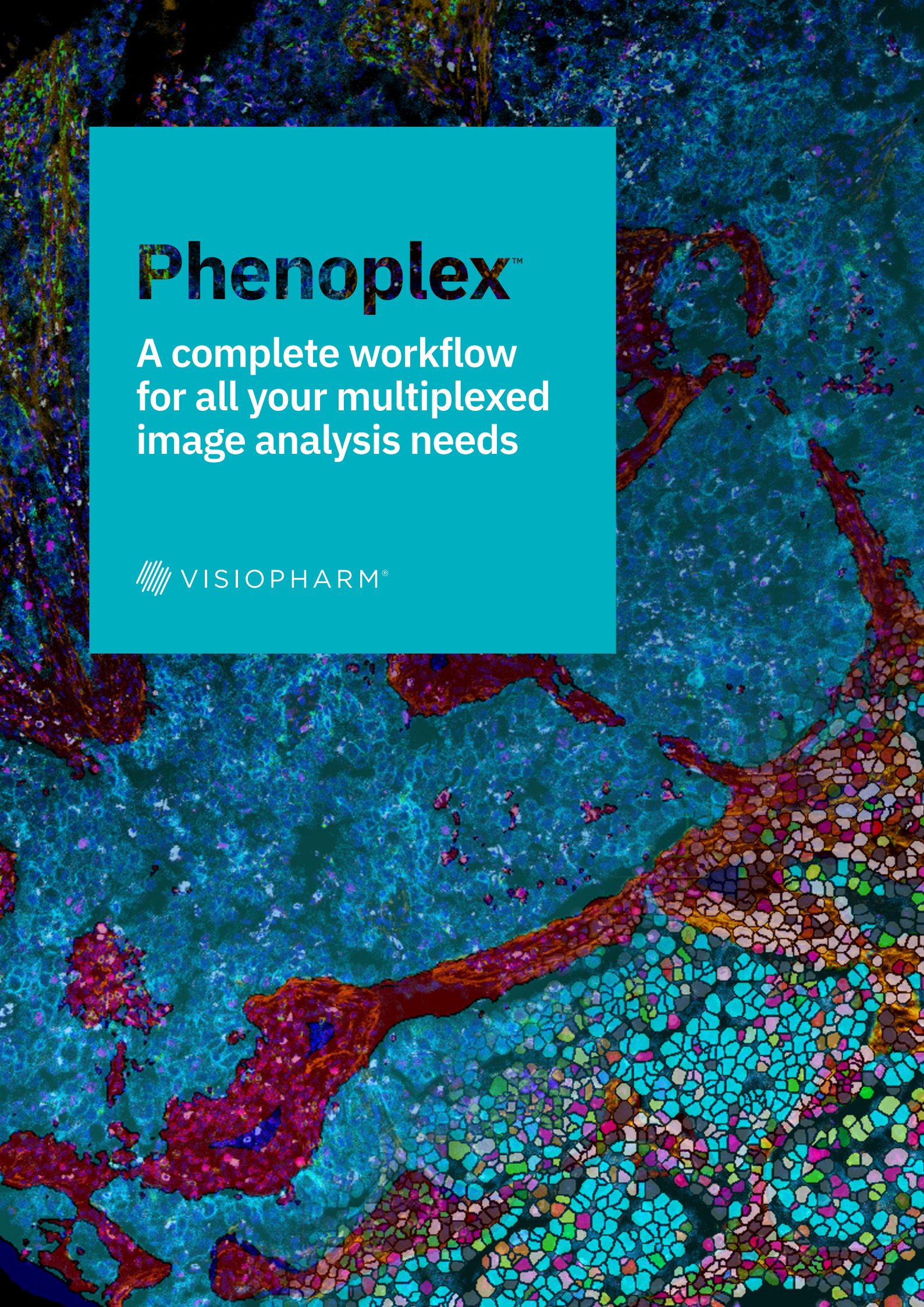




Phenoplex™

A complete workflow
for all your multiplexed
image analysis needs

 VISIOPHARM®

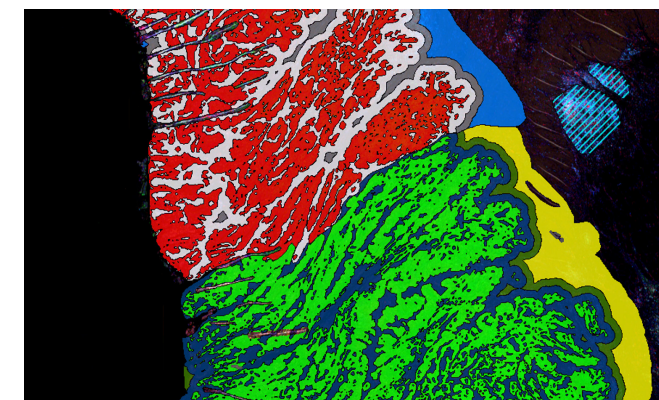
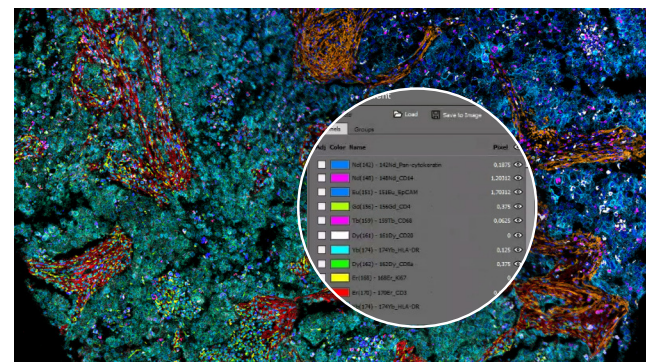


Spatial Biology deserves image analysis software designed for multiplex images. Phenoplex is a complete analysis workflow for multiplex tissue images, built on Visiopharm's best-in-class AI, with interactive verification steps throughout. Make new discoveries, measure spatial neighborhood metrics, compare between cohorts, and re-analyze past experiments. Phenoplex's comprehensive workflow allows you to rapidly confirm observations, no matter the 'plex level, so you can confidently move on to the next multiplex experiment.

Phenoplex is compatible with all major multiplex imaging instruments including: Akoya Biosciences, Standard BioTools, Lunaphore, Rarecyte, Ionpath, Canopy Biosciences, Olympus, Zeiss, Leica, and more.



Visual inspection is always a challenge with multiplex data sets. **Phenoplex's channel manager lets you set multiple color channel combinations to easily display cell types/markers into logical groups.** Assign color palettes to individual channels or channel groups. Review each channel to make sure it contains appropriate data for inclusion into downstream multiplex analysis.



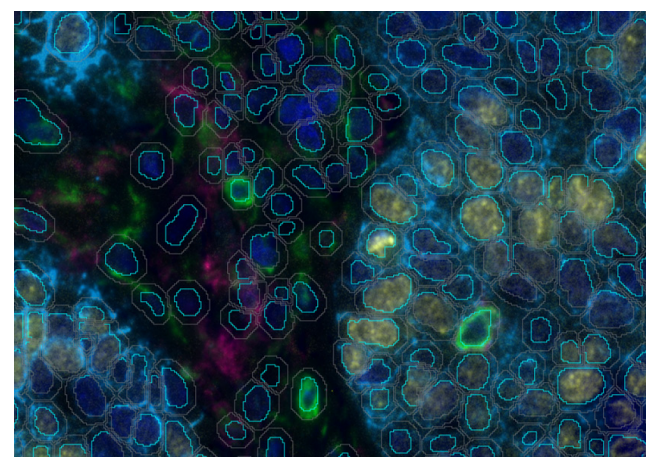
MAP TISSUE

Mapping tissue samples into independent compartments of analysis will unlock even more spatial data from multiplex images. Isolate tissue structures like tumor, stroma, necrosis, among others; subdivide into one or more marginal zones; and exclude non-relevant areas like artifacts, out of focus areas, tissue folds, etc. Improve and apply the simplified Paint-to-Train AI capabilities of Phenoplex to find these compartments across all your multiplex images.



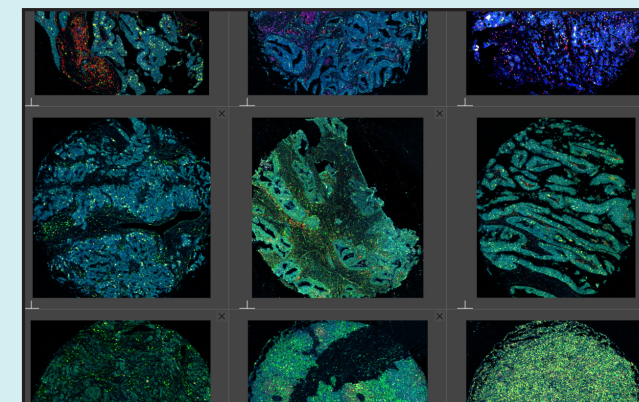
DETECT CELLS

Our superior pre-trained AI algorithms for nuclear detection and cell segmentation are optimized for multiplex instruments using fluorescence markers like DAPI and SYTO13, as well as imaging mass cytometry with Iridium. These algorithms are designed to precisely delineate the majority of cells accurately on the first attempt, ensuring high reliability and efficiency in complex imaging workflows. Yet they are modifiable by simply incorporating more biomarkers from the panel, add irregularly shaped cells to the training set, or add some morphological rules. You have the control to generate a custom segmentation method that is highly accurate and robust for your tissue.

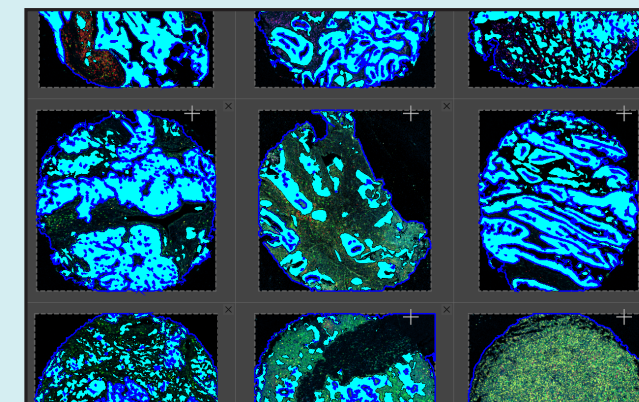


Interactive Multiview Analysis

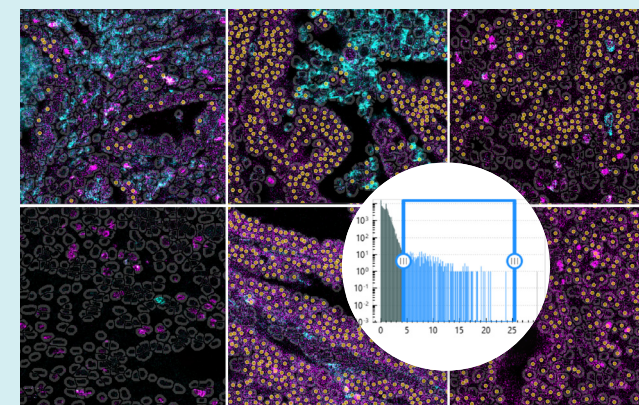
Multiplex image data sets are typically large, but Phenoplex allows you to review multiple images as you move through the workflow using a unique Multiview display. Multiview is crucial because settings applied at each step can have unexpected effects across the entire data set, which might be missed if you focus on just one area. Phenoplex enhances efficiency and confidence by employing a Multiview approach, letting you see the impact of settings across images, including positive and negative controls. Navigate each panel individually or synchronize views, display colors, pan and zoom, and review overlays. Multiview ensures the validity of your settings, speeds up result curation, and boosts confidence in downstream spatial analysis.



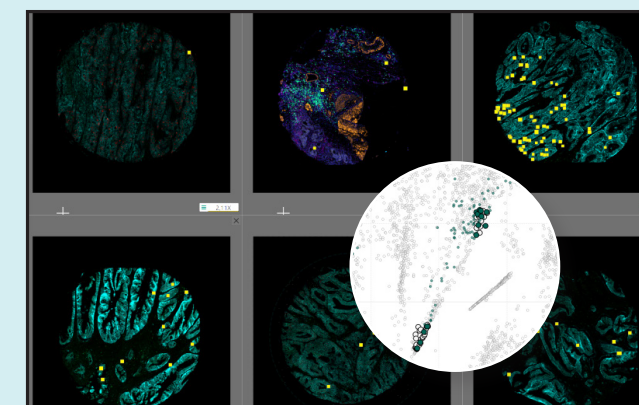
Visualize multiple slides in parallel and review different groups of biomarkers to learn about the distribution of phenotypes in your data set. Identify images of different staining intensity that may need individual positivity settings during data analysis to limit false positive or false negative results.



Train tissue and cell segmentation on different images to increase robustness of the detection. Save time by performing detection on multiple images at the same time.



Gated phenotyping: Set gates in the histogram of biomarker expression levels and get direct feedback of your selection in all open images (yellow dots). Optimize biomarker thresholds across images to harmonize and speed up biomarker phenotype analysis where possible.

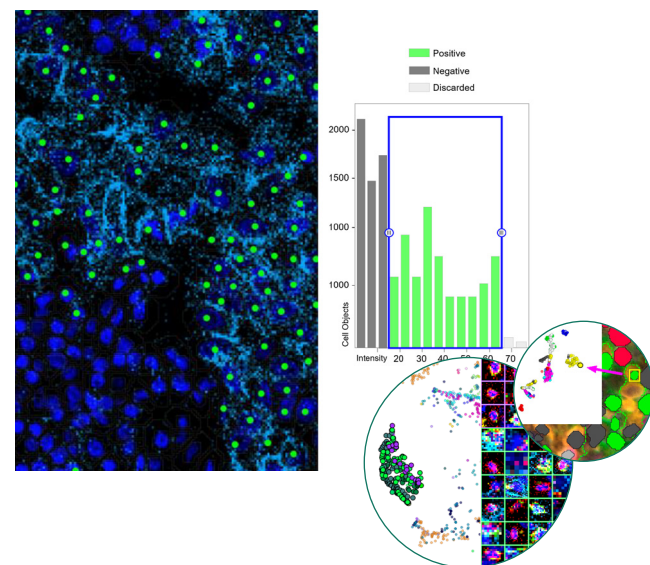
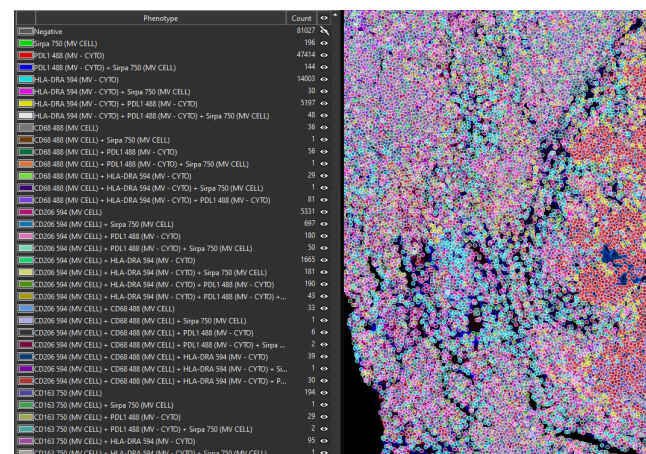


Interactive Plotting is synchronized with Multiview, providing real-time updates of highlighted cells based on selected data points in the plots or graphs. This feature allows you to instantly see the spatial context of selected points as they are highlighted within the image they originated. Multiview will allow you to quickly recognize similar and different trends across the image data set.



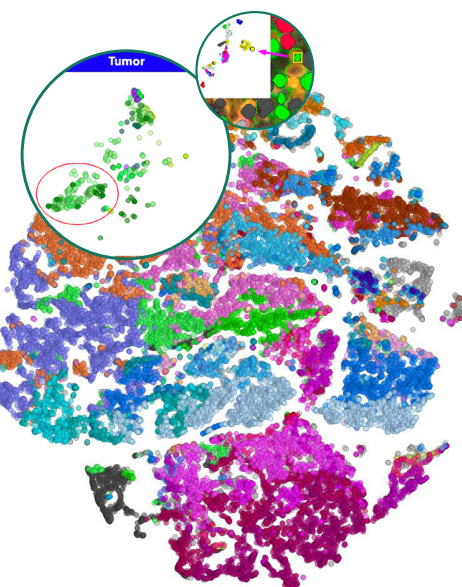
PHENOTYPE

Identifying cell types by their biomarker expression is the first step in spatial quantification. Phenoplex offers several methods to identify all cells in the tissue, giving you complete control over phenotyping. You can define cell types manually, let Phenoplex AI provide an initial guess, or use our guided workflow to set biomarker positivity thresholds across multiple or single images using the biomarker expression histogram. The Phenotype list contains each phenotype with counts and has individual display controls to create the imagery you need for publications. For staining variability, set differential biomarker thresholds across large datasets.



VERIFY

Trusting your results means viewing them from multiple perspectives, whether it's a quick review or a detailed exploration. Phenoplex provides multiple ways to review data throughout the analysis process, helping you quickly separate relevant information from noise. Plots link directly to corresponding cells in the image, allowing for fast verification at each step. Selected data points in your plots will link back to the respective cells in the image to quickly verify the results of each step. Phenoplex integrates graphs and t-SNE plots with recolored image views to confirm phenotyping results. All graphical interfaces are interactive and customizable, allowing you to compare or focus on specific tissue compartments, phenotypes, biomarkers, or cells enabling swift verification at each step.



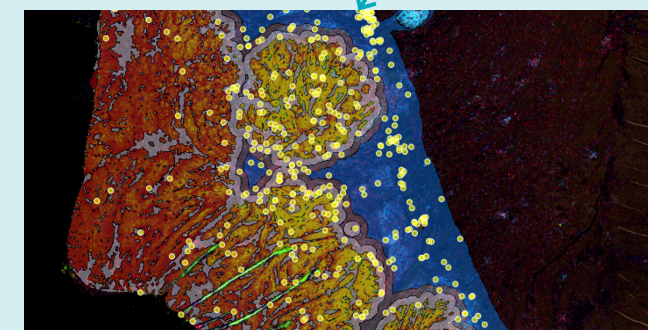
EXPLORE

Once cellular phenotypes are confirmed across tissue maps, it's time to fully explore the multiplex data. Phenoplex offers a comprehensive suite of bidirectional evaluation plots to delve deeper into your results. Extract detailed data from each cell, calculate spatial relationships and distances between phenotypes and tissue boundaries, identify areas of varying density, and analyze cellular neighborhoods. You can pool data across cohorts to compare control and test populations, responders and non-responders, and more. Phenoplex's interactive tools simplify the complexity of multiplex data exploration, enabling you to make new discoveries, test hypotheses, and plan future experiments.

Interactive plotting

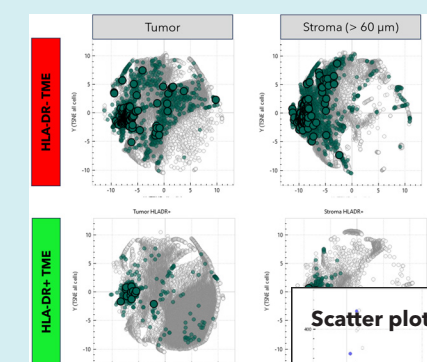
Linking bioinformatics to cellular insights through interactive plotting. Understanding complex multiplex data solely through bioinformatics can be challenging without linking it back to the originating cells. Interactive plotting helps assess the quality of initial Phenoplex stages and enables deeper interrogation of curated data. This visual approach, integrating bioinformatic spatial data with plots and images, provides the most effective means to grasp the complexities of multiplex data sets.

	CD163 (MV CELL)	CD20 (MV - NUC)	CD3 (MV - NUC)	CD31 (MV CELL)	CD34 (MV CELL)	CD45 (MV CELL)
Total	7587	7450	56020	7826	15685	44133
CD163 (MV CELL)	7587	156	2035	117	1061	2456
CD20 (MV - NUC)	156	7450	3856	89	582	7002
CD3 (MV - NUC)	2035	3856	56020	765	2185	28847
CD31 (MV CELL)	117	89	765	7826	4156	1835
CD34 (MV CELL)	1061	582	2185	4156	15685	2878
CD45 (MV CELL)	2456	7002	28847	1835	2878	44133
CD45-RA (MV - NUC)	1270	6823	10419	1501	2514	18052

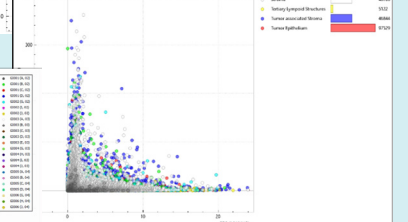


Confirm positivity settings using the **Co-occurrence Matrix** to check for co-localization of biomarkers. Numbers in the matrix are the total number of cells positive for the combination. Selecting a box in the matrix will highlight the dual positive cells in the image.

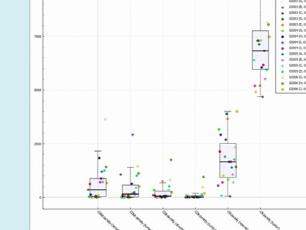
tSNE plots



Scatter plots



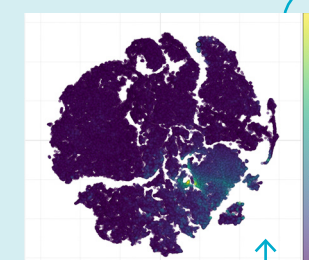
Box plots



Interactive plots and graphs

Phenoplex combines graphs and t-SNE plots with recolored image views to better confirm the phenotyping results.

Graphs and plots



Cell gallery

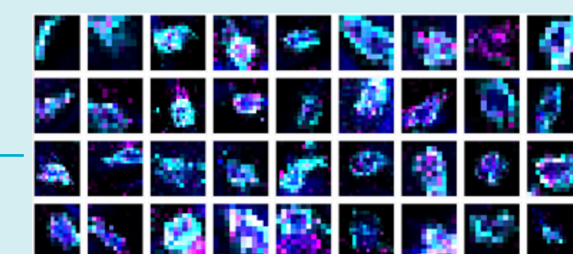
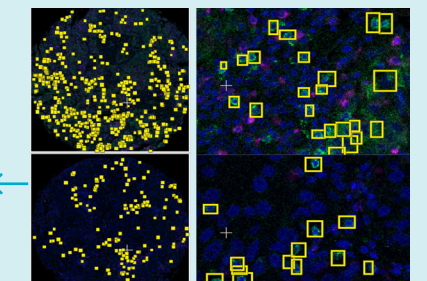
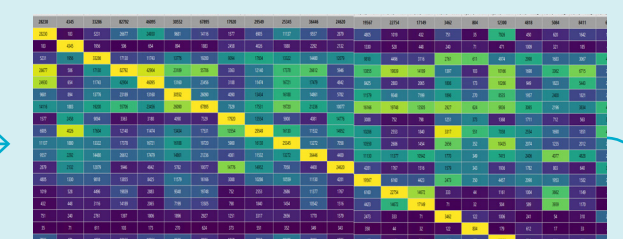


Image visualizations



Co-expression matrix



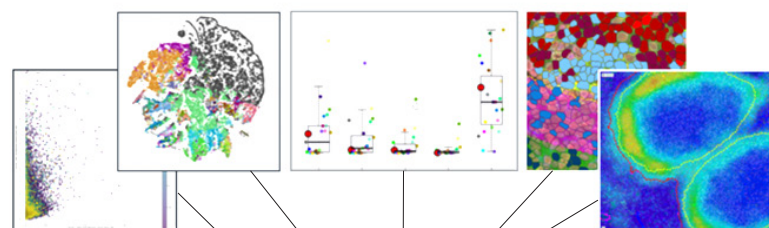
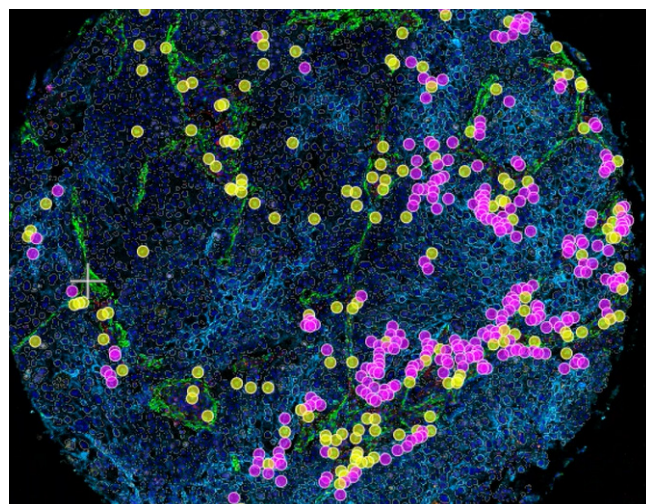
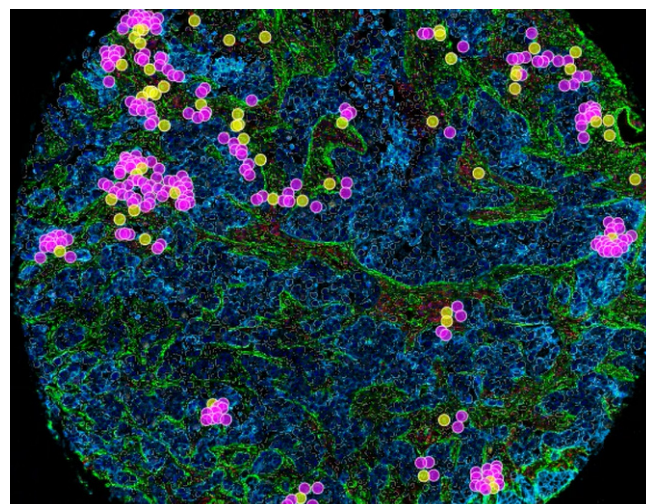


EXPLORE

Spatial Neighborhood Analysis

Spatial Neighborhood Metrics enable scientists to examine the relationships and distances between specific cell populations, revealing the complexity of tissue microenvironments in both normal and disease states. In immuno-oncology, understanding the neighborhood composition of tumor and immune cells in the tumor microenvironment is crucial for developing novel precision

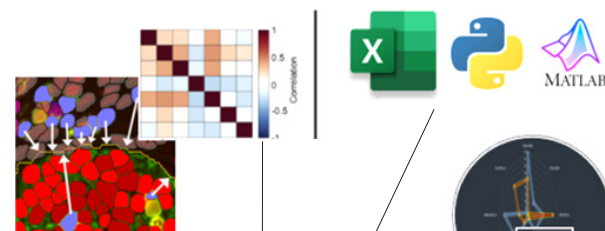
diagnostics. And there are parallels to this across all fields of study. The Phenoplex workflow now includes hypothesis-driven spatial analysis options, allowing for cellular proximity counts and nearest neighbor distance calculations. With our intuitive workflow, users can define target cells and analyze their neighbors within a specified radius.



RESULTS VISUALIZATION

PUBLISH

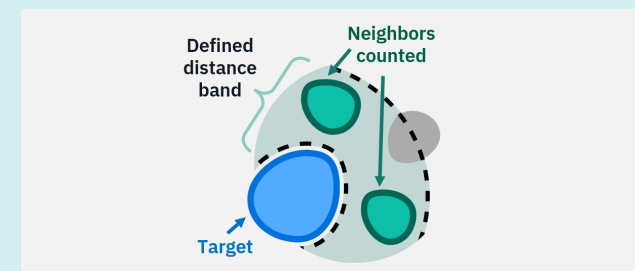
Every stage of Phenoplex offers rich visualizations to help you confirm hypotheses or uncover new discoveries. Utilize the channel manager, phenotype maps, and interactive plots to continue shaping your scientific narrative. Designed as a comprehensive workflow for spatial biology metrics, Phenoplex also integrates seamlessly with existing data and bioinformatics pipelines. **All data and images are exportable in publication-ready formats or for further analysis.** Just remember to reference Phenoplex from Visiopharm as your trusted multiplex analysis workflow assistant.



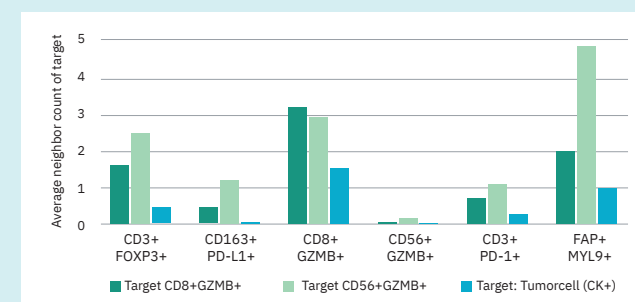
DATA EXPORT

Spatial neighborhood metrics

Proximity Profile Analysis:



Set defined distance bands (dotted line) around your selected target cell (blue). Phenoplex will quantify all specified neighbors (green) inside this radius and generate mean, median and min/max readouts. Different distances can be set for different neighbors.



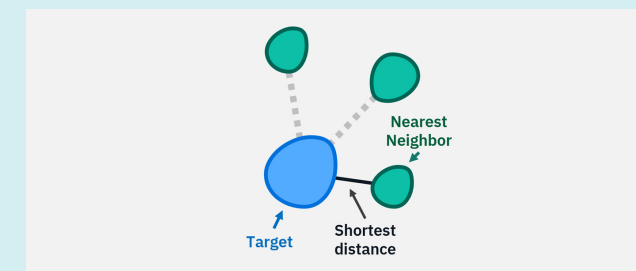
	CD3+ FOXP3+	CD163+ PD-L1+	CD8+ GZMB+	CD56+ GZMB+	CD3+ PD-1+	FAP+ MYL9+
Target CD8+ GZMB+						
Target CD56+GZMB+						
Target: Tumorcell (CK+)						

Know which cells are interacting

Understanding the tissue context of specific cellular populations provides insights into cellular niches. Knowing which cells interact can be of high importance in immune oncology. Use our intuitive visualization tools to get an easy overview of neighborhood relationships.

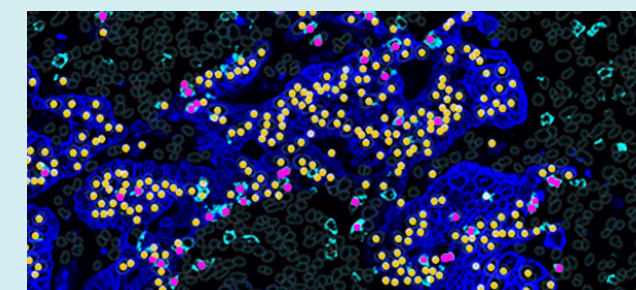
Displayed on top: Which target has the most neighbors of those specific cell populations within the specified radius?

Nearest Neighbor Analysis:



Set your target (blue) in any ROI and receive comprehensive statistical analysis about the distances of the respective nearest neighbors (green) for each target cell. Define a maximum distance to limit the search for a nearest neighbor.

Interactive exploration of neighbor quantifications



Review the selected targets (yellow, here: CK) and their respective neighbors (magenta, here: CD8+GZMB+) within 30µm radius around each target cell. Visible channels: Cyan = CD8, dark blue = CK

Mean	Distance To From Target To Nearest Neighbor [µm]					
	Distance To From Target [µm]			Distance To Nearest Neighbor [µm]		
	Calculate					
Filter By:						
30-plex_human_FFPE_CRC_ba						
Save						
CD8 (MV - NUCL)						
GranzymeB (MV CELL)						
inside Stroma, Tumor						
CD8 (MV - NUCL)	27.2	24.5	72.7	32.0	74.5	94.4
GranzymeB (MV CELL)	52.1	80.1	14.4	83.4	87.7	118.3
inside Stroma, Tumor						

The fully interactive table shows the mean distance in µm for each neighbor (columns) to the targets (rows). The view also allows to show mean with standard deviation or min - max values of counts or distance values. Export the tables through an easy copy and paste action to Excel or any other data analysis tool to generate figures of your results.

Visiopharm

We are a leading provider of AI-driven precision pathology software for research and diagnostics.

In research, we are a technology leader providing tools that help scientists, pathologists, and image analysis experts produce accurate data for all types of tissue-based research.

In diagnostics, we are a leader within clinical applications, with no fewer than nine diagnostic algorithms cleared under IVDR for EU/UK customers. These applications provide

diagnostic decision support and can be easily activated and integrated into existing lab workflows.

Founded in 2002, we are privately owned and operate internationally with over 750 customer accounts in more than 40 countries. Our headquarters are located in Denmark's Medicon Valley, with legal entities in Sweden, the UK, Germany, the Netherlands, and the United States, and local representation in France and China.

Tissue research /



Turning expert knowledge into reliable results through intuitive image analysis

IHC Quality Assurance /



Standardization of tissue biomarker staining

Spatial Biology /



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Diagnostic /



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precision
pathology

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+40
countries

+100

employees

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of the top 20
big pharma

7

patents

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