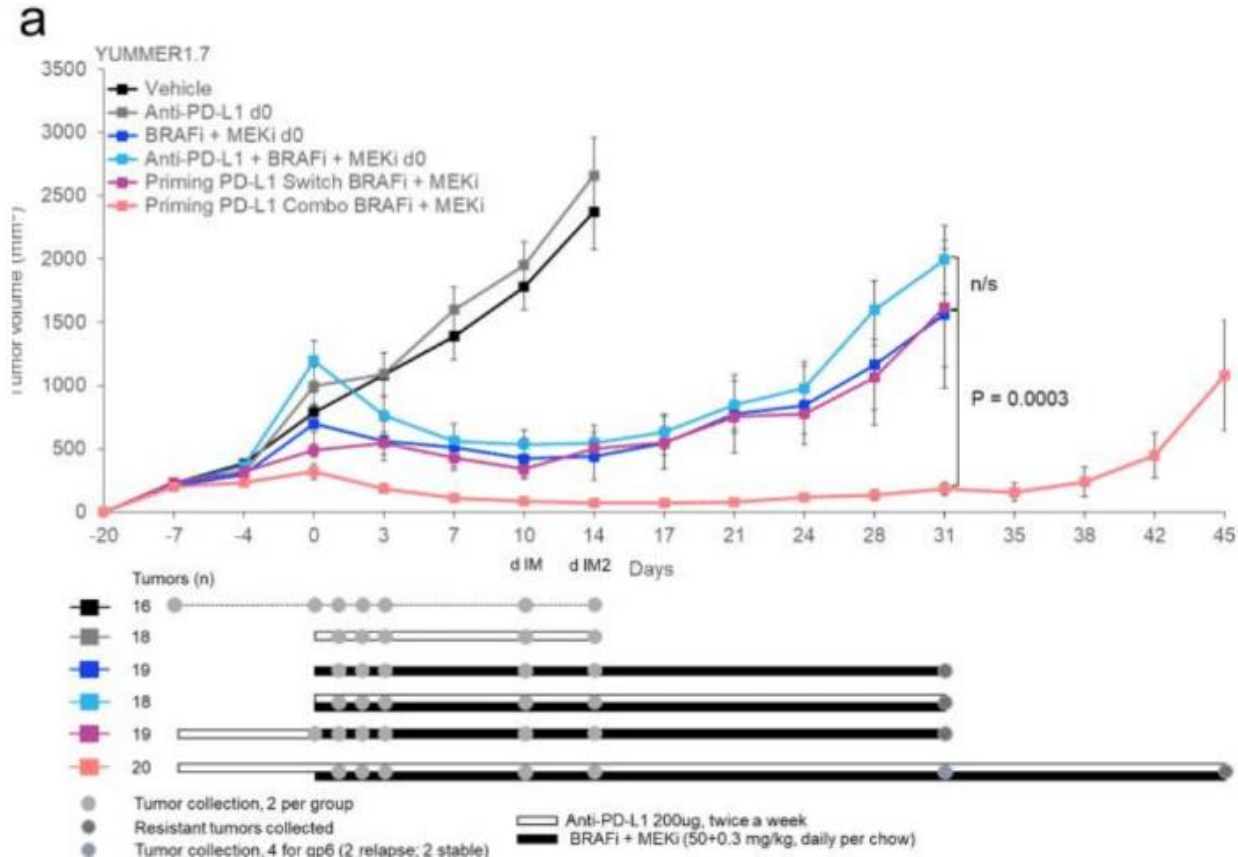


DBiT-seq Image Processing and Analysis

Pelin Garbioglu, Mohitveer Kahlon
August 15, 2024

The U54 Project

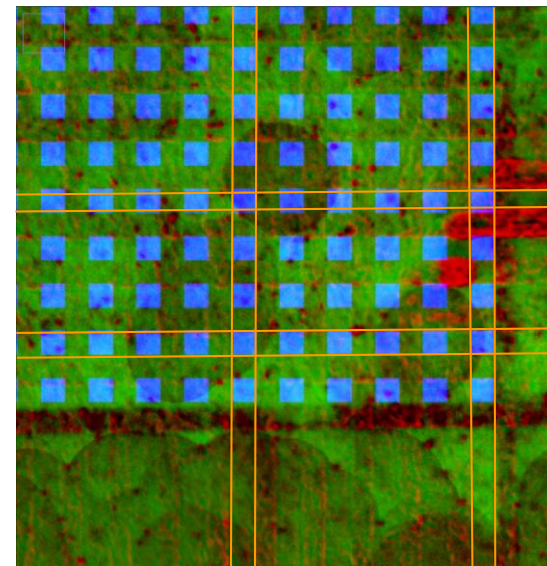
- Our work is part of a larger study to create predictive models that can help design combination therapies
- In collaboration with UCLA and Yale
- Using spatial omics to better understand the environment in melanomas at different periods of treatment



Anti-PD-1/L1 lead-in before MAPK inhibitor combination maximizes antitumor immunity and efficacy - Wang, Y., et al.

Techniques and Terminology

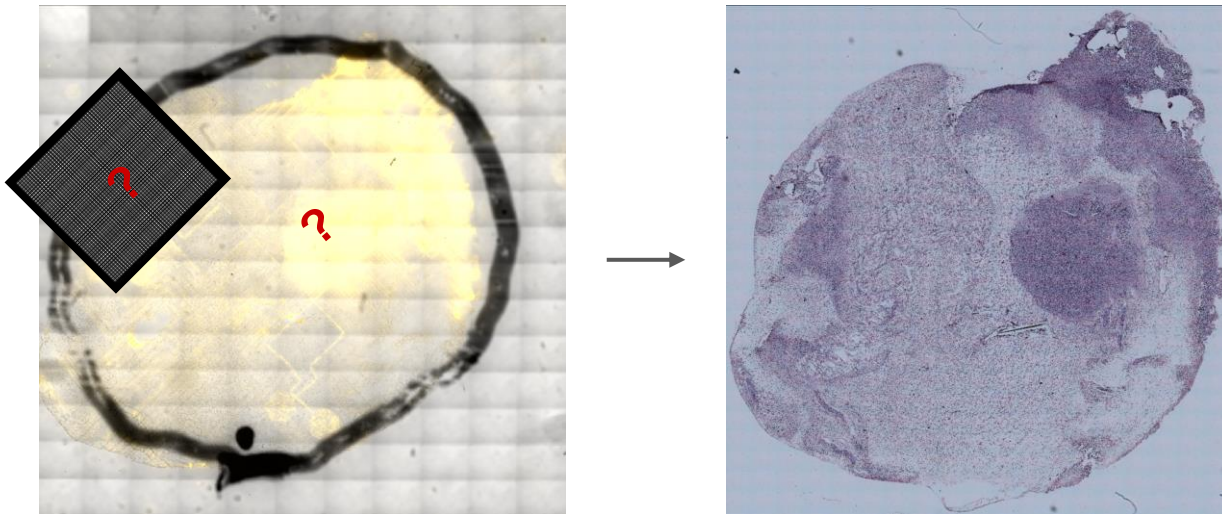
- Deterministic Barcoding in Tissue for Spatial Omics Sequencing (DBiT-seq)
 - Allows for tracking of cells by placing specific DNA barcodes on them
 - Measures gene expression in the context of the cells' environment
- Flowcell
 - Used to deliver microfluidic barcodes (DBiT-seq) to tissue in region of interest
 - Tixel - junction of where flow channels meet
 - AtlasXomics
- Single Cell ATAC-seq
 - Locates open chromatin in individual cells
 - Single cell sequencing shows the diversity of the cells and what they're doing instead of an average of cell behavior



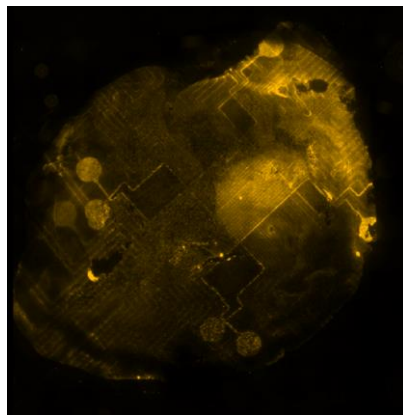
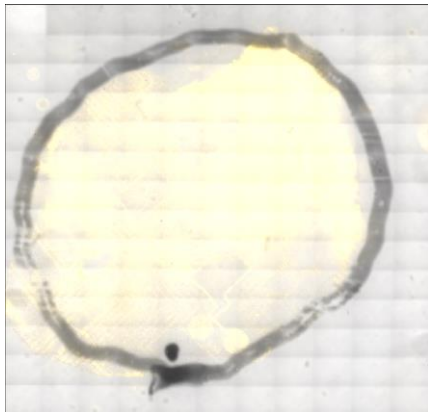
Alternating dye channels indicate tixel location

The Problem

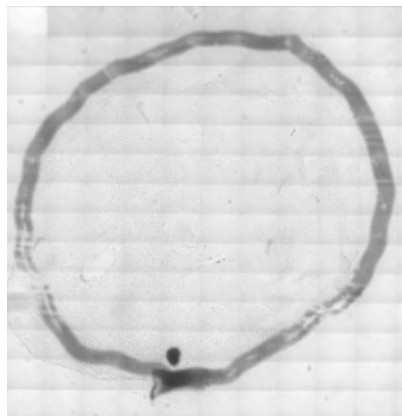
- Need to map flowcell model to tissue sample, but we don't have the exact coordinates of the tixels
 - Tools for mapping and analyzing spatial omic data aren't very developed yet– need to map flowcell manually
- Images must also be fit for registration with H&E tissue



**Process Both
Layers of Image**

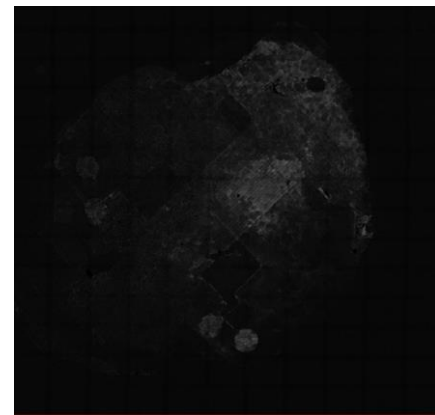


Dye Channel Layer

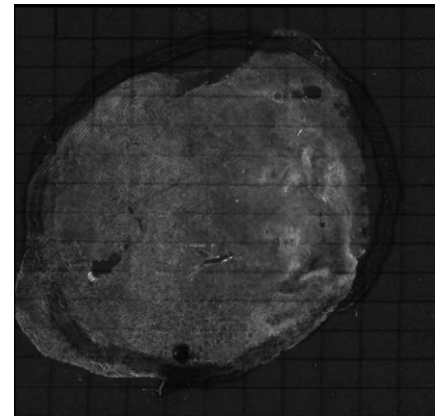


Melanoma Layer

**Invert
color,
adjust
intensity,
and reduce
noise in
image**

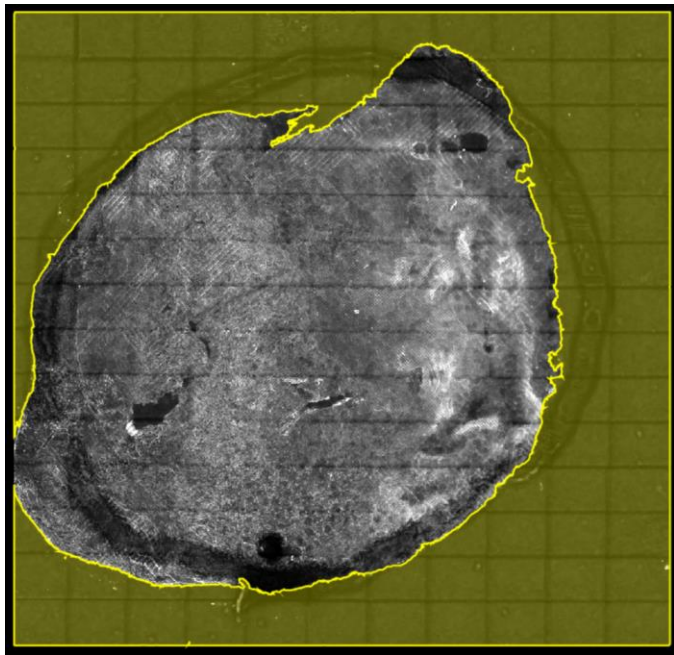


Processed Dye Channel

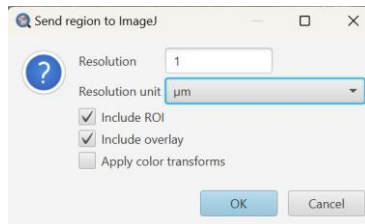


Processed Melanoma

Background Noise Reduction



Use wand tool in QuPath to outline the perimeter of each individually processed DBiT-seq tissue layer



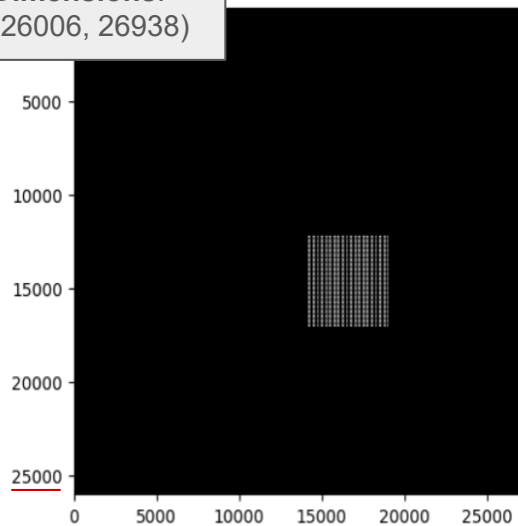
Use ImageJ extension in QuPath to send the annotated region to ImageJ



Use Fill tool while annotation is selected to remove background. This is done on both DBiT-seq layers

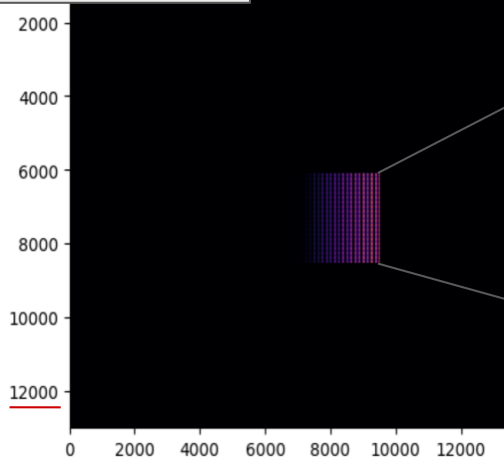
Generating Flowcell Mask

Dimensions:
(26006, 26938)

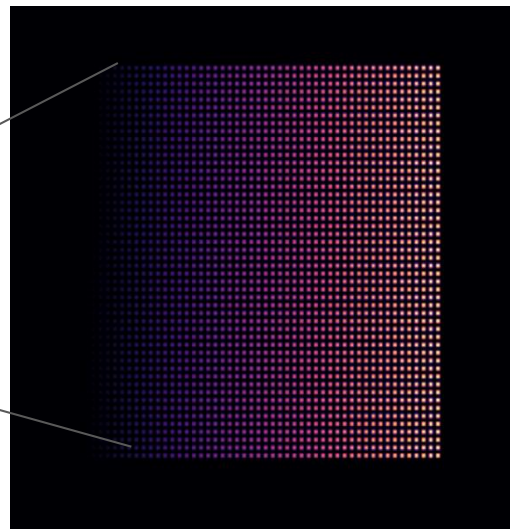


Flowcell Model on 20X
Magnification

Dimensions:
(13003, 13469)

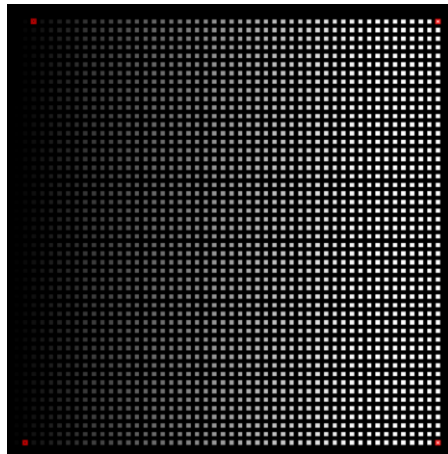
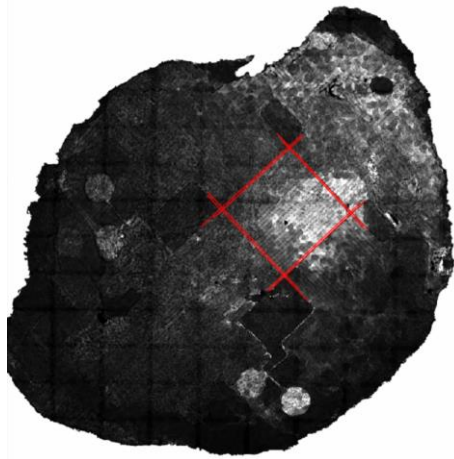


Flowcell Mask on 10X
Magnification



Zoomed in View of Flowcell
Mask

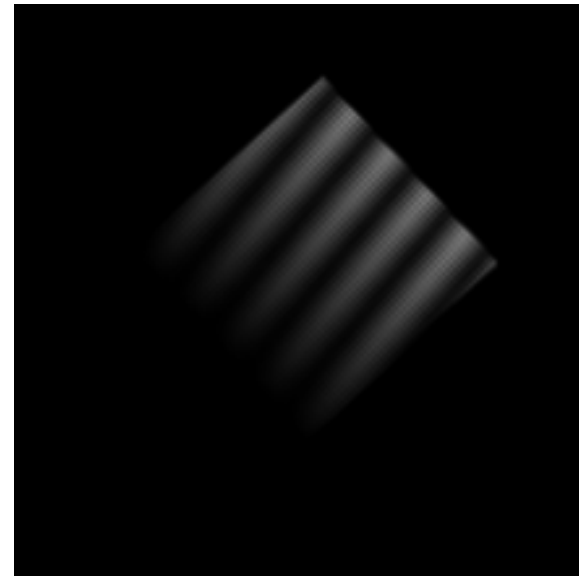
Transform Flowcell Mask to Align with DBiT-seq Tissue



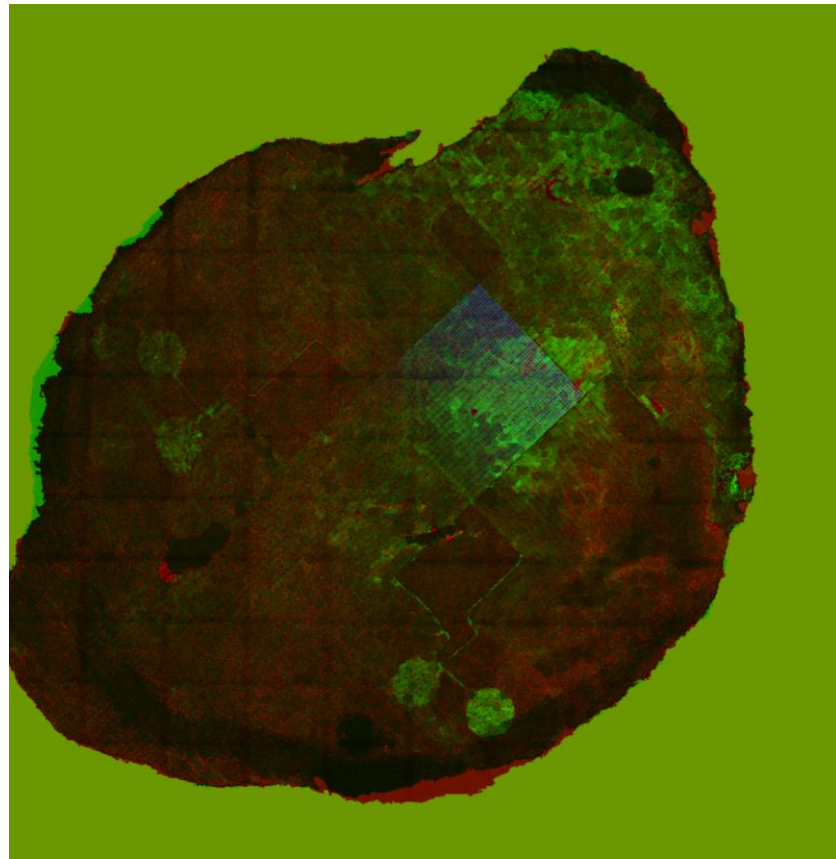
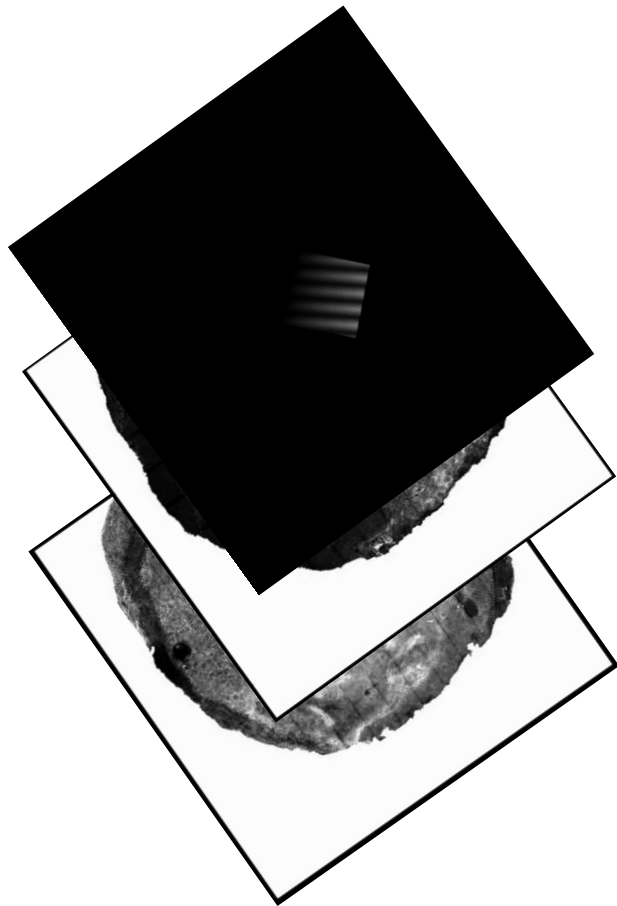
Coordinates of tissue and flowcell
mapped in QuPath

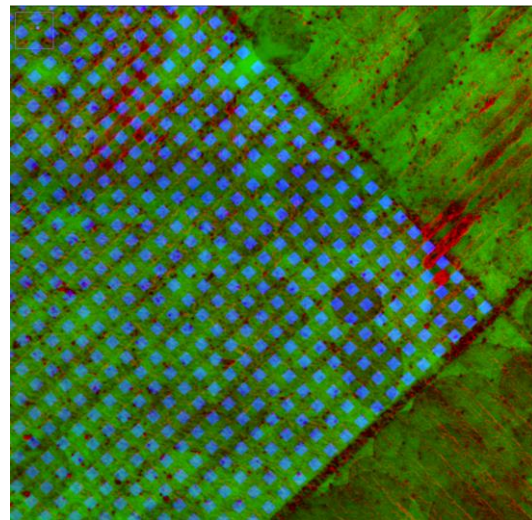
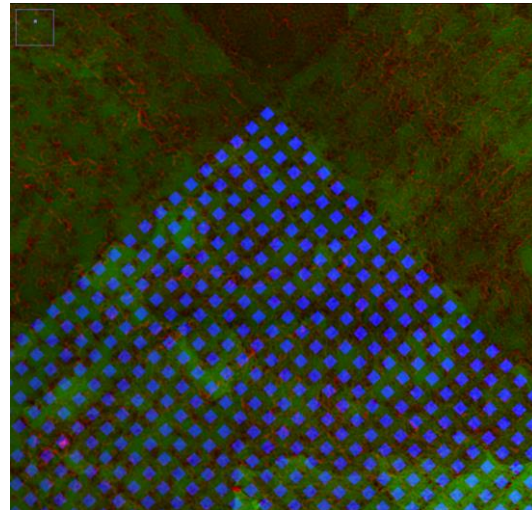
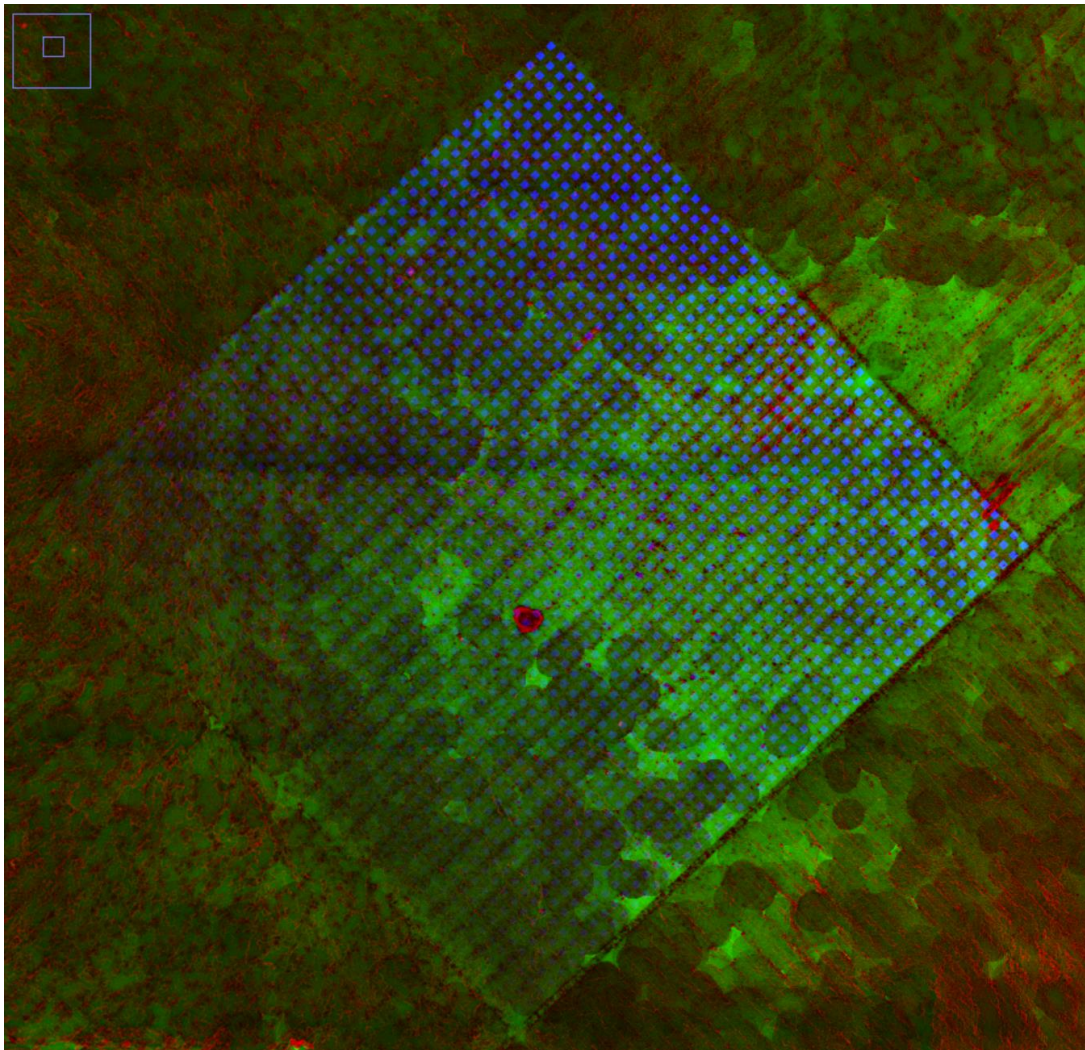


Affine (linear) transformation on the
tixel grid based on tissue
coordinates



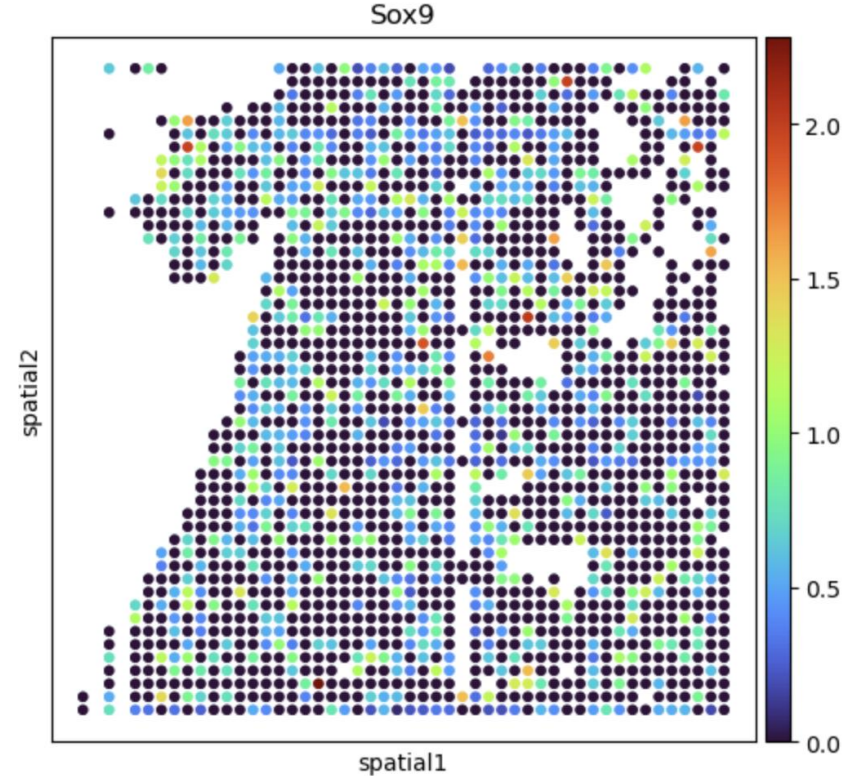
Stacking the Images



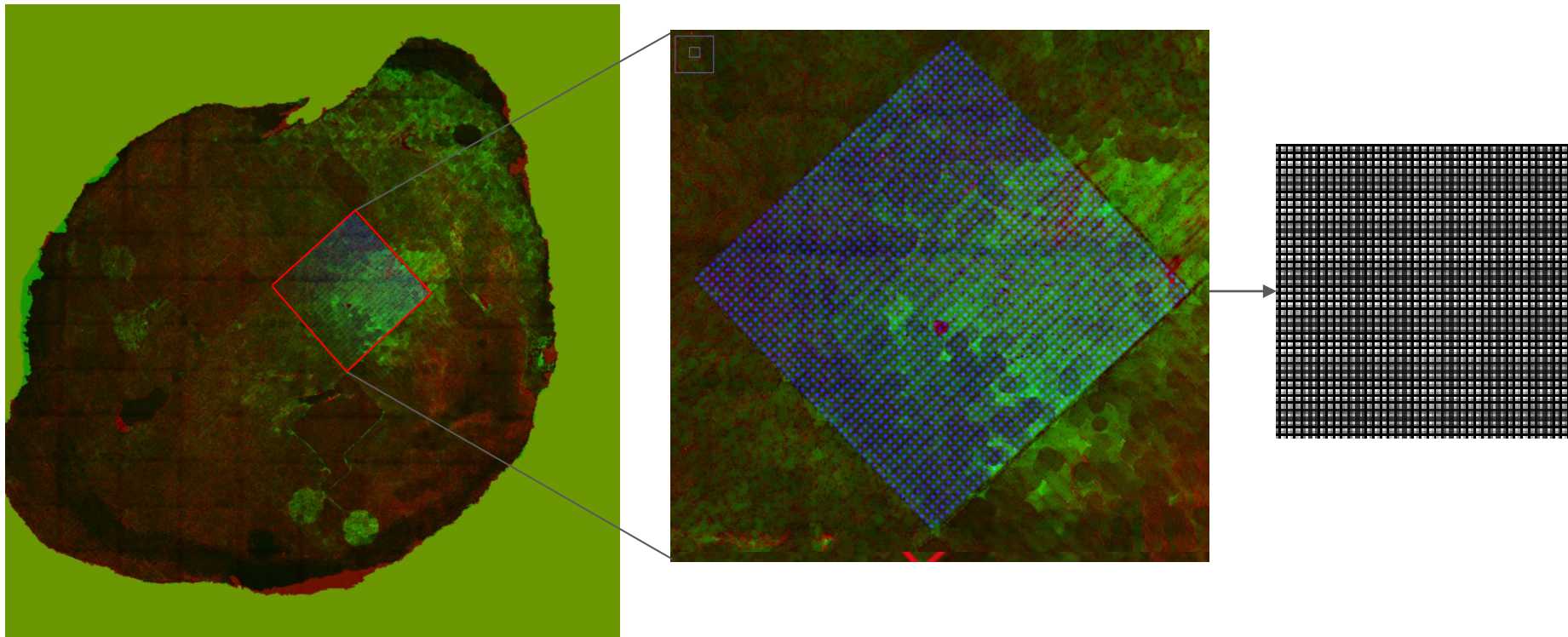


Challenges & Next Steps

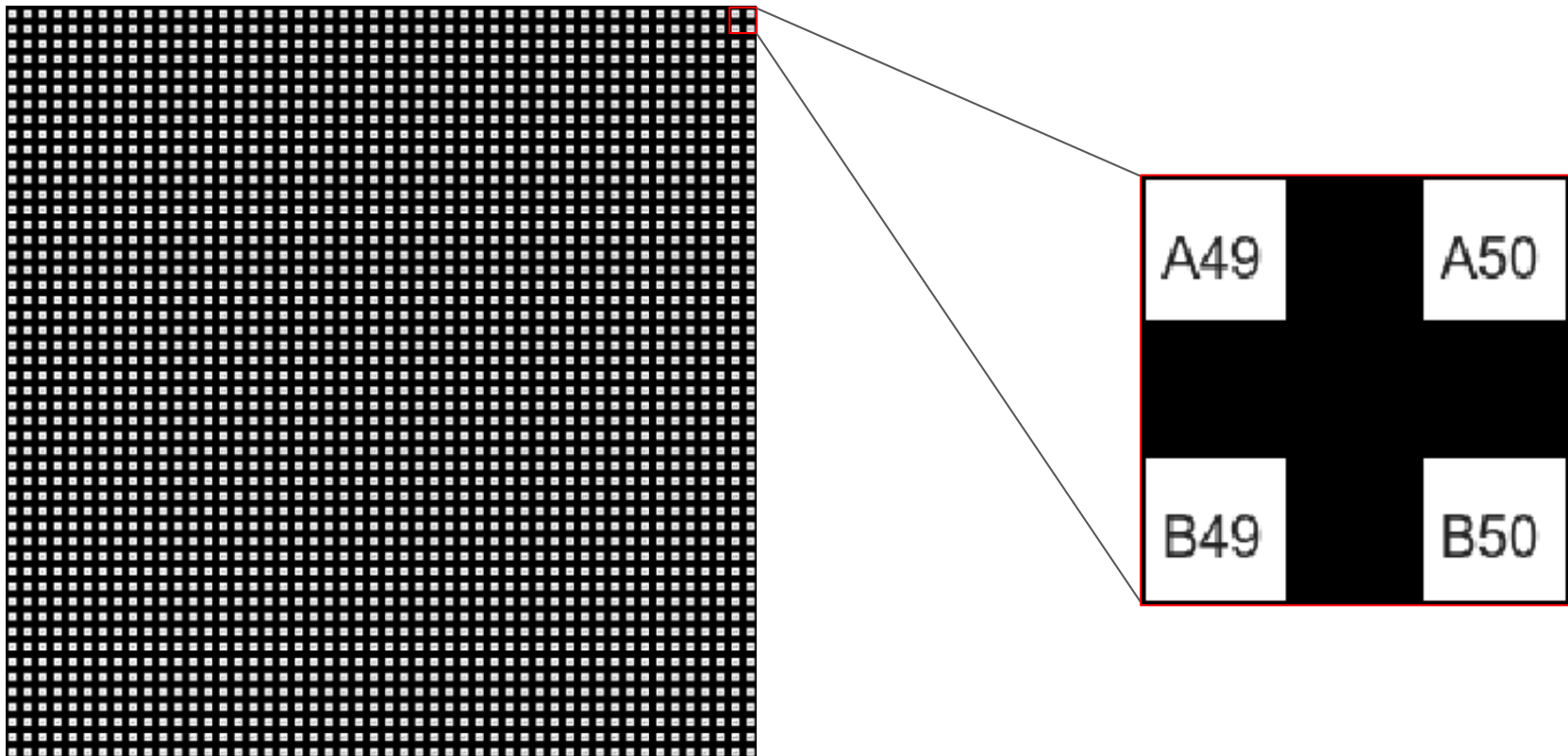
- Registration with the H&E images
- Alternating dye channels
- Verify presence of immune cells with single-cell ATAC-seq analysis



Flowcell model mask segmentation

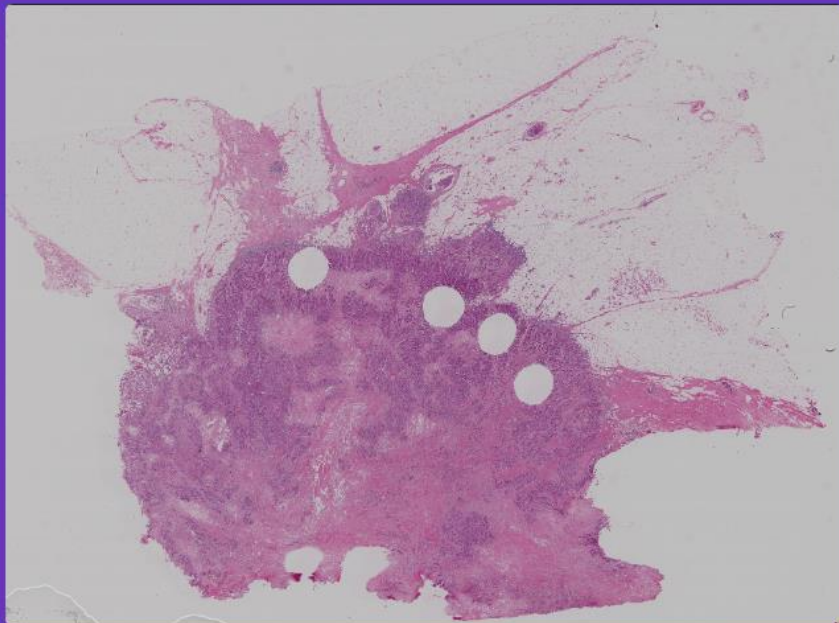


Tixel Mask

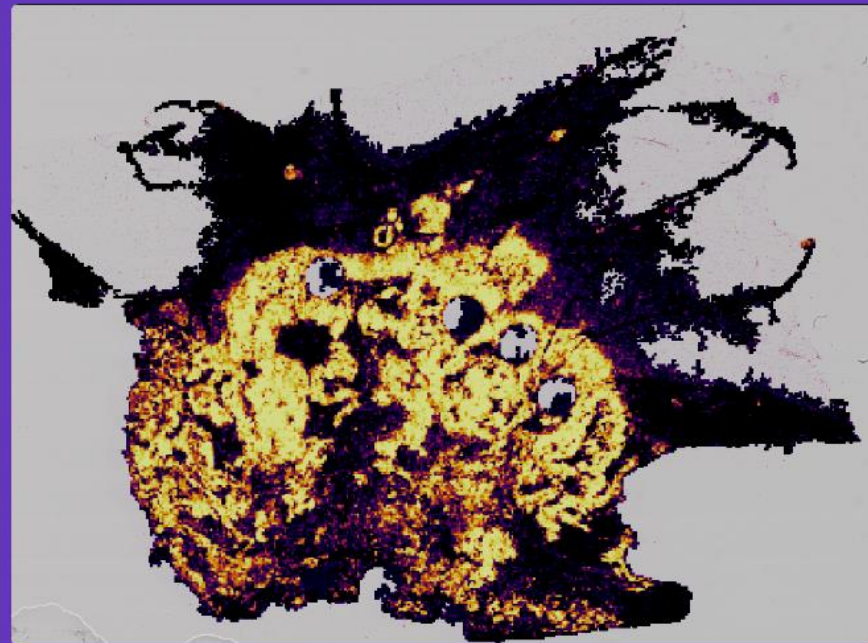


Background on WSInfer

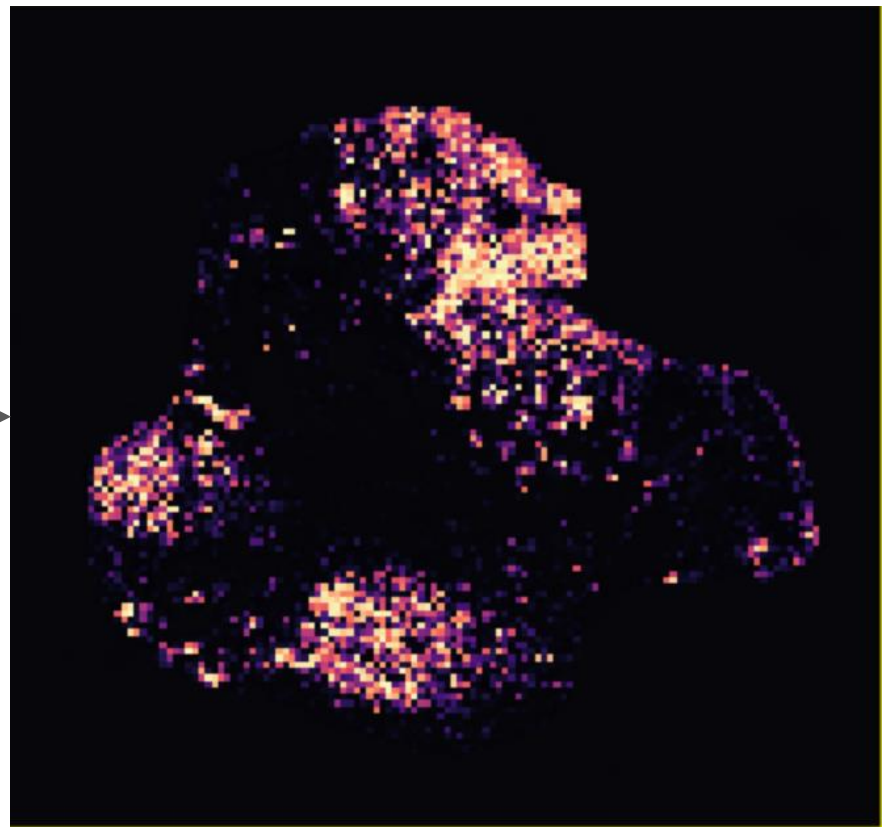
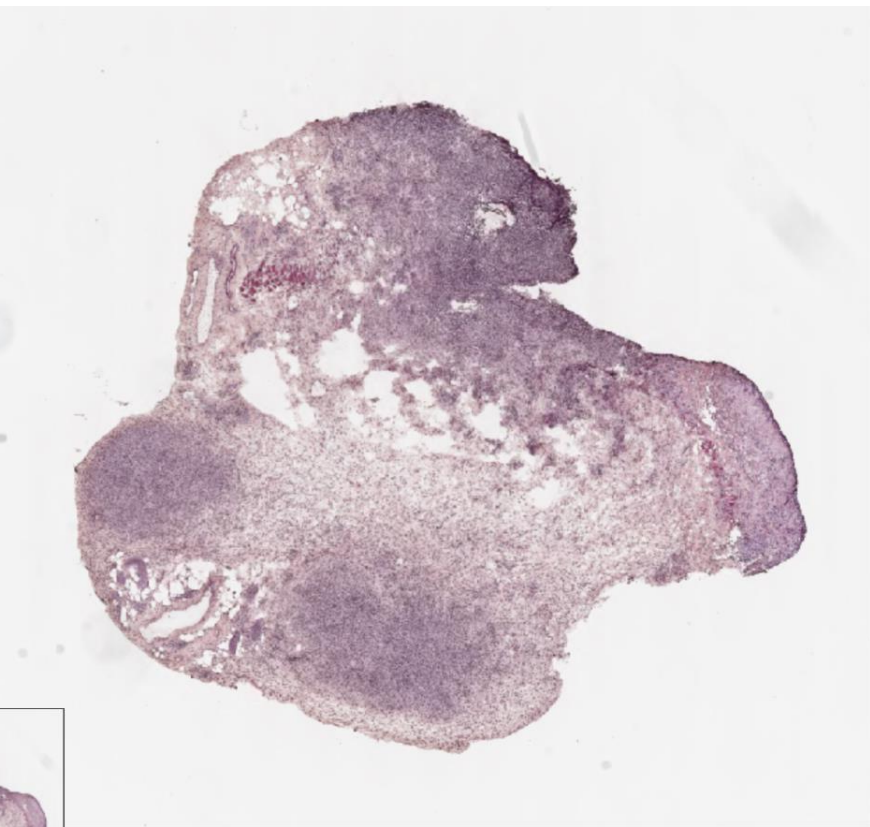
Original H&E



Heatmap of Tumor Probability



H&E G5D3 → G5D3 H&E HEATMAP Using WSInfer



Running the model

WSInfer

Run Inference

Select a model

pancancer-lymphocytes-inception...

Create or select an annotation

or

Select all Annotations Tiles

Run

No annotation or detection selected

View Results

Measurement Maps Results Table

Overlay Opacity

Additional Options

Preferred device: cpu

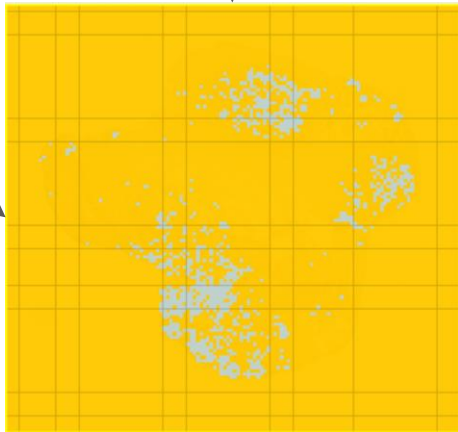
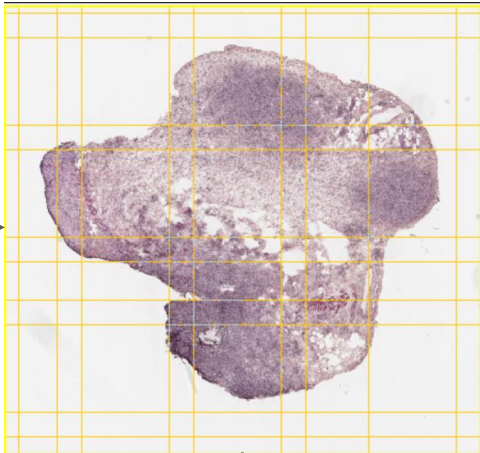
Batch size: 4

Number of parallel tile loaders: 4

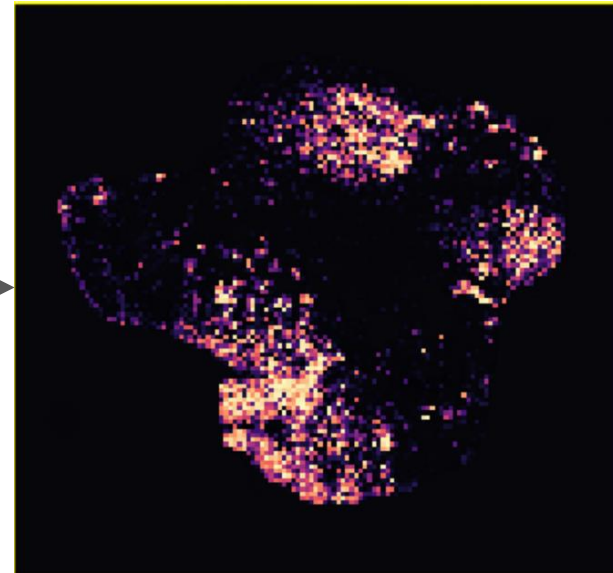
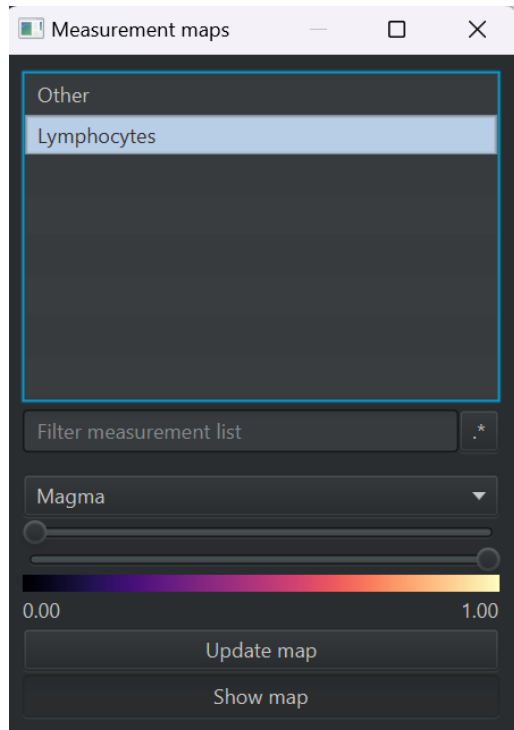
Downloaded model directory:

C:\Users\mohit\QuPath\v0.5\wsinfer

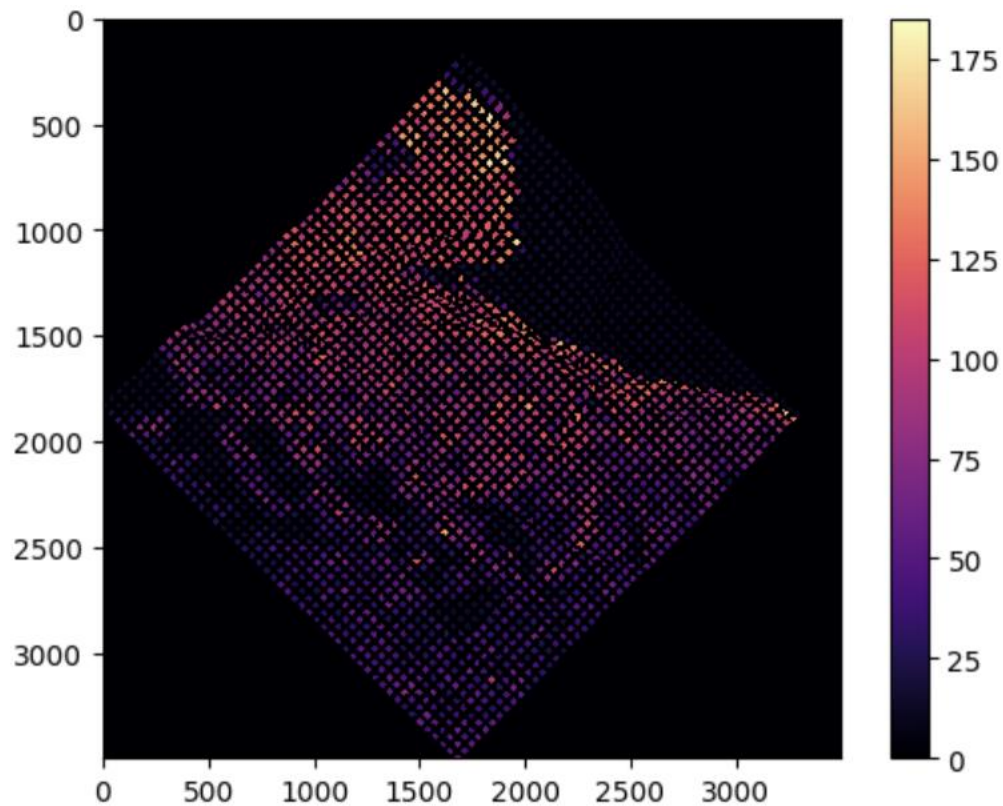
pancancer-lymphocytes-inception...



Generating the Heatmap



Plotting the WSIInfer probabilities onto tixels



Future

Automating the heatmap process

Using other models to make predictions like finding a tumor or normal cell

Updating the mask to a better set of labels

Challenges

New to Python and coding in general

Learning new libraries; scikit-learn, pandas, matplotlib etc.

Our mentors



David Gibbs
Vesteinn Thorsson

Thank you

Special thanks

Shmulevich Lab
Heath Lab

Chong Xia
Juho Kim