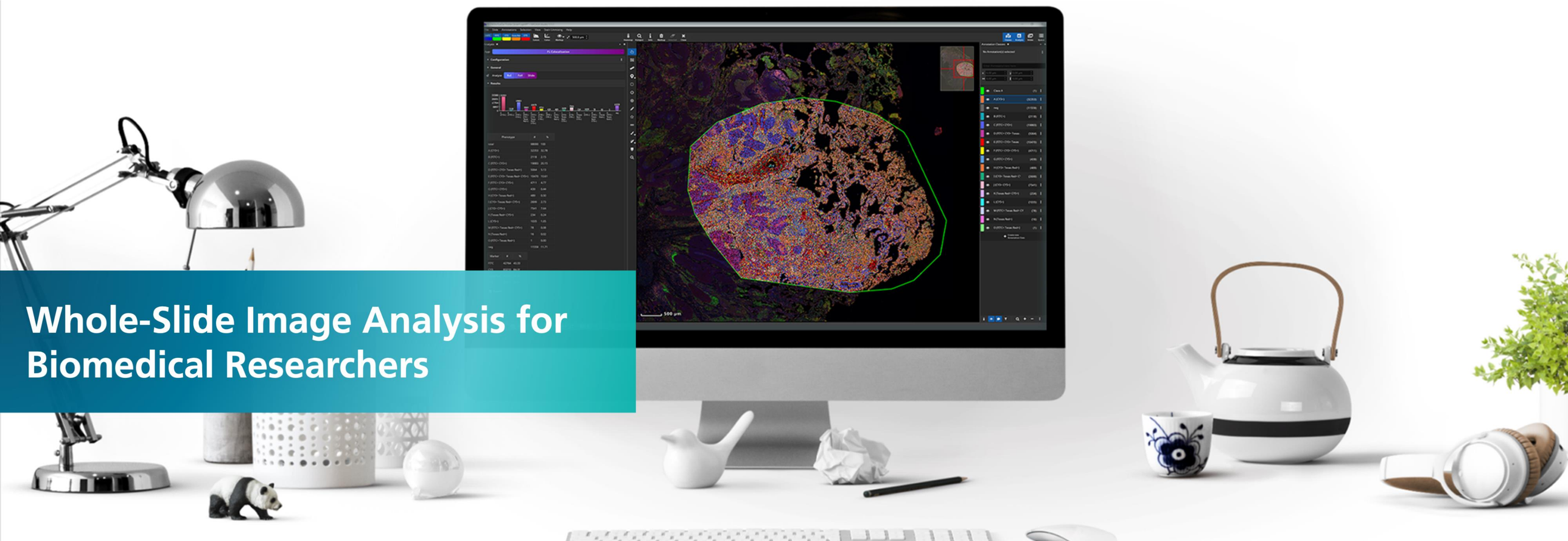
MIKAIA®

Fraunhofer IIS



Whole-Slide Image Analysis for Biomedical Researchers

7 Reasons to Use Digital Image Analysis in your Histopathology Research

Conduct quantitative analyses that are **simply not possible manually.**
E.g., counting all cells, statistics about cell-cell connections, accurate measurements, tissue composition, predict mutations from standard stains.

Analyze **more data** to get higher statistical significance.
Evaluate larger ROIs, an entire whole-slide image (WSI), or an entire data set comprising hundreds of WSIs.

Analyze slides faster: more data in the same amount of time.
You only have limited time for your research. Use it efficiently and do not waste time on manual evaluations. Analyzing entire data sets takes hours, not days.

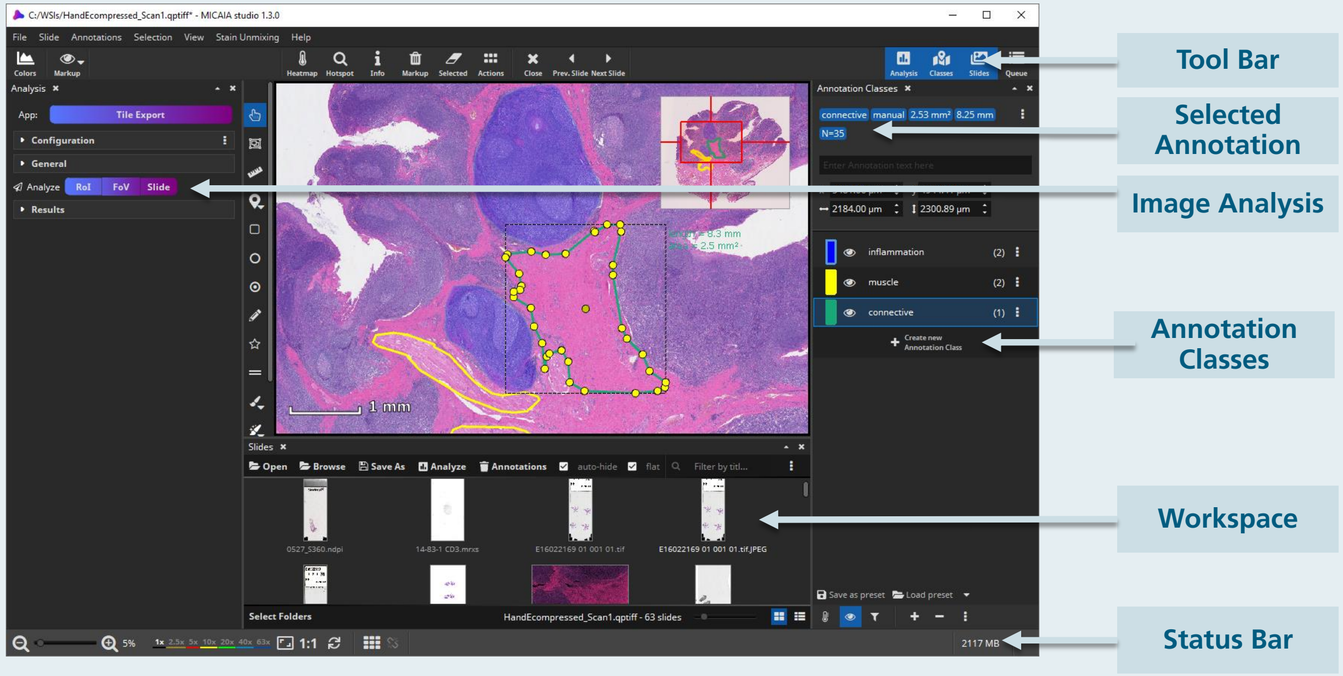
Analyze slides faster: same data in less time. Be the **first to publish your discovery**. The clock is ticking. Don't let others beat you to the punch.

Increase chances of **getting your paper accepted** by using latest digital technologies and AI.
When scoring manually, reviewers will challenge you and ask why no Digital Image Analysis was used.

Eliminate Bias, Inter- and Intra-Observer Variance.
Digital analysis will yield identical results regardless of time and stress. It will not subconsciously change its evaluation criteria over the course of a larger analysis.

Do not waste your talent and intelligence on tedious tasks.
Let the computer collect the raw data. You draw the conclusions and focus on complicated cases.

MIKAIA® Overview



Designed for researchers in academia / biotech / pharma.

Lightweight. Local Windows software. Simple installation. No need to upload WSIs into the cloud.

Developed specifically for (batch) analysis of whole-slide images.

Addressed Use Cases

Annotation & dataset creation

Quantitative image analysis of brightfield & immunofluorescence (efficacy studies, ROI detection, hotspotting, cell-cell interactions, …)

Batch-analysis of entire datasets

AI Authoring for tissue segmentation

App API for plugging in your own AI*

*coming soon

MIKAIA® in Your Workflow

FFPE or Fresh Frozen Sample

Prepare and stain sample, e.g., H&E, IHC, or IF.

Digitize glass slide(s). Most scanners are supported.

Alternatively capture one or multiple field-of-views with a camera-equipped microscope.

Dataset of whole-slide images (WSI)

or "flat" field-of-views.

Build analysis workflow by utilizing off-the-shelf analysis apps or train AI.
First analyze sub-cohort and gain confidence in quality.
Then batch-analyze entire study cohort.

Export quantitative results to CSV (spreadsheet).

Downstream statistical analysis; correlation with clinical data; creation of plots, e.g., with Excel, Python, Matlab, or R.

Draw conclusions. Publish Paper/ Write Study Report

MIKAIA® lite

Available for free download at www.mikaia.ai/

- Supports most scanners
  Leica, 3DHistech, Hamamatsu, Zeiss, Olympus, Roche, Motic, Akoya, Lunaphore, NanoString, Precipoint, OME-TIF, DICOM …
- Annotation I/O round-trip with QuPath, Leica, import from 3DHistech.
  Safe Annotations: undo / redo, auto-save, class presets, …
- Decentralized annotation storage (in sibling files).
  Safe to relocate your WSIs at any time.
- Convert and crop any input WSI format to SVS or DeepZoom format.
- Free apps: Tissue Detection, Tile Export, Annotation-to-Image (incl. segmentation mask generation for AI training)
- Live Stain Unmixing, Density Heatmap, Hotspot Search

MIKAIA® studio

- Large set of additional image analysis apps
- Combine apps to build powerful analysis workflows
- App REST API for integrating your own AI
- Batch-process entire datasets.
  Create comprehensive CSV file with all results in one place.
- Competitive pricing
- Distributors in Europe, US, and Asia:
  www.smartinmedia.com/mikaia
  www.benestartech.com/mikaia
  www.scientialux.bio/mikaia

The App Center

- Various use-case-specific analysis apps are available in the App Center and ready to be used off-the-shelf.
- Apps can be filtered by tags HE, IHC, FL or by full text search.
- More apps will be added in future updates.

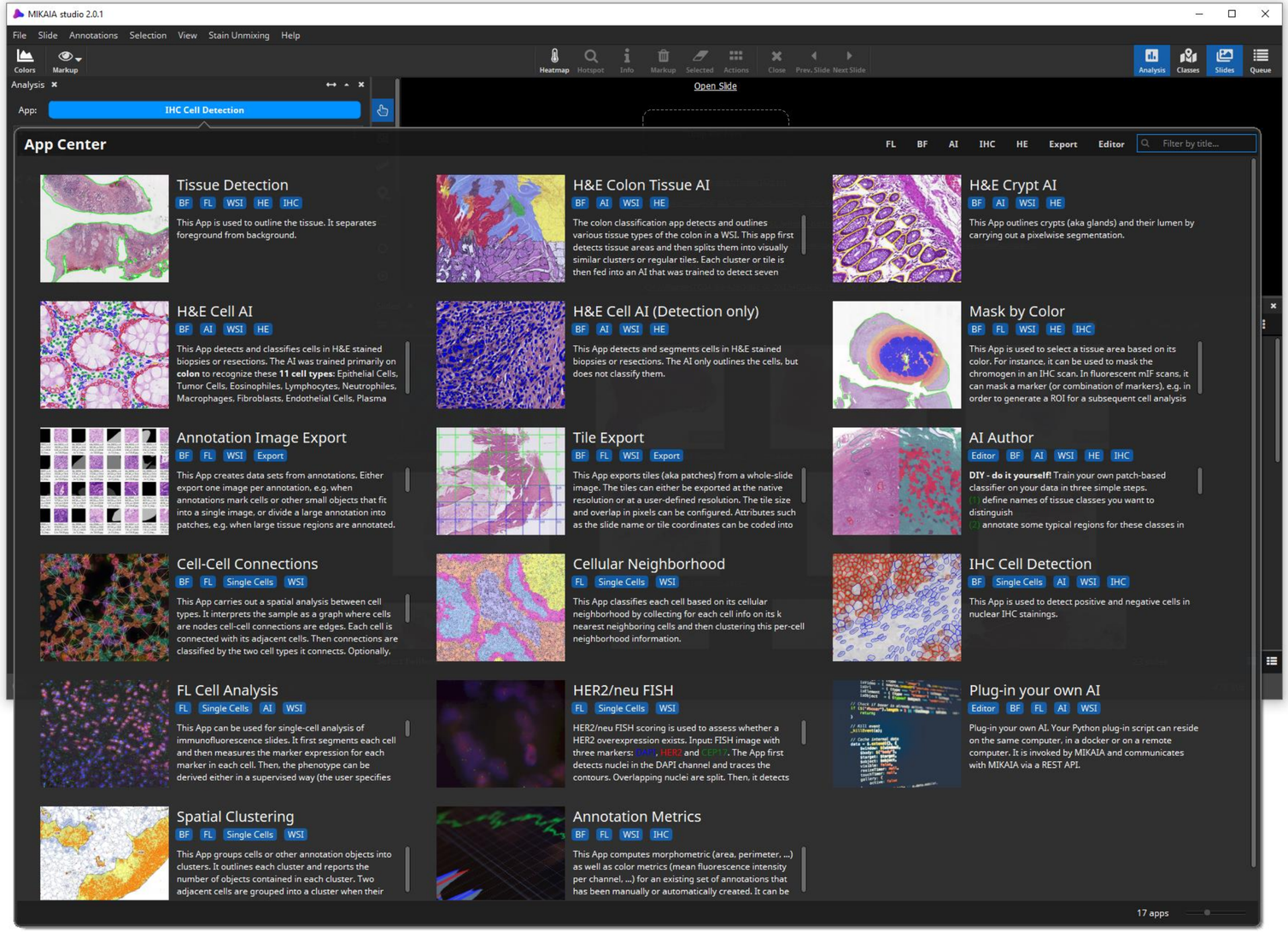
App 1 → **App 2** → **App 3** → **...**

Each app adds annotations to the WSI.
These can serve as input to the next app.

Extensible

Users can easily add further apps using the AI Author.
No technical or AI knowledge required.

Data scientists can plug in in their own AIs using a REST API.
This could, e.g., be a Python script residing on the same computer, in a docker, or on a network computer



MIKAIA® Apps and AIs

MIKAIA® apps are available in three feature sets:

- MIKAIA® lite
- MIKAIA® studio
- MIKAIA® studio + AI Add-on Bundle.

	MIKAIA® lite	MIKAIA® studio	AI Add-on Bundle	BF	FL	AI	
IHC Cell Detection		●		✓		✓	
FL Cell Analysis		●			✓	✓	
Mask by Color		●		✓	✓		
H&E Cell AI (Detection only)		●		✓		✓	
Cell-Cell Connections		●		✓	✓		
Cellular Neighborhood		●		✓	✓		
Spatial Clustering		●		✓	✓		
FISH HER2/neu		●			✓		
Annotation Metrics		●		✓	✓		
Plug-in your own AI		●		✓	✓	✓	
AI Author			●	✓		✓	
H&E Cell AI			●	✓		✓	
H&E Crypt AI			●	✓		✓	
H&E Colon Tissue AI			●	✓		✓	
Tissue Detection	●			✓	✓		free
Annotation Image Export	●			✓	✓		
Tile Export	●			✓	✓		

Tissue Detection App

Outline tissue, TMA de-arraying, divide slides into scan areas.

Tissue Detection

Tissue Analysis Apps (find ROIs)

Analyze tissue and create masks that can serve as ROIs for further analysis.

Mask by Color

AI Author

H&E Colon Tissue AI

Cell / Spot / Object Detection Apps (per ROI)

Detect cells or other objects and assign them to a ROI.

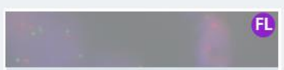
IHC Cell Detection

FL Cell Analysis

H&E Cell AI

H&E Cell AI (Detection only)

H&E Crypt AI



HER2/neu FISH

Detected Objects Analysis Apps

Analyze already detected objects (spatial, color, or morphometric analysis).

Cell-Cell Connections

Spatial Clustering

Cellular Neighborhood

Annotation Metrics

Data Export

Export slide level results, markup and images

Quantitative results

Each app exports to CSV (Excel)

Markup

GeoJson (for QuPath), Aperio XML, CSV, MIKAIA ANO

Image Export Apps

Annotation Image Export

Tile Export

Further tools

- batch processing
- search hotspots
- density heatmap
- create concentric margins
- annotation set operations (fuse, subtract, intersect, clip)
- import/export annotations
- crop / export WSI
- live stain unmixing
- stain estimation
- undo / redo annotations
- edit annotations
- auto-save
- …

Plug-in your own AI

I can recommend MIKAIA® to anyone who wants to step up their research. My PhD students and I use it to analyze various IHC and H&E datasets and extract quantitative data for our publications. MIKAIA® is at the sweet spot between ease-of-use and flexibility.«

PD Dr. med. Carol-Immanuel Geppert MIAC, Senior Pathologist
Head of Cytology & Digital Pathology
University Hospital Erlangen-Nuremberg, Bavaria, Germany

Our References

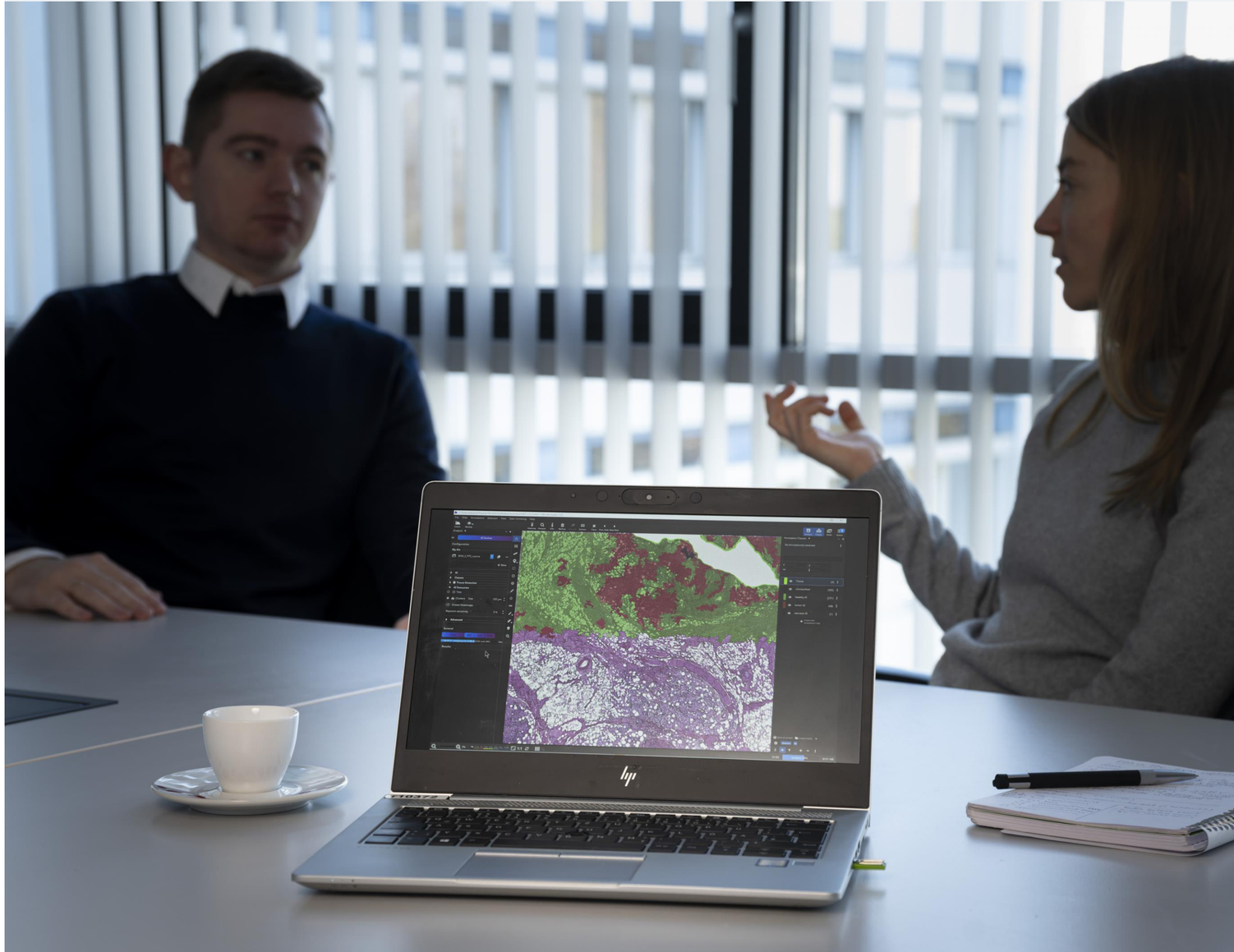
Current Partners

- Charité Universitätsmedizin Berlin
- Paul-Ehrlich-Institut PEI
- Helmholtz Munich
- Max Delbrück Center for Molecular Medicine
- Bernhard Nocht Institute for Tropical Medicine (BNITM)
- University Hospital Erlangen-Nuremberg
- Fraunhofer Institute for Cell Therapy and Immunology IZI
- Fraunhofer Institute for Toxicology and Experimental Medicine ITEM
- Fraunhofer Institute for Digital Medicine MEVIS

… and more

Past Partners

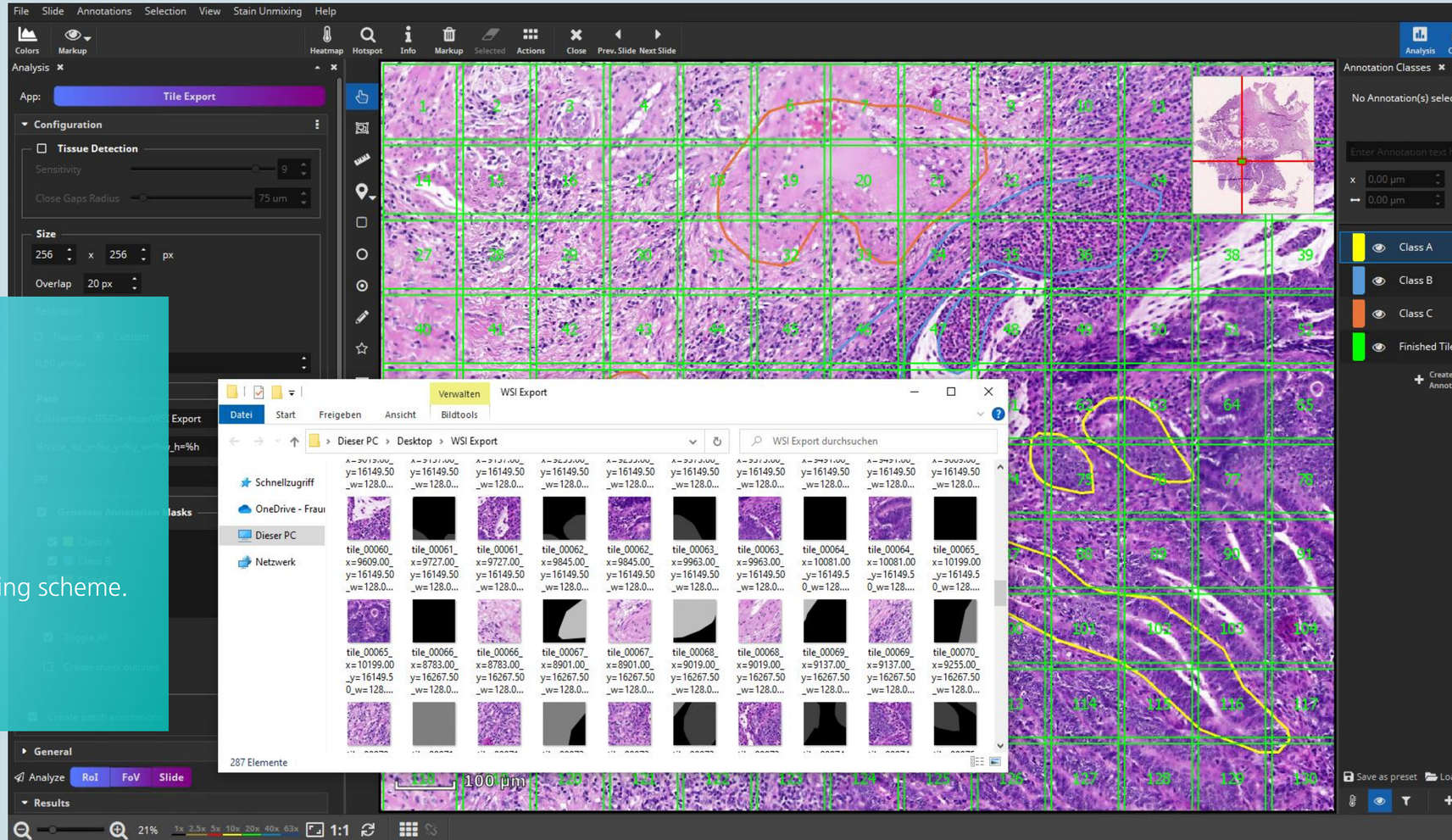
- Definiens AG
- PreciPoint GmbH
- University of Regensburg
- Technical University of Munich
- Bilkent University, Ankara
- Universitätsklinikum Frankfurt



Don’t waste time on the first step of every Histo AI Development:
Use MIKAIA® to create datasets for AI Training.

Dataset Creation Workflow

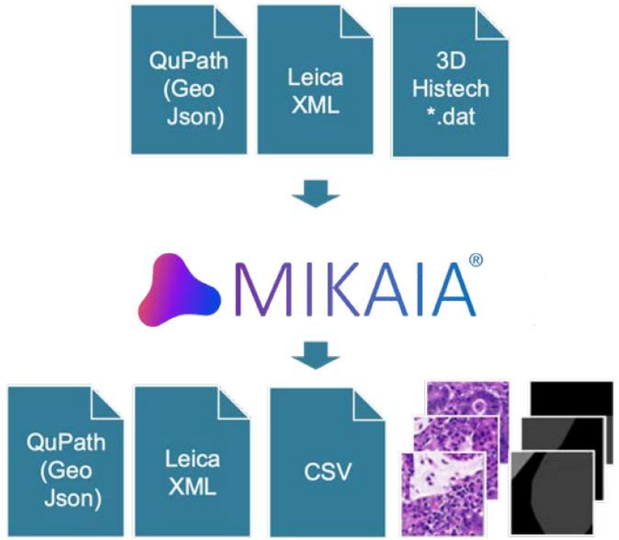
1. Create classes A, B, C (optionally from template).
2. Annotate by hand or use one of the analysis apps.
3. Select *Tile Export* app in App Center.
4. Set up desired tile size (px), overlap, resolution (µm/px), naming scheme.
5. App will export tiles and segmentation masks to the disk.
6. Train AI outside of MIKAIA®.



Create Data Sets

The right annotation tool can save many hours of work. The wrong tool can cost many hours.

We have the highest standards when it comes to annotation tools, and we use MIKAIA® ourselves to annotate training data for our AI models.



Auto-save
Every few minutes the annotation file is automatically saved in the background. Like you are accustomed to in Microsoft Office Applications: If the software or computer shuts down unexpectedly, the recovery file is detected at the next program start.

Undo / redo support
You can undo and redo the creation / deletion of annotations. When drawing a large polygon, you can rewind the last segments by clicking the right mouse button and then continue drawing from there.

Ergonomic Annotation
While drawing with the Pen tool, you can release the mouse button intermittently to rest your finger. If you prefer to draw by clicking at each segment point, that's also possible.

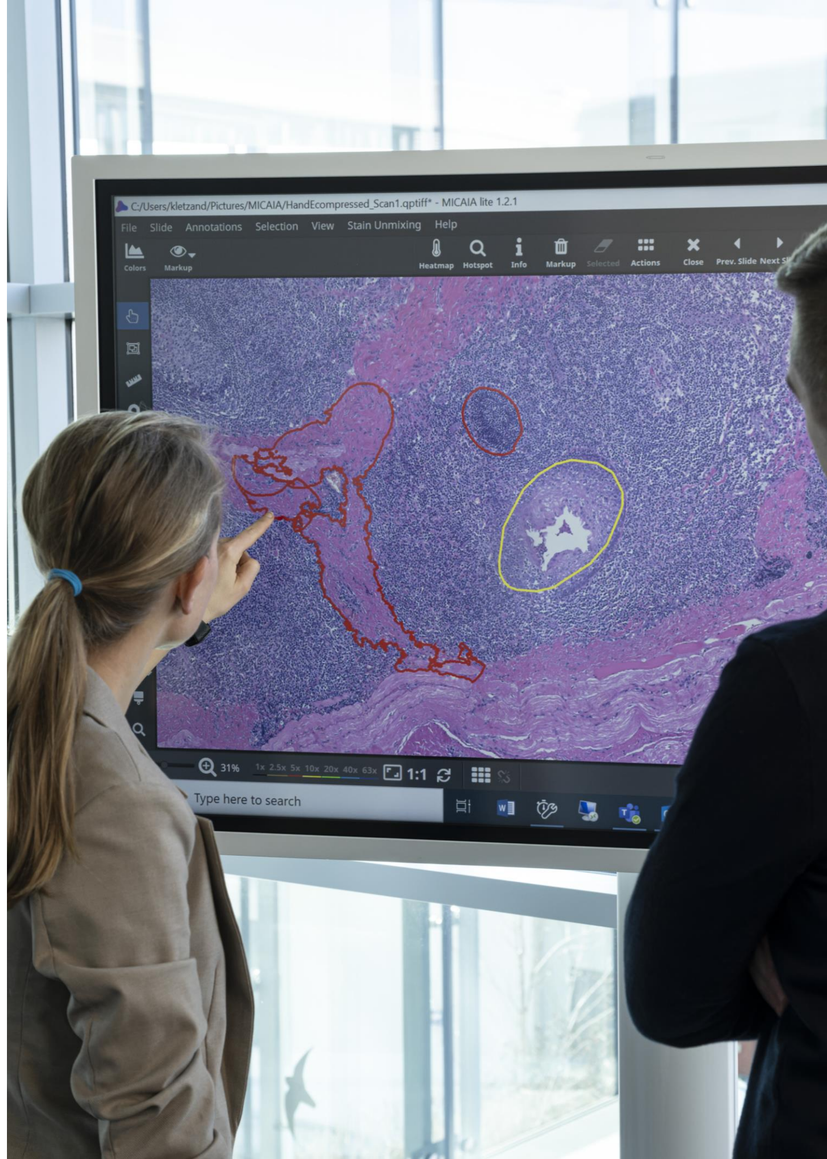
Navigate while drawing
Sometimes it is necessary to annotate at the highest resolution. Your annotation may soon reach the edge of the current field of view. You can easily navigate with the mouse or keyboard arrows without interrupting the annotations.

Touch support
You can use a stylus pen on a touchscreen (e.g., a WACOM tablet) for drawing.

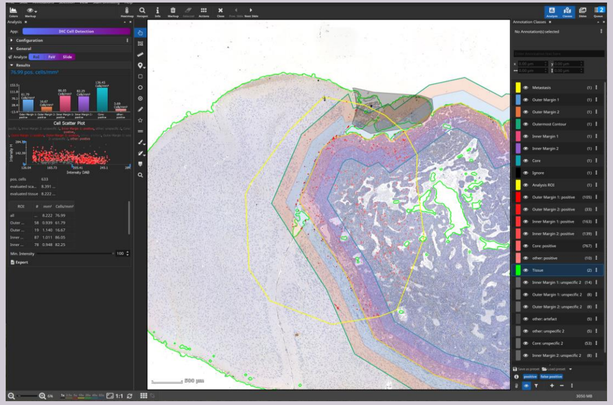
Create Class Presets
Typically, you will annotate multiple WSIs, and multiple annotators might be involved. Often you end up with different annotation class names, e.g., "Tumor", "tumor", "tumour", "tumor", and they will sometimes be red, green, or blue. In MIKAIA® you can simply create a list of annotations classes, configure the appearance of each class, and then save this as a preset.

Smart Annotation
The magic brush and magic eraser support image-content aware annotating. The circular shape will clip to the image content. You can configure the radius and color-tolerance. The brush works well in conjunction with synthetic views, e.g., you can annotate an optical density visualization or the unmixed DAB component of a H-DAB stain.

Create Cell Datasets
The class label tool is handy to create cell classification datasets.
1. Outline cells using the pen tool, magic brush, or HE Cell Detection App.
2. Assign the unlabeled cells to the current class simply by selecting it with the class changer tool.



IHC Cell Detection App



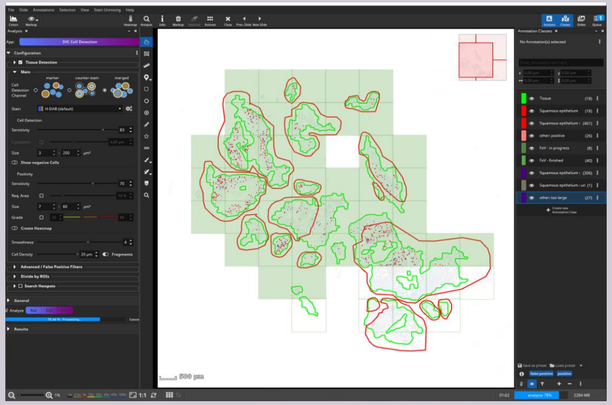
CD3+ T-Cell analysis in metastasis

Objective: measure T-cell density inside and outside metastasis and near the invasive margin.

1. Annotate metastasis by hand (or with the Mask-by-Color App)
2. Used “add margins” feature to add 2 concentric margins inside and 2 outside, 100 µm diameter each, to cover the tumor microenvironment.
3. Draw ROI to be analyzed in yellow. Optionally, mark areas to be ignored (here, black “ignore“ class). Cells picked up in this area will be discarded
4. Start IHC analysis and select that pos. cells (here CD3) should be grouped by ROI, selecting the 4 margins and metastasis core. The remaining area will be implicitly added as “other“.

In the class side bar, it is visible how pos. cells are divided into six different classes with different shades of red.

The bar diagram shows the cell density in cells/mm² per ROI. Each point in the scatter plot represents one cell. Clicking on a point will center the viewer on the cell. The H- and DAB intensity, as well as the size can be plotted.

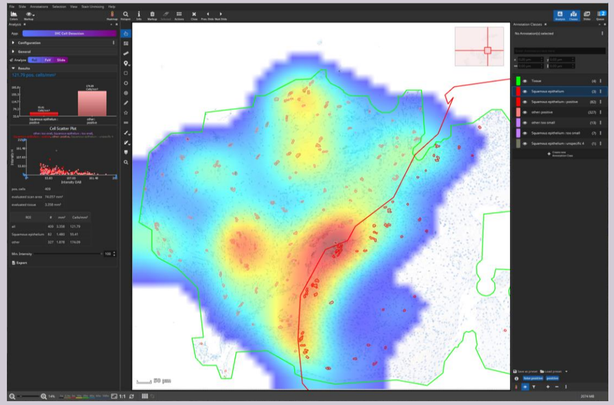


CD117+ mast cell analysis

Objective: measure mast cell density inside and outside squamous epithelium.

1. Batch-run Tissue Detection App on entire dataset to detect tissue (green). Manually correct, where necessary.
2. Manually outline squamous epithelium in all slides (red). Since they will be automatically intersected with the green tissue outline during the analysis, the outlines outside the tissue can be drawn sloppily.
3. Batch-run IHC analysis on entire dataset and configure that pos. cells (here, mast cells) should be grouped by ROI, selecting here only the “squamous epithelium“ class.

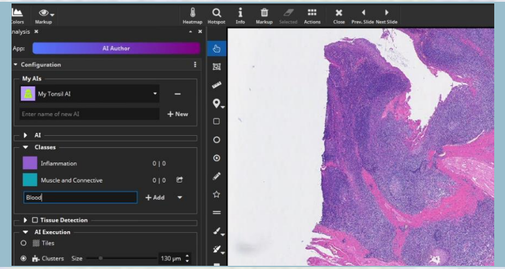
The above screenshot is taken during the analysis. Green filled tiles have already been analyzed; green unfilled tiles are currently being processed in parallel. Detected positive cells are drawn in shades of red, depending on the ROI they belong to.



Zoom-in on an analyzed slide with CD117 mast cell staining. Detected cells are outlined in red. The density heatmap visualization is activated and shows clusters with a relatively high density of mast cells.

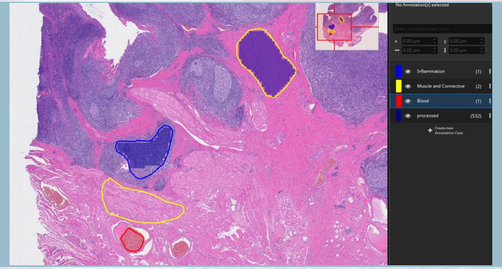
AI Authoring Workflow

By design, researchers have unique questions they want to answer.



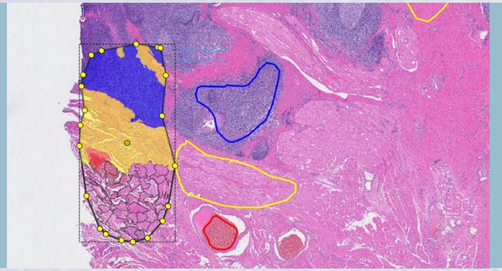
1

Select “AI Author“ app, create new AI, and configure classes (here 3).



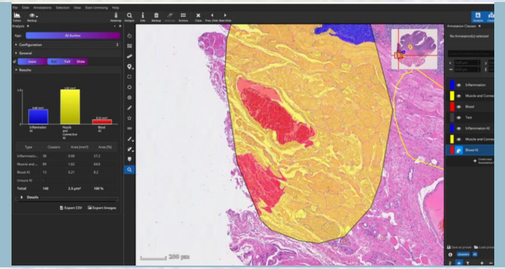
2

Outline example regions for each class and train AI on them. AI will analyze them patch by patch (blue squares).



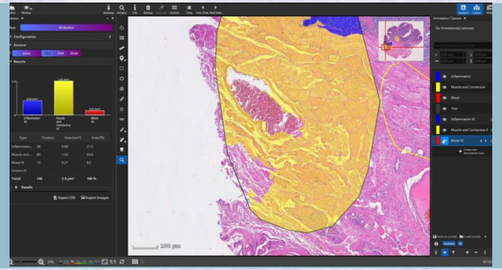
3

Test AI on ROI (black). The ROI is analyzed patch by patch or optionally cluster by cluster.



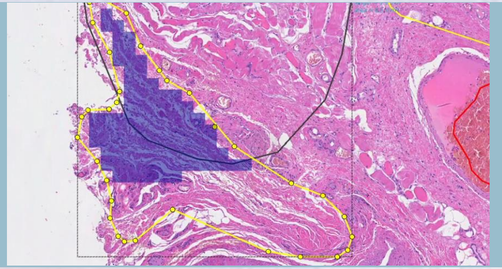
4

Inspect quality of result. Here a region is classified as the red class but should be yellow.



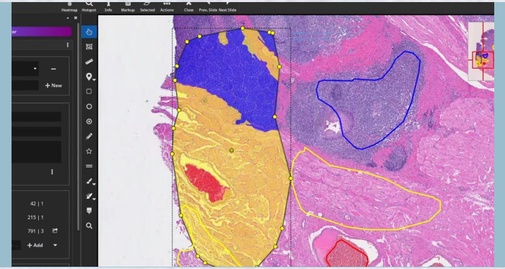
5

The mistake looks plausible. The misclassified red region has a texture similar to the true red region.



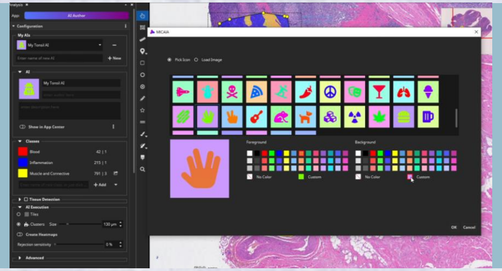
6

Add a new training annotation to teach the AI that the tissue contained in the misclassified area belongs to the yellow class.



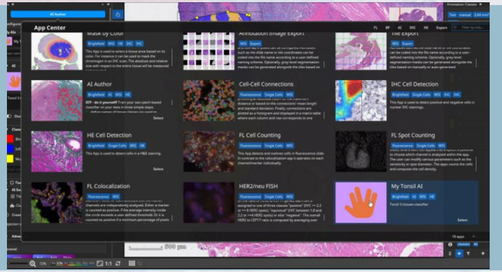
7

Repeat the analysis of the test-ROI. Now the result looks correct. The trained AI is ready to be used now.



8

Pick an icon, add a description text, and enable “Show in App Center“.



9

The trained AI is now available as a new App in the MIKAIA® App Center. It can also be shared with other MIKAIA® users.

Viewing and Analyzing Immunofluorescent Scans

Proteomics

Immunofluorescent multi-plex or high-plex slides comprise multiple channels that mark targets such as DNA or proteins. MIKAIA® has full support for visualizing plexed slides.

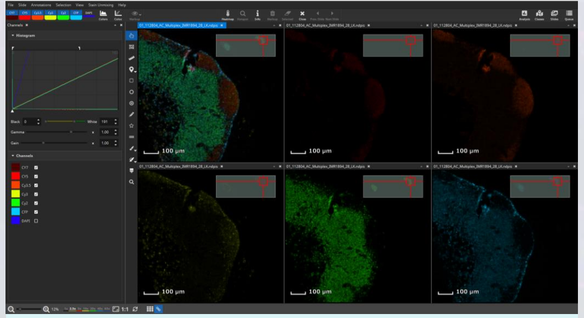
- ✓ **Toggle channels on and off (on/off, solo, only others).**
- ✓ **Configure visualization (levels, gamma, gain, pseudo color) individually for each channel.**
- ✓ **Rendering with 16 bit precision.**
- ✓ **Multiple formats supported (OME-TIFF, Zeiss CZI, Hamamatsu NDPIS, Olympus VSI, and more).**

1. **Get fast insights: Channel-wise correlation**
   The correlation module shows an interactive scatter plot for each pair of two channels and sorts them by descending correlation (Pearon's correlation coefficient). This analysis takes < 5 seconds for a whole-slide image scan.

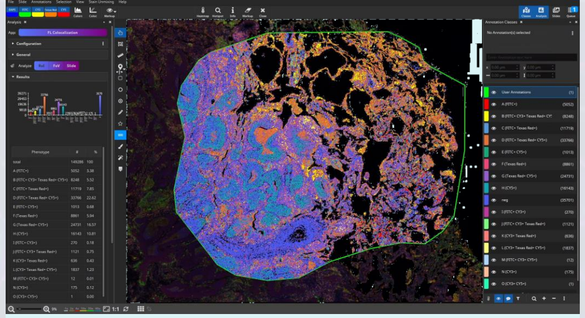
2. **Cell analysis**
   The FL Cell Analysis App will first detect and outline all nuclei in an ROI or FoV by analyzing the cell marker channel (usually DAPI). Each nucleus annotation is then grown by a user-defined margin to include the cytoplasm. The app will then decide which markers are expressed in each cell. From this, cell phenotypes are derived and visualized in separate annotation classes. The interactive density heatmap can optionally be enabled to show hotspots of certain cell types.

3. **Spatial Analysis**
   In the next step, the FL Cell-Cell Connections App operates on the classified cell annotation objects and connects each cell to its neighbors. From the resulting graph, various metrics can be derived such as
   - Bystander analysis: which cell types are neighbored. "On average, a cell of type A has 2.3 neighbors of type B".
   - Proximity analysis: what is the average distance between cell types. "On average, the distance between cell types A and B is 10.5 µm ± 2.1".

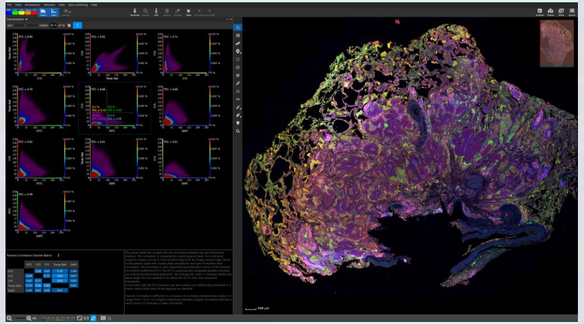
   Again, the density heatmap can be enabled and operates on edges (cell connections) to show hotspots where certain cell types are neighbored.



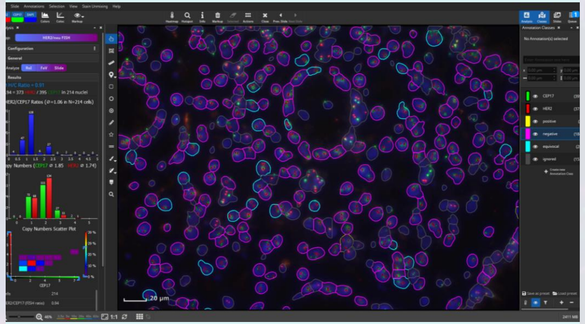
Synchronized **side-by-side** viewing of markers.
Mouse pointer is mirrored



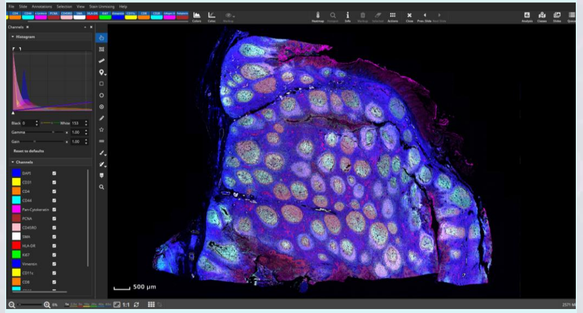
Single cell marker expression with **FL Cell Analysis App**



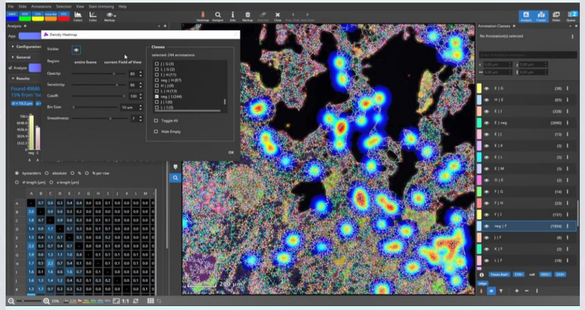
Correlation analysis of marker pairs
(Pearson correlation + scatter plot)



FISH Spot Analysis App. Here: HER2/neu



Good support for high-plex **proteomics** scans
Above: 15-plex Tonsil by Akoya Biosciences



The **Cell-Cell-Connections App** connects cells to their neighbors in a graph.

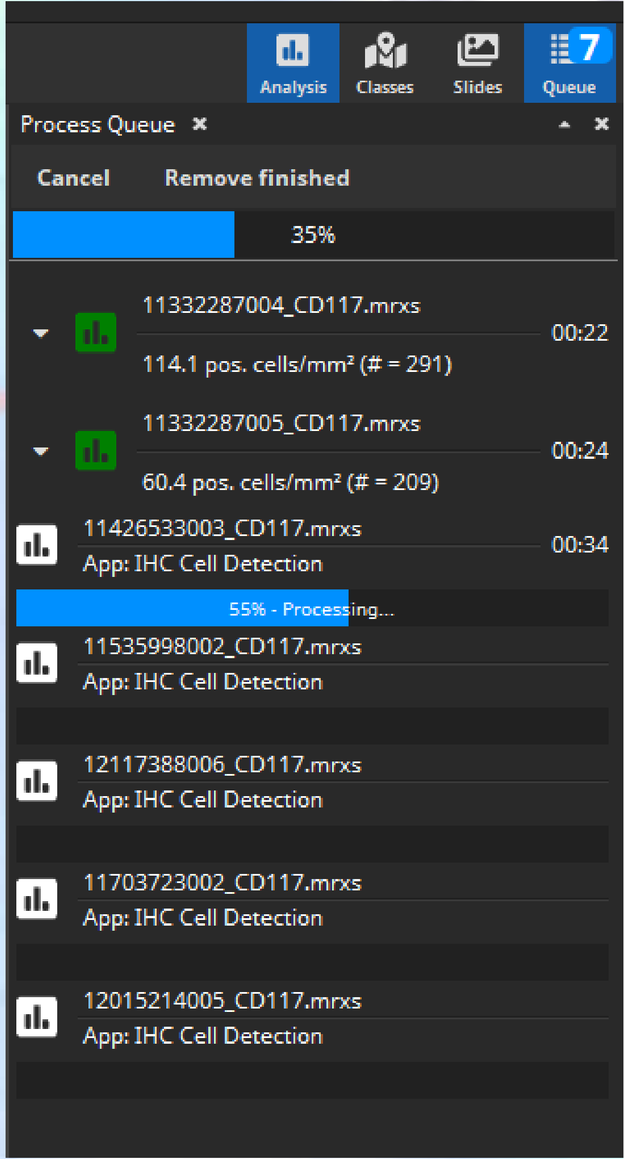
Batch Analysis of Multiple WSIs

Find parameters → Evaluate on test set → **Batch analyze entire dataset** → Manual corrections → Obtain and process summary report

1. **Find parameters**
   Pick a few regions or WSIs in order to experiment with the app settings and find good parameters. Save the parameters as a preset, e.g., "CD3 mouse liver", so that the parameters can be easily restored throughout the project.
2. **Evaluate on test set**
   It is good practice to evaluate the performance (accuracy, time) on a small test set in a quantitative or qualitative fashion. After a successful validation, the analysis can be "rolled out" to the entire dataset.
3. **Batch analyze entire dataset**
   A large batch analysis is started simply by loading the folder containing all WSIs into the workspace, selecting them all, and clicking the "Batch" analysis button. One job per WSI (using current app with current settings) will then be added to the job queue and processed one after the other. As soon as a WSI is processed, the WSI results (markup file, CSV spreadsheet, low-res preview images with and without markup) will be written to the result folder.
4. **Manual Corrections**
   When you inspect the results by stepping through the analyzed WSIs, you may spot errors, e.g., a cell that should have been picked up, but was missed, or a false cell annotation that should not have counted. You can simply correct such mistakes by deleting false annotations or adding missing annotations. Afterwards, you will want to update the app-specific result CSV files. Simply repeat the batch in "Do not analyze. Load annotations." mode and obtain an updated set of result files.
5. **Obtain and process summary report**
   As WSIs are processed, a summary CSV file containing all results from all WSIs is continuously updated and stored in the results folder. After the batch has finished, this file will contain all quantitative data. For each WSI, it contains rows for different "entities":
   - for each cell
   - summary for each ROI, for each class and for the entire WSI

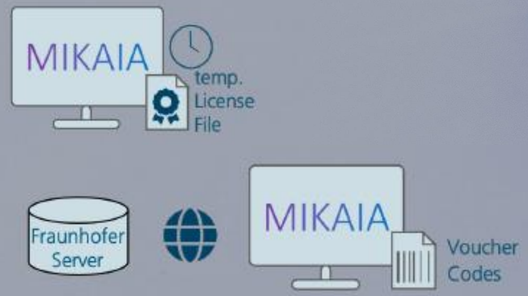
   In other words, it contains both the row data (e.g., location, size, and mean intensity for each single cell) and summary statistics. The granularity is modifiable by only showing rows of a certain type and hiding all other rows.

   The CSV file can be simply opened with Microsoft Excel but is structured in a way that it can also be easily imported into other statistics tools, such as R, Python, or Matlab. In this way, researchers can use their favorite tool to correlate the quantitative MIKAIA® output with clinical data or create custom plots.

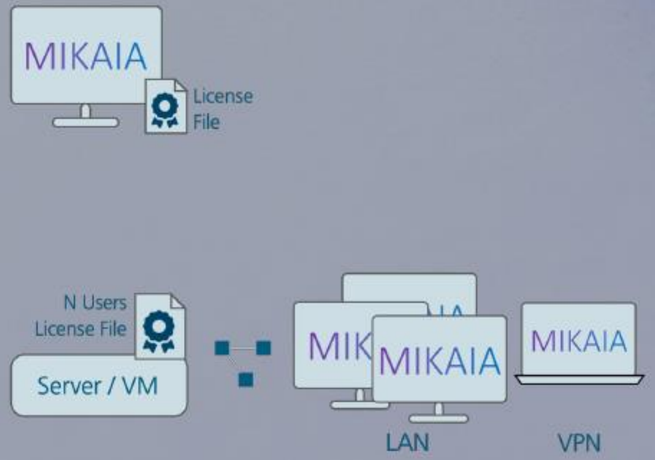


Evaluation & Activation

Evaluation



Permanent Activation



Evaluation

Temporary license file
Same as "Activation with node-locked license", except that MIKAIA® is only temporarily unlocked.

Voucher codes
1. Download MIKAIA® from www.mikaia.ai and install.
2. Import voucher codes.
3. One voucher is consumed for each execution of an app (requires internet connection).

Permanent Activation

Node-locked single-user license

1. Download MIKAIA® from www.mikaia.ai and install.
2. Create license request file. It contains the computer's unique fingerprint.
3. Receive an activation file and import it into MICAIA®. MICAIA® is now unlocked.

The name changes from ***MIKAIA® lite*** to ***MIKAIA® studio.***

Floating or multi-user license

Server installation
1. Download MIKAIA® from www.mikaia.ai and install it on server (can be a VM).
2. Create license request file. It contains the server's unique fingerprint.
3. Receive an activation file and import it into MIKAIA®.
4. Open CodeMeter Control Center, open WebAdmin, enable „Server" to make the server discoverable from other computers.

Client Installation
Download MIKAIA® onto any client computer and install it.
If client and server are in the same LAN, client will auto-detect server and MIKAIA® will be auto-magically unlocked right away.
If client is connected via VPN (e.g., when working from home), open Codemeter WebAdmin and add the server name or IP to the server search list first. If the server license permits 10 parallel users, the first 10 users can start MIKAIA® studio on their computer. The 11th will get a notice that all slots are in use.

Hardware Requirements

MIKAIA® is a native 64bit Windows software that runs locally on your computer.
A CUDA-enabled GPU is recommended for all AI apps.

Keep your data in safe hands – in yours!
Bring AI to the data, not data to the AI.

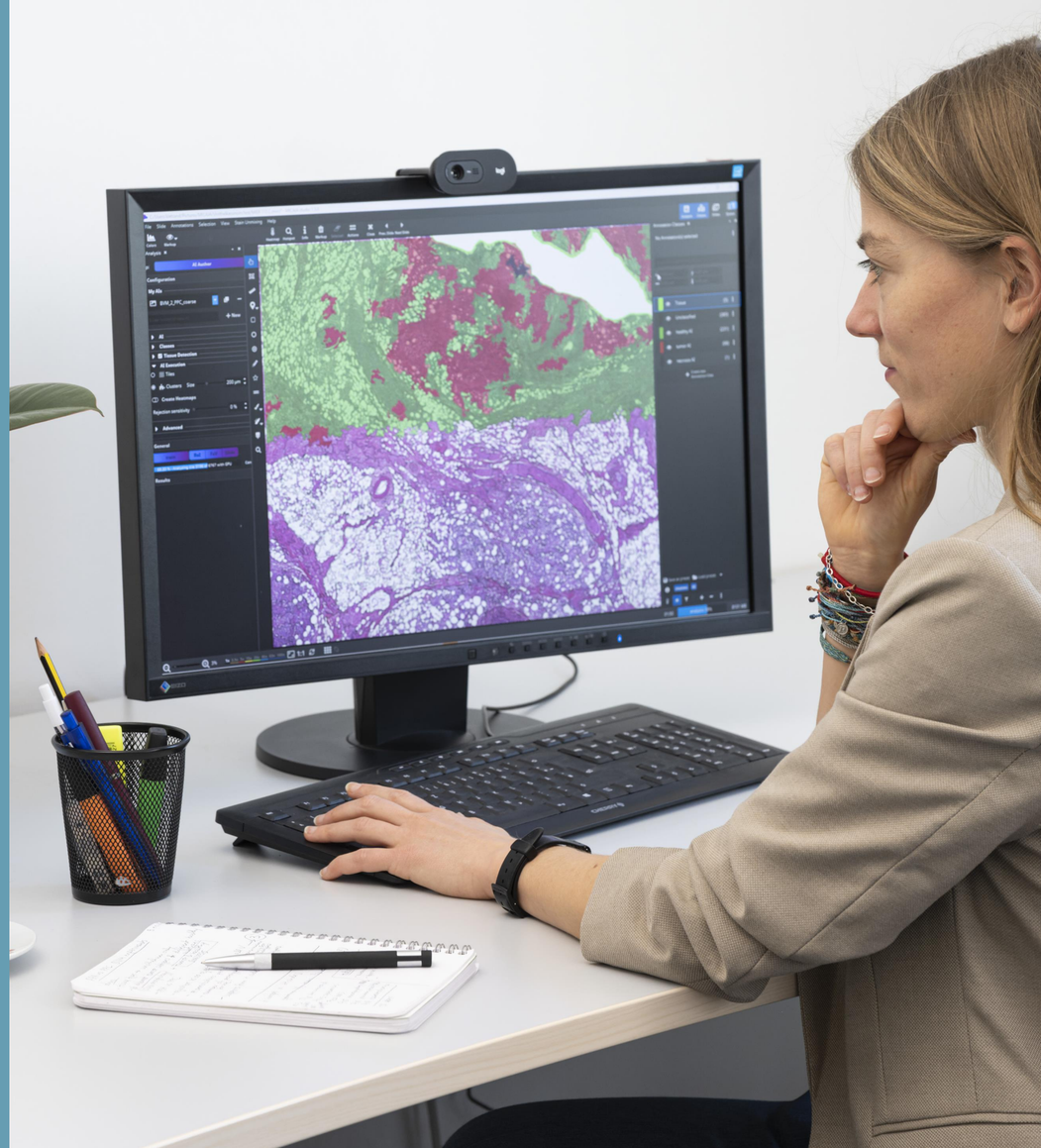
WSIs are often larger than 1 GB. Uploading this data to the cloud can take a long time and require a lot of expensive cloud disk space.

Instead, analyze your data locally and load WSIs into MIKAIA® directly from your network or local folder.

Easy Installation – start in less than a minute
You can simply download MIKAIA® from www.mikaia.ai.

Start using it right away. No need to wait for a technician to set up an on-site server, which would also entail involvement of your local IT department. MIKAIA® is available as

- a regular installer or
- a portable version (zip file, no admin rights required).



MIKAIA®

Free Download of MIKAIA® lite:
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MIKAIA® University on our blog *SMART SENSING insights*