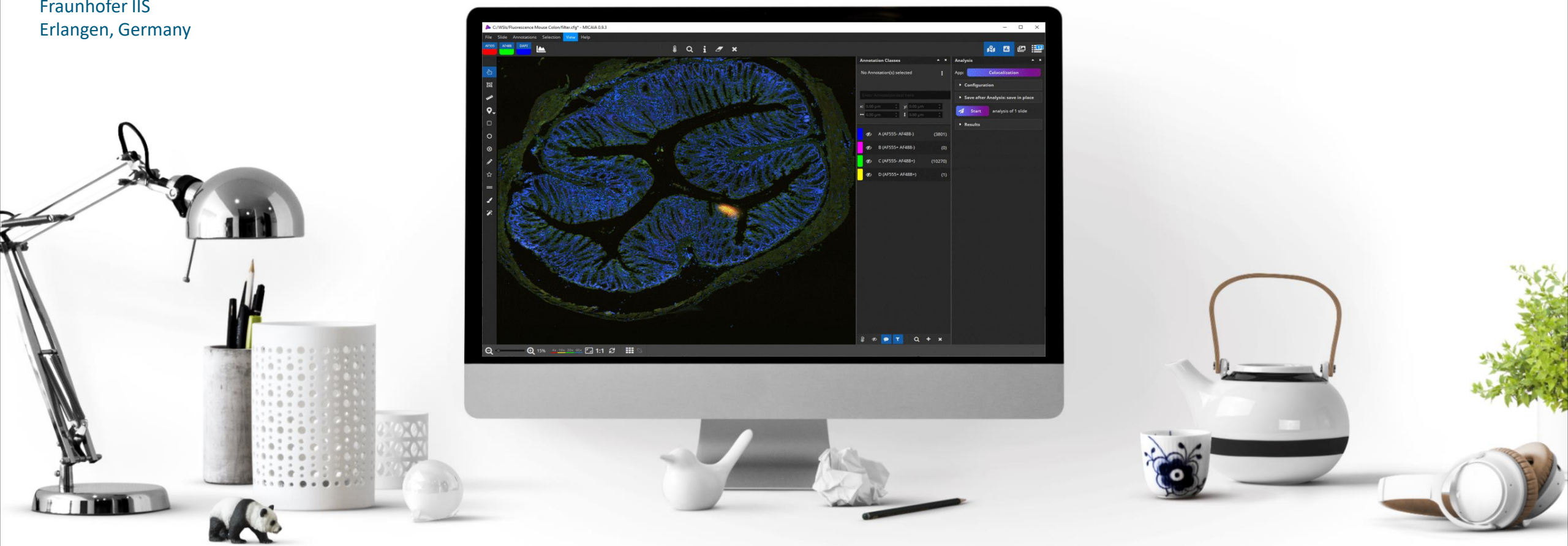
Do it yourself - Programming-free Spatial Proteomics Image Analysis for Biologists

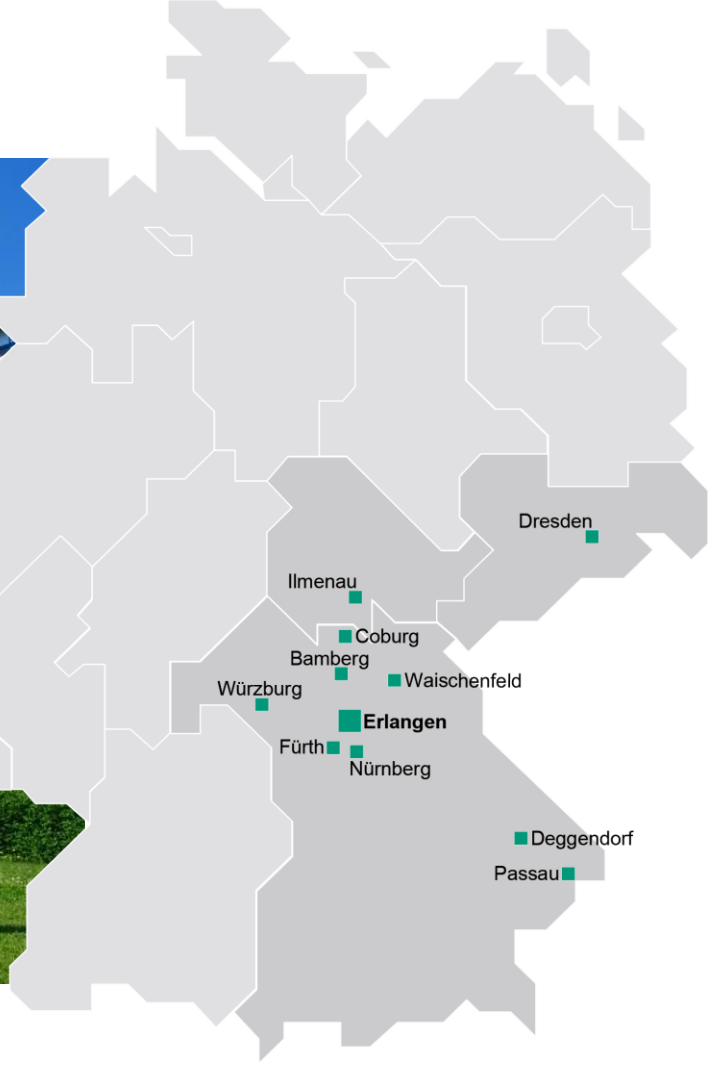
Dr. Volker Bruns
Head of Medical Image Analysis Research Group
Fraunhofer IIS
Erlangen, Germany

Fraunhofer IIS

Fraunhofer Institute for Integrated Circuits IIS



Fraunhofer Institut for Integrated Circuits IIS



- **Fraunhofer Society**: European largest Research Organization for **Applied** Sciences
- **Fraunhofer IIS:** founded in 1985, ~1200 employees, ~€ 250m budget, largest Fraunhofer Institute, 14 sites in 11 cities
  home of mp3, AAC, 5G/6G, IoT, XXL CT, JPEG XS, digital cinema, MIKAIA…
- Focus on commercialization of research results: **RnD contracting, IP licensing, joint research projects**

Fraunhofer IIS

Spatial Biology Instruments

PhenoCycler Fusion
by Akoya Biosciences

Comet
by Lunaphore

Cell DIVE Imager
by Leica Microsystems

MACSima
by Miltenyi Biotec

Cell Scape
by Canopy Biosciences

Orion
By RareCyte

Hyperion
By Standard BioTools

G4x
by Singular Genomics

Xenium
by 10x Genomics

merscope
by vizgen

CosMx
by Nanostring

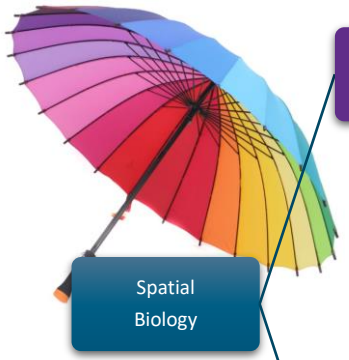
MALDI-TOF MS
by Shimadzu

FLEX series
by Bruker

Fraunhofer IIS

»Spatial Biology« is an umbrella term for many technologies

employed technologies



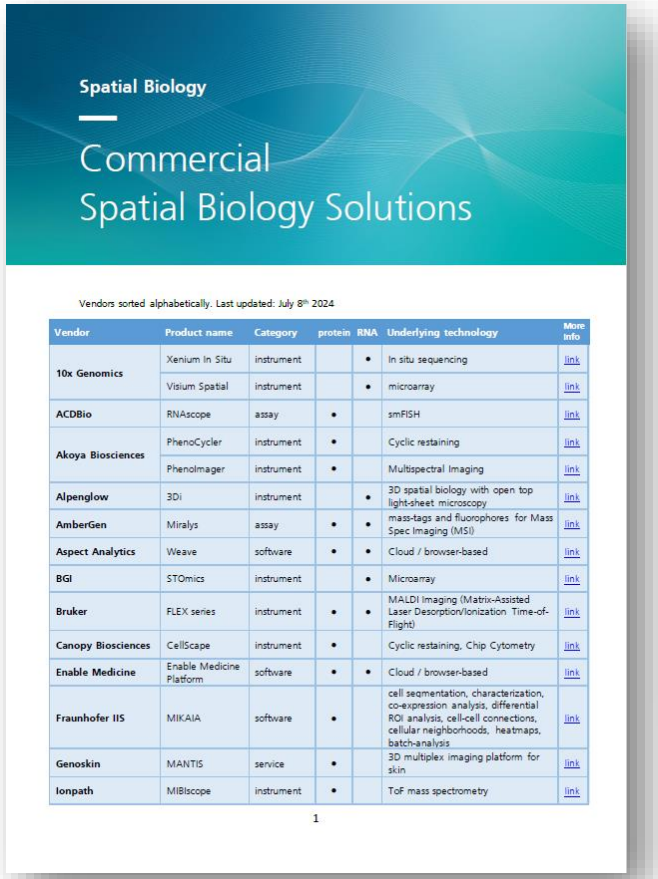
- Spatial Biology
  - Spatial Proteomics
    - Cyclic Restaining (seqIF)
      - PhenoCycler by Akoya Biosciences
      - Comet by Lunaphore
      - MACSima by Miltenyi Biotec
      - CellScape by Canopy Biosciences
      - Cell Dive by Leica Microsystems
    - Multispectral Imaging (MSI)
      - PhenoImager by Akoya Biosciences
      - Orion by RareCyte
    - Mass Cytometry Imaging (MCI)
      - Imaging Mass Cytometry by Time of Flight (CyToF)
        - Hyperion by Standard BioTools
      - Multiplexed Ion Beam Imaging (MIBI)
        - MIBIscope by Ionpath
    - Mass Spectrometry Imaging (MSI)
      - Matrix Assisted Laser Desorption / Ionization (MALDI)
        - FLEX series by Bruker
        - MALDI series by Shimadzu
        - SYNAPT & Xevo by Waters
  - Spatial Transcriptomics
    - Imaging-based
      - In situ *hybridization (ISH)*
        - CosMx by Nanostring
        - MERFISH by VizGen
        - Tissue Cartography by Resolve Biosciences
        - SeqFish by Spatial Genomics
        - RNAscope by ACDbio
      - *in situ* sequencing (ISS)
        - Xenium by 10x Genomics
        - G4x by Singular Genomics
    - Seguencing-based
      - Microdissection
        - GeoMx by Nanostring
      - Microarray
        - Visium Spatial by 10x Genomics
        - STOmics (stereo-seq) by BGI

Technologies and instruments offer different trade-offs

Scanning time	minutes ↔ days
Resolution	sub-cellular ↔ multiple cells
Field of View	mm ↔ cm
Analytes	proteins, RNAs, metabolites, …
Panel size	<10 genes ↔ whole exome
Costs	instrument, consumables, maintenance

Vendor lists

Free download on our blog



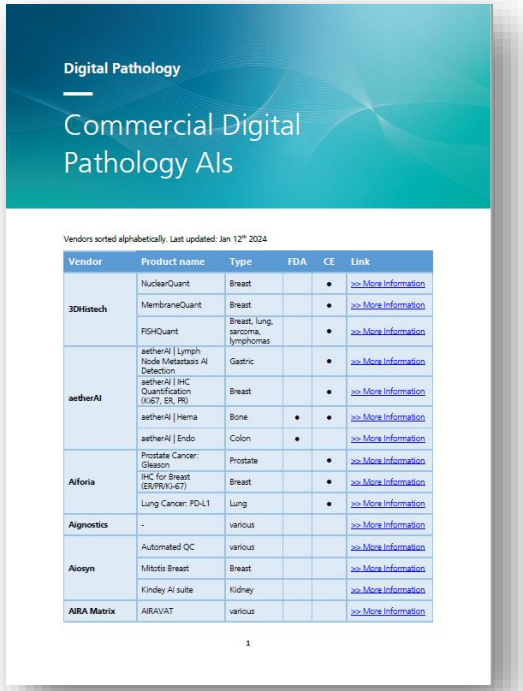
Spatial Biology

Commercial Spatial Biology Solutions

Vendors sorted alphabetically. Last updated: July 8th 2024

Vendor	Product name	Category	protein	RNA	Underlying technology	More info
10x Genomics	Xenium In Situ	instrument		•	In situ sequencing	link
	Visium Spatial	instrument		•	microarray	link
ACDBio	RNAscope	assay	•		smFISH	link
Akoya Biosciences	PhenoCycler	instrument	•		Cyclic restaining	link
	PhenoImager	instrument	•		Multispectral Imaging	link
Alpenglow	3Di	instrument		•	3D spatial biology with open top light-sheet microscopy	link
AmberGen	Miralys	assay	•	•	mass-tags and fluorophores for Mass Spec Imaging (MSI)	link
Aspect Analytics	Weave	software	•	•	Cloud / browser-based	link
BGI	STOmics	instrument		•	Microarray	link
Bruker	FLEX series	instrument	•	•	MALDI Imaging (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight)	link
Canopy Biosciences	CellScape	instrument	•		Cyclic restaining, Chip Cytometry	link
Enable Medicine	Enable Medicine Platform	software	•	•	Cloud / browser-based	link
Fraunhofer IIS	MIKAIA	software	•		cell segmentation, characterization, co-expression analysis, differential ROI analysis, cell-cell connections, cellular neighborhoods, heatmaps, batch-analysis	link
Genoskin	MANTIS	service	•		3D multiplex imaging platform for skin	link
Ionpath	MIBIscope	instrument	•		ToF mass spectrometry	link

1



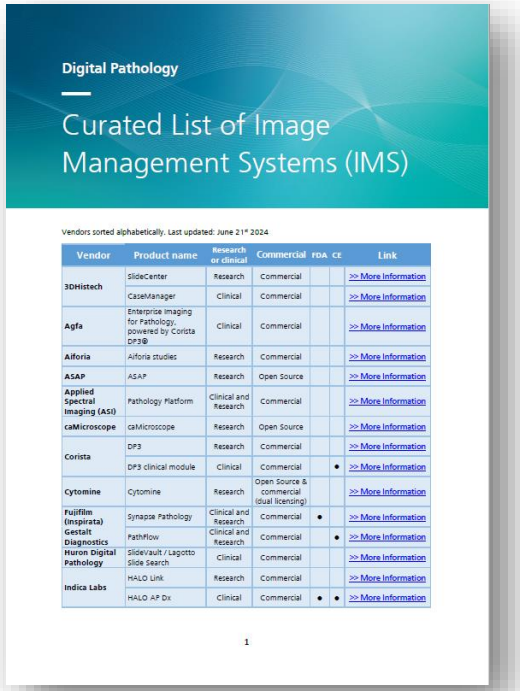
Digital Pathology

Commercial Digital Pathology AIs

Vendors sorted alphabetically. Last updated: Jan 12th 2024

Vendor	Product name	Type	FDA	CE	Link
3DHistech	NuclearQuant	Breast		•	>> More Information
	MembraneQuant	Breast		•	>> More Information
	FISHQuant	Breast, lung, sarcoma, lymphomas		•	>> More Information
aetherAI	aetherAI | Lymph Node Metastasis AI Detection	Gastric		•	>> More Information
	aetherAI | IHC Quantification (Ki67, ER, PR)	Breast		•	>> More Information
	aetherAI | Hema	Bone	•	•	>> More Information
	aetherAI | Endo	Colon	•		>> More Information
Aiforia	Prostate Cancer: Gleason	Prostate		•	>> More Information
	IHC for Breast (ER/PR/Ki-67)	Breast		•	>> More Information
	Lung Cancer: PD-L1	Lung		•	>> More Information
Aignostics	-	various			>> More Information
Aiosyn	Automated QC	various			>> More Information
	Mitotis Breast	Breast			>> More Information
	Kindey AI suite	Kidney			>> More Information
AIRA Matrix	AIRAVAT	various			>> More Information

1



Digital Pathology

Curated List of Image Management Systems (IMS)

Vendors sorted alphabetically. Last updated: June 21st 2024

Vendor	Product name	Research or clinical	Commercial	FDA	CE	Link
3DHistech	SlideCenter	Research	Commercial			>> More Information
	CaseManager	Clinical	Commercial			>> More Information
Agfa	Enterprise Imaging for Pathology, powered by Corista DP3®	Clinical	Commercial			>> More Information
Aiforia	Aiforia studies	Research	Commercial			>> More Information
ASAP	ASAP	Research	Open Source			>> More Information
Applied Spectral Imaging (ASI)	Pathology Platform	Clinical and Research	Commercial			>> More Information
caMicroscope	caMicroscope	Research	Open Source			>> More Information
Corista	DP3	Research	Commercial			>> More Information
	DP3 clinical module	Clinical	Commercial		•	>> More Information
Cytomine	Cytomine	Research	Open Source & commercial (dual licensing)			>> More Information
Fujifilm (inspirata) Gestalt Diagnostics	Synapse Pathology	Clinical and Research	Commercial	•		>> More Information
	PathFlow	Clinical and Research	Commercial		•	>> More Information
Huron Digital Pathology	SlideVault / Lagotto Slide Search	Clinical	Commercial			>> More Information
Indica Labs	HALO Link	Research	Commercial			>> More Information
	HALO AP Dx	Clinical	Commercial	•	•	>> More Information

1

www.smartsensing.blog

MIKAIA® in Digital Pathology & Spatial Biology Research

Fraunhofer IIS

MIKAIA studio 1.6.1

File Slide Annotations Selection View Stain Unmixing Help

Colors Coloc Markup | Heatmap Hotspot Info Markup Selected Actions | Close Prev. Slide Next Slide | Analysis Classes Slides Queue

Analysis

App: FL Cell Analysis

Configurati

General

Analyze R

Results

Annotation Classes

No Annotation(s) selected

Slides

Open B

Select Folders

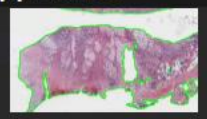
27 of 49 slides

Save as preset Load preset

984 MB

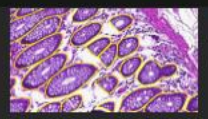
App Center

FL BF AI IHC HE Export Editor Filter by title...

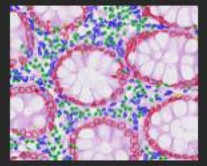


This App is used to outline the tissue. It separates foreground from background.

The colon classification app detects and outlines various tissue types of the colon in a WSI. This app first detects tissue areas and then splits them into visually similar clusters or



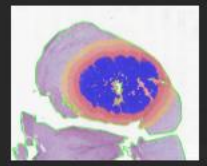
This App outlines crypts (aka glands) and their lumen by carrying out a pixelwise segmentation.



H&E Cell AI

Brightfield AI WSI HE

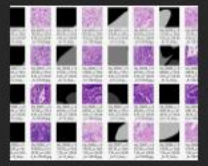
This App detects and classifies cells in H&E stained histological images. The AI was trained to recognize these 10 cell types: Epithelial Cells, Tumor Cells, Eosinophiles,



Mask by Color

Brightfield Fluorescence WSI HE IHC

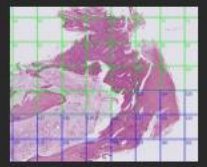
This App is used to select a tissue area based on its color. For instance, it can be used to mask the chromogen in an IHC scan. In fluorescent mIF scans, it can mask a marker (or



Annotation Image Export

WSI Export

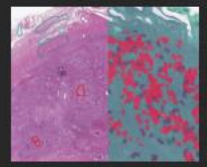
This App creates data sets from annotations. Either export one image per annotation, e.g. when annotations mark cells or other small objects that fit into a single image, or divide a



Tile Export

WSI Export

This App exports tiles (aka patches) from a whole-slide image. The tiles can either be exported at the native resolution or at a user-defined resolution. The tile size and

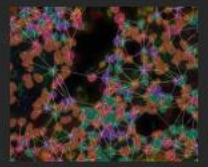


AI Author

Editor Brightfield AI WSI HE

DIY - do it yourself! Train your own patch-based classifier on your data in three simple steps.

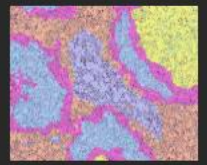
(1) define names of tissue classes you want to distinguish



Cell-Cell Connections

Fluorescence Single Cells WSI

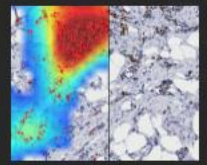
This App carries out a spatial analysis between cell types. It interprets the sample as a graph where cells are nodes cell-cell connections are edges. Each cell is connected with its



Cellular Neighborhood

Fluorescence Single Cells WSI

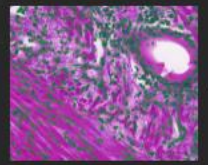
This App classifies each cell based on its cellular neighborhood by collecting for each cell info on its k nearest neighboring cells and then clustering this per-cell



IHC Cell Detection

Brightfield Single Cells WSI IHC

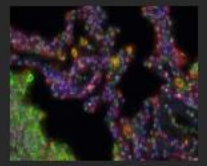
This App is used to detect positive and negative cells in nuclear IHC stainings.



H&E Cell Detection

Brightfield Single Cells WSI HE

This App is used to detect cells in a H&E staining.



FL Cell Analysis

Fluorescence WSI

This App can be used to analyze immunofluorescence slides to carry out a co-expression study. It requires a DAPI channel plus one or more additional markers. For each detected cell,

HER2/neu FISH

Fluorescence Single Cells WSI

HER2/neu FISH scoring is used to assess whether a HER2 overexpression exists. Input: FISH image with three markers: DAPI, HER2 and CEP17. The App first detects nuclei in the

Plug-in your own AI

Editor AI WSI

Plug-in your own AI. Your Python plug-in script can reside on the same computer, in a docker or on a remote computer. It is invoked by MIKAIA and communicates with MIKAIA via a

Spatial Clustering

Fluorescence Single Cells WSI

Annotation Metrics

Brightfield Fluorescence WSI IHC

17 apps

MIKAIA mIF analysis workflow

mIF WSI

Image source: https://singulargenomics.com/g4x/

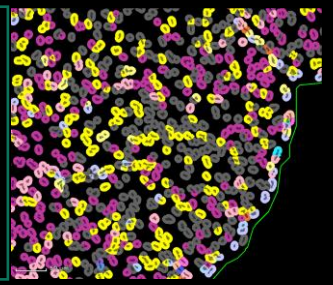
WSI analysis

ROI analysis

ROI creation
- manual
- thresholding

Differential ROI analysis

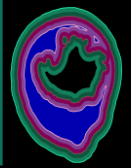
Cell Segmentation
Cell Qualification
Cell Typing
Annotations



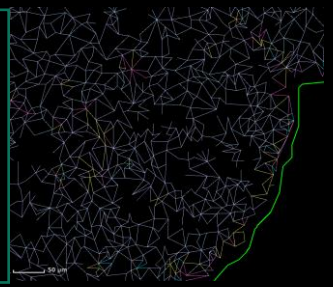
Density Heatmaps

Proximity Analysis

concentric margins

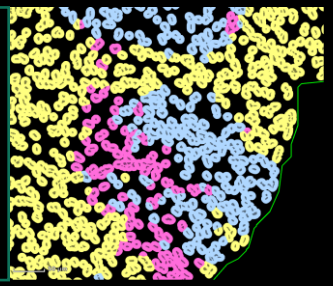


Cell-Cell Connections Analysis



Density Heatmaps

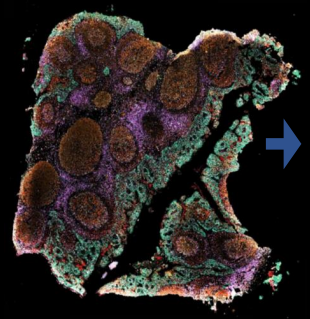
Cellular Neighborhood Analysis



Batch analysis of many WSIs → *.csv *.png *.ano

MIKAIA mIF analysis workflow

mIF WSI



CD3 CD4 CD8

Image source: https://singulargenomics.com/g4x/

Cell Segmentation

Computer Vision

or

CellPose AI

2 contours: nucleus + cytoplasm

Cell Qualification

0,8
0,2
0,6
0,1
0,0
0,0

Cell Typing

supervised

> t_{user} → $CD3^+$ $CD4^+$ $CD8^-$

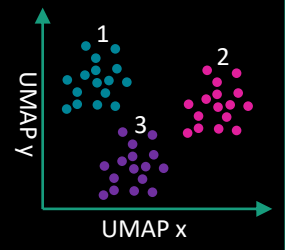
or

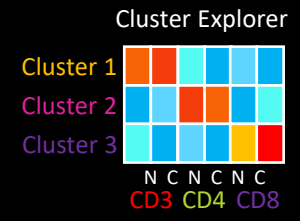
unsupervised clustering

k-means

or

DBSCAN





Generate Annotations

per marker

or

co-expression

1:N

1:1

user naming scheme

$CD3^+$ $CD4^+$	„T helper cells“
$CD3^+$ $CD8^+$	„Cytotoxic T-Cells“
...	...

1:1

Annotation Classes

„CD3“
„CD4“

„T helper cells“
„Cytotoxic T-Cells“
„neg“

„Cluster 1“
„Cluster 2“
„Cluster 3“

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File Slide Annotations Selection View Stain Unmixing Help

Colors Coloc Markup | Heatmap Hotspot Info Markup Selected Actions | Close Prev. Slide Next Slide | Analysis Classes Slides Queue 2

Open Slide

Drop File here

C:/WSIs/PhenoCycler-Fusion_tonsil.qptiff

C:/WSIs IHC/Aidpath_Breast_1634.svs

C:/.../Desktop/FISH kompozitne slike LaMP/M923-24 KBC ZG 11553-24 1-8 15 min EGFR Zv 3.jpg

C:/WSIs/HandEcompressed_Scan1.qptiff

C:/WSIs/LuCa-7color_Scan1.qptiff

C:/.../Desktop/FISH kompozitne slike LaMP/M250-24 KBC ZG 2519-24 1-2 13 min Kr10 Zv 3.jpg

C:/.../Desktop/FISH kompozitne slike LaMP/M370-24 KBC ZG 1481-24 2 16 min MYCC Zv 2.jpg

C:/.../Desktop/FISH kompozitne slike LaMP/M250-24 KBC ZG 2519-24 1-2 13 min Kr10 Zv 1.jpg

C:/WSIs/kidney_betatest.qptiff

Annotation Classes

No Annotation(s) selected

Enter Annotation text here

x 0.00 µm y 0.00 µm

0.00 µm 0.00 µm

Filter classes...

Create new Annotation Class

Slides

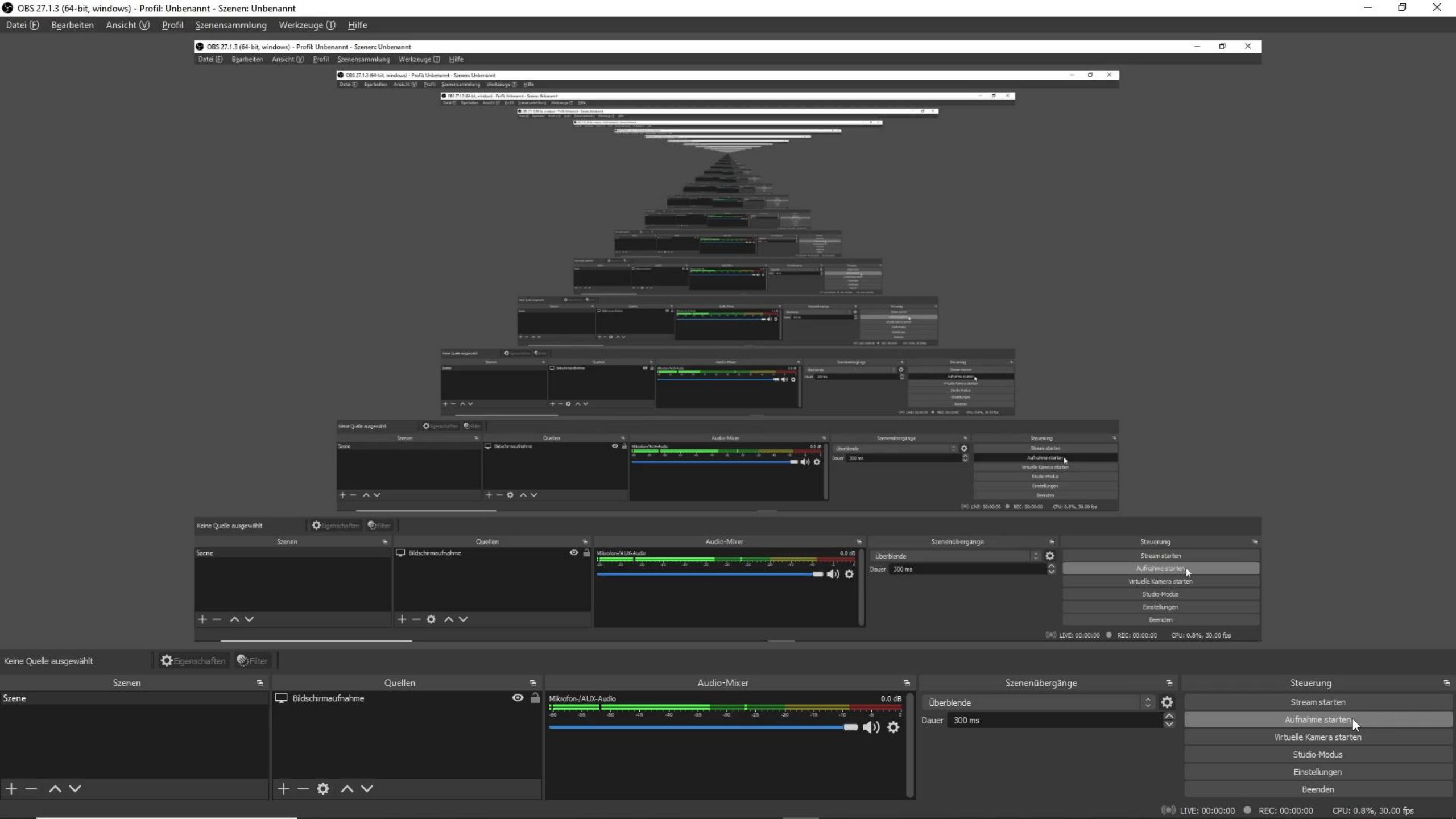
Open Browse Export... Analyze Annotations... auto-hide flat Filter by title...

Save as preset Load preset

Select Folders

27 of 45 slides

4263 MB


OBS 27.1.3 (64-bit, windows) - Profil: Unbenannt - Szenen: Unbenannt
Datei (F) Bearbeiten Ansicht (V) Profil Szenensammlung Werkzeuge (T) Hilfe
Keine Quelle ausgewählt
Eigenschaften
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Szene
Quellen
Bildschirmaufnahme
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Dauer
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Steuerung
Stream starten
Aufnahme starten
Virtuelle Kamera starten
Studio-Modus
Einstellungen
Beenden
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REC: 00:00:00
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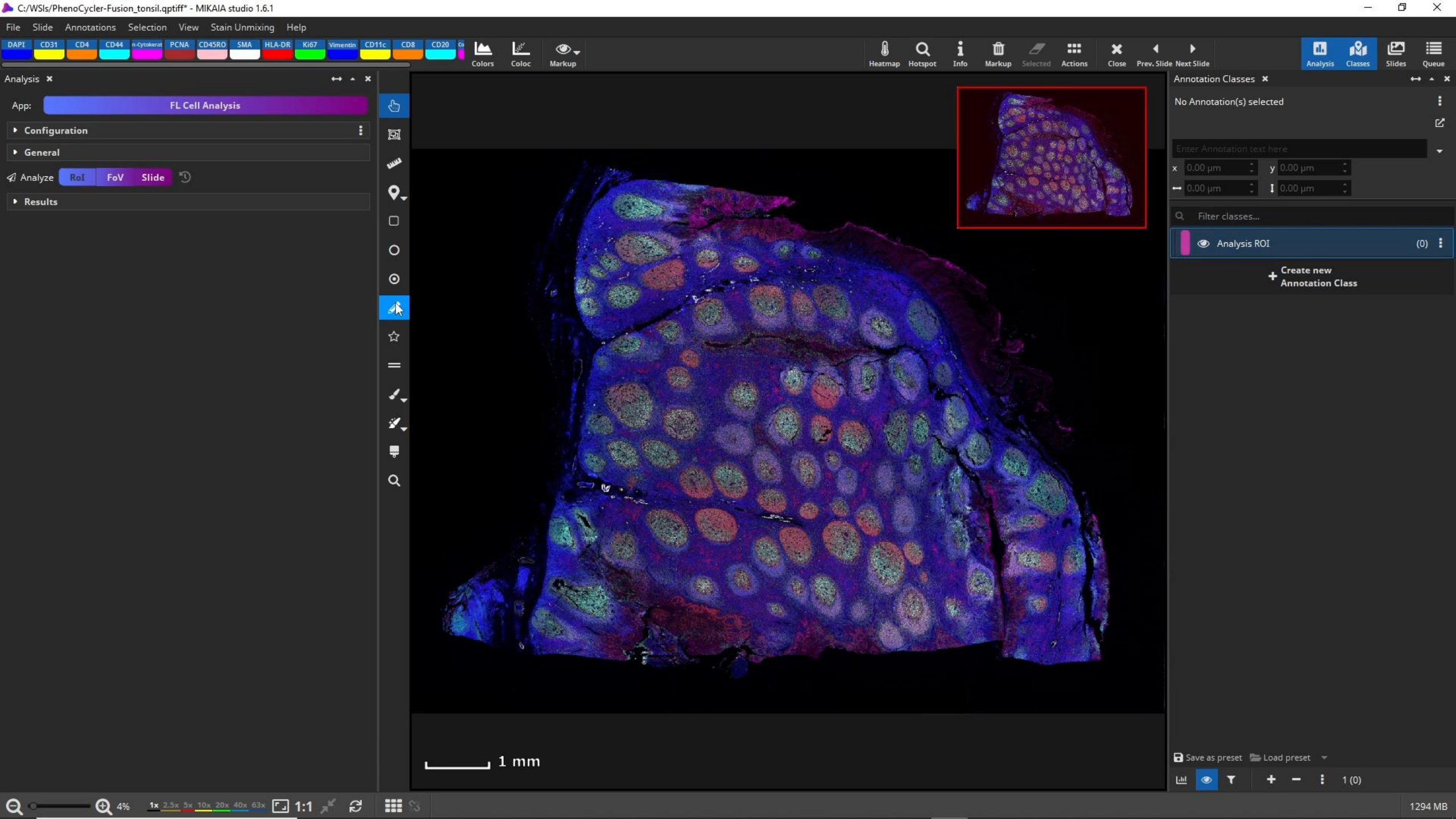
C:/WSIs/PhenoCycler-Fusion_tonsil.qptiff* - MIKAIA studio 1.6.1
File
Slide
Annotations
Selection
View
Stain Unmixing
Help
DAPI
CD31
CD4
CD44
n-Cytokerat
PCNA
CD45RO
SMA
HLA-DR
Ki67
Vimentin
CD11c
CD8
CD20
Colors
Coloc
Markup
Heatmap
Hotspot
Info
Markup
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Next Slide
Analysis
Classes
Slides
Queue
Analysis
App:
FL Cell Analysis
Configuration
General
Analyze
RoI
FoV
Slide
Results
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y 0.00 µm
0.00 µm
0.00 µm
Filter classes...
Analysis ROI
(0)
Create new Annotation Class
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Save as preset
Load preset
1 (0)
4%
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1:1
1294 MB

C:/WSIs/PhenoCycler-Fusion_tonsil.qptiff - MIKAIA studio 1.6.1

File Slide Annotations Selection View Stain Unmixing Help

DAPI CD31 CD4 CD44 n-Cytokerat PCNA CD45RO SMA HLA-DR Ki67 Vimentin CD11c CD8 CD20

Colors Coloc Markup Heatmap Hotspot Info Markup Selected Actions Close Prev. Slide Next Slide Analysis Classes Slides

Analysis

App: FL Cell Analysis

Configuration

Tissue Detection

Cell Typing

Annotate Markers Marker Co-Expression Cluster

Clusters Fixed 7 Dynamic 0.10

Cell Type Mapping Akoya Tonsil ...

Cell Marker: DAPI

Signal Range Fixed 1 - 255 take from viewer

Sensitivity 100 1

Smoothness 3

Cell Density 4 µm segment stronger

Area 2 - 9999 µm²

Cytoplasm 4.56 µm (0.00)

Positivity: Average Intensity Positive Area Ratio

CD31 N+C

CD4 N+C

CD44 N+C

Pan-Cytokeratin N+C

PCNA N+C

CD45RO N+C

SMA N+C

HLA-DR N+C

Ki67 N+C

Vimentin N+C

CD11c N+C

CD8 N+C

200 µm

Annotation Classes

"Analysis ROI" Polygon manual 10.84 mm² 13.27 mm

Enter Annotation text here

x 4160.92 µm y 2722.43 µm

3688.60 µm 3944.25 µm

Filter classes...

Analysis ROI

GC

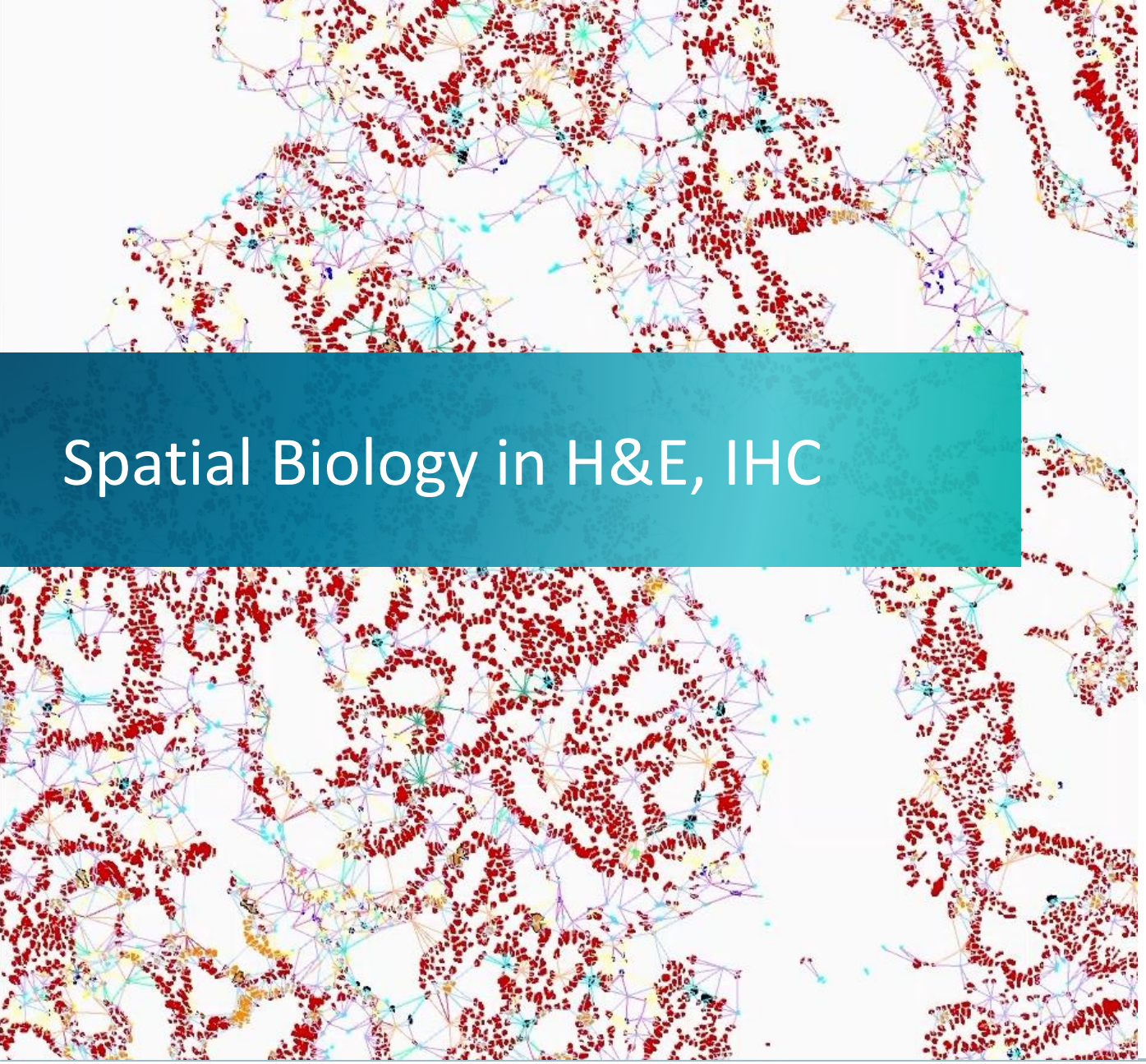
Create new Annotation Class

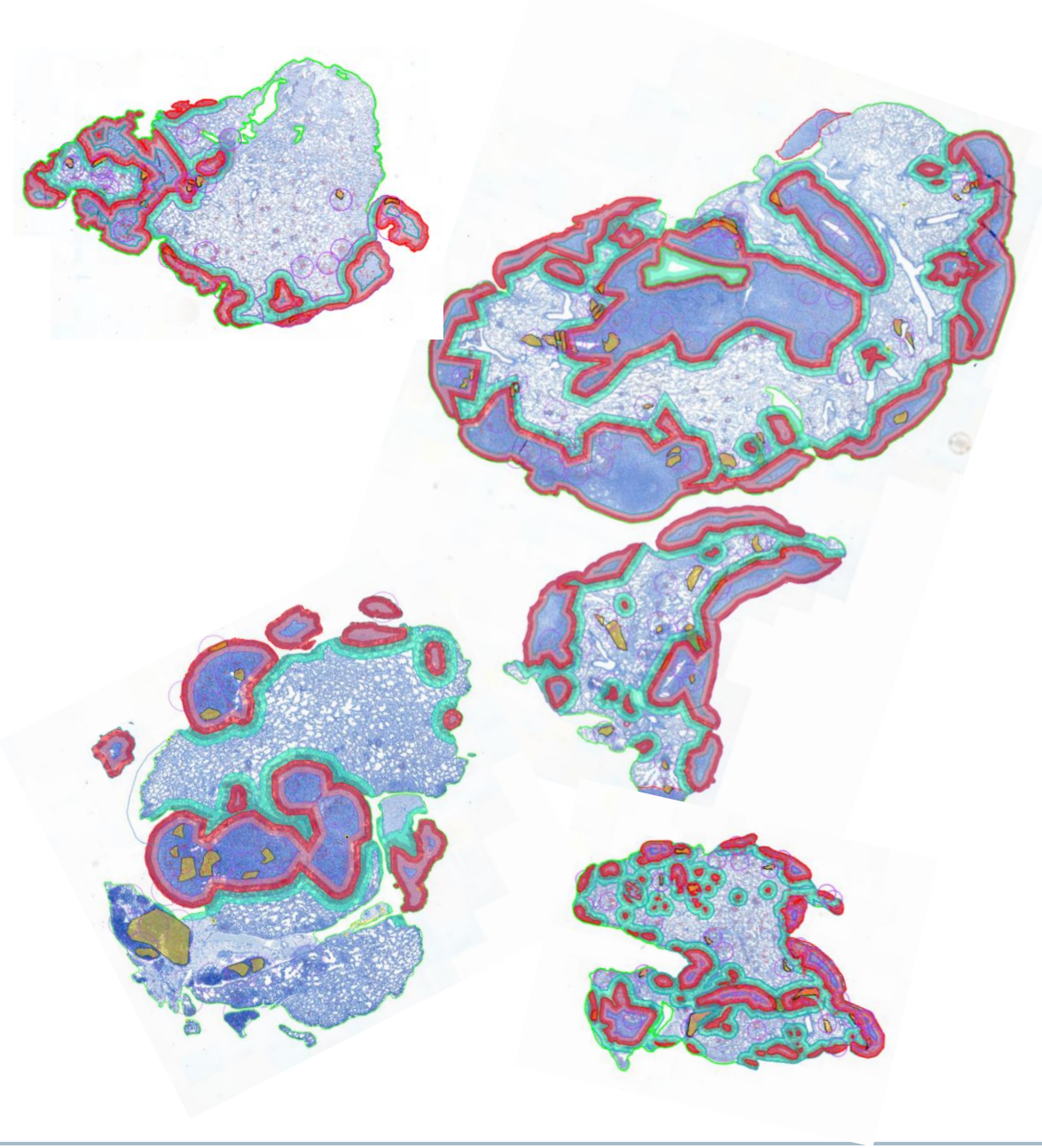
Save as preset Load preset

1 (1)

23% 1:1

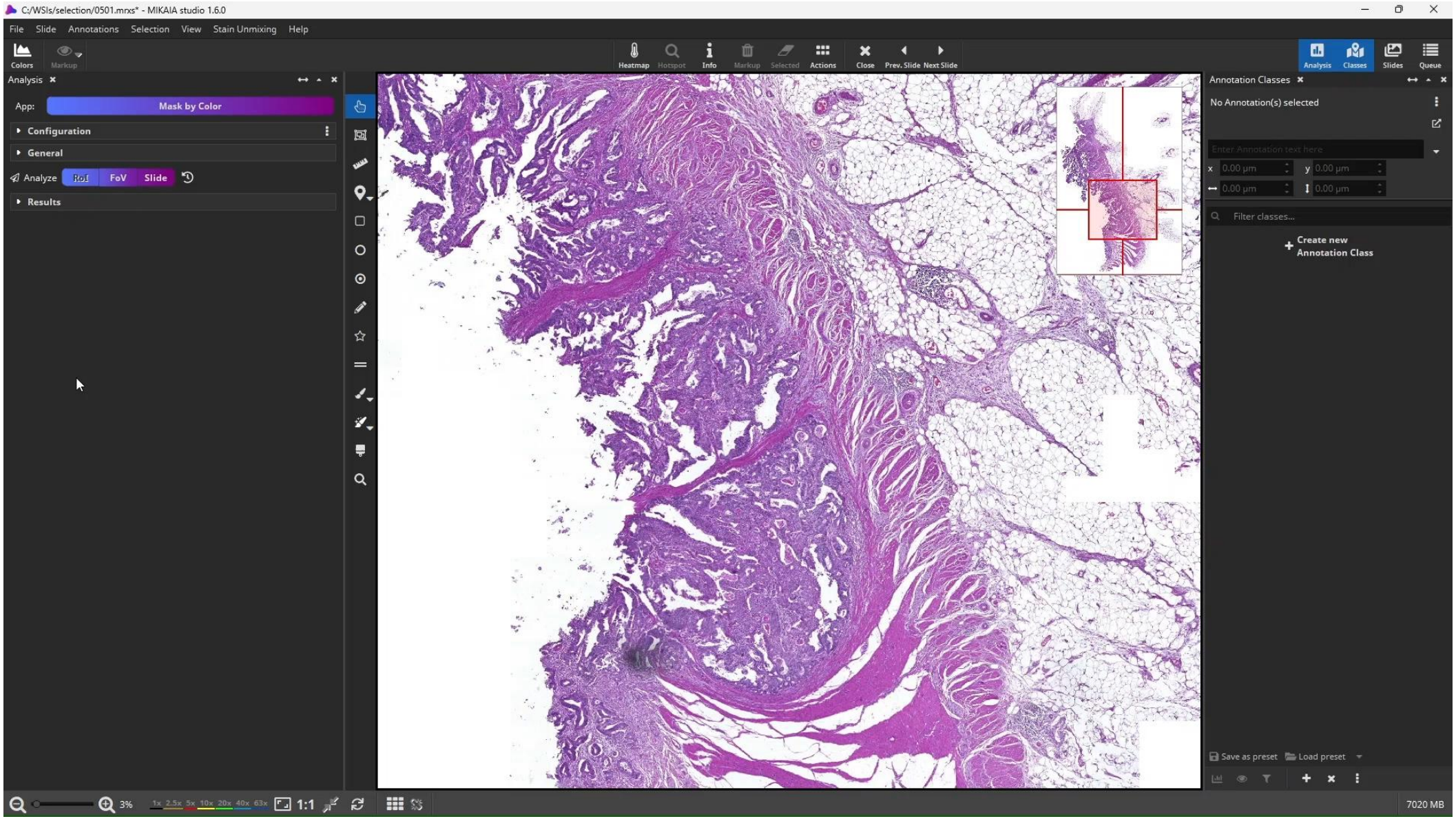
Spatial Biology in H&E, IHC





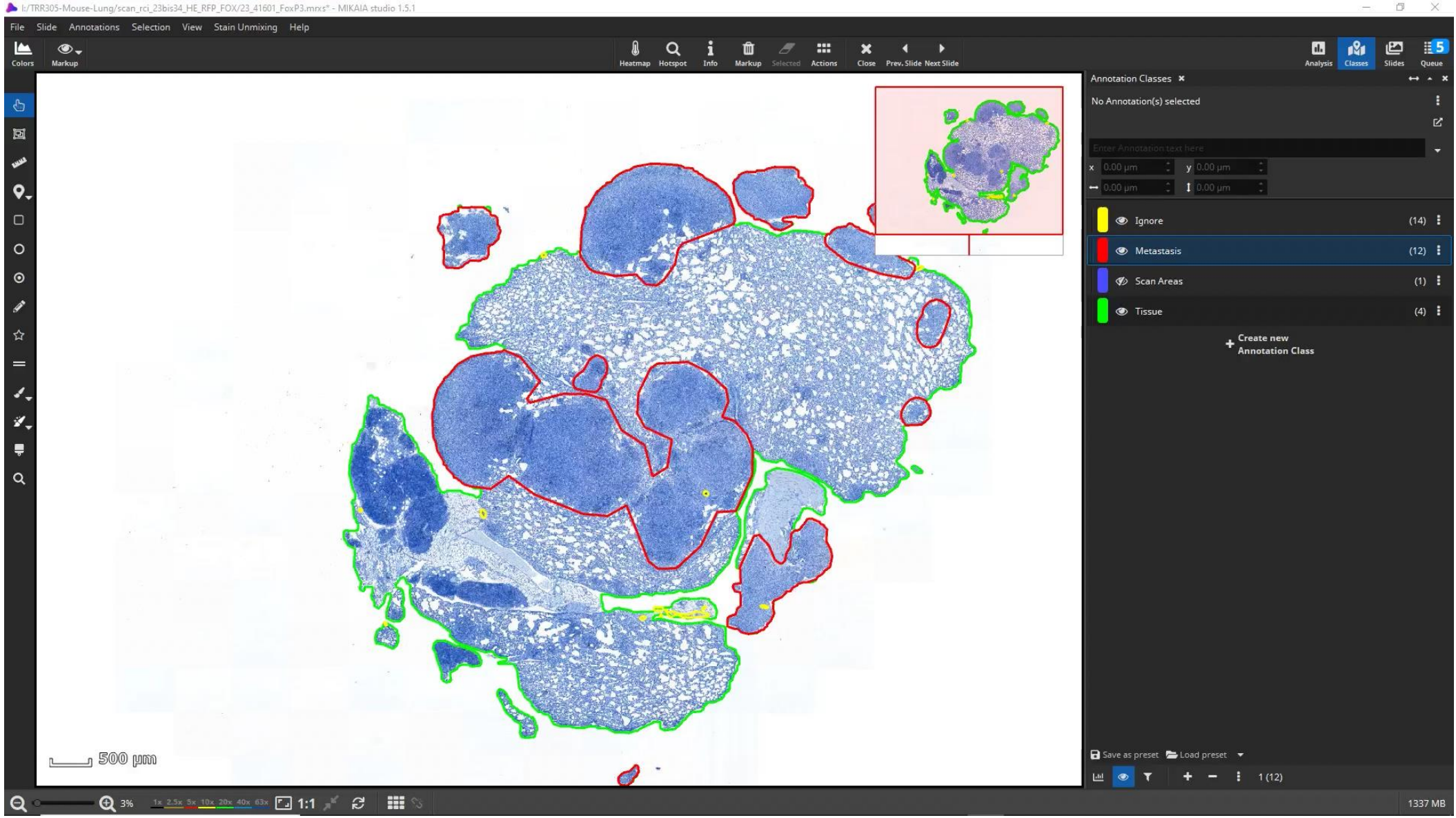
Fraunhofer IIS

1. Tissue detection
2. Tissue Layer Detection
3. Cell Detection
4. Spatial Neighborhood Analysis



Fraunhofer IIS

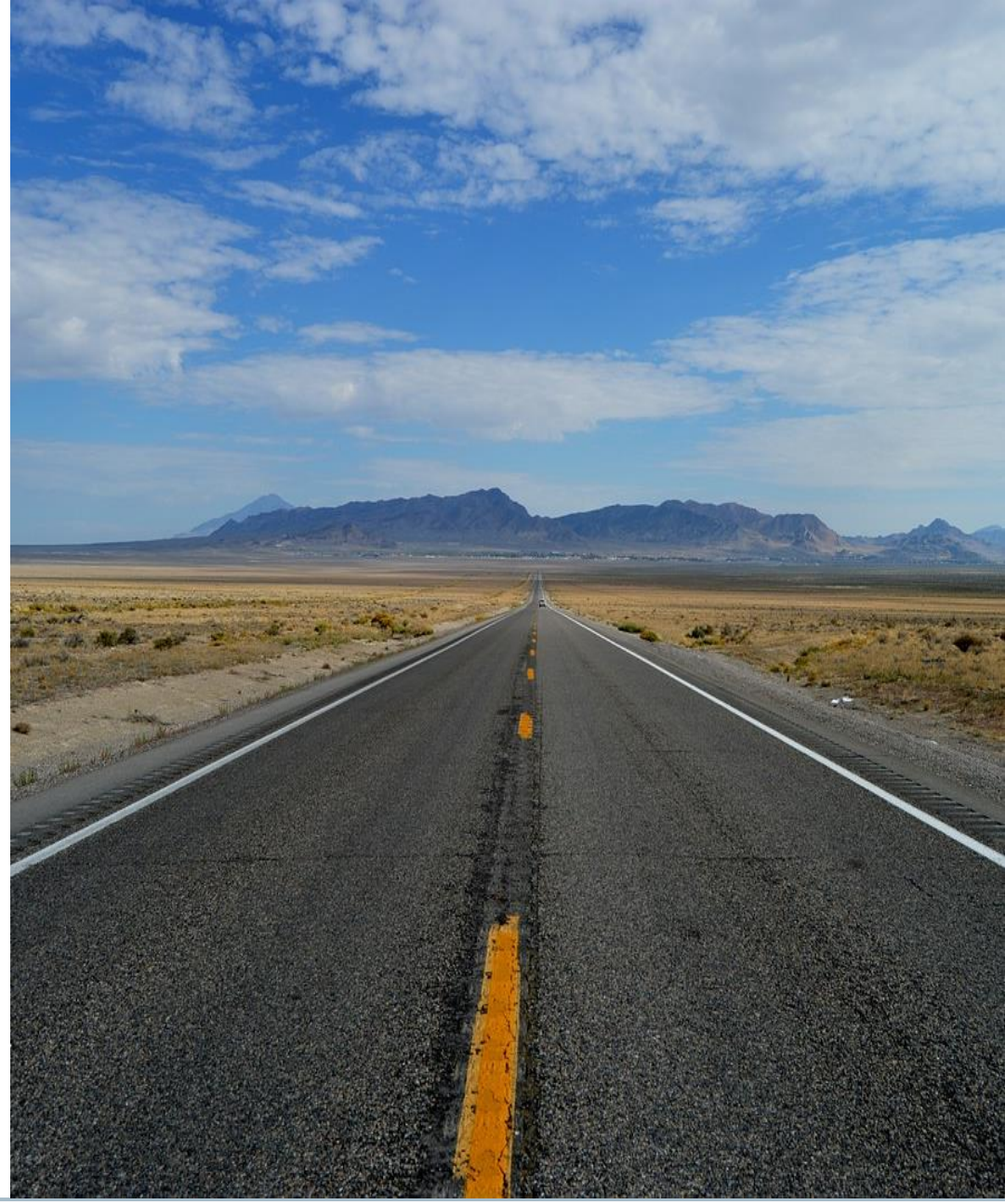
1. • **Tissue detection**
2. • Division into **Scan Areas**
3. • **Subdivision into ROIs**
4. • **Concentric ROI distance margins**
5. • **Cell detection**
6. • **Cell qualification**
7. • **False positive** filtering
8. • **Hotspots search**
9. • **Spatial clustering**
10. • **Visualization /** Heatmaps
11. • **export to CSV**
12. • export of extra files (doc & repeat)



Fraunhofer IIS

Outlook

- Combination of mIF + FISH
- Speed / performance
- Co-registration for „manual seqIF“: merge N low-plexes into 1 high-plex
- Epithelial Segmentation
- Save clustering models and apply on other ROIs / scans
- More clustering algorithms
- Expression levels on | off -> off | low | high
- Gating
- Generate report
- Object-to-Interface Proximity Analysis
- Spatial Transcriptomics analysis
- …




Thank you for your Attention
Questions?

Contact

Dr. Volker Bruns
Head of Medical Image Analysis (MIA) Group
volker.bruns@iis.fraunhofer.de
www.iis.fraunhofer.de/digital-pathology
www.mikaia.ai