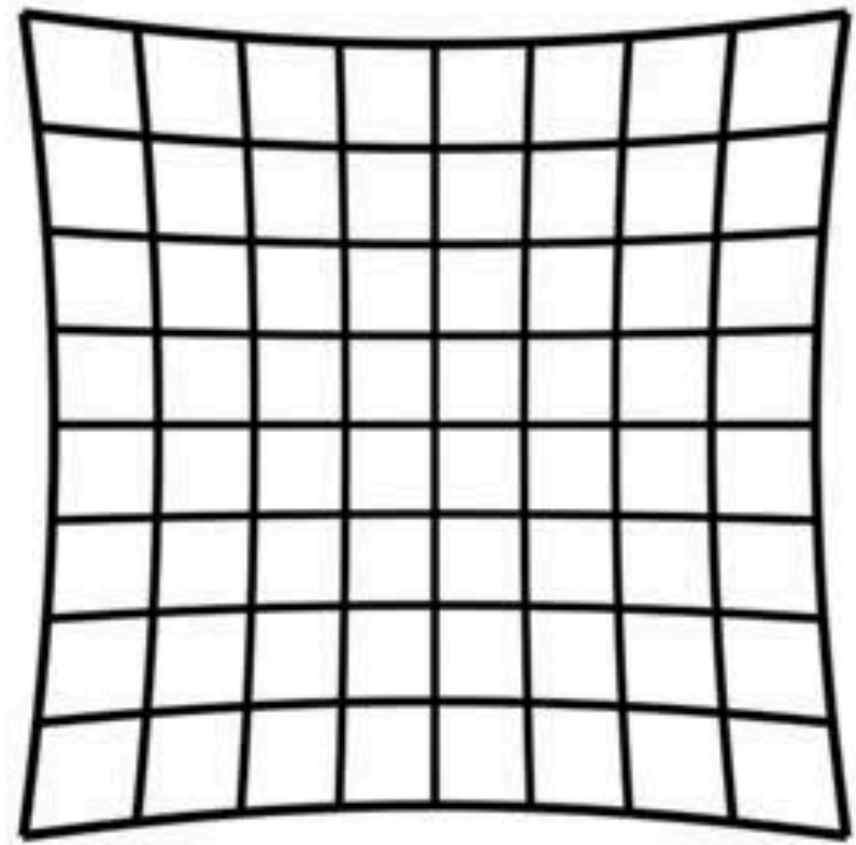 - Robust illumination intensity measurements *in situ*
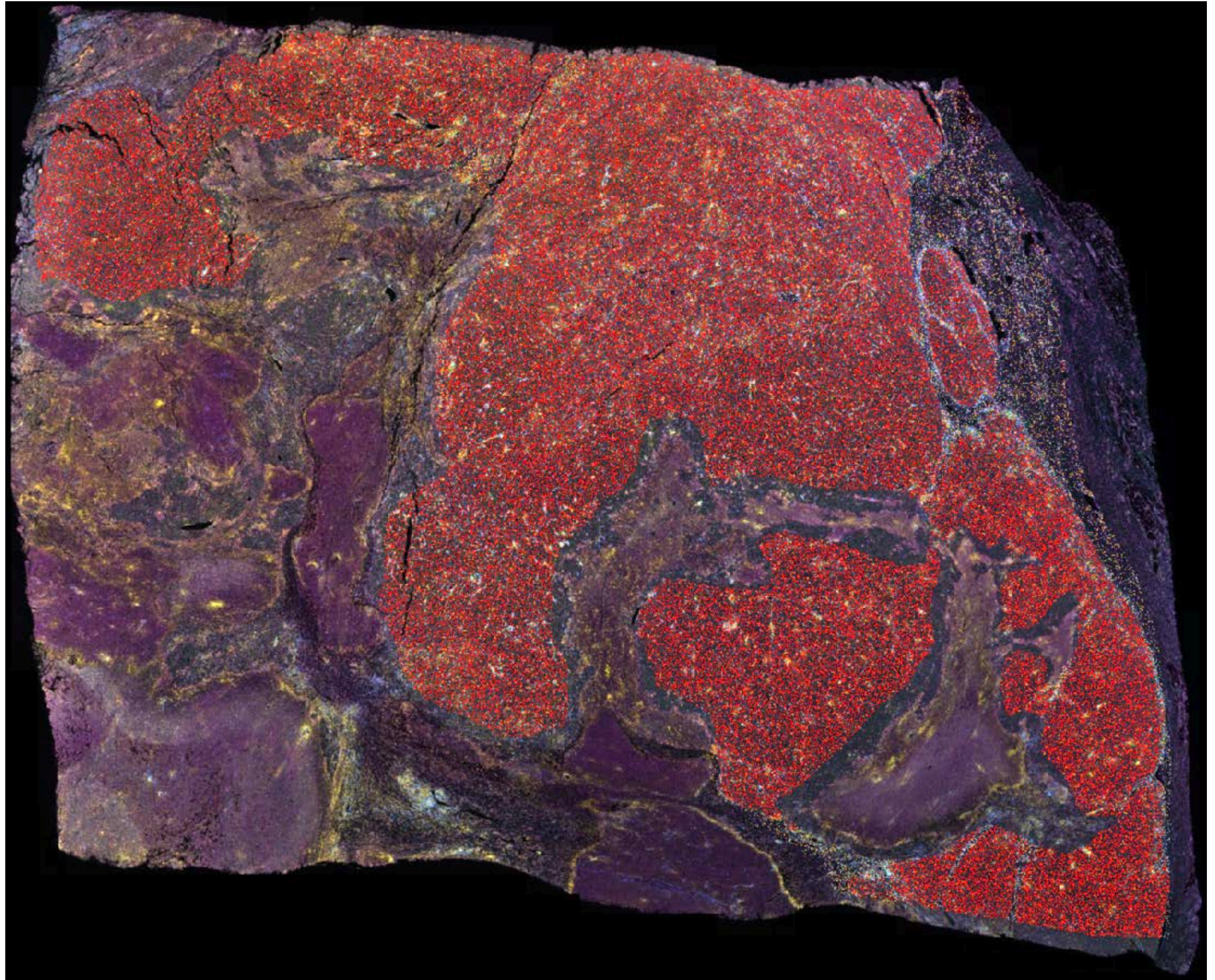
AstroPath Goals and Data

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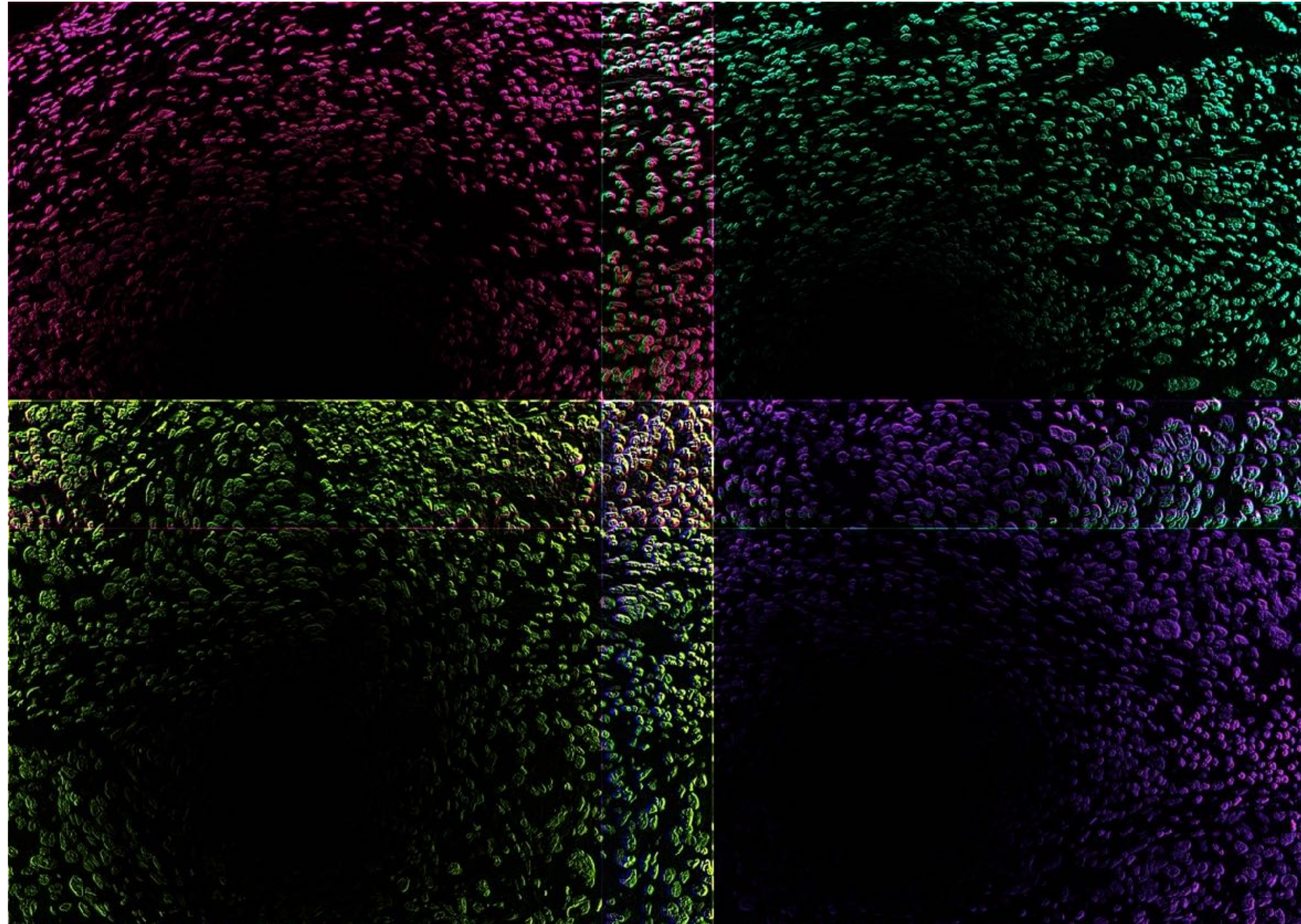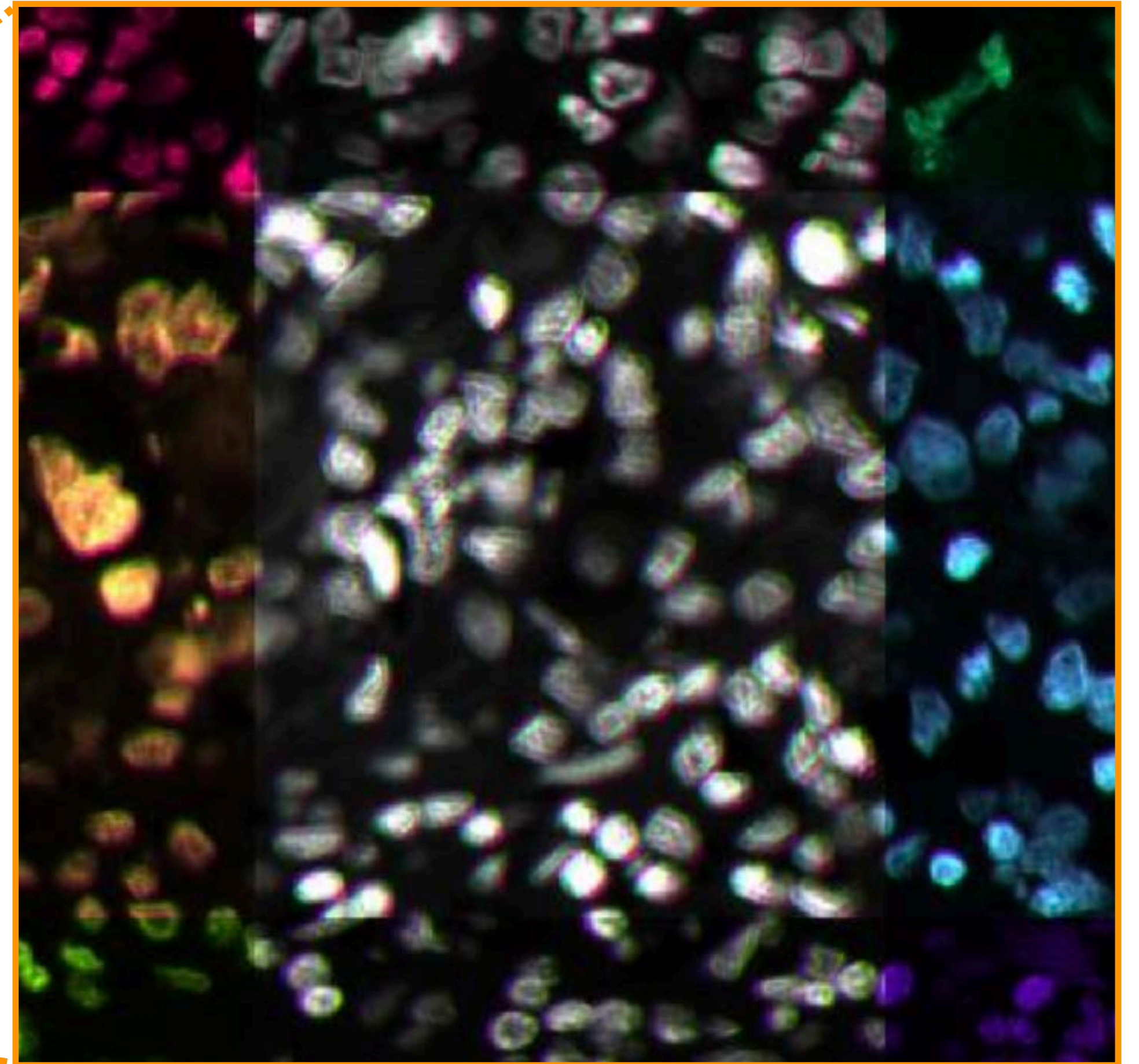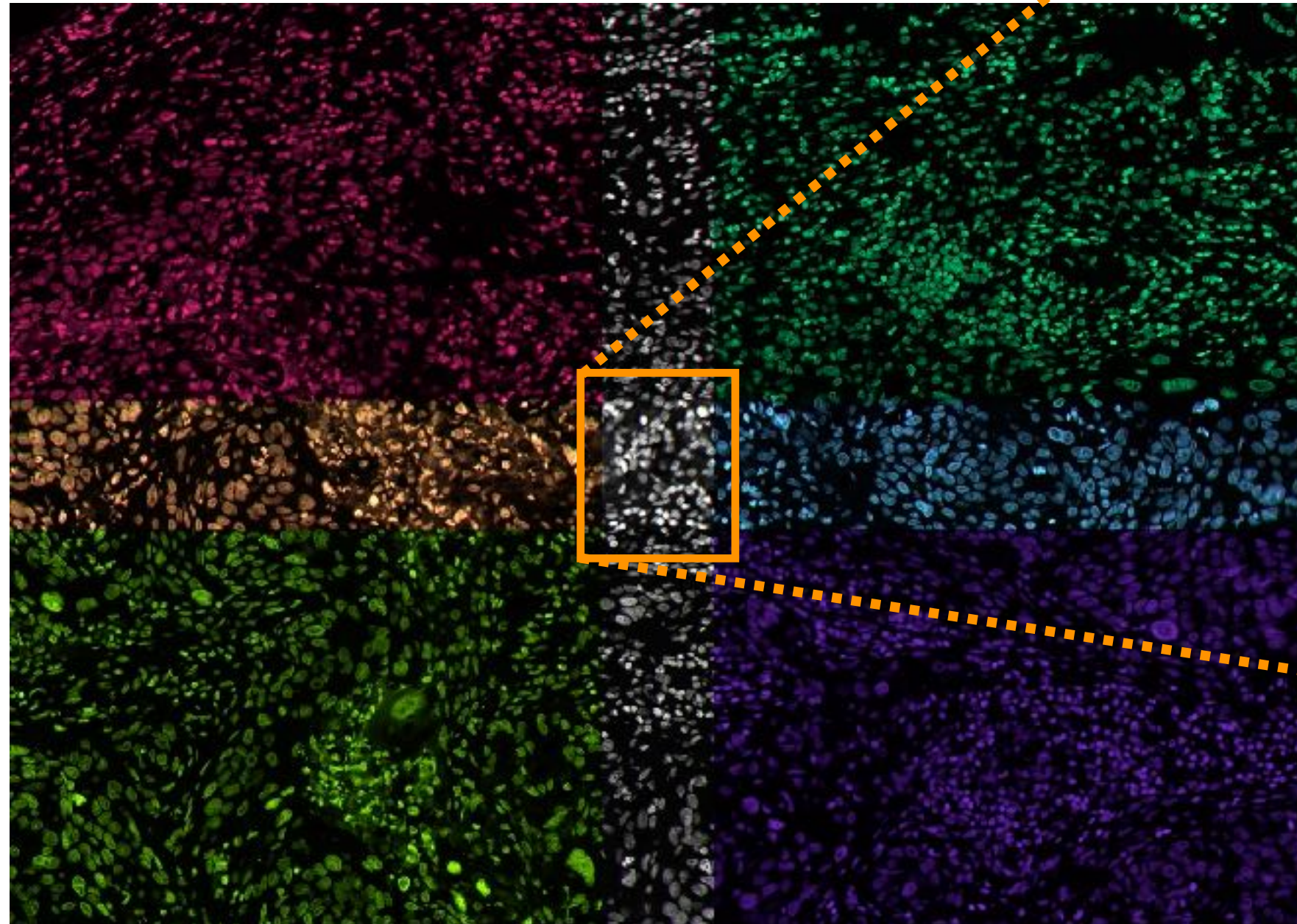
Warping Effects

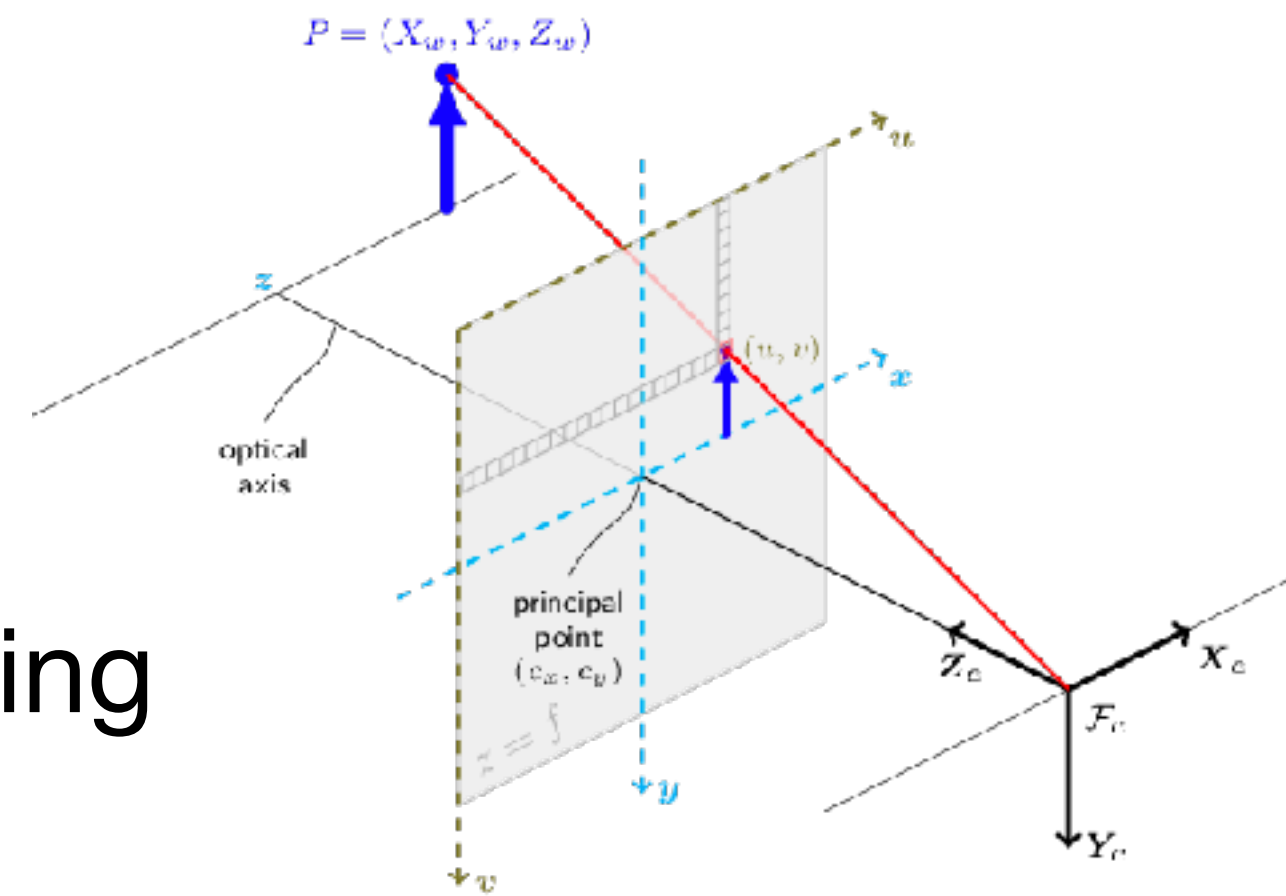- Pincushion distortion from objective & camera lenses


Pincushion Distortion
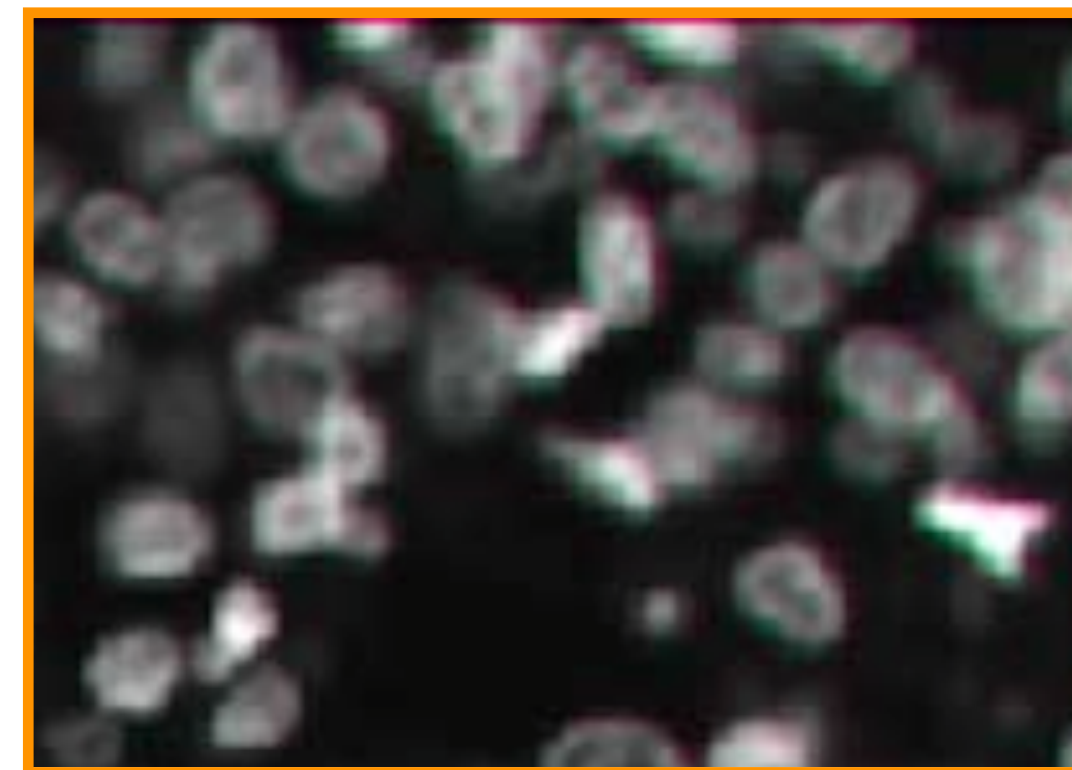
Warping Effects

- Pincushion distortion from objective & camera lenses

- Model using OpenCV camera calibration

  - camera matrix + radial distortion parameters

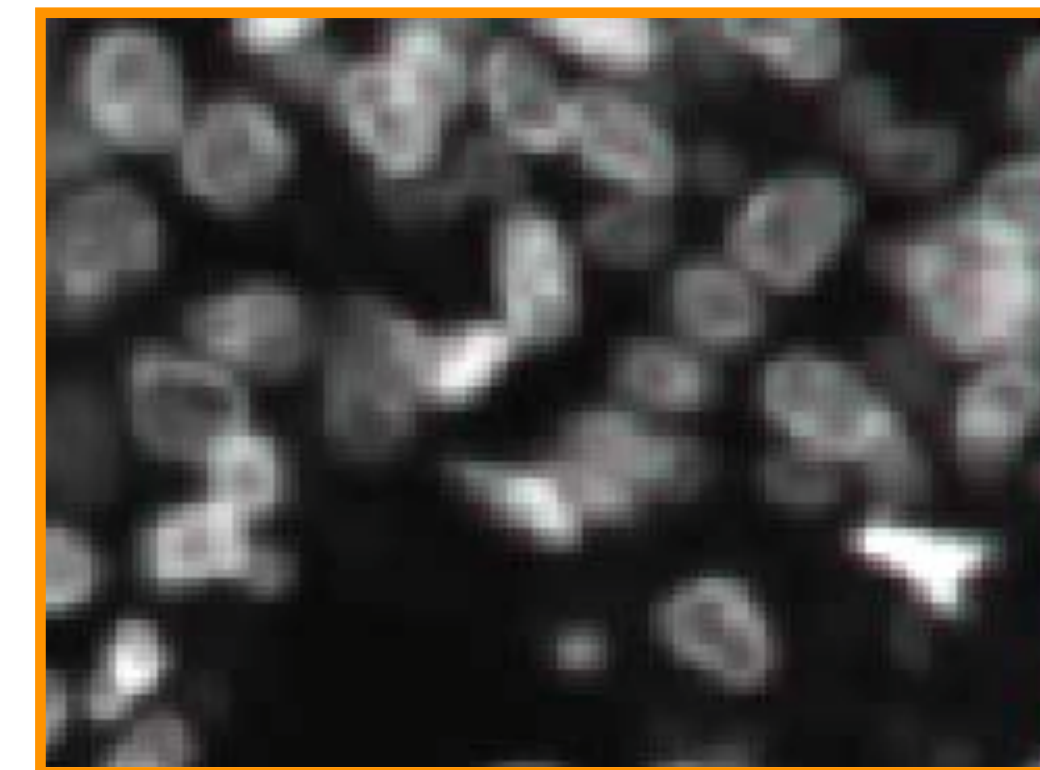- Minimize MSE between overlapping image regions

$$\begin{bmatrix} x'' \\ y'' \end{bmatrix} = \begin{bmatrix} x' \frac{1+k_1 r^2+k_2 r^4+k_3 r^6}{1+k_4 r^2+k_5 r^4+k_6 r^6} + 2p_1 x' y' + p_2(r^2+2x'^2) + s_1 r^2 + s_2 r^4 \\ y' \frac{1+k_1 r^2+k_2 r^4+k_3 r^6}{1+k_4 r^2+k_5 r^4+k_6 r^6} + p_1(r^2+2y'^2) + 2p_2 x' y' + s_3 r^2 + s_4 r^4 \end{bmatrix}$$
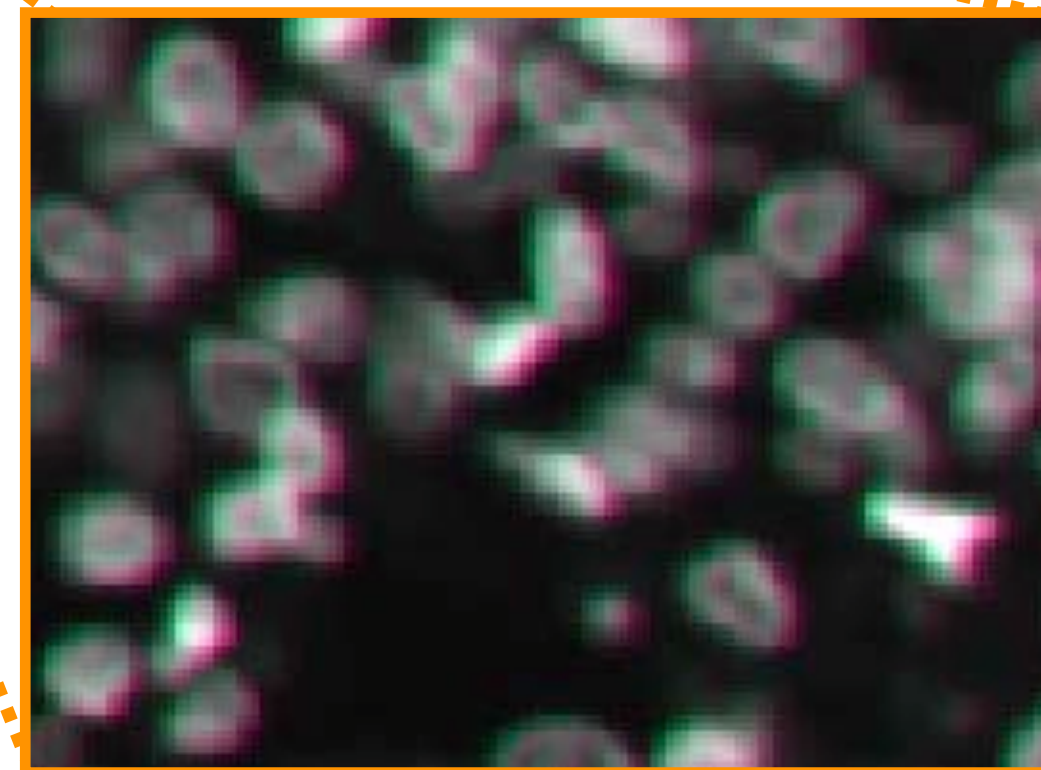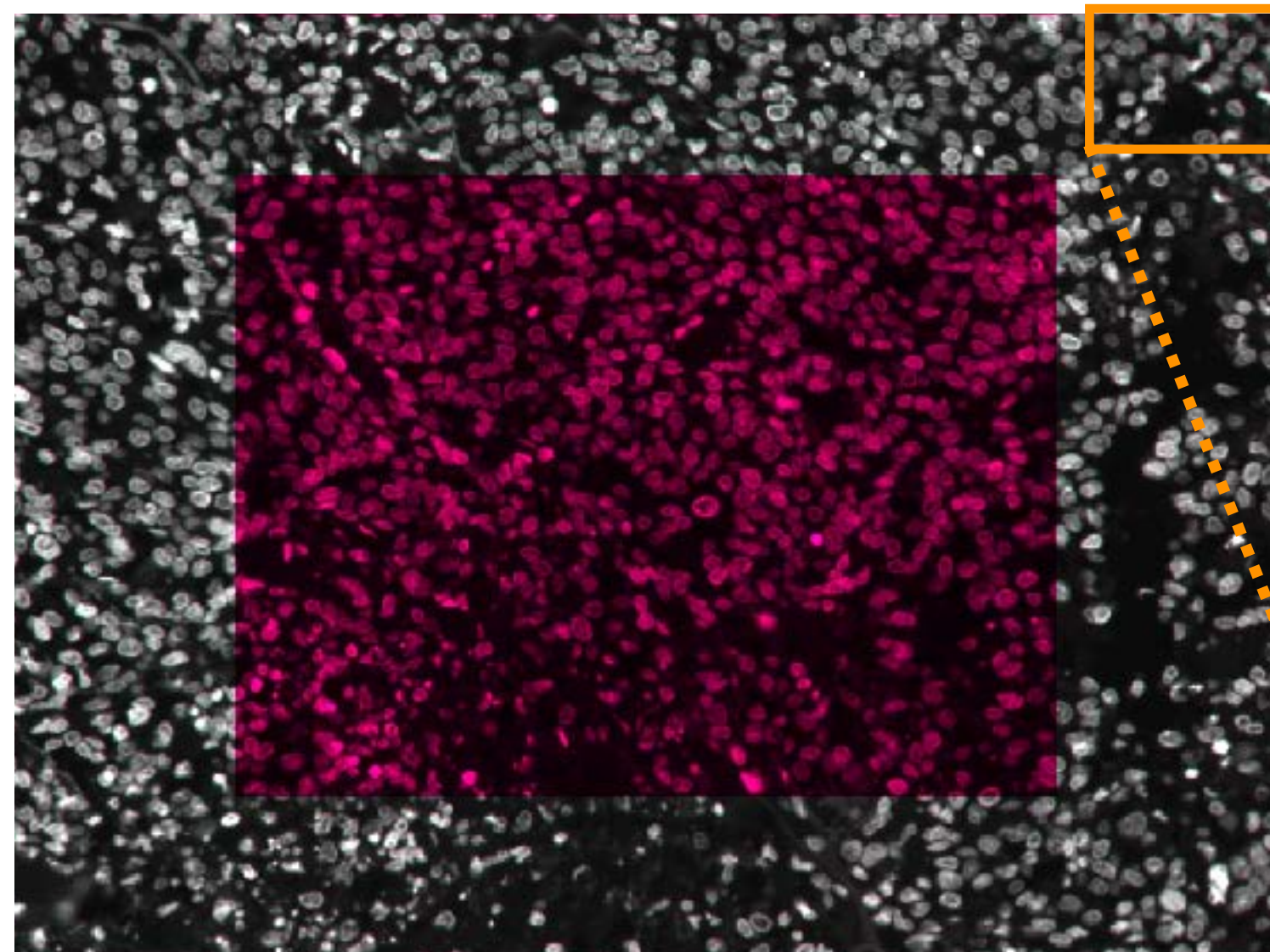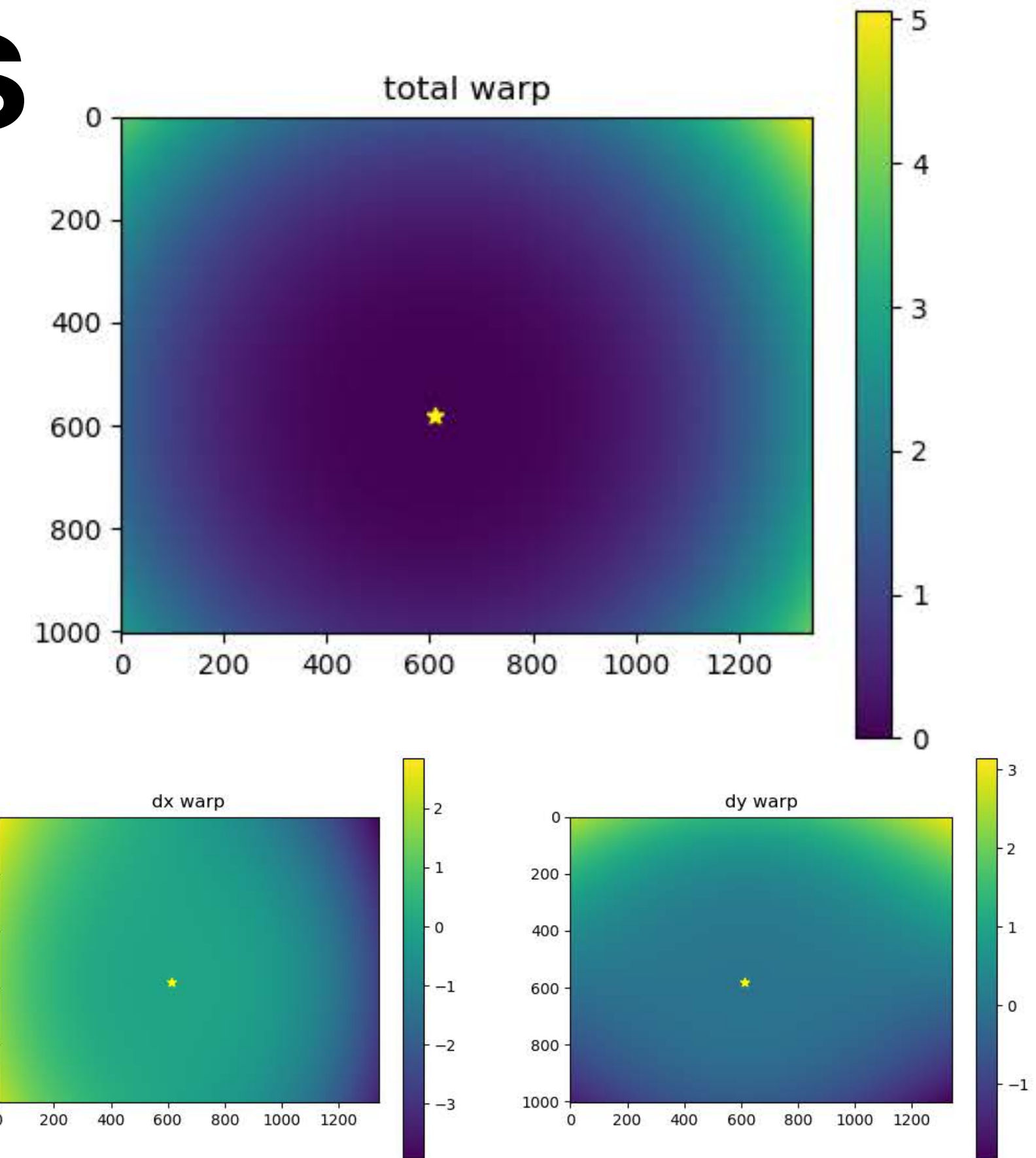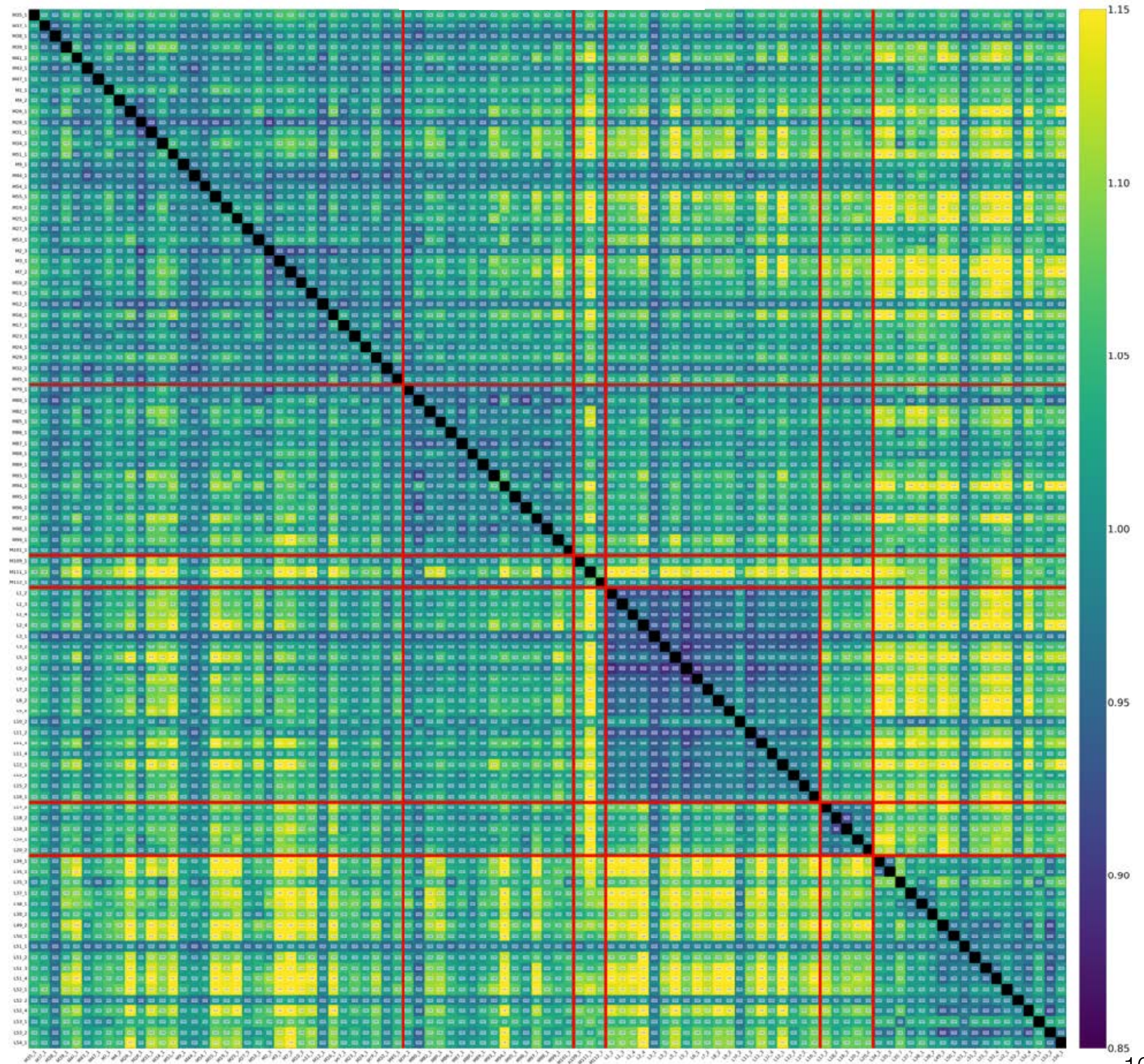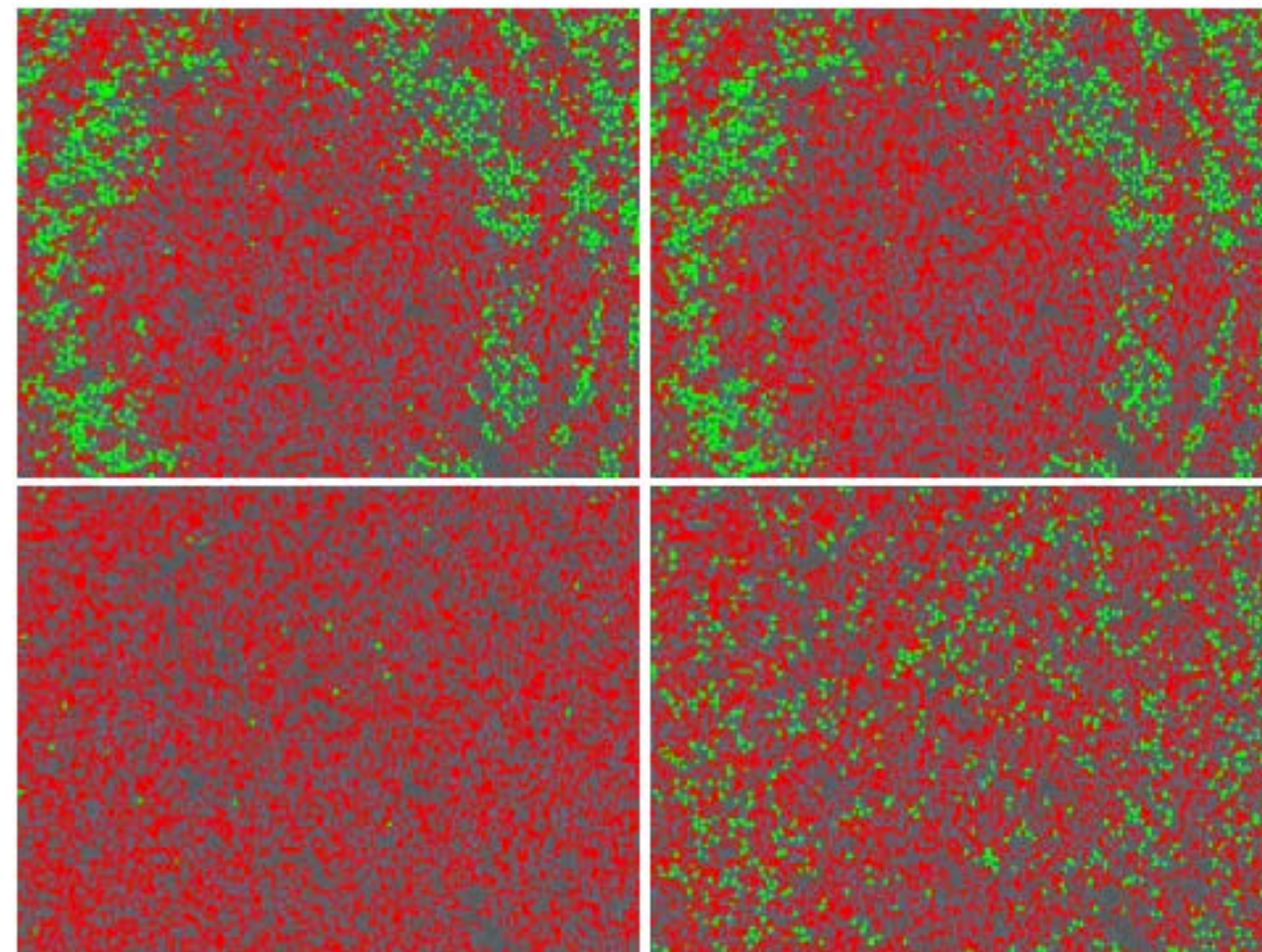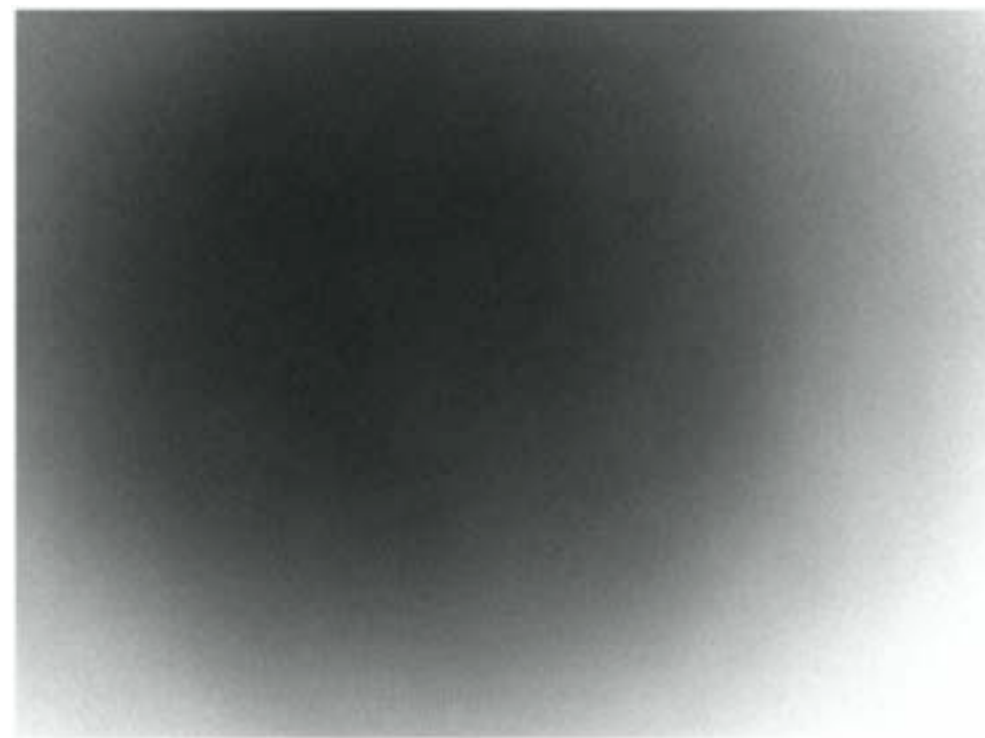
raw, not aligned **aligned (only) MSE=0.010** **unwarped + aligned MSE=0.003**
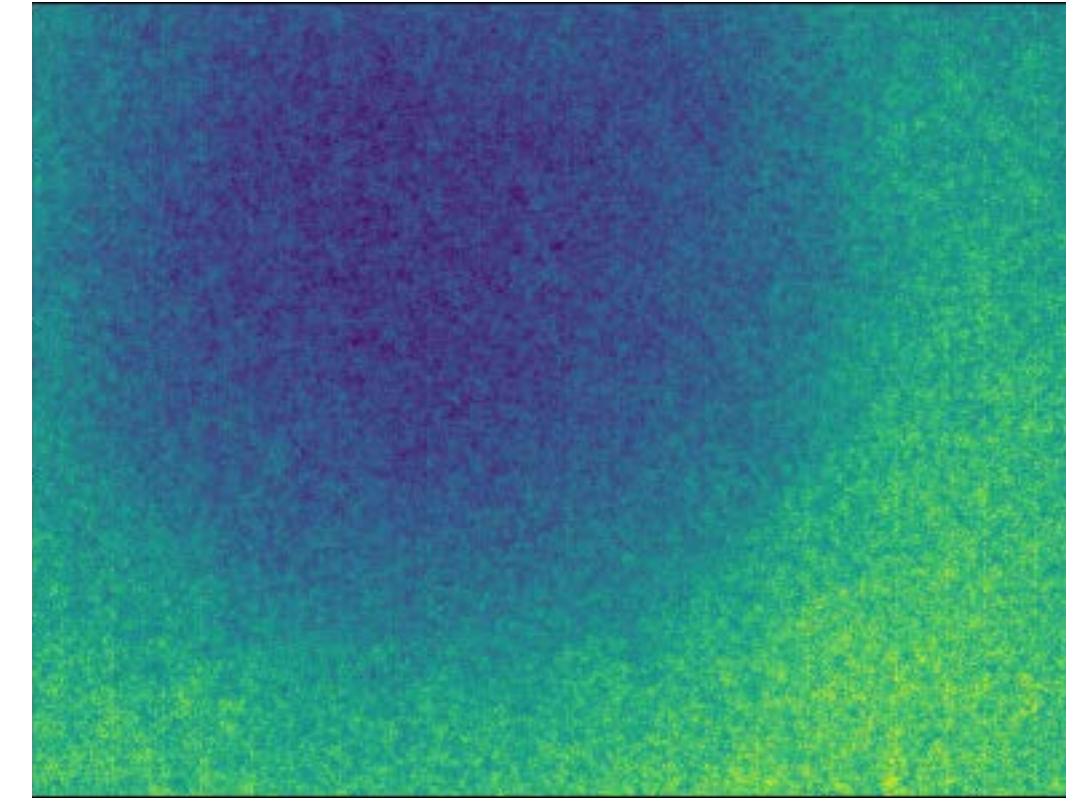
Flatfielding Effects

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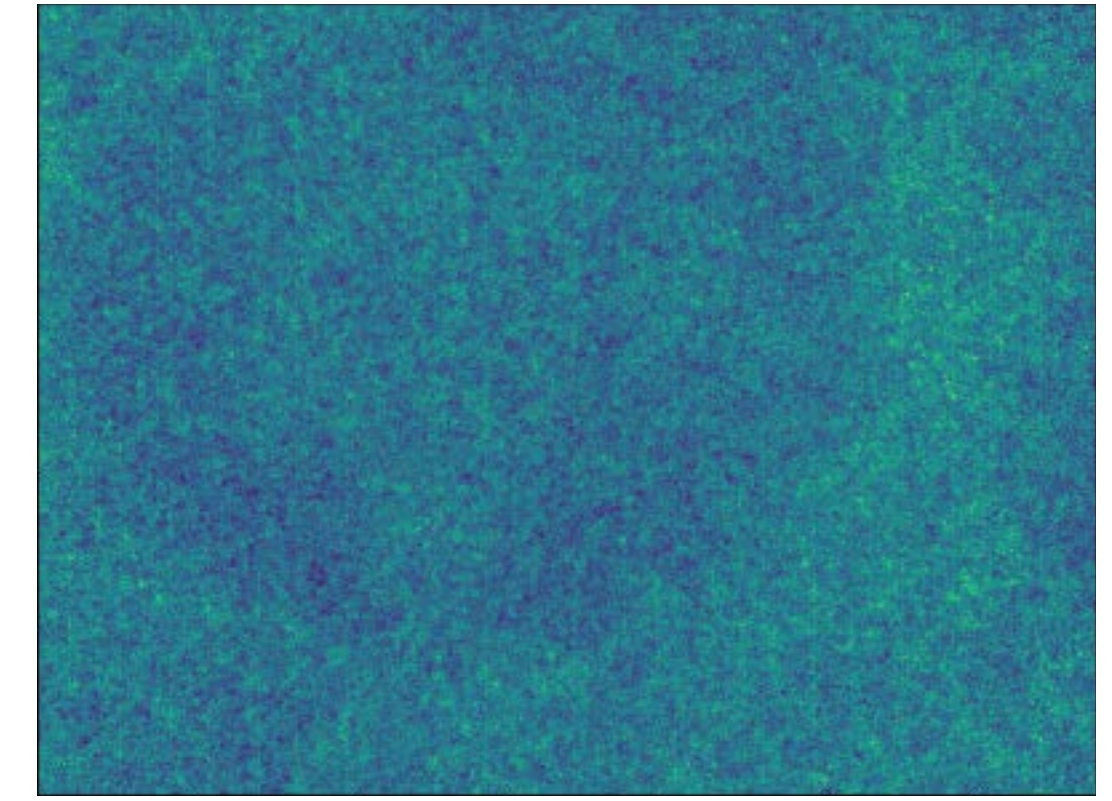 - Changes over time/per-microscope

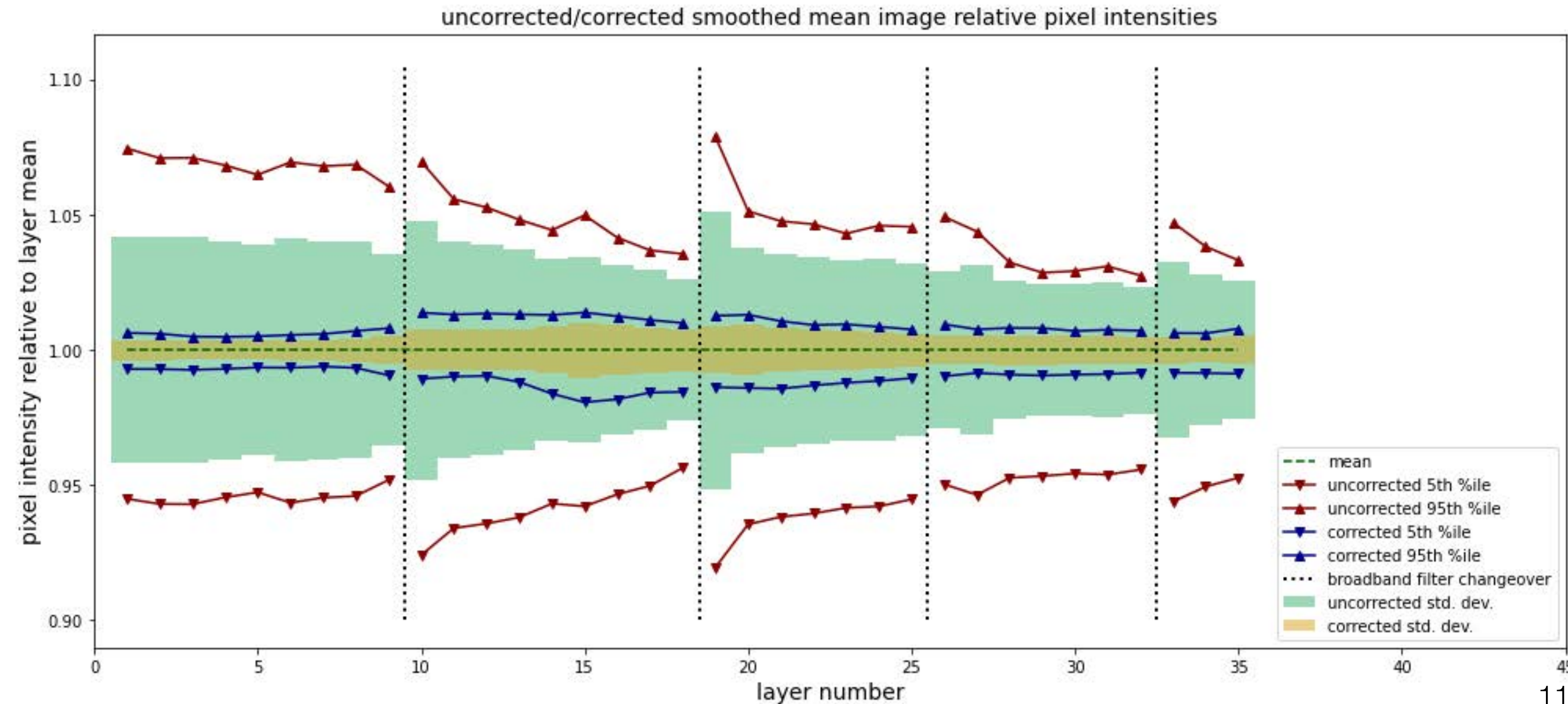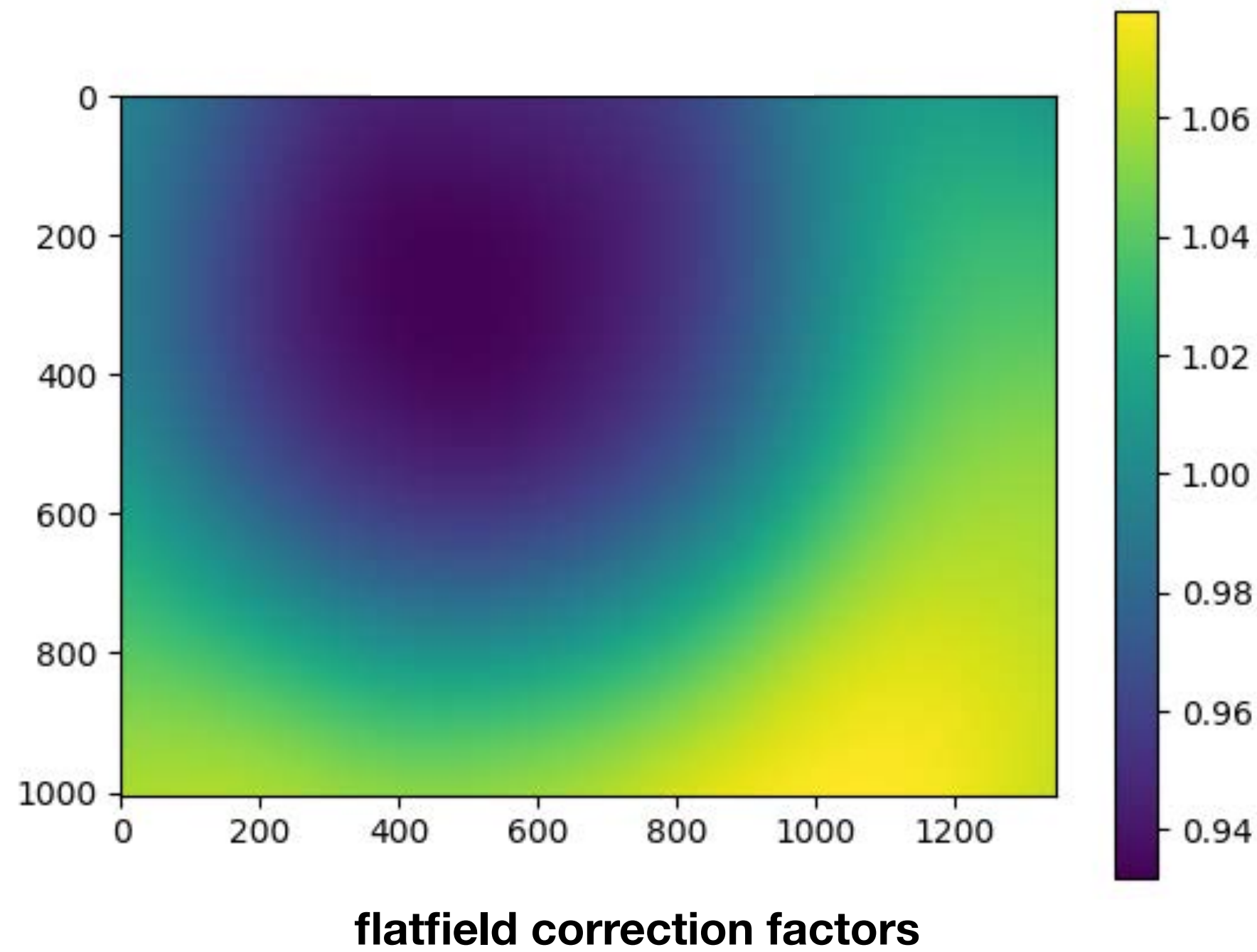# Flatfielding Effects

- 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

uncorrected/corrected smoothed mean image relative pixel intensities
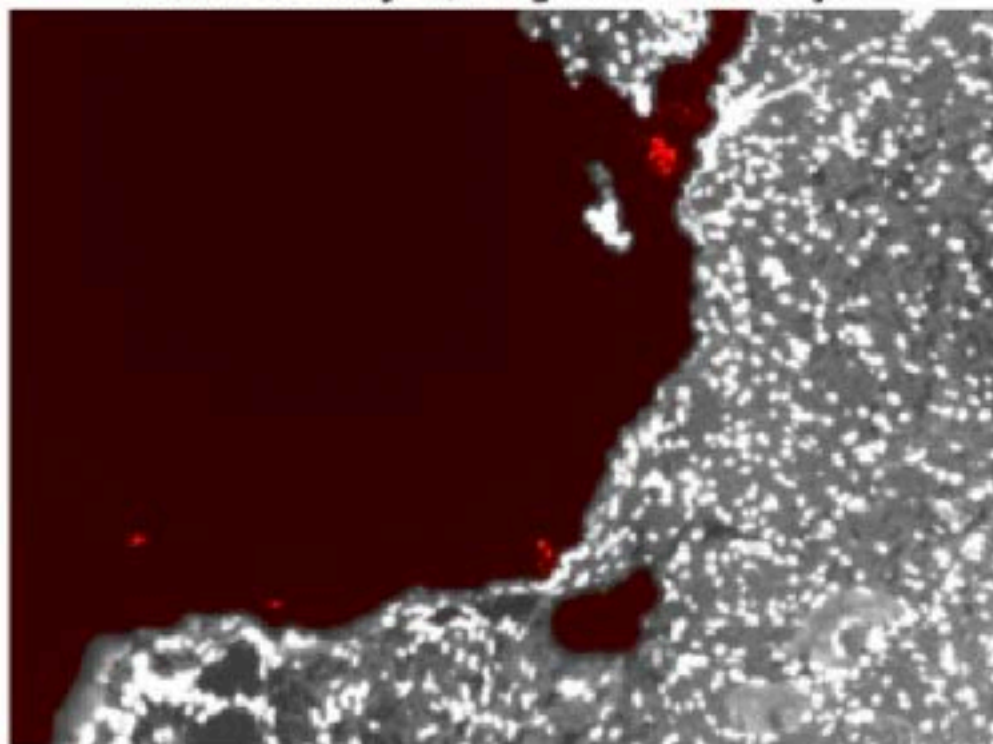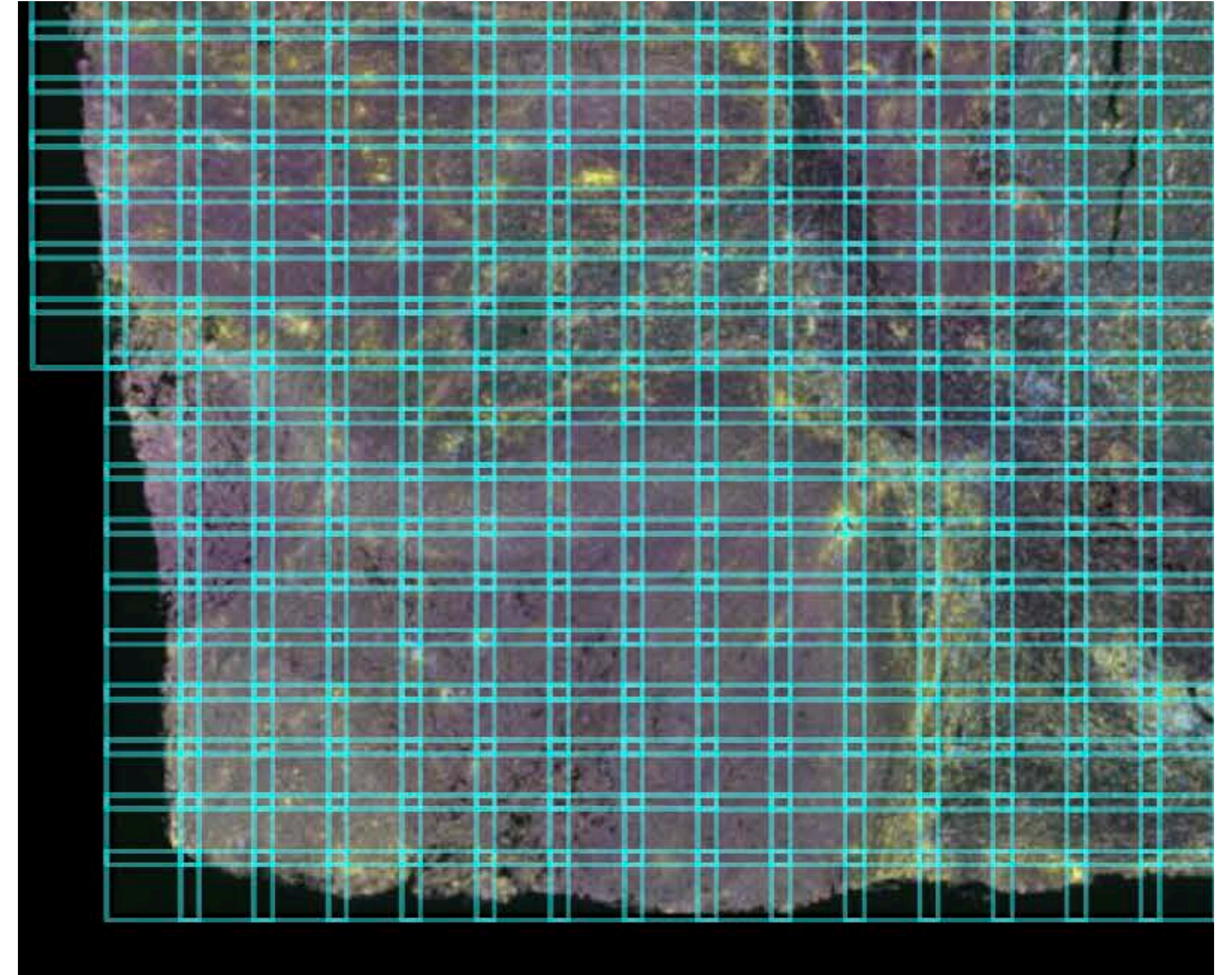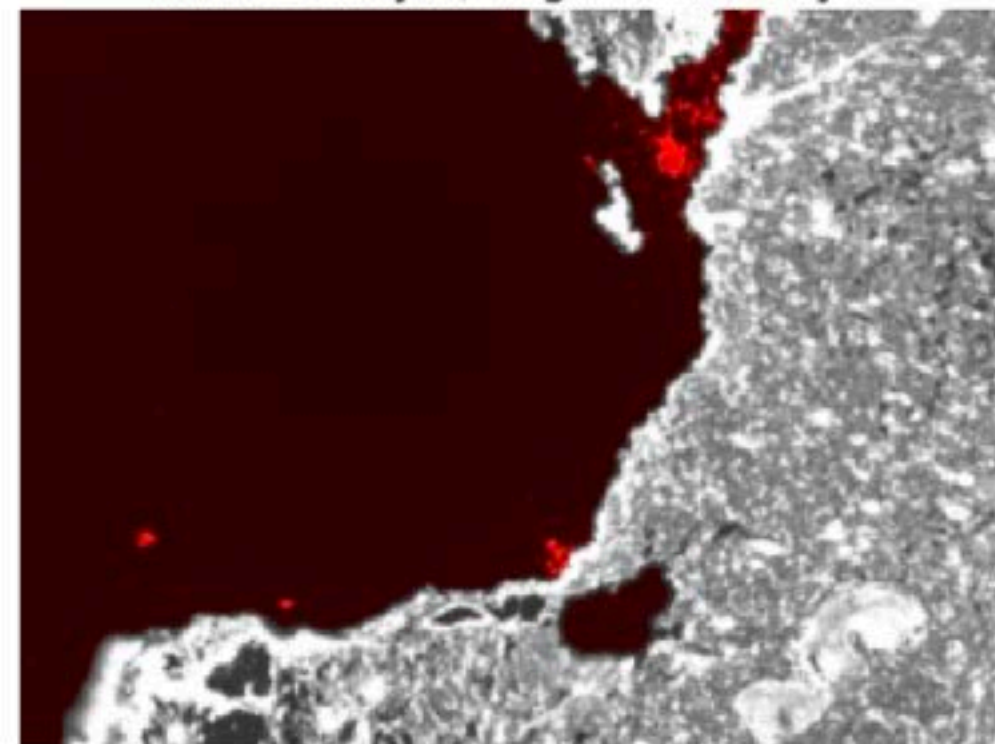
Flatfielding Effects

- Practical considerations
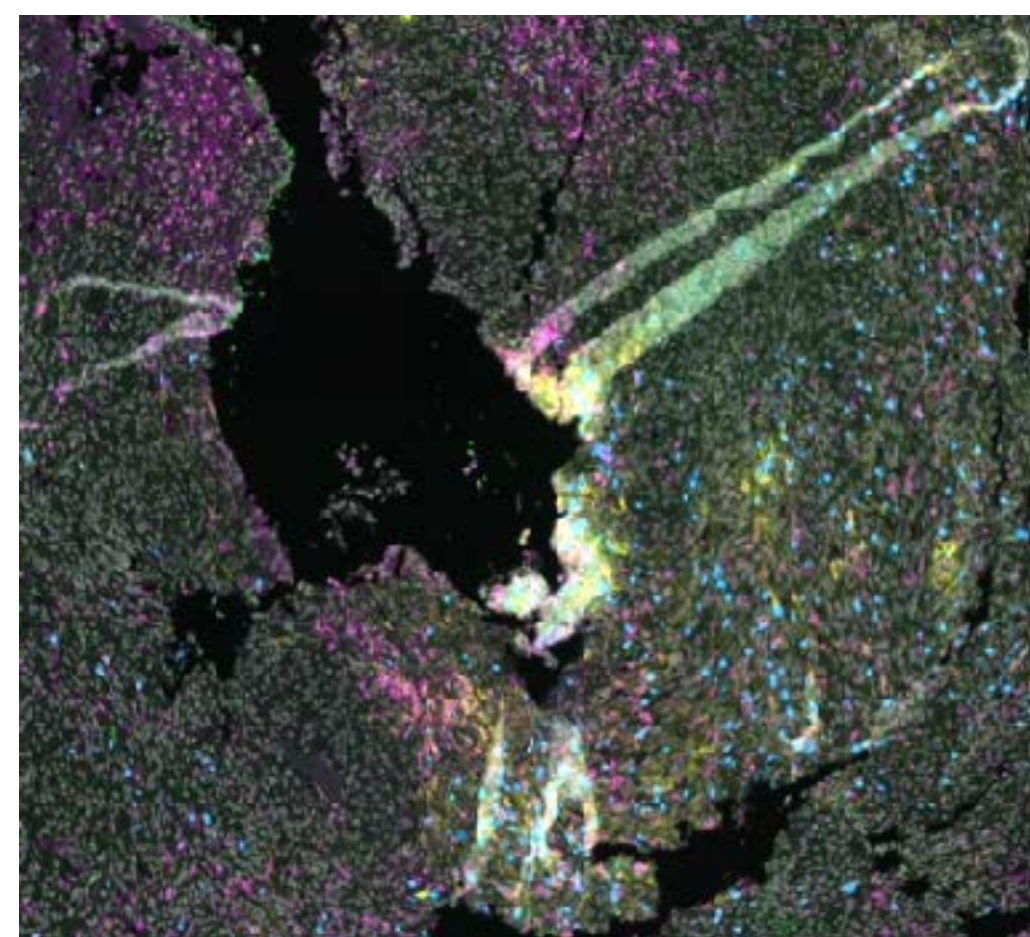 - Leave out empty background

mask overlay w/ brightest DAPI layer

mask overlay w/ brightest FITC layer

Flatfielding Effects

- Practical considerations

  - Leave out empty background

  - Image artifacts

- Image masks

  - Improve corrections

  - Useful to have in the database
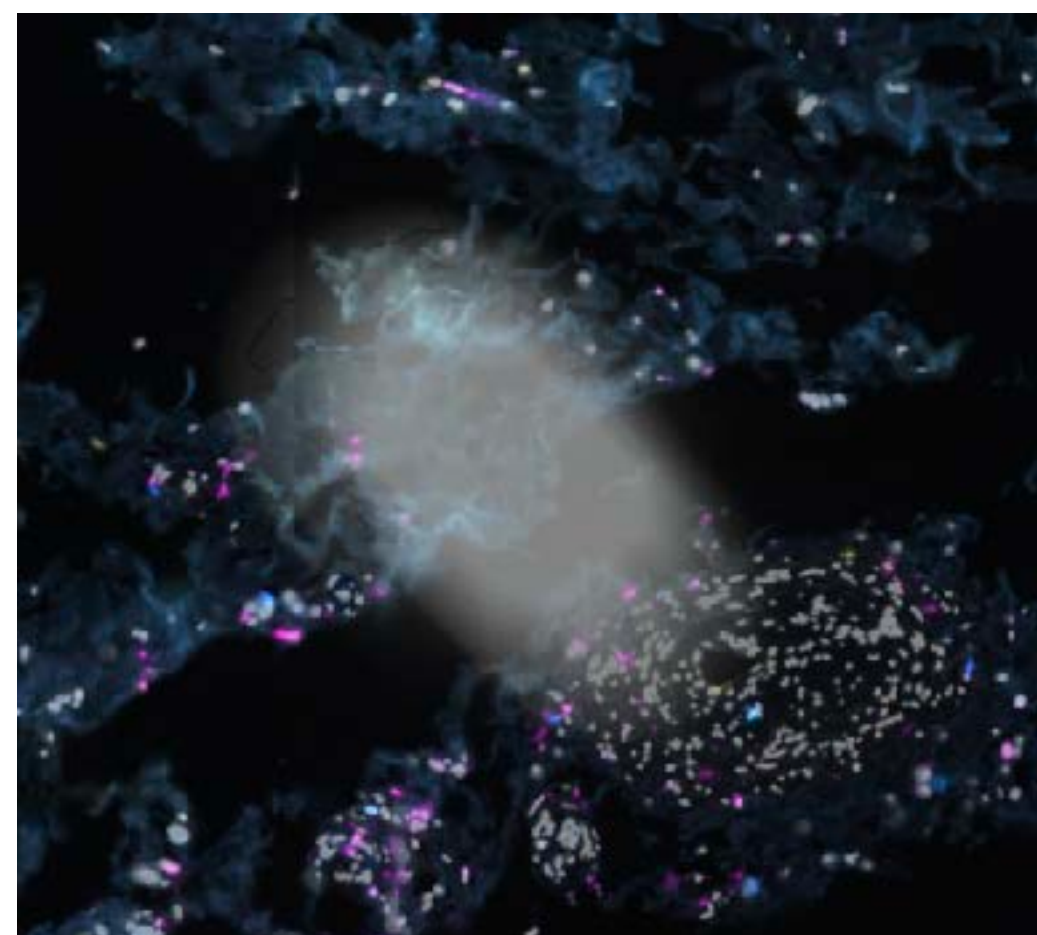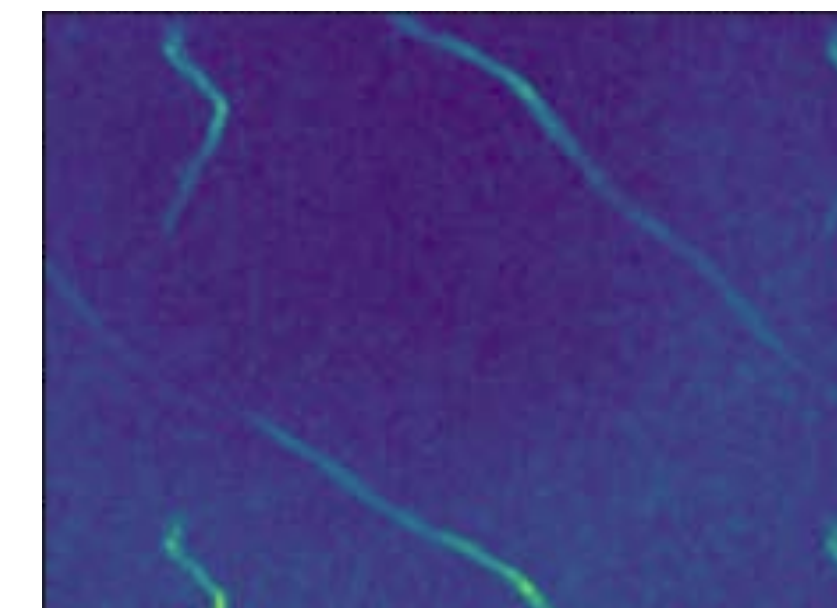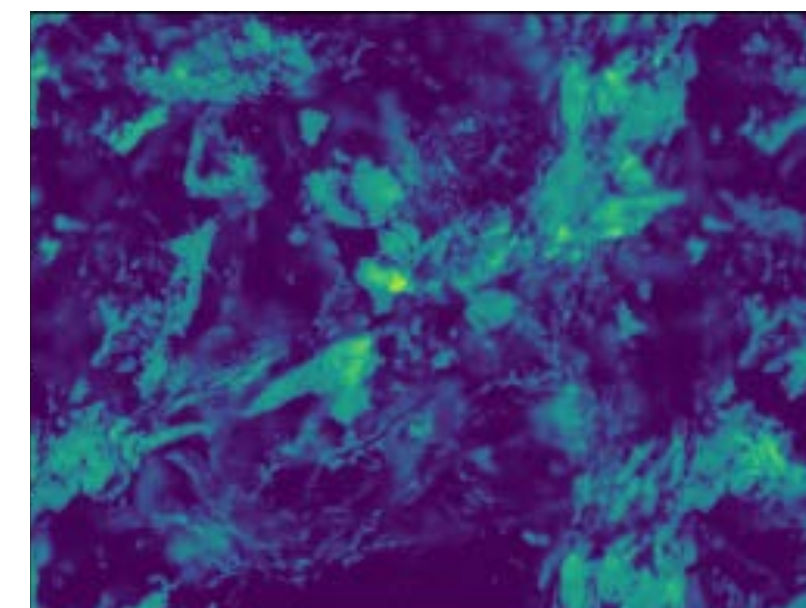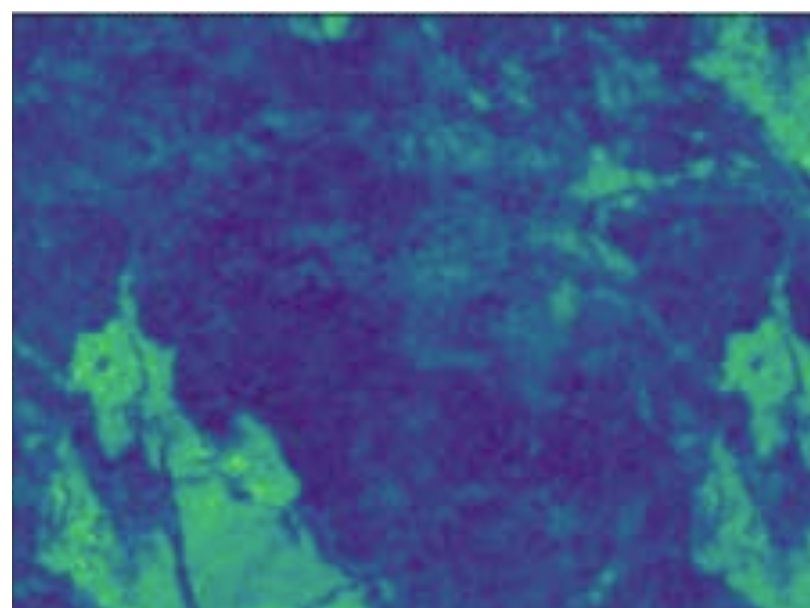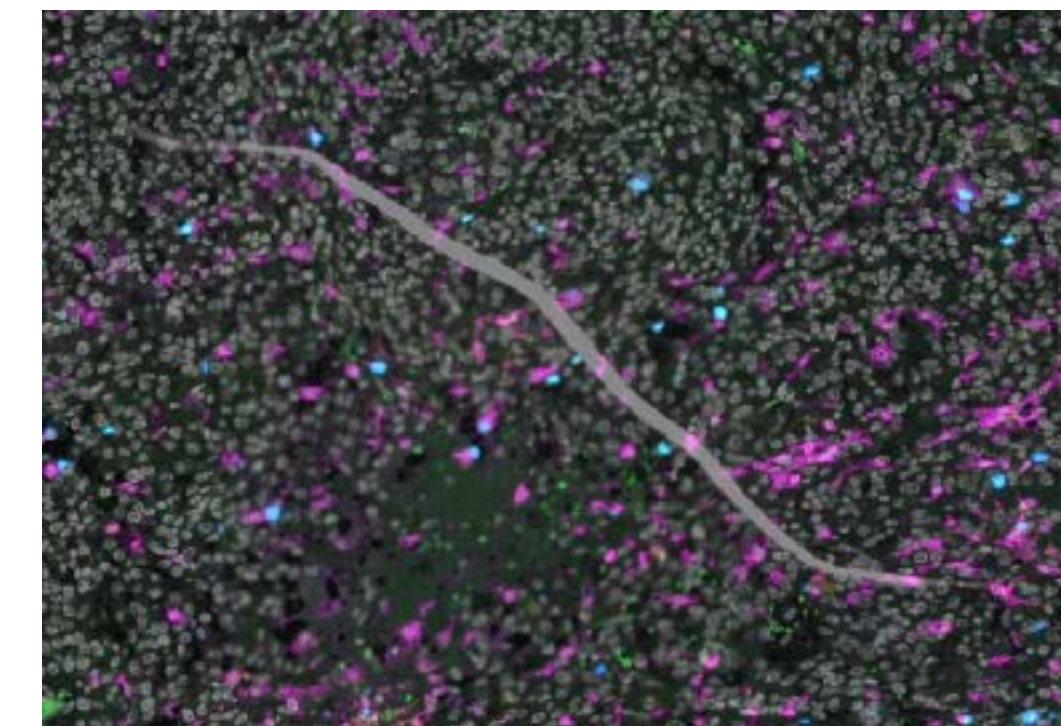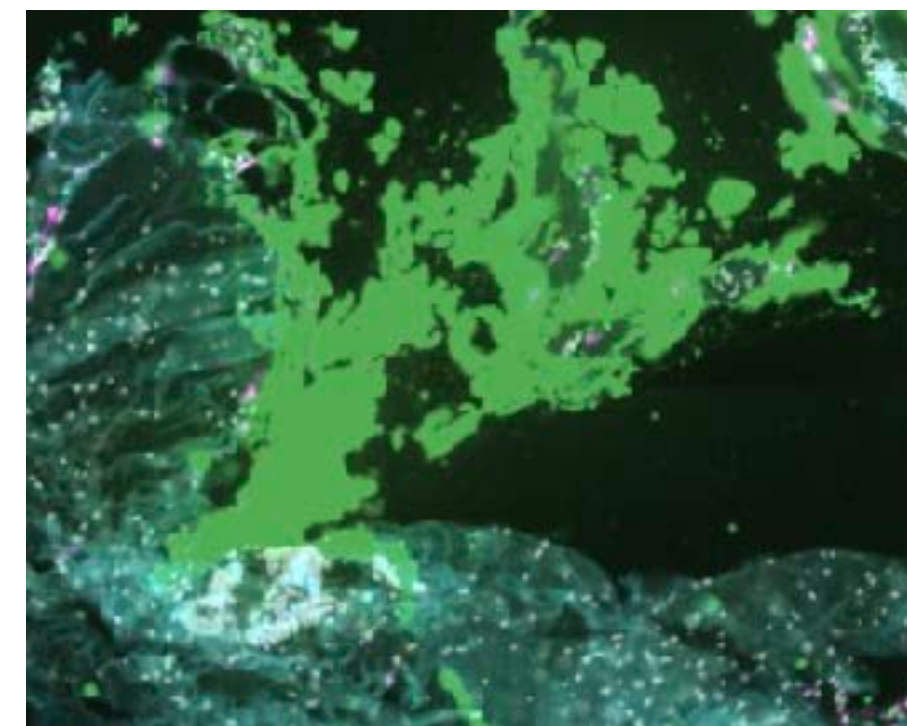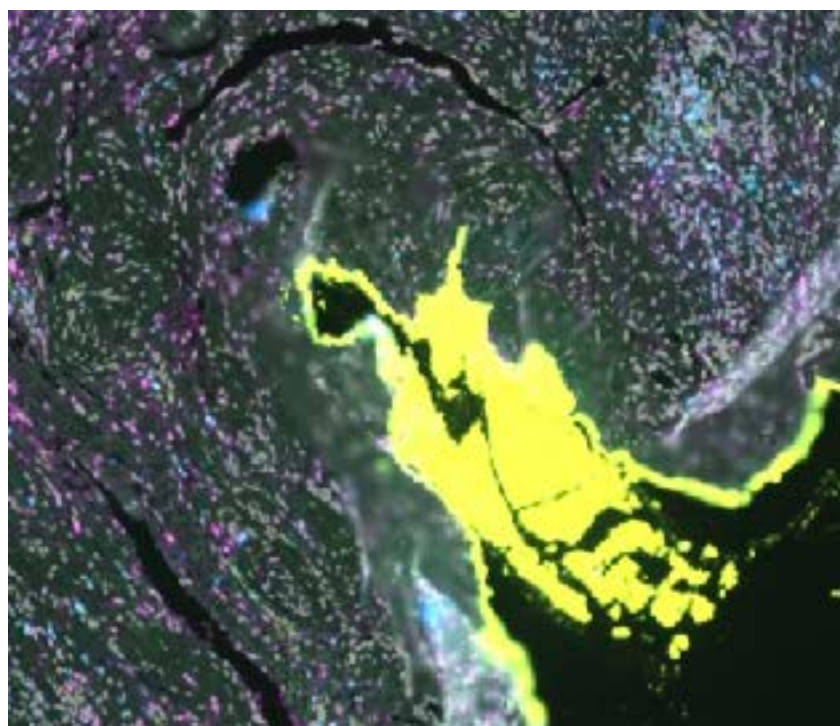
flatfield model	uncorrected illumination variation	corrected illumination variation	reduction in illumination variation
no masking	11.6%	3.20%	**72.4%**
with masking	10.6%	1.95%	**81.6%**

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

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- Nicholas Giraldo, MD, PhD

IDIES

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- Dmitry Medvedev
- Nate Eisenberg (undergrad)

AI / Computer Vision

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- Seyoun Park, PhD
- Yixiao Zhang (PhD student)

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  - Evan Lipson, MD
- Akoya
  - Cliff Hoyt, MS
  - Chi Wang
- Bristol Myers Squibb
  - Robin Edwards, MD

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The Mark Foundation® for Cancer Research