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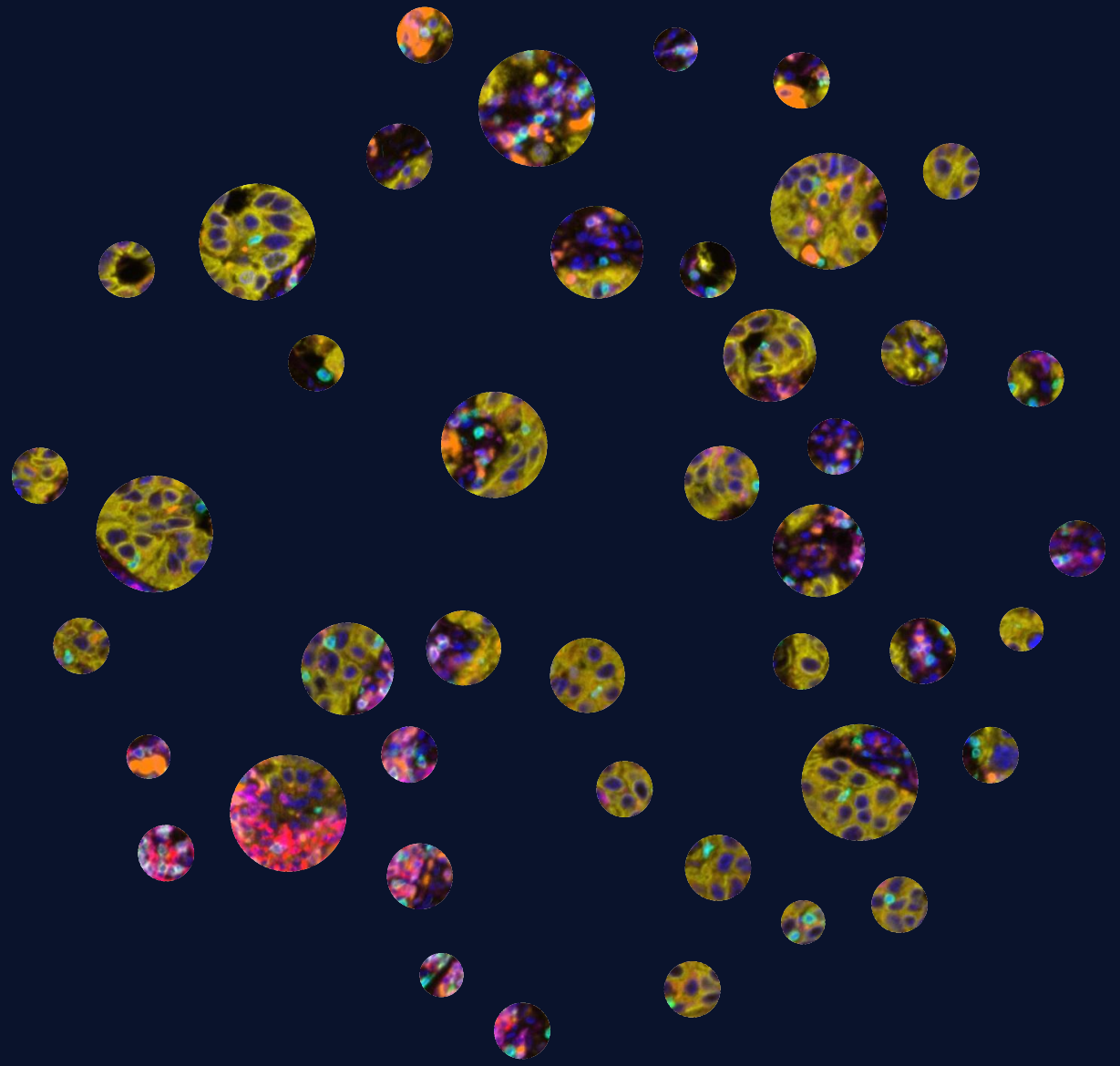
PhenoImager Data Analysis using QuPath and Python

Software Workflow

Aditya Pratapa, Ph.D.

Akoya Biosciences

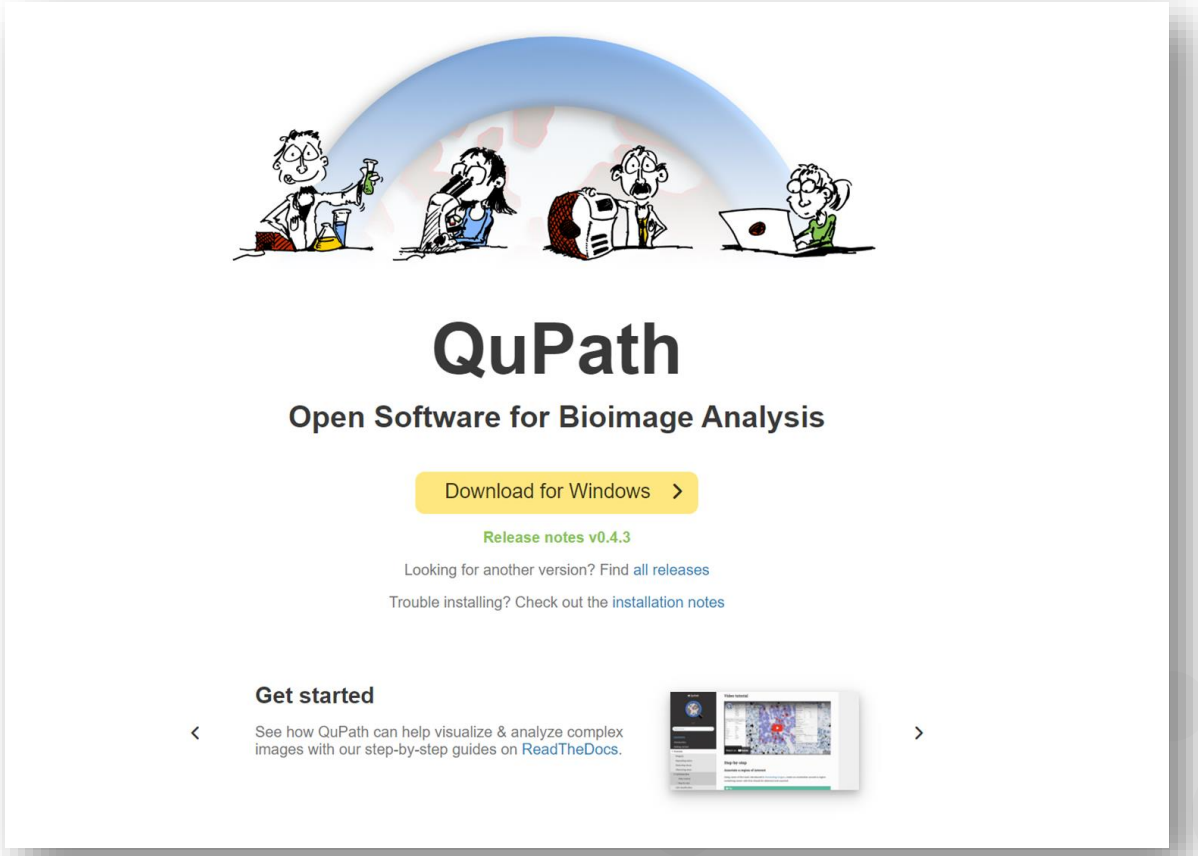


Learning Objectives

- QuPath
  - QuPath project set-up
  - Cell segmentation using StarDist
  - Data export
- Python
  - Anaconda environment set-up
  - Data import
  - Automated phenotyping
  - SpatialScore computation

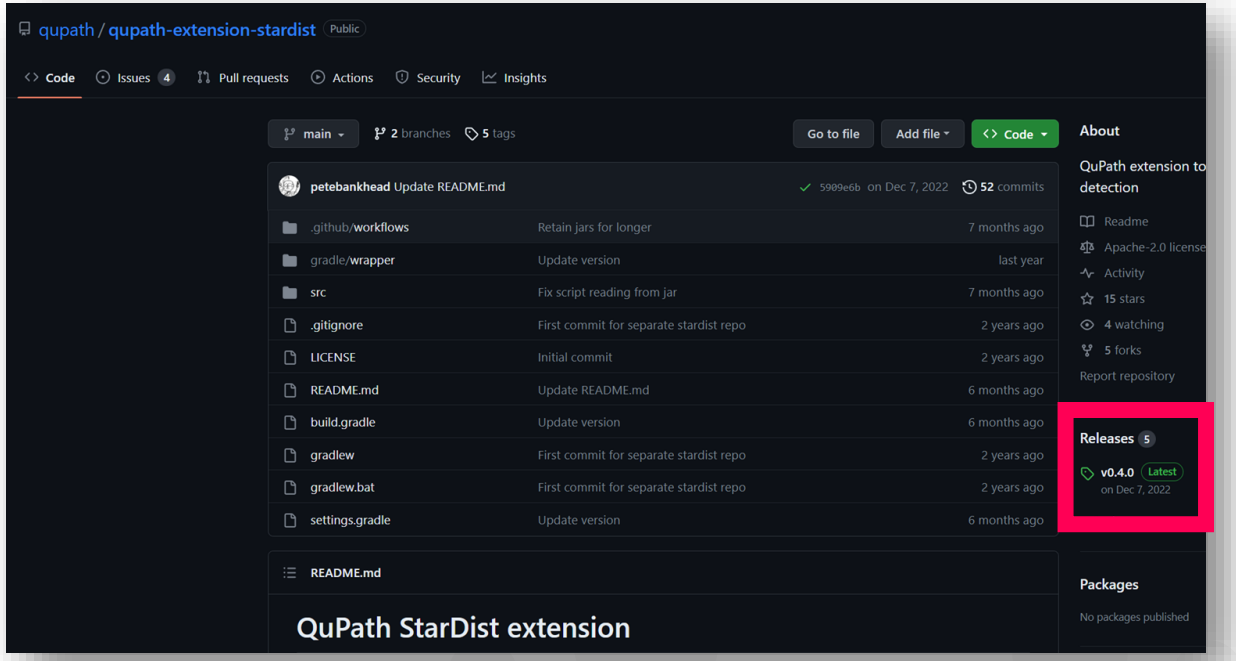
QuPath set-up

- Basic QuPath:
  - Grab the latest version of QuPath v0.4.3
  - https://qupath.github.io



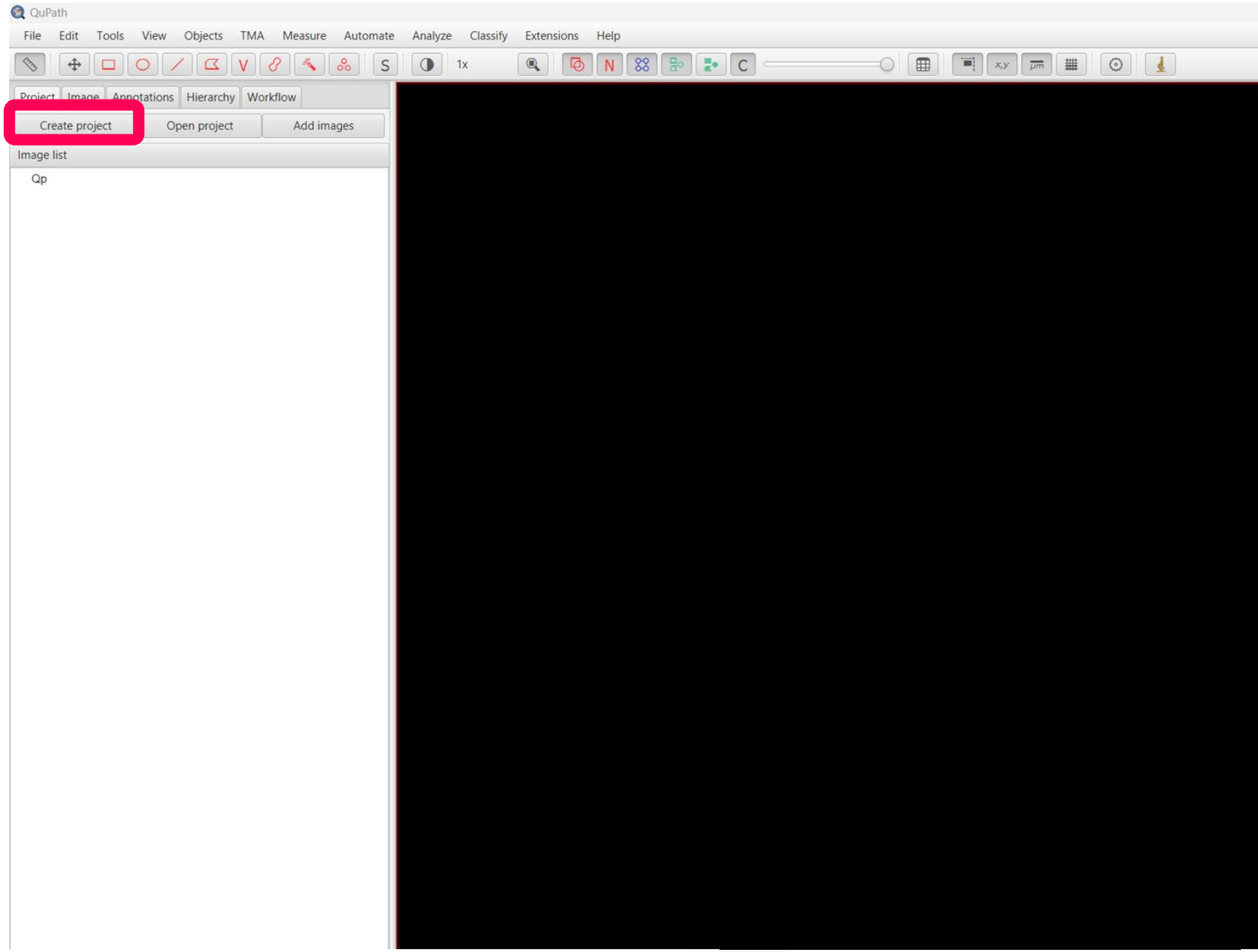
QuPath set-up

- Basic QuPath:
  - Grab the latest version of QuPath v0.4.3
  - https://qupath.github.io
- For Cell Segmentation
  - QuPath's StarDist extension
  - https://github.com/qupath/qupath-extension-stardist
  - To install the StarDist extension, download the latest qupath-extension-stardist-[version].jar file from releases and drag it onto the main QuPath window.



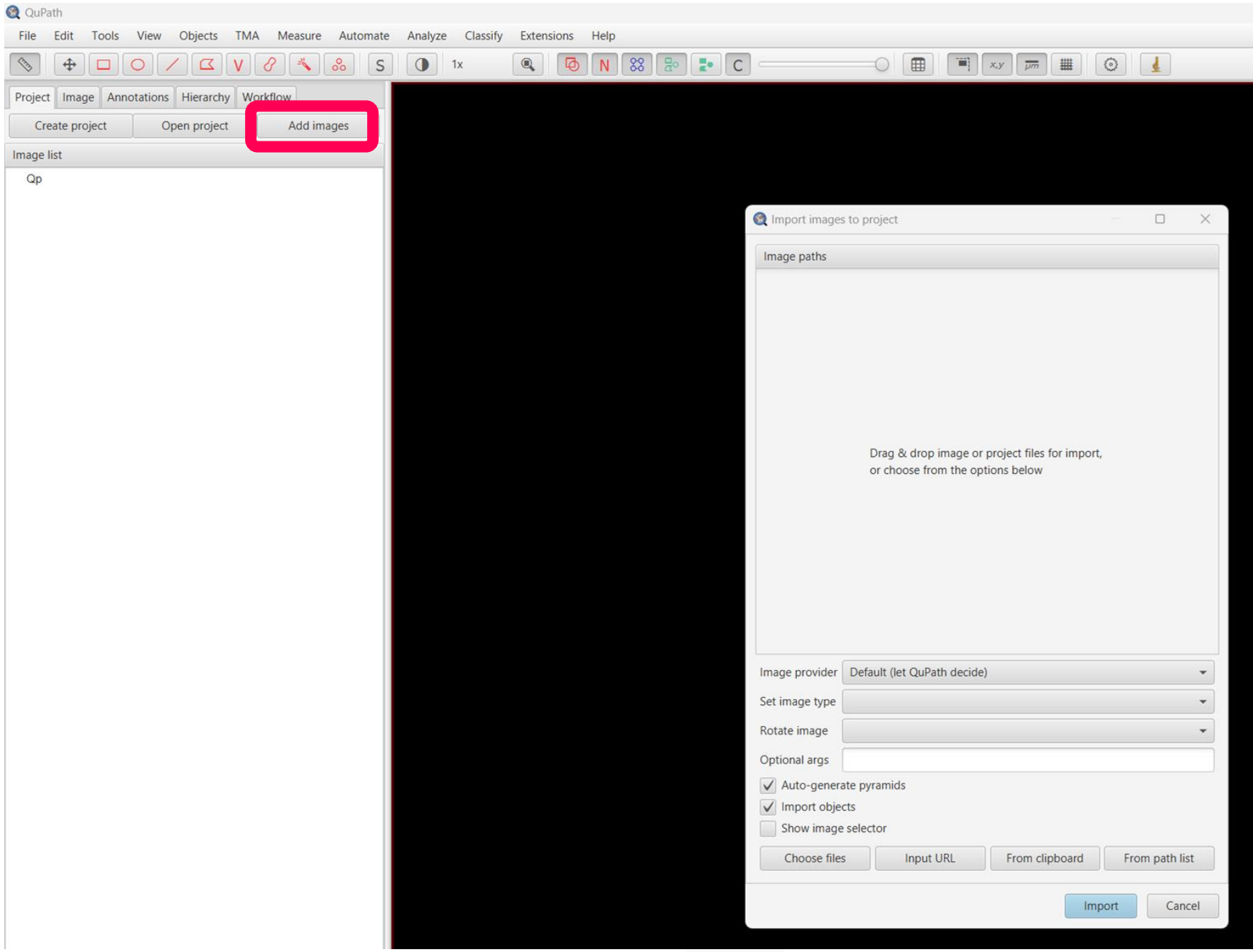
Create Project

- Create a new QuPath project in an empty folder



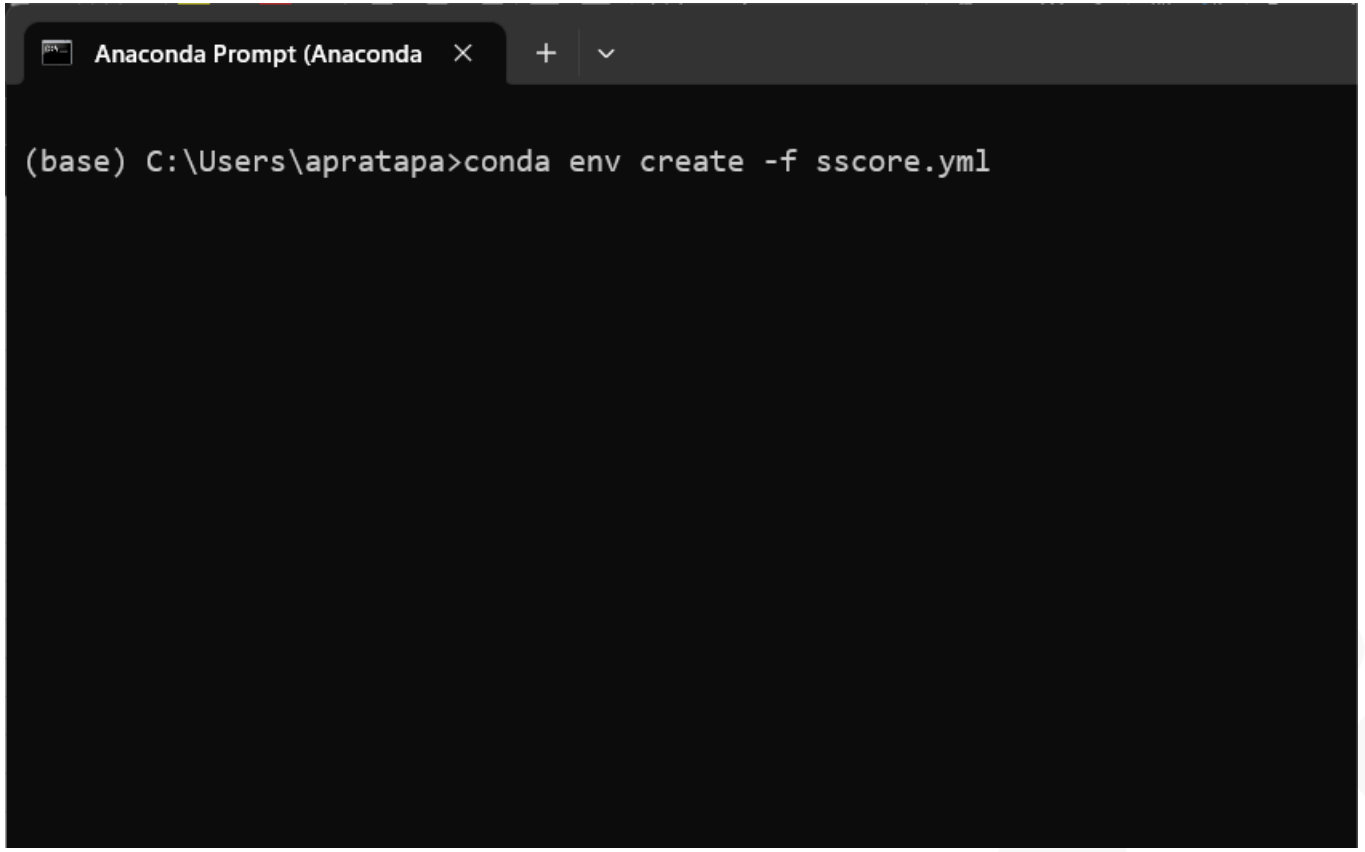
Add images

- Add unmixed ome.tif files



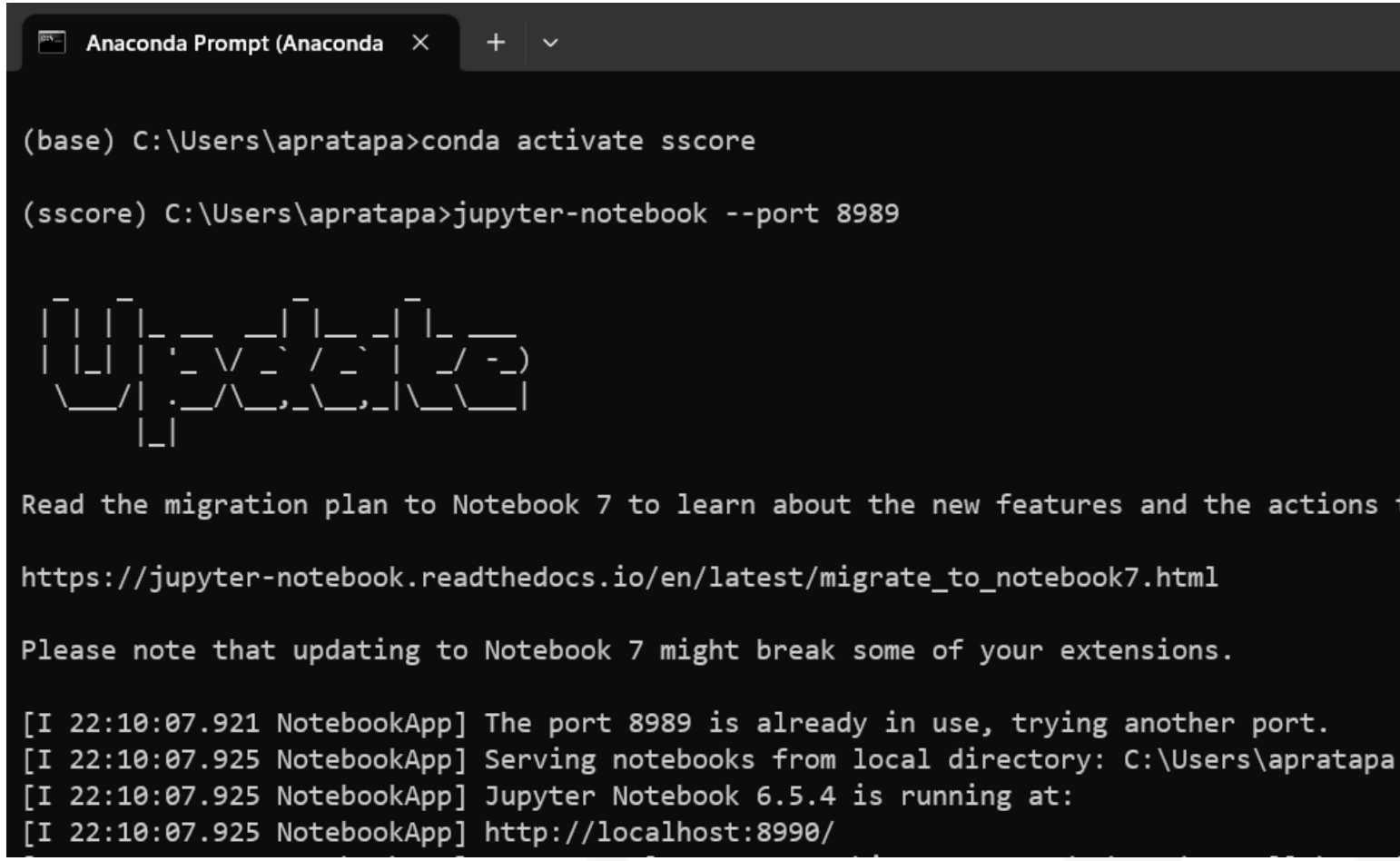
Python Environment Set-up

- Download and install Anaconda
  - https://anaconda.org/
- Anaconda prompt
  - Run ‘conda env create –f sscore.yml’



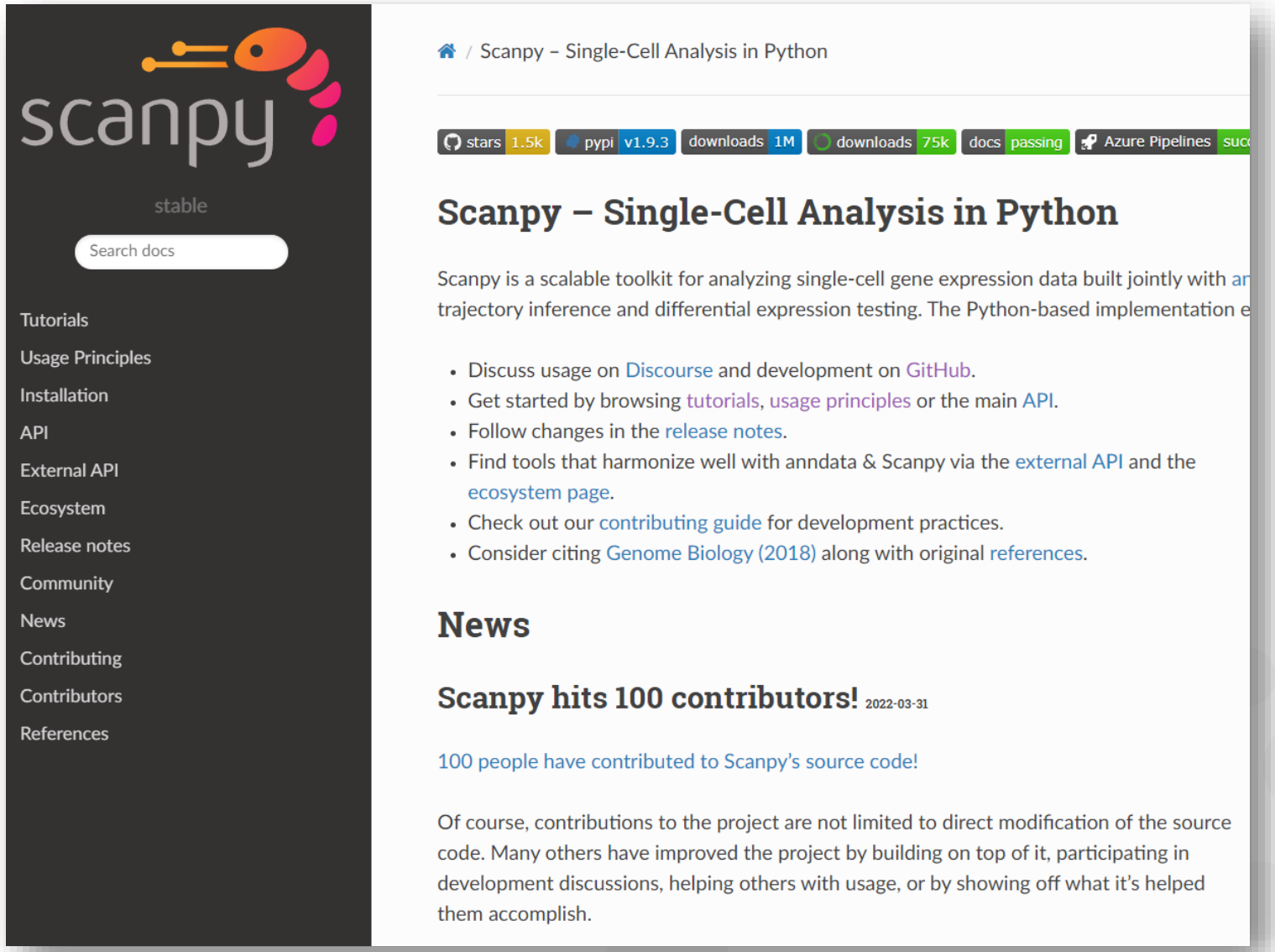
Start the notebook server

- Activate newly installed environment
  - conda activate sscore
- Start a jupyter notebook server
  - jupyter-notebook --port 8989



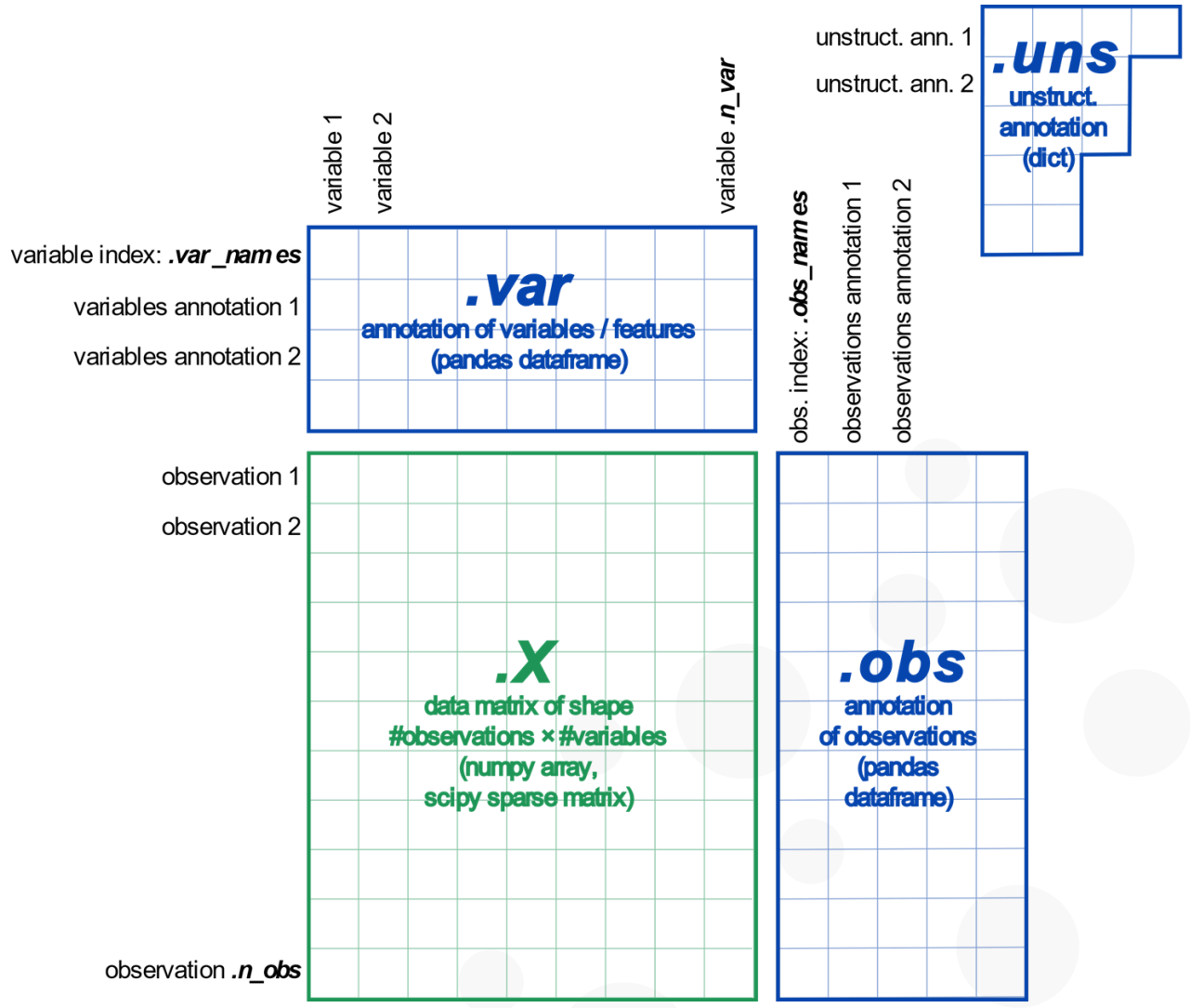
Step-1: Data Import

- Scanpy library for single-cell analysis
- Read measurements.tsv and create an AnnData object



Step-1: Data Import

- AnnData
  - Expression data: .X
  - Spatial data: .obs
  - Cell metadata: .obs



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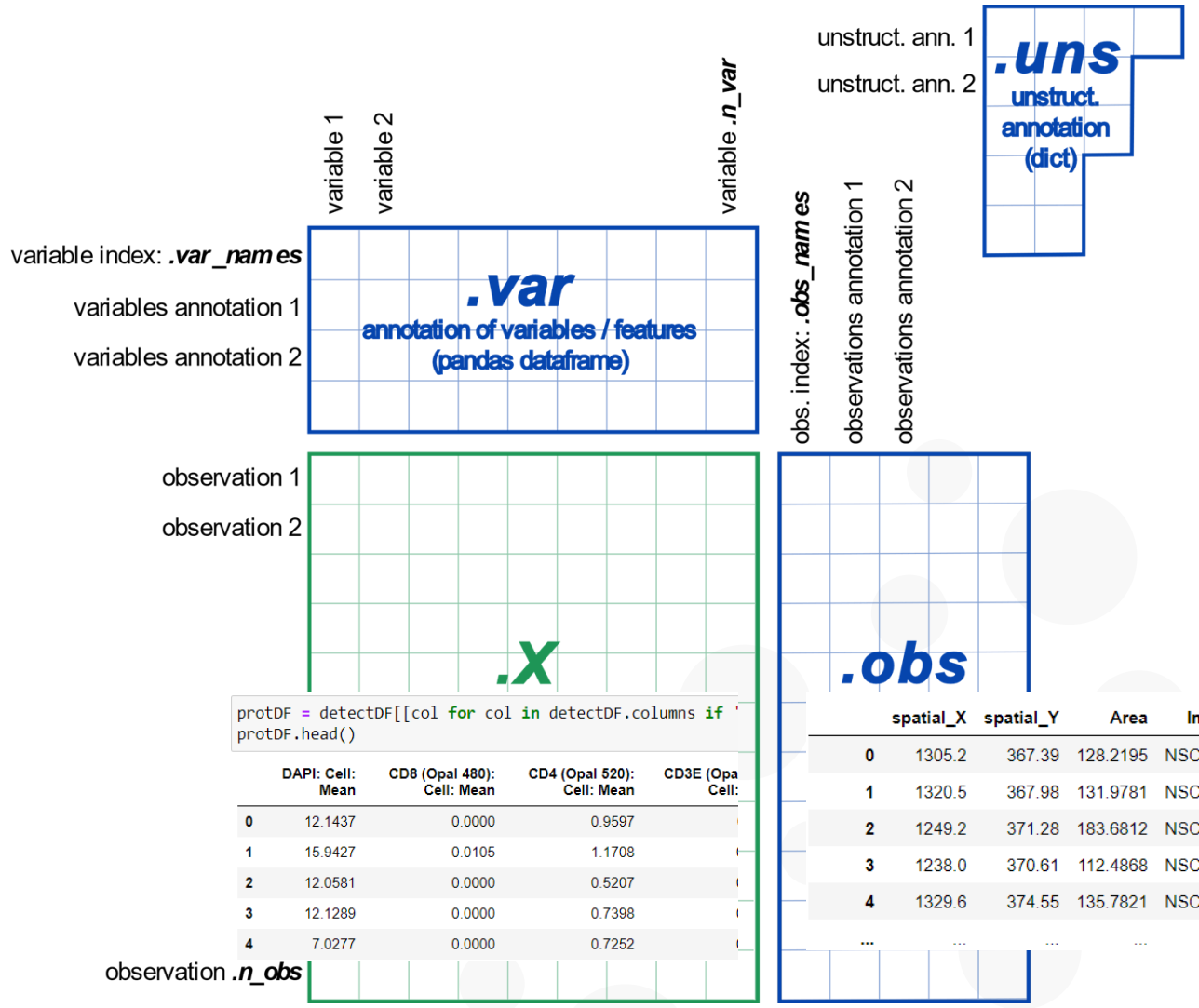
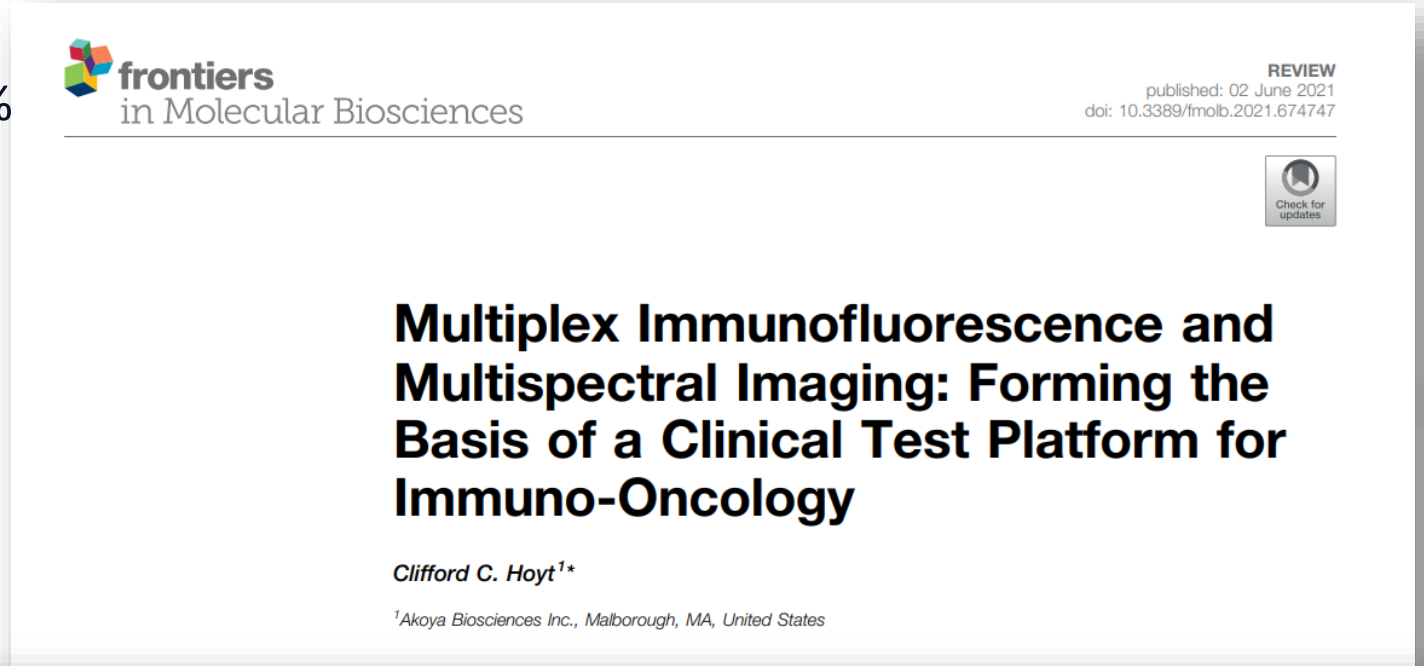


Step-2: Evaluate Staining Quality

- Compute Mean of top 20 cells and divide it by the mean of the bottom 10% of the cells of each marker
- Use ratio of top20:bottom10 as the signal-to-background(noise) ratio
- Ideally, greater than 10
  - Typically > 100



frontiers in Molecular Biosciences

REVIEW
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doi: 10.3389/fmolb.2021.674747

Multiplex Immunofluorescence and Multispectral Imaging: Forming the Basis of a Clinical Test Platform for Immuno-Oncology

Clifford C. Hoyt[1]*

[1]Akoya Biosciences Inc., Malborough, MA, United States

Dynamic Range Our standard approach assessing dynamic range is to calculate a signal-to-background (SNR) ratio by dividing the average of the top 20 brightest cells by the average intensity of the weakest 10% of cells. An SNR of 10 or more supports reliable image analysis, including accurate counting of positive cells and quantifying expression levels. While we recommend an SNR of 10 or greater, typical ratios are well in the 100s with high-performing antibodies, or as low as 3-to-1 that still provide analytical value.

Step-2: Evaluate Staining Quality Control

- Compute Mean of top 20 cells and divide it by the mean of the bottom 10 percentile of each marker
  - computeTop20Btm10(AnnData)
- Use ratio of top20:bottom10 as the signal-to-background(noise) ratio
- Ideally, greater than 20
  - Inf indicates zero background

ImageID	Protein	Top20/btm10
NSCLC_S1	CD8	inf
NSCLC_S1	CD4	inf
NSCLC_S1	CD3E	3306.3506
NSCLC_S1	CD20	771.2482
NSCLC_S1	PanCK	436.5684
NSCLC_S1	CD68	959.6335
NSCLC_S2	CD8	inf
NSCLC_S2	CD4	73434.0859
NSCLC_S2	CD3E	1826.7528
NSCLC_S2	CD20	64.1688
NSCLC_S2	PanCK	1425.5354
NSCLC_S2	CD68	276.1166

Step-3: Phenotyping

SCIMAP

Search

GitHub
1.0.0 39 14

Home Getting Started Tools Shortcut SCIMAP Functions Tutorials

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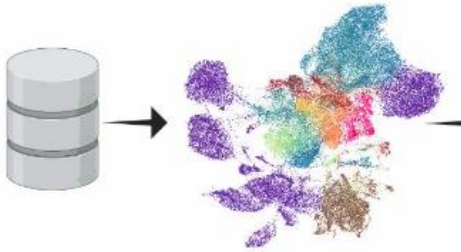
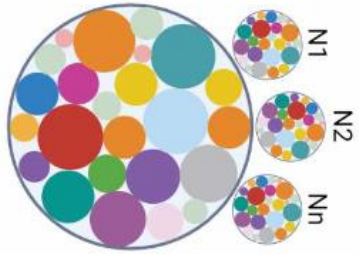
Spatial Data
.csv
.h5ad

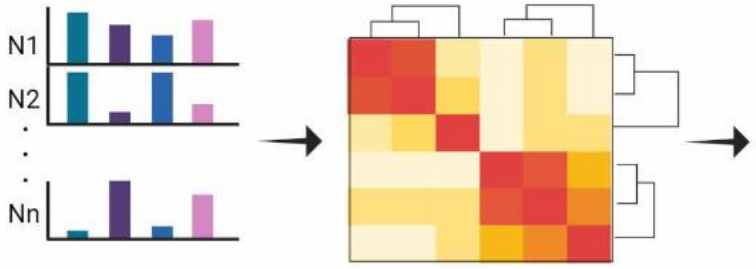


Gating,
Clustering and
Phenotyping

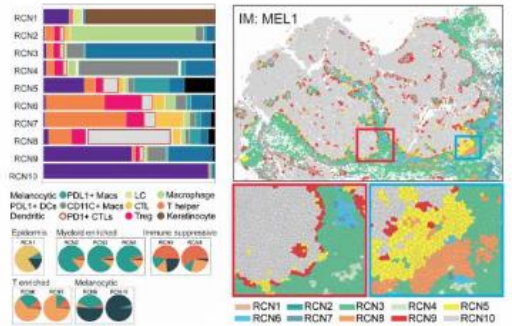
Build
Neighbourhood
Graph



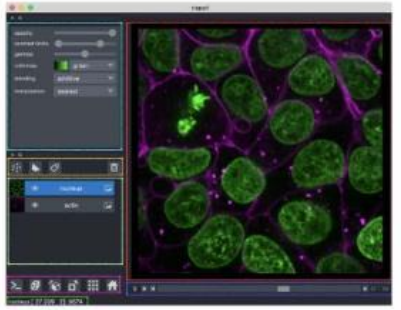
Spatial-LDA and similar methods
for identifying Reccurent Cellular
Neighbourhood



Spatial Distances and
Neighbourhood Frequencies



Voronoi
Diagram's
and other
plotting
tools

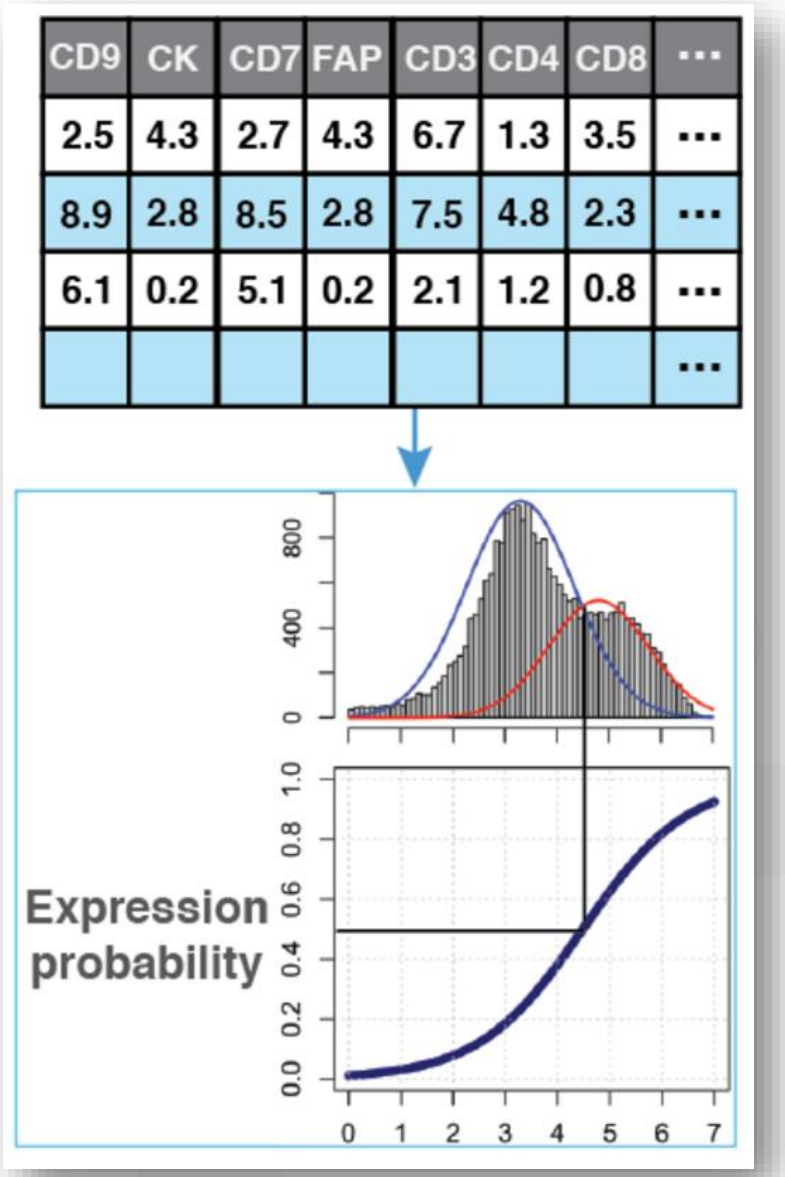


Napari
Visualization

www.scimap.xyz

Step-3: Phenotyping

- Step 3a: Rescale data using GMM
  - Fit a 2-class Gaussian Mixture Model
  - Fully automated
  - Converts raw intensities to probabilities, i.e., values between 0-1
  - Value >= 0.5 indicated ‘positive’ for a given marker
  - sm.pp.rescale(method=‘by_image’)

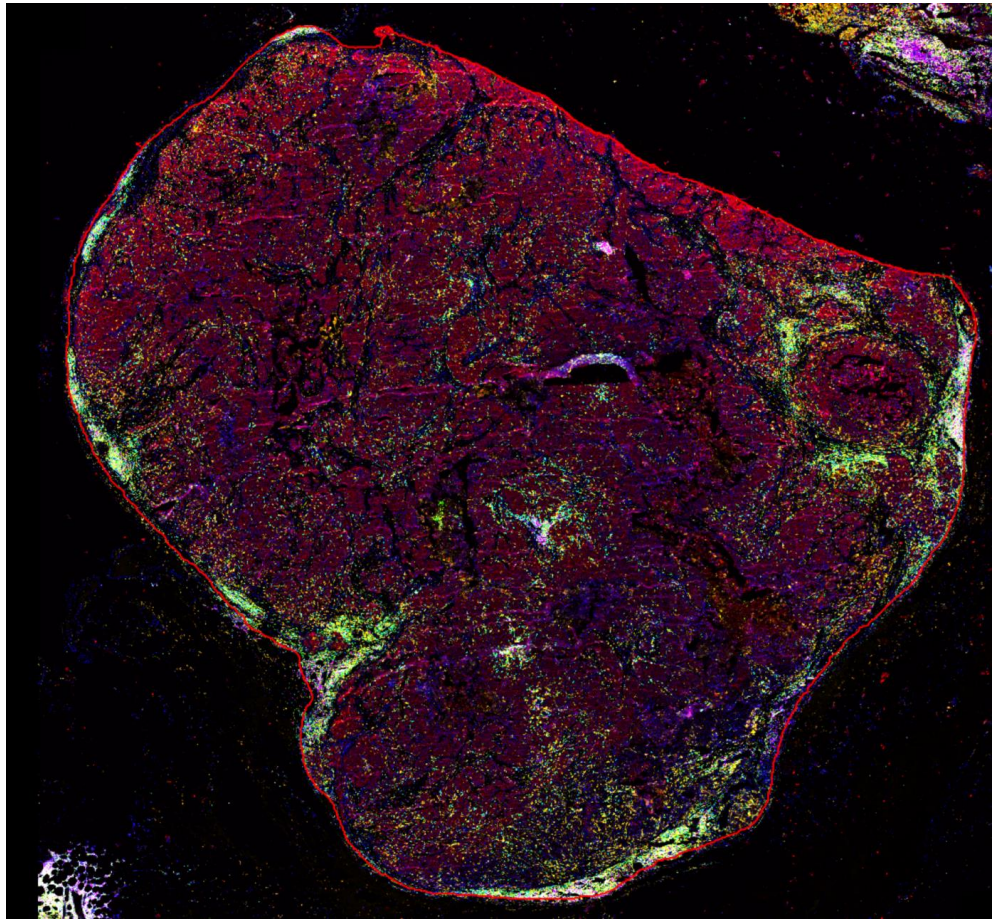


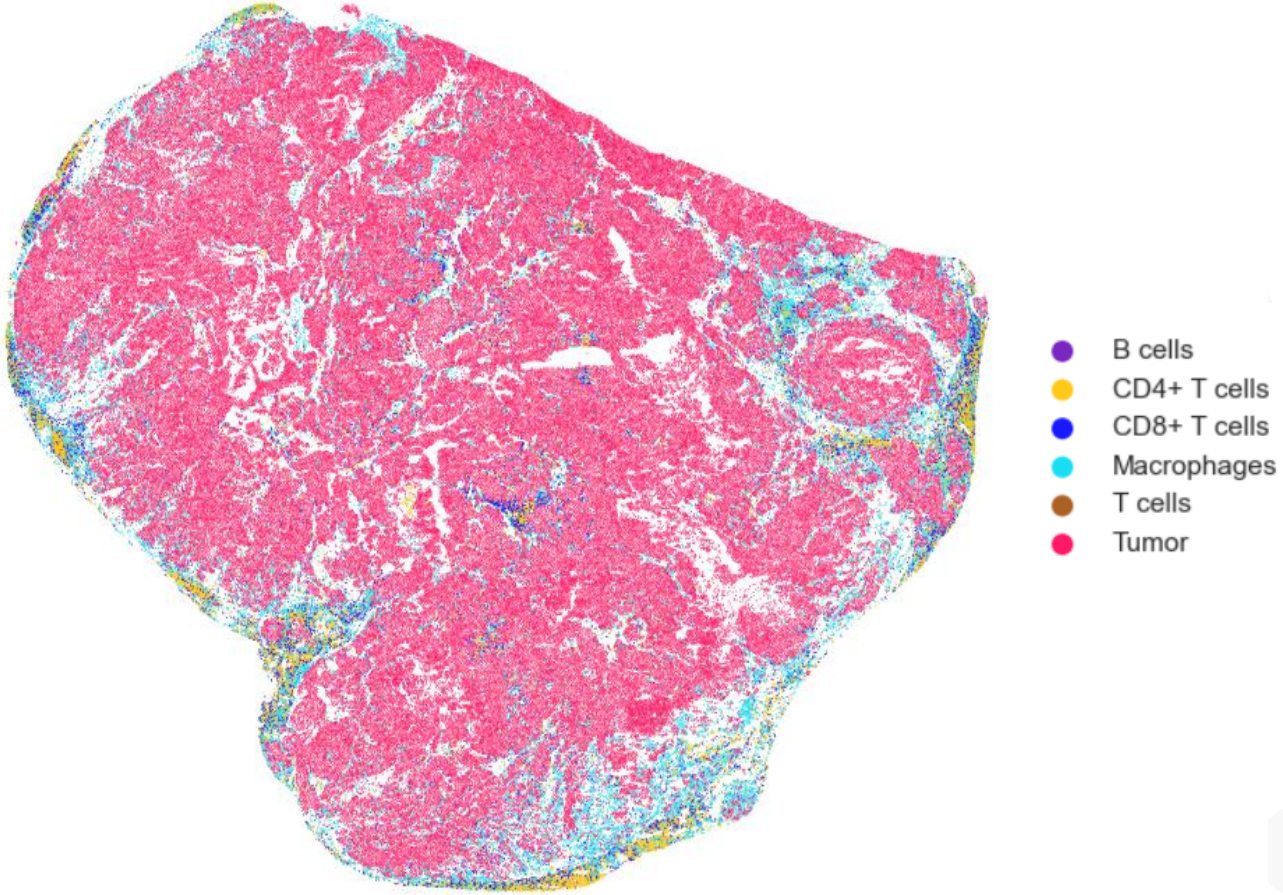
Step-3: Phenotyping

- Step 3b: Rule-based phenotyping
  - Value >= 0.5 indicated ‘positive’ for a given marker
  - If ‘pos’ -> assign a cell type
    - **CD20+** : B cells
    - **PanCK+**: Tumor
    - …
    - **CD3E+ CD8+**: Cytotoxic T cells
  - sm.tl.phenotype_cells()

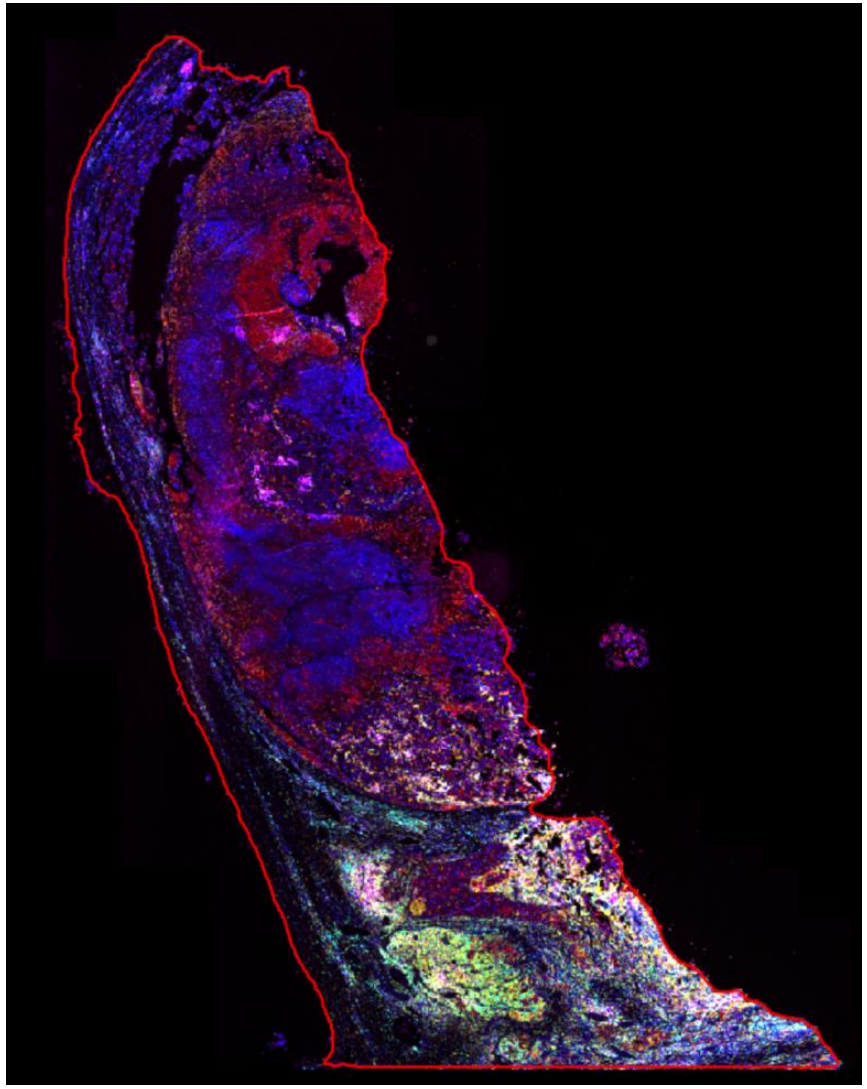
parent	child	CD3E	CD20	PanCK	CD68	CD8	CD4
all	B cells		pos				
all	T cells	pos					
all	Tumor			pos			
all	Macrophages				pos		
T cells	CD8+ T cells					pos	
T cells	CD4+ T cells						pos

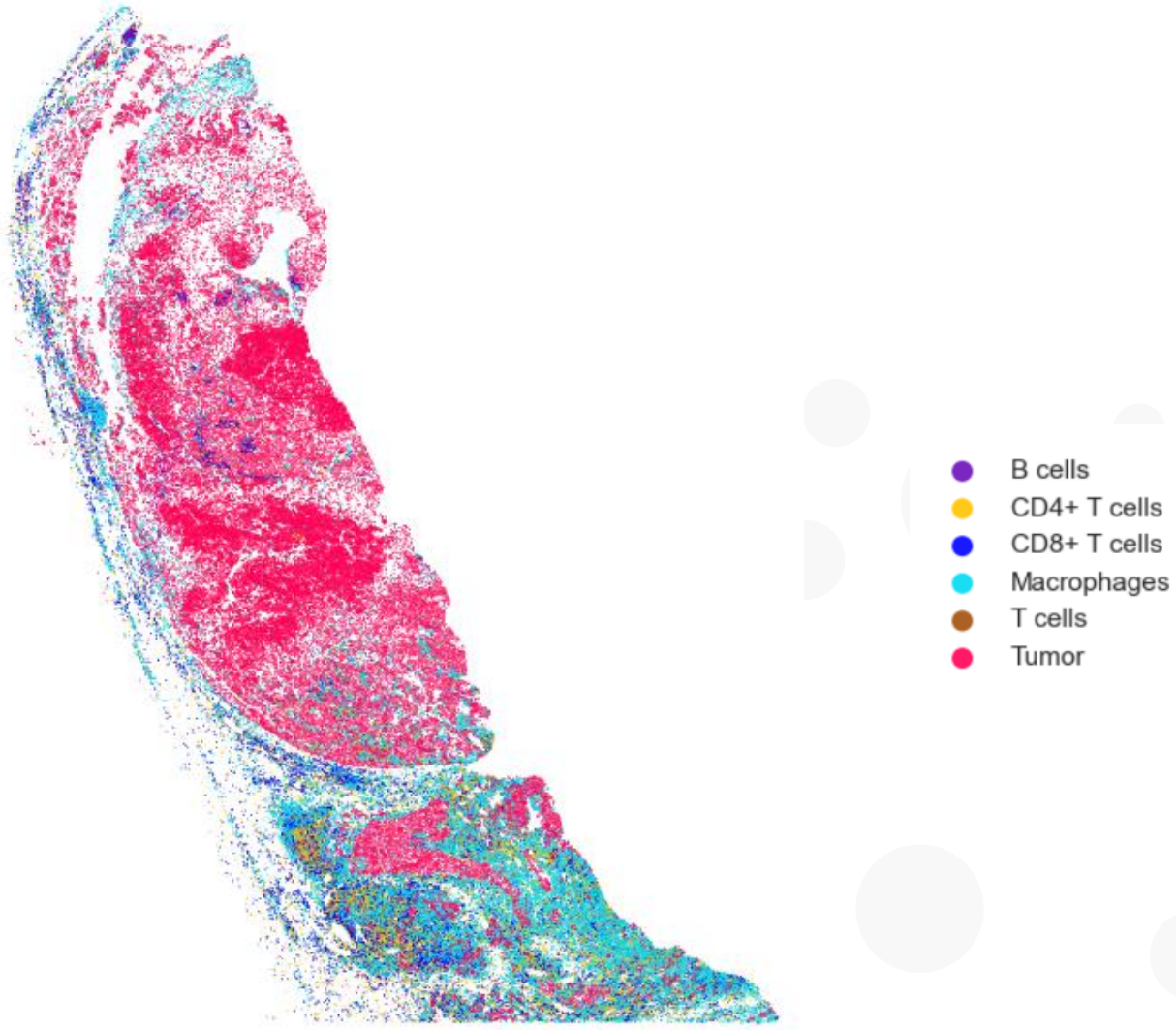
Step-3: Phenotyping





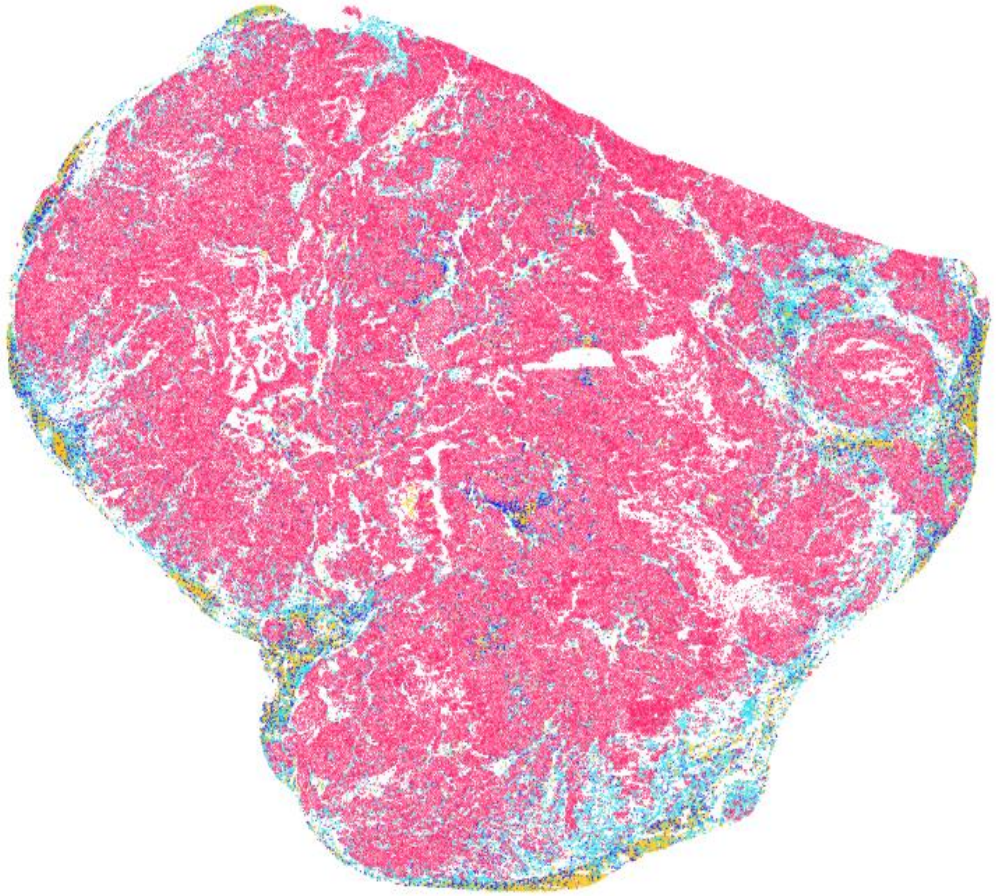
Step-3: Phenotyping

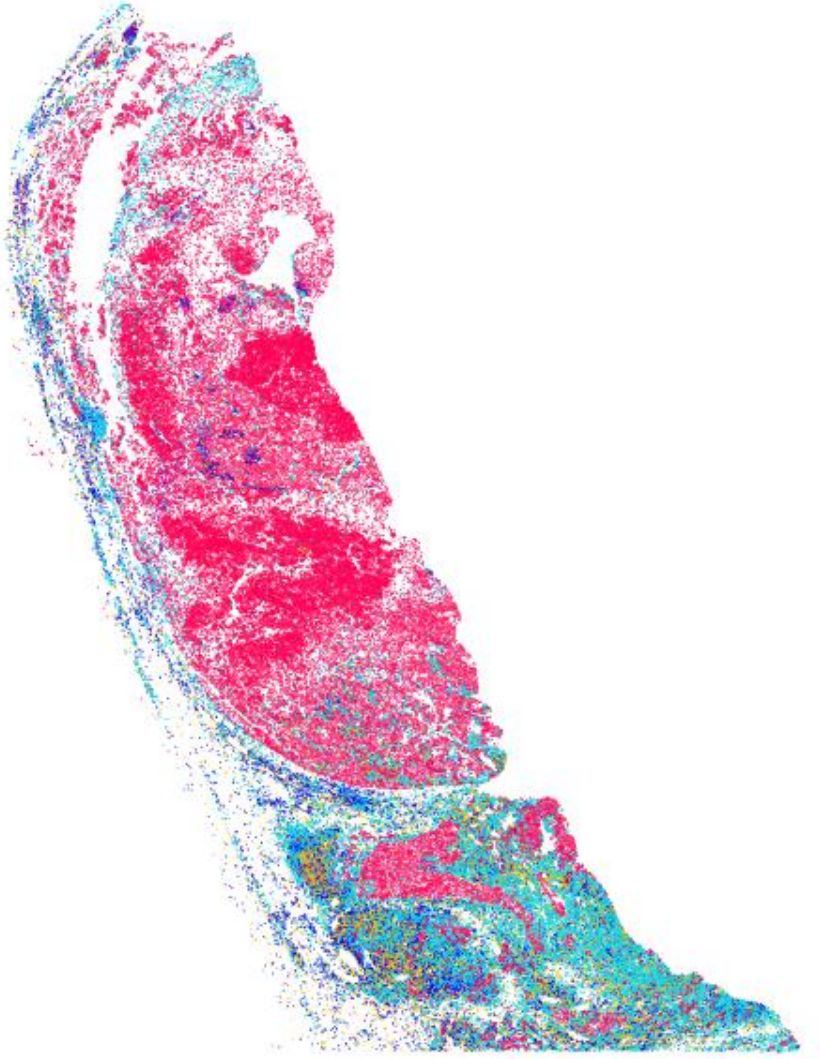




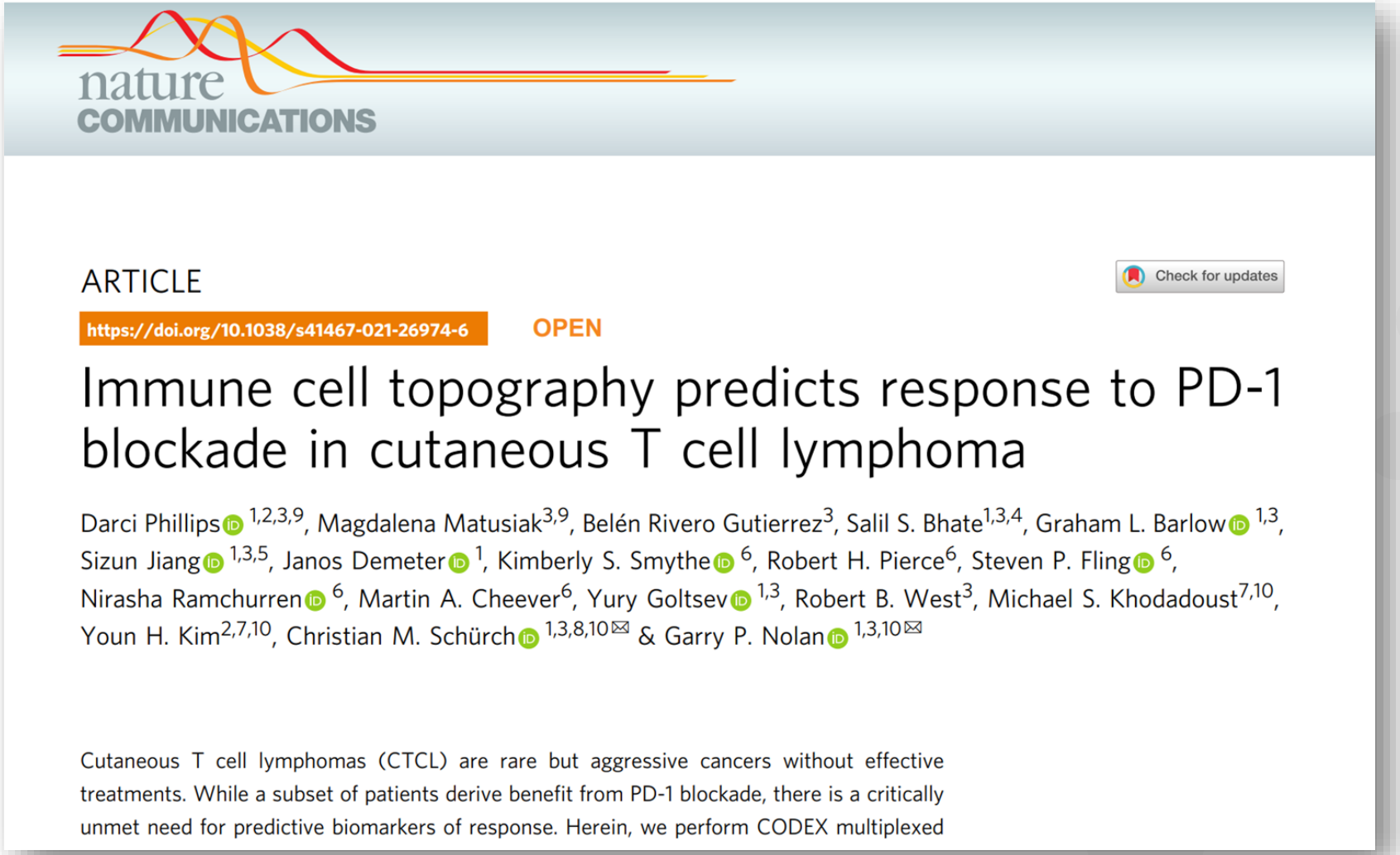
Step-3: Phenotyping

Responder vs. **Non-responder**





Step-4: SpatialScore



nature COMMUNICATIONS

ARTICLE

Check for updates

https://doi.org/10.1038/s41467-021-26974-6 OPEN

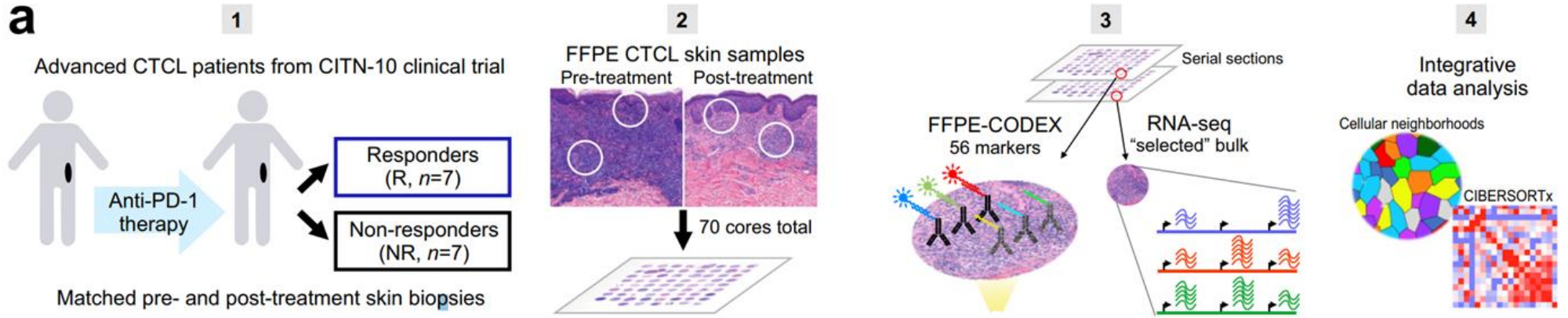
Immune cell topography predicts response to PD-1 blockade in cutaneous T cell lymphoma

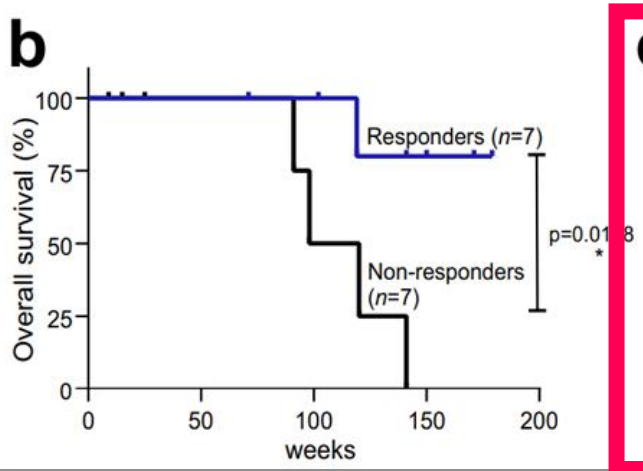
Darci Phillips[1,2,3,9], Magdalena Matusiak[3,9], Belén Rivero Gutierrez[3], Salil S. Bhate[1,3,4], Graham L. Barlow[1,3], Sizun Jiang[1,3,5], Janos Demeter[1], Kimberly S. Smythe[6], Robert H. Pierce[6], Steven P. Fling[6], Nirasha Ramchurren[6], Martin A. Cheever[6], Yury Goltsev[1,3], Robert B. West[3], Michael S. Khodadoust[7,10], Youn H. Kim[2,7,10], Christian M. Schürch[1,3,8,10]✉ & Garry P. Nolan[1,3,10]✉

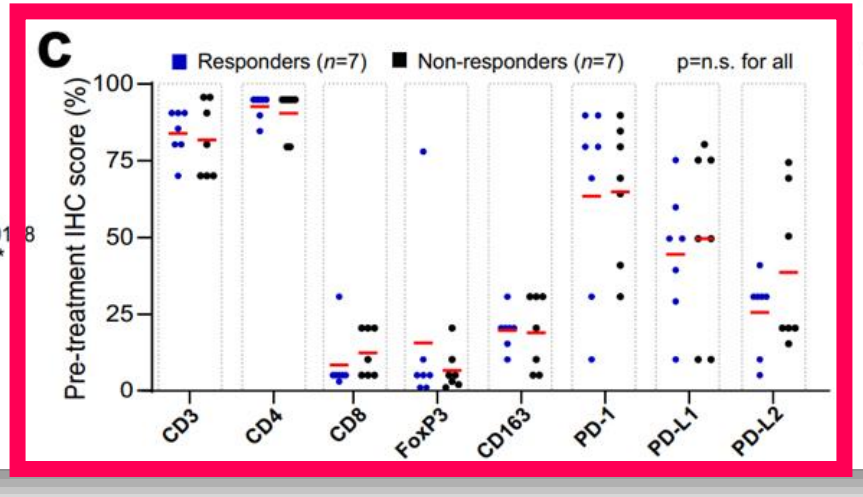
Cutaneous T cell lymphomas (CTCL) are rare but aggressive cancers without effective treatments. While a subset of patients derive benefit from PD-1 blockade, there is a critically unmet need for predictive biomarkers of response. Herein, we perform CODEX multiplexed

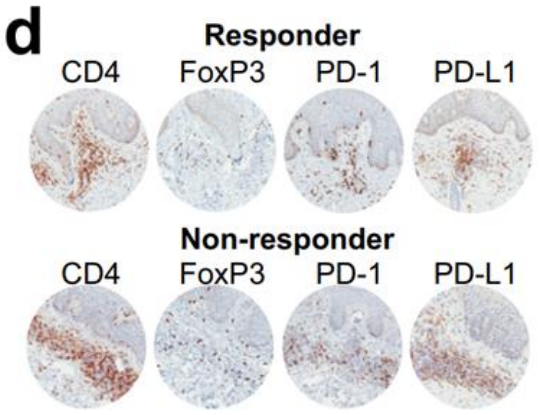
Step-4: SpatialScore

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-021-26974-6 ARTICLE



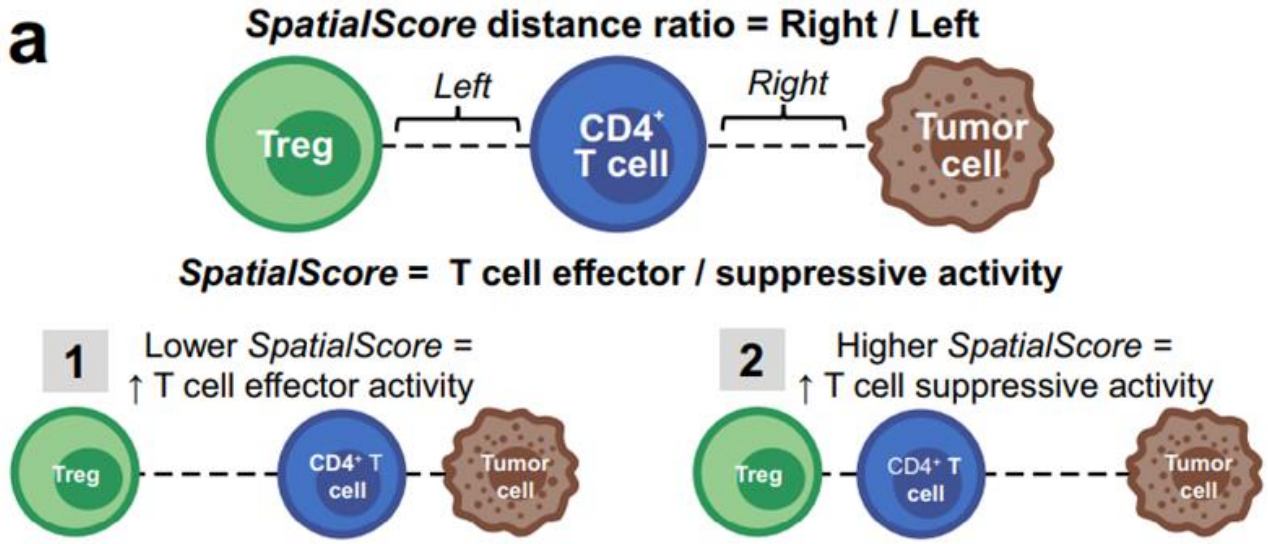






Step-4: SpatialScore

NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-021-26974-6



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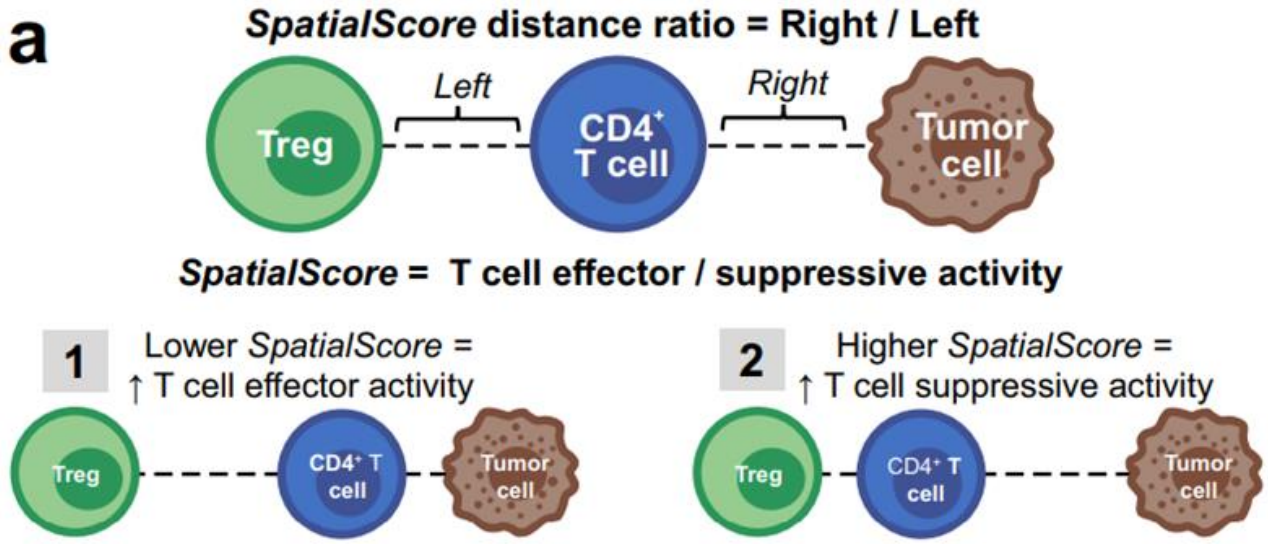
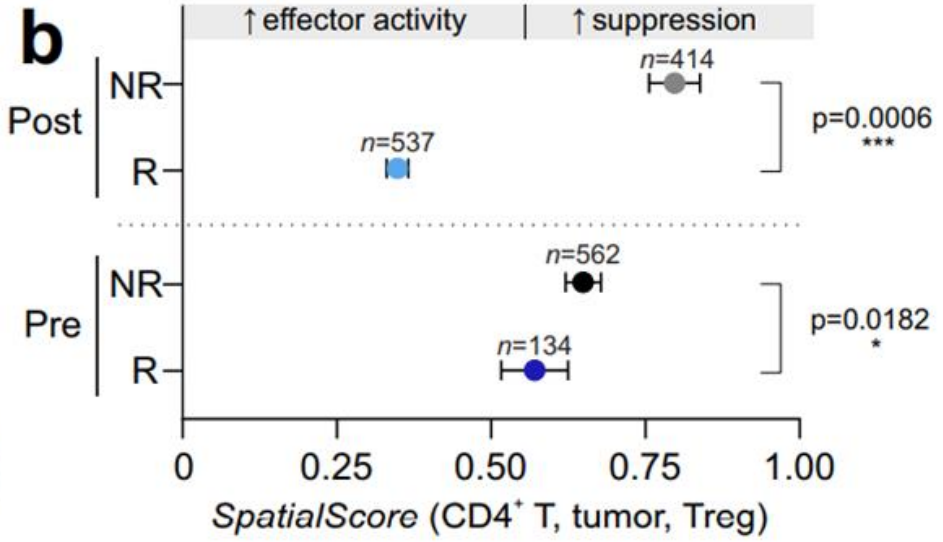
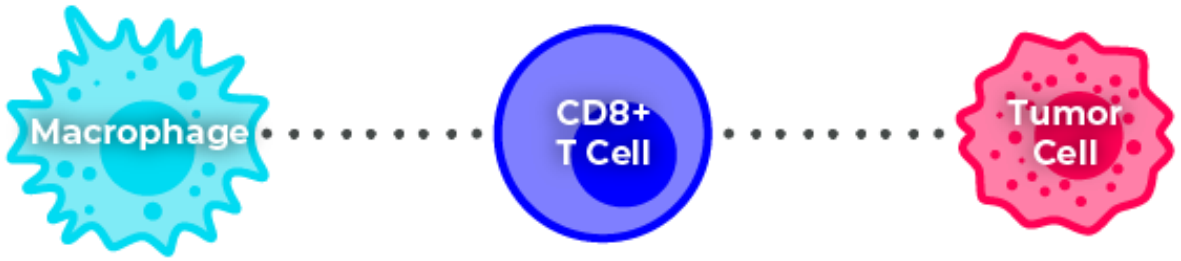




Step-4: SpatialScore

SpatialScore distance ratio = CD8+ T Cell to Tumor Cells / Macrophage to CD8+ T Cell



Low spatial score: High T cell *effector* activity; better outcome
High spatial score: High T cell *suppressive* activity; poor outcome

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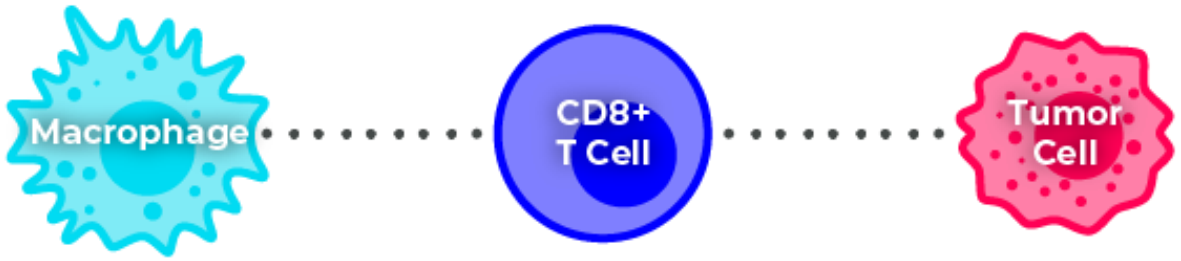

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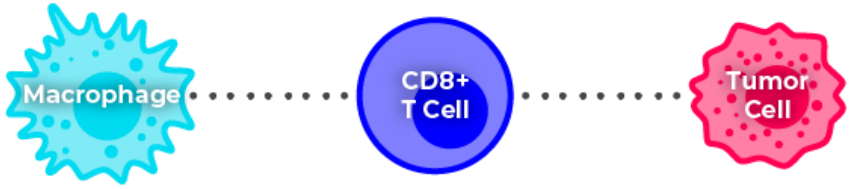
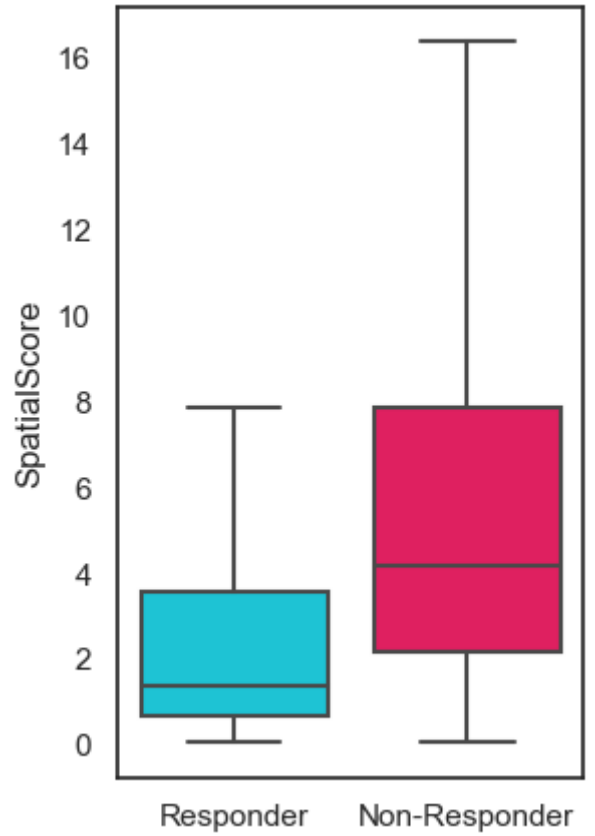


Low spatial score: High T cell *effector* activity; better outcome
High spatial score: High T cell *suppressive* activity; poor outcome

- **computeSpatialScore(left='Macs',middle='CD8',right='Tumor')**
- **Compute and plot value for each CD8+ T cell**



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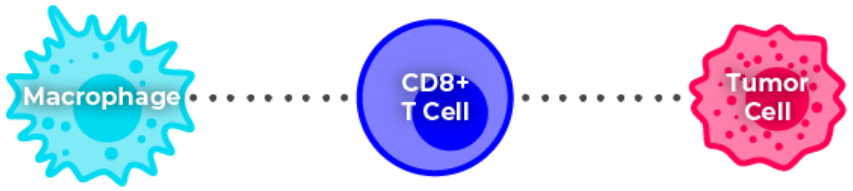
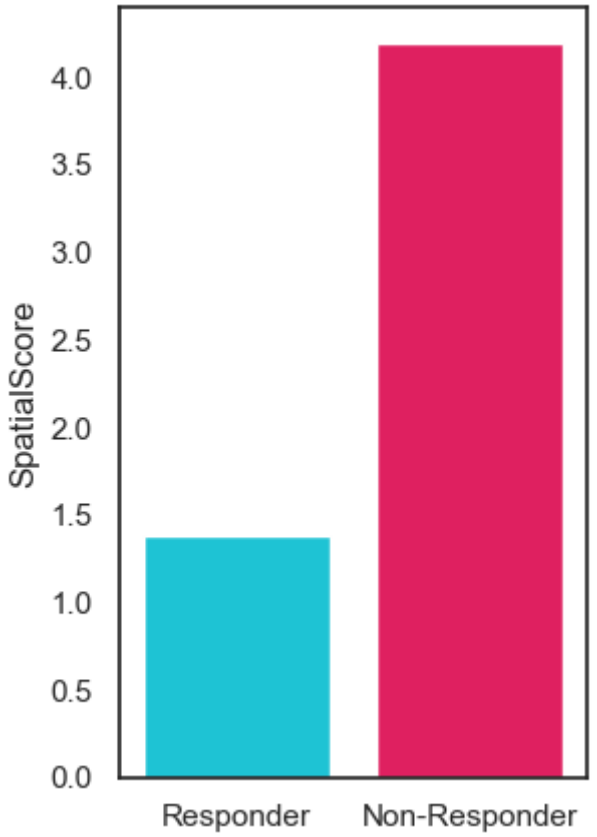


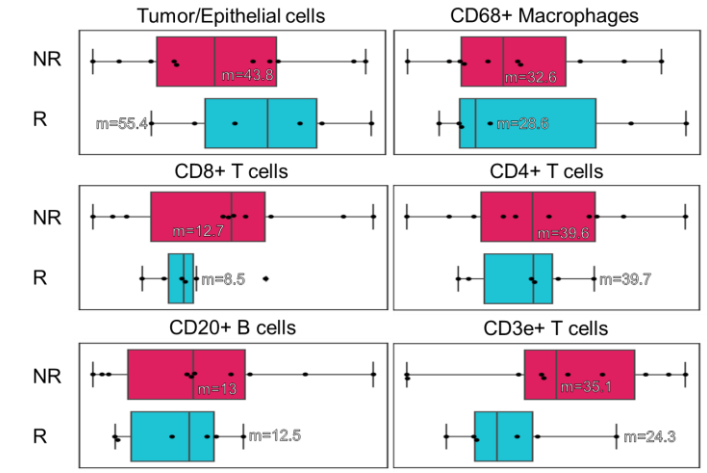
Low spatial score: High T cell *effector* activity; better outcome
High spatial score: High T cell *suppressive* activity; poor outcome

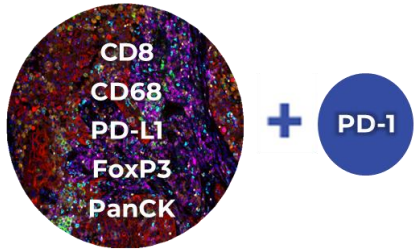
- **computeSpatialScore(left='Macs',middle='CD8',right='Tumor')**
- **Compute and plot median value per sample**



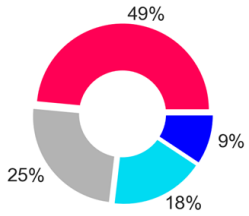
6-plex Spatial Phenotyping with PSP Validates Immune Landscape but Rules out Differences in Immune Cell Composition

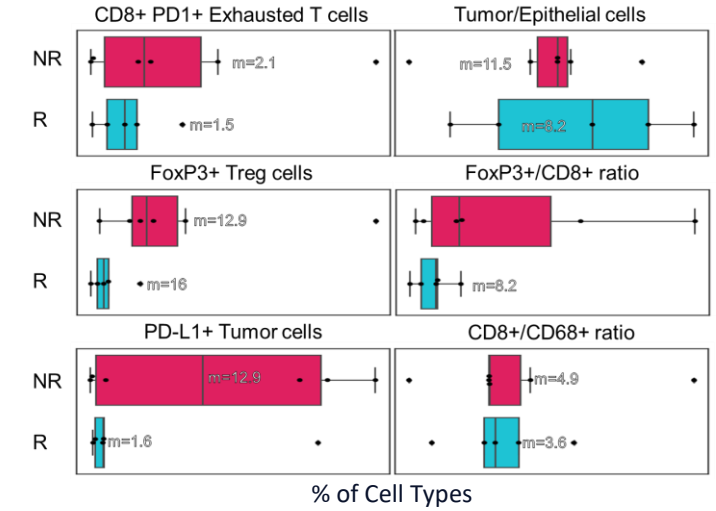
- CD4+ T cells
- CD8+ T cells
- B cells
- Tumor cells
- Macrophages
- T cells





- Macrophages
- CD8+ T-cells
- CD68+ PDL1+
- CD8+ PD1+
- Tumor cells
- FoxP3+
- FoxP3+ PDL1+
- PD1+
- PDL1+
- Tumor CD68+
- Tumor PDL1+
- Tumor PDL1+ CD68
- CD8+ PDL1+





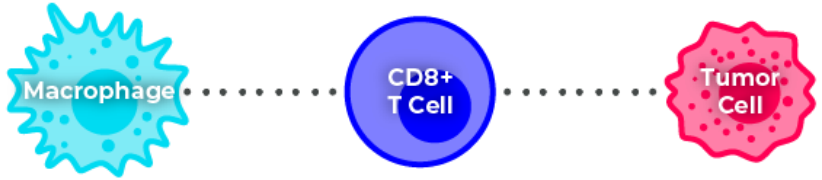
% of Cell Types

A Predictive Spatial Signature for Treatment Outcomes

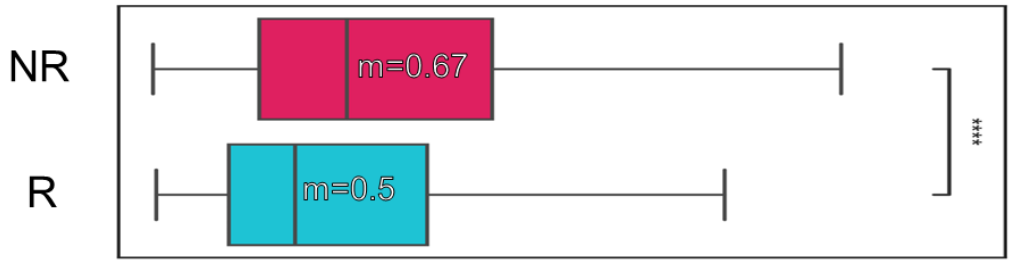
SpatialScore

SpatialScore distance ratio = CD8+ T Cell to Tumor Cells / Treg to CD8+ T Cell

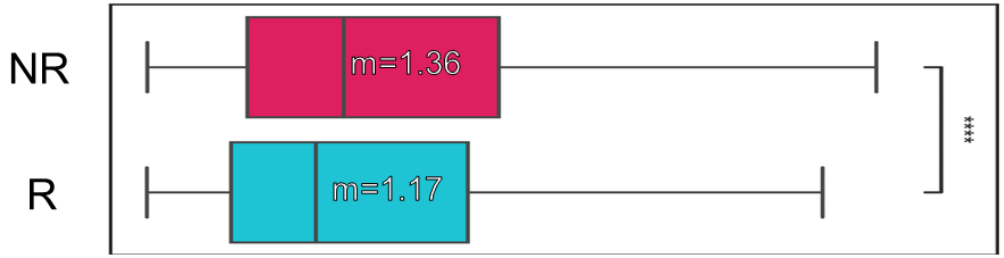
SpatialScore distance ratio = CD8+ T Cell to Tumor Cells / Macrophage to CD8+ T Cell



Prediction of ICI Response



SpatialScore (CD8+ T cells, tumor, Treg)



SpatialScore (CD8+ T cells, tumor, Macrophages)

Conclusion

- QuPath and Python-based workflow for PhenoImager analysis
  - QuPath: Visualization, segmentation
  - Python: Data processing, phenotyping, spatial metrics
- SpatialScore helps identify spatial insights into your PhenoImager data
- **Data and code available at: https://bit.ly/akoya_0622**

Questions from Zoom Webinar

Question	Answer
Hello! Is this webminar recorded? Can I follow it again in my own time?	Yes, this webinar will be avialable in a few days on the Akoya webpage under Resources and Akoya Academy.
Please also provide a programming note for the software used.	The code and data can be found here: https://bit.ly/akoya_0622
Qupath is not compatible with the latest Mac system Ventura. Any solution?	The latest version of QuPath is compatible with Ventura (see: https://twitter.com/petebankhead/status/1681554982464544768). The Python scripts are compatible with data from the older version of QuPath (<0.4) as well, so the general workflow remains the same.
The `modeltoPath` step is unclear.	QuPath provides helpful documentation on getting the segmentation model, please refer to QuPath's StarDist extension
Are there any plugins/scripts/software available to create a membrane mask based on membrane markers?	You can try CellPose: https://github.com/MouseLand/cellpose/
If I have to analyze the whole image, sometimes there are some artifacts in the tissue, can we excluded some areas?	Yes, you can include/exclude ROIs using QuPath's Annotation tool.
Can the whole workflow be replicated in R instead of Python?	Yes, instead of Scanpy/Scimap, you can use Seurat/CELESTA for automated phenptyping. Additional resources are linked on the Github repo.
Is the distance measured in microns?	Yes, QuPath stores cell coordinates in microns, so all distance calculations are in microns as well.
Is it possible to also say negative while tagging. In order to eliminate mistakes during scanning. especially when one dye bleed into another.	Yes, refer to Scimap's documentation for all the combination of rules you can set.
How do you convert the QuPath data formatting into AnnData - What if you already phenotyped in QuPath?	QuPath's export measurements will store the Classification information under the Class variable. You can include that column while creating the AnnData object.
Is there a difference in phenotyping by using QuPath's sequential classifiers compared to scimap?	QuPath's classifier does not include the normalization step so the results may vary.
How do you generate a fully unmixed WSI that you are using - are you doing any stitching?	Yes, stitching can be done with the QuPath's script here: https://gist.github.com/petebankhead/b5a86caa333de1fdcff6bdee72a20abe
Is the geojson file same as the .tsv format export from your inference?	No, geojson stores additional information like the cell's outline as a polygon, which isn't included in the tsv/csv.
What would be the clear adavantage of using QuPath versus ScanPy or SquidPy?	Scanpy does not contain cell segmentation module. However, if one is interested in running cell segmentation programmatically, they can use Stardist's Python implementation directly.