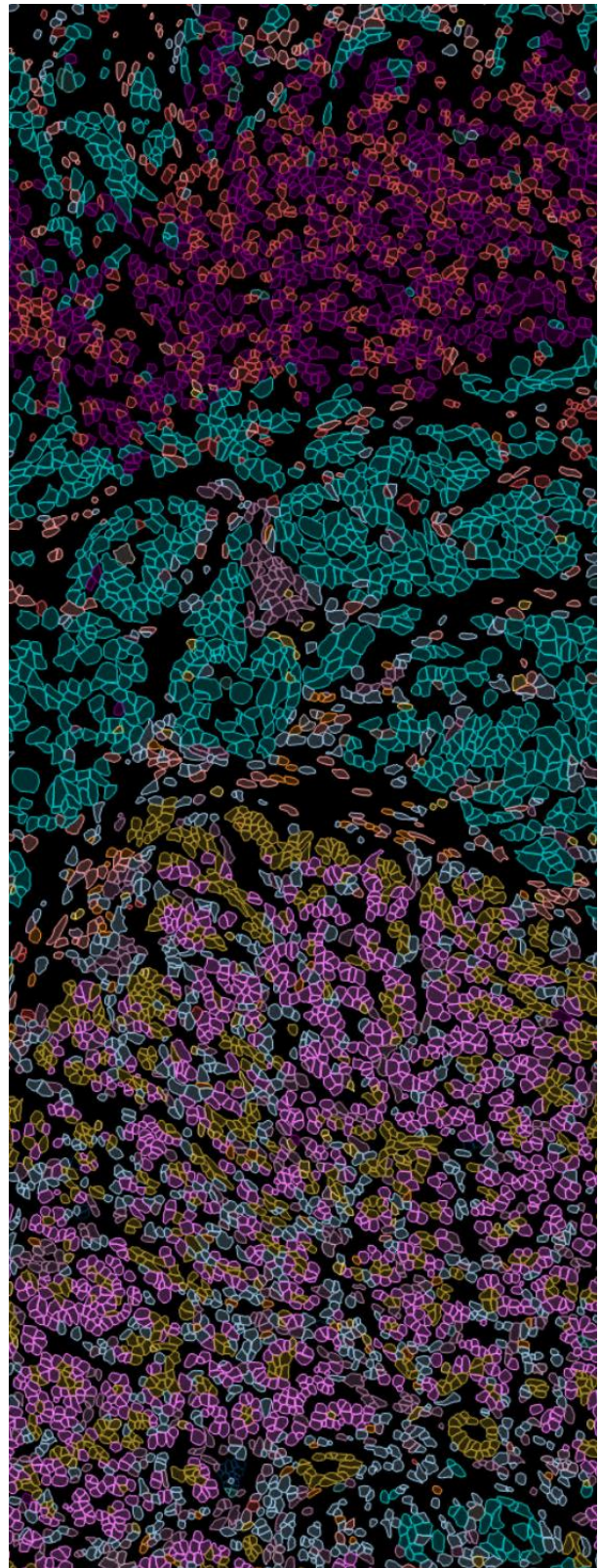


From Eye to Insight



Aivia 14.1

Release Notes



30-channel multiplexed pancreatic ductal adenocarcinoma tissue section imaged on Cell DIVE by Dr. Melinda Hill, Leica Microsystems

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Release Documentation for Aivia 14.1

This document describes the version 14 release of the Leica Microsystems Aivia software for image analysis. Please read this document before installing a copy of this software.

All reasonable steps have been taken to ensure that this publication is correct and complete. Should any user be in doubt about any detail, clarification may be sought from Leica Microsystems CMS GmbH, or their accredited representatives. The information in this document is subject to change without notice and should not be construed as a commitment by Leica Microsystems CMS GmbH. Leica Microsystems CMS GmbH accepts no responsibility for any errors that may appear in this document.

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Date: September 16, 2024 applying to Aivia 14 release, r41384.

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Technical Requirements

System Requirements

Check to make sure your computer meets the Minimum Computer Requirements to run Aivia before you install the software. We suggest following the Recommended or High-End Computer Configurations to ensure Aivia runs smoothly. **Actual requirements are directly linked to the type and size of image/images in hand plus the analysis required - for hardware recommendations for your specific application, please [contact us](#).**

For the most up to date recommended specs, please visit our website ([link](#))

Minimum Computer Requirement (Sufficient for Smaller Data sets)

- Operating System: Microsoft Windows 10 or 11 Professional (64-bit)
- CPU: 2 GHz (Intel or AMD) dual-core processor
- RAM: 8 GB or more
- Storage: Hard Disk with at least 100 GB free space
- GPU: NVIDIA GeForce GTX 1060 with 6 GB VRAM

Recommended Computer Configuration (Good for Most Applications)

- Operating System: Microsoft Windows 10 or 11 Professional (64-bit)
- CPU: 3.5 GHz (Intel or AMD) 6-to-12 core processor
- RAM: 16 to 64 GB
- Storage: 500 GB SSD drive (or larger) for cache and image; and storage disk (HDD or SSD) with at least 100 GB of free space
- GPU: NVIDIA GeForce GTX 1080 Ti with 10+ GB VRAM

High End Computer Configuration

- Operating System: Microsoft Windows 10 or 11 Professional (64-bit)
- CPU: 4.0 GHz (Intel or AMD) 16-core processor
- RAM: 192 GB or more
- Storage: Two 2 TB+ m.2 SSD drives or cache and image; and storage disk (HDD or SSD) with at least 100 GB of free space
- GPU: NVIDIA GeForce RTX 4080 with 16 GB VRAM or NVIDIA RTX A6000 with 48 GB VRAM

For 2D Multiplexed (up to 100 Channels) and/or Large Data (85K X 57K in Size)

Recommended for Best Multiplexed Experience:

- Operating System: Microsoft Windows 10 or 11 Professional (64-bit)
- CPU: Intel i9-14900K (24 Core) or better
- RAM: 192 GB DDR5 or more
- Storage: 4 TB+ m.2 SSD drive space for cache and images; and storage disk (HDD or SSD) with at least 100 GB of free space
- GPU: NVIDIA GeForce RTX 4080 with 16 GB VRAM or better

Leica Available Machine:

CPQ: 11640665 Gold Workstation

HP Z4G4 PC

- Operating System: Windows 10 Pro 64 Workstations Plus
- Processor: Intel XeonW-2245 3.9GHz 8C 165W CPU
- Main Memory: 192GB (6x32GB) DDR4 2933 DIMM ECC Registered WW Memory
- Graphics Board: NVIDIA RTX A5000 24GB (4) DP or equivalent
- System Drive: 512GB SSD SATA
- Temp Drive: 8TB SSD RAID0 (HP Z Turbo Drive Quad Pro with 4x2TB NVMe)
- Data Drive: 4TB SSD RAID0 (2x 2TB NVMe)

Additional Hardware Requirements

For Virtual Reality (VR) Visualization

- HTC Vive Virtual Reality Headset and Controllers
- NVIDIA GeForce GTX VR-ready GPU

For Deep Learning

- CUDA 10 compatible NVIDIA GPU with 8 GB VRAM

Additional Requirements

- Internet connection (with admin rights) is needed for license verification and the use of AiviaWeb

Aivia 14.1 is a patch release containing 41 bug fixes, usability improvements, and security updates since Aivia 14.0 release on June 12, 2024. Powered by AI, Aivia 14.1 offers easy to use spatial insight generation tools for 3D multiplexed images (15 channels for simultaneous visualization and multiplexed cell detection analysis) to go from eye to insight faster.

Aivia 14 and 14.1 Key Features Summary

All Aivia Packages:

- Visualize 3D multiplexed images (15 channels for simultaneous visualization)
- Group biologically relevant channels to simultaneously turn on-off all the channels and automatically populate the Phenotyper with the phenotype names and channels
- Automatically classify any of the detected 3D objects in Aivia (cells, neurons, meshes) using one of the 4 available phenotyping methods in Aivia (Object Classifier) using any number of intensity and/or morphological measurements
- Ability to further sub-cluster a cluster of interest for deeper insights using the Object Classifier
- Ability to visualize the unclassified class for the Phenotyper and the PhenoGraph-Leiden method for deeper exploration of the objects that do not confirm to the training examples or user-specific automatic clustering parameters
- All the classifiers now output confidence values for an object's membership into a particular class. These confidence values can be used for quality control of the generated phenotypes, for retraining low-confidence objects, for selecting high-confidence objects for focused downstream analysis, for plotting the range of confidence values for different phenotypes using the violin plot, or for visualizing low vs high confidence objects in the images for spatial insights
- 2-sided violin plot for visualizing the distribution of two different measurements for object sets or phenotypes

- Multi-channel auto-region of interest (ROI) detection for 2D, enabling users to input multiple channels for accurate delineation of the ROI
- Sample animation key frames for 3D images to immediately generate demo reels
- Camera Sync option for visualizing the same objects in 2D and 3D
- Viventis ome.tiff file import support

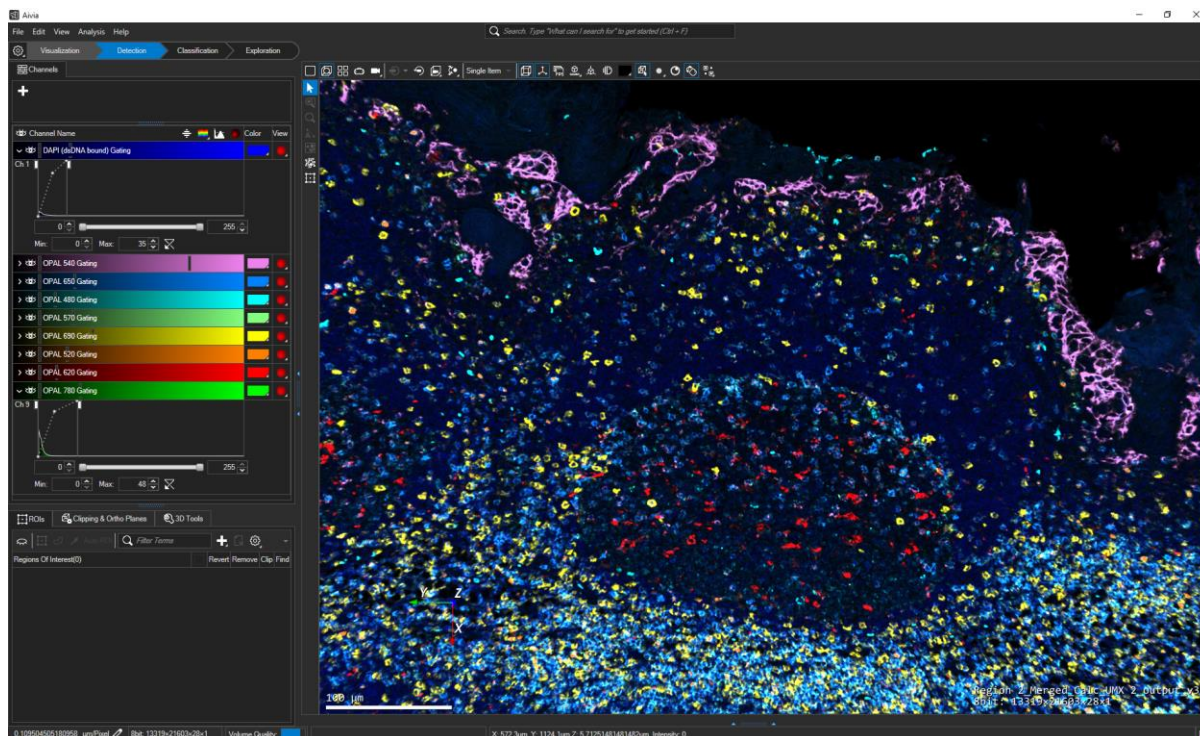
For Elevate and Apex packages only:

- New 3D Multiplexed Cell Detection deep-learning based recipe for detecting cells without nuclear markers with up to 15 biomarker inputs for the membrane
- Automatically classify any of the detected 3D objects in Aivia (cells, neurons, meshes) using any of the four available phenotyping methods in Aivia (Object Classifier, Phenotyper, k-means automatic clustering, PhenoGraph-Leiden automatic clustering) using any number of intensity and/or morphological measurements
- Ability to further sub-cluster a cluster of interest for deeper insights using any of the 4 available classifiers
- Multiple interactivity features for the Marker-Cluster Dendrogram for spatial exploration of 3D multiplexed cells

14.0 Features (Released on June 12, 2024)

15+ Channels Simultaneous Visualization in 3D

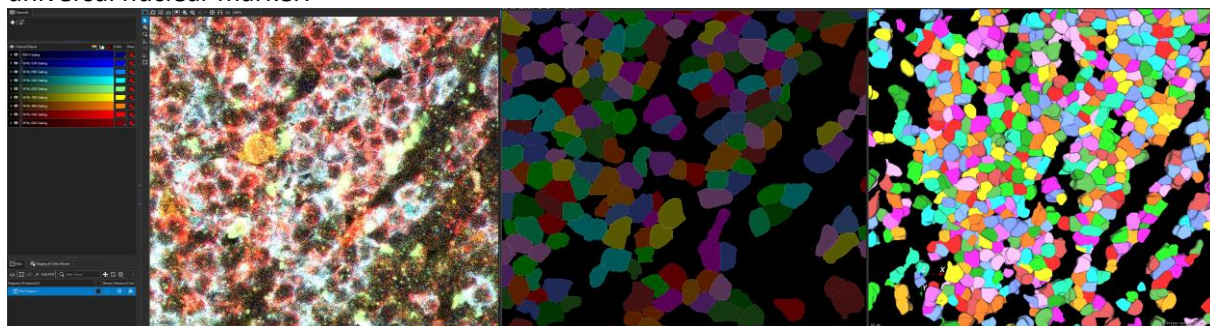
Aivia 14 enables visualizing 15+ channels simultaneously in 3D, enabling visualization of multiplexed cells.



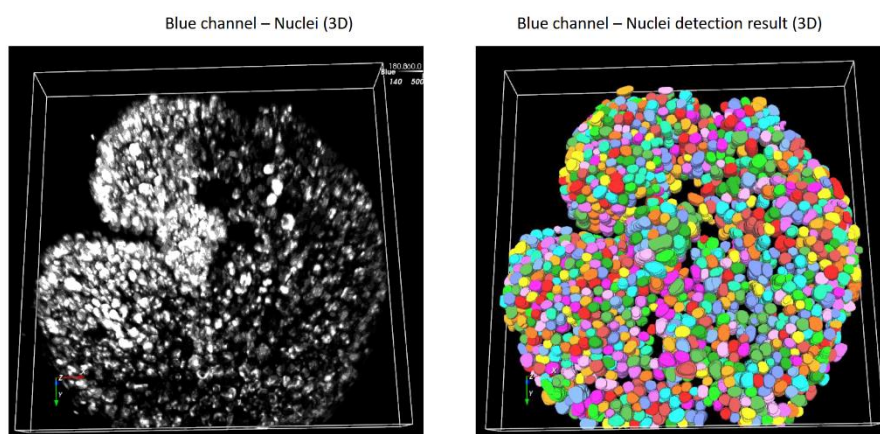
A 9-channel multiplexed tonsil data acquired on Leica STELLARIS by Dr. Tatjana Straka of Leica Microsystems.

3D Multiplexed Cell Detection

Aivia 14 introduced 3D Multiplexed Cell Detection recipe which is modified version of the Cellpose generalist deep-learning cell segmentation algorithm (Stringer et al, 2020)¹, optimized for 3D multiplexed images for accurate segmentation and partitioning of cells. This recipe is designed for common scenarios in 3D multiplexed images in which multiple biomarkers in a single slide to gain insights about the interaction of the biomarkers in their spatial context. This recipe is optimized for experimental setups in which there are several membrane biomarkers for the membrane but no universal nuclear marker.



3D Multiplexed Cell Detection Recipe ran on 6 membrane markers without nuclear markers. Original image (top left) with segmentation results (2D - top middle, 3D - top right), image acquired on a Leica STELLARIS by Dr. Tatjana Straka.



The 3D Multiplexed Cell Detection recipe is based on the Cellpose deep learning algorithm to handle images with varying degrees of intensity, shape and size (left), image courtesy of Dr. Andreas Moor at ETH Zurich.

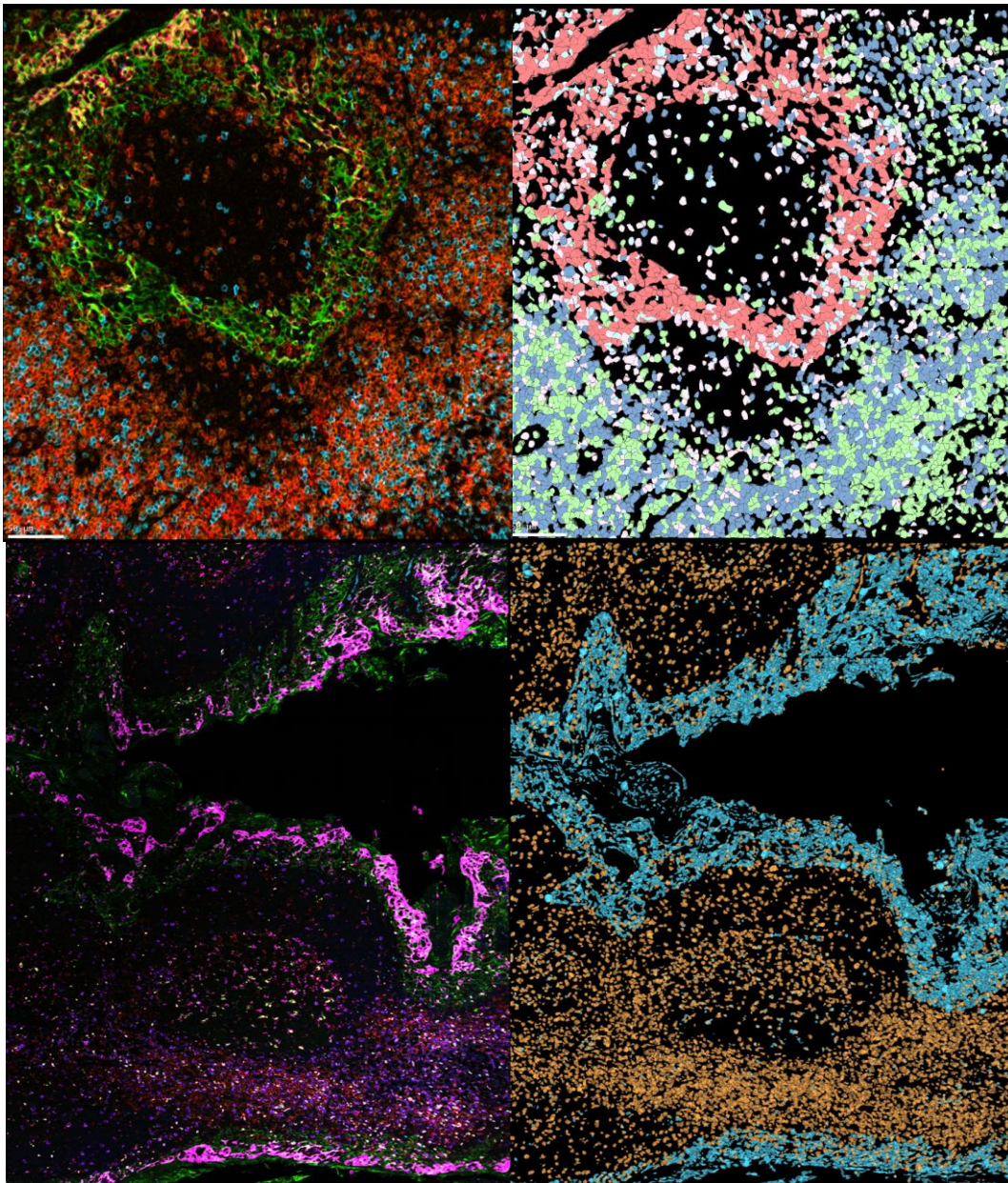
This recipe was tested up to 15 biomarkers although the real limit of the total number of channel inputs for the recipe is the number of channels Aivia can open in one instance which depends on the GPU, and the image size.

This recipe is our fastest (by up to 78% compared to our previous detection methods) and most accurate cell segmentation method to date. This recipe can be used for single channel or multiple channel inputs.

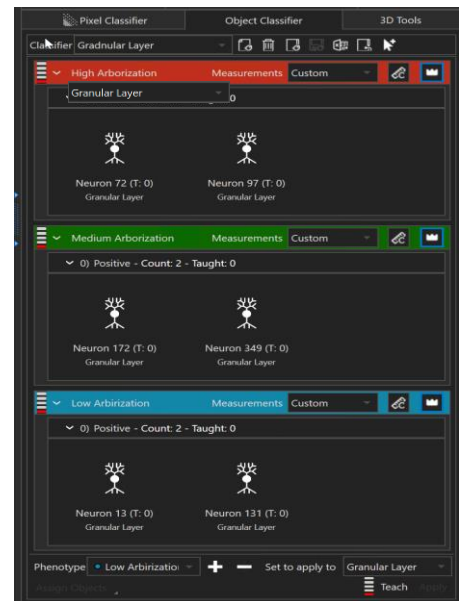
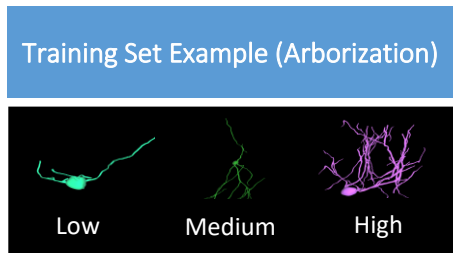
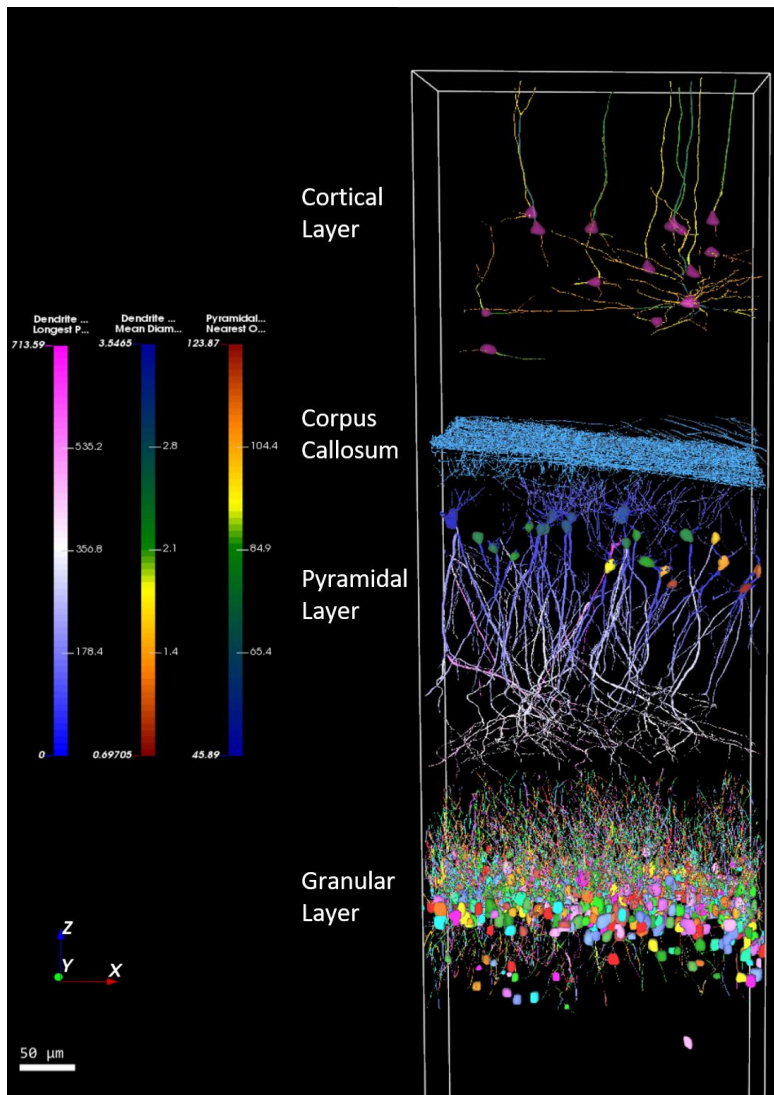
Supervised and Unsupervised 3D Phenotyping Powered by AI

Aivia 14 makes all 4 available methods of phenotyping fully 3D compatible and users can phenotype using intensity and/or morphological measurements either using expert knowledge about the phenotypes (Object Classifier, or Phenotyper) or automatically (k-means, or PhenoGraph-Leiden).

	Object Classifier	Phenotyper	Autoclassifier (K-means)	Autoclassifier (PhenoGraph-Leiden)
Requires user-selected examples for classifier training	Yes	Yes	No training required	No training required
User-defined number of classes	Yes	Yes	Yes	User-defined Max Cluster number
All classes have the same measurements	Yes	No	Yes	Yes
Every object is assigned to a class	Yes	No	Yes	No* (if the user-specified "Min Object per Cluster" is not met for the Cluster)
An object can belong to multiple classes	No	Yes	No	No
Usage Example	Classify all apoptotic cells into 4 known classes using the same set of measurements with user selection of examples per class. Every cell belongs to a single class.	Classify immune cells into 4 known classes using different measurements per class with user selection of examples. Cells can belong to multiple (or no) classes.	Automatically classify all dividing cells into a user-defined number of classes based on selected number of measurements.	Automatically classify all neurons based on user-selected measurements (recommended for multiplexed data or larger number of measurements)



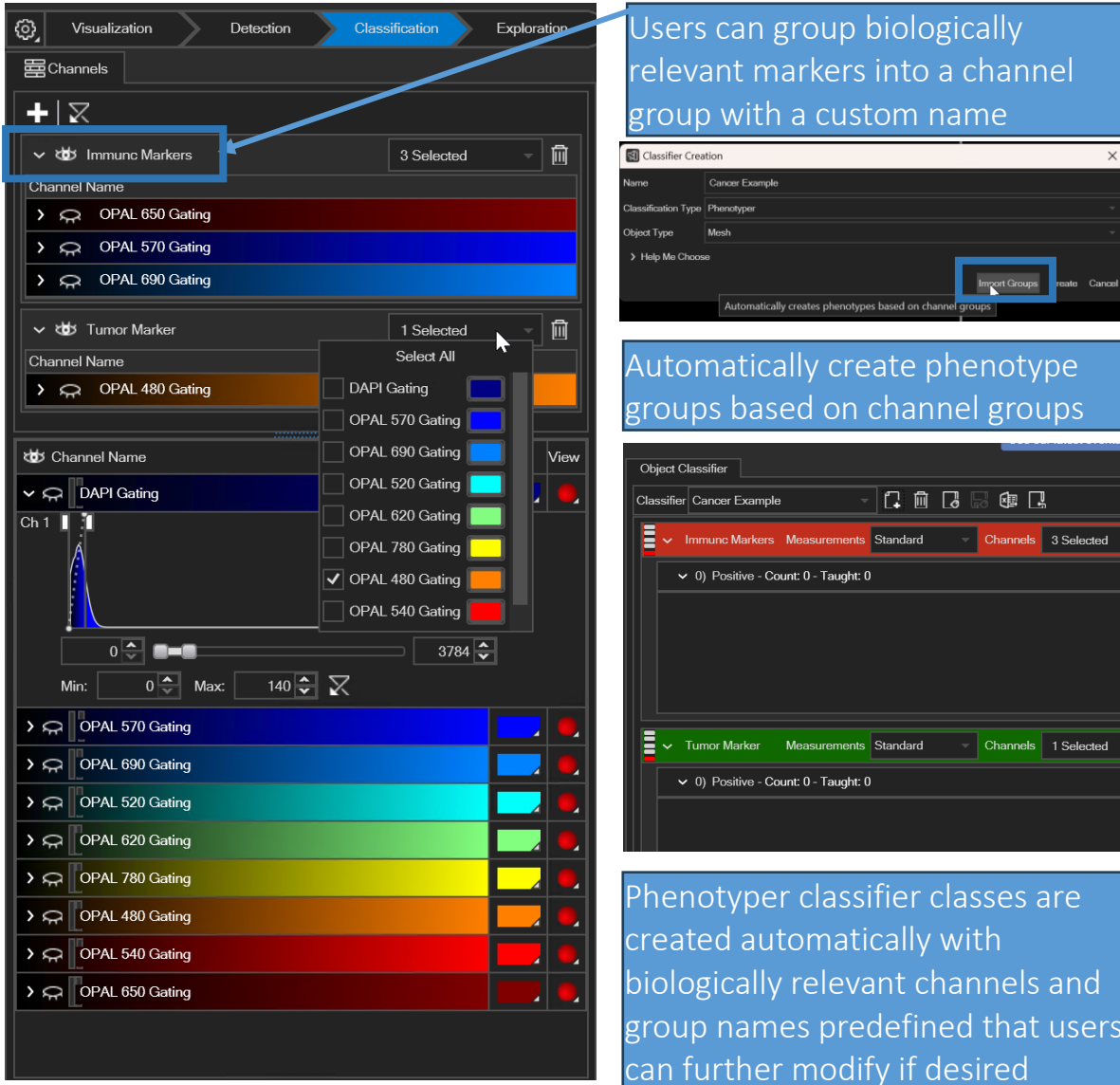
Intensity-based phenotyping (right panels) for 3D multiplexed images of tonsils (left panels) acquired on a Leica STELLARIS by Dr. Tatjana Straka of Leica Microsystems



Granular neurons in the cleared mouse cortex were segmented using Cellpose for soma detection and dendrites were traced. The neurons in the granular layer were used for training using morphological measurements of the dendrites (e.g. dendritic total length, dendritic branching numbers, tortuosity) in the Phenotyper with two neuron examples in each class which could effectively classify 546 neurons in this image. Image courtesy of Dr. Hu Zhao of Texas A & M.

Group Biologically Relevant Channels

Biologically relevant channels can be grouped together for visualization (simultaneous turning on-off of the channels), import into the Phenotyper for automated class definition and channel selection, and visualization in the Marker-Cluster Dendrogram for grouping all the training channels for each phenotype together for assessing how well the training channels worked for phenotyping.

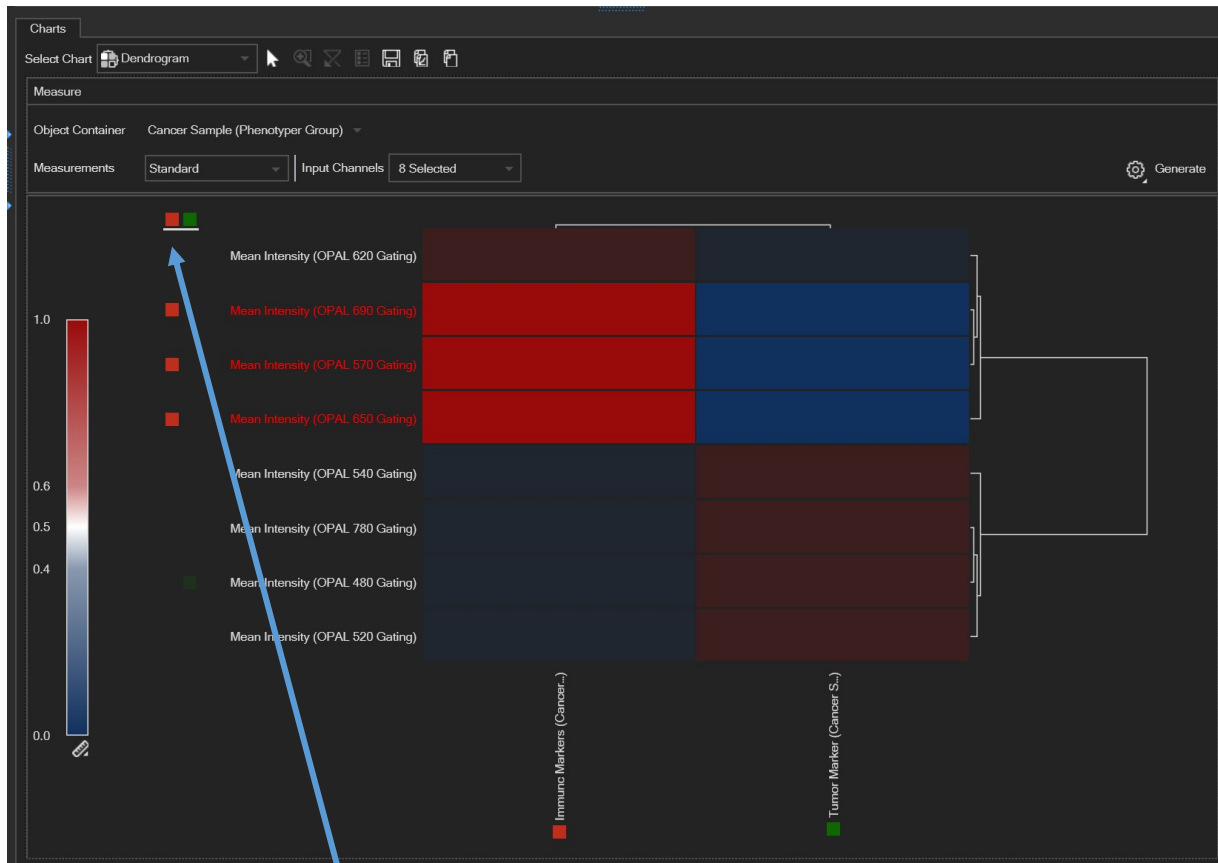


The image displays a software interface for channel management and classifier creation. The main window shows a list of channels grouped into 'Immune Markers' (3 selected) and 'Tumor Marker' (1 selected). A 'Channel Name' dropdown menu is open, showing a list of channels with checkboxes and color swatches. A histogram for 'Ch 1' is visible, showing a peak at 3784. The 'Classifier Creation' dialog box is open, showing 'Name: Cancer Example', 'Classification Type: Phenotyper', and 'Object Type: Mesh'. The 'Import Groups' button is highlighted. The 'Object Classifier' window shows two classifier classes: 'Immune Markers' (3 Selected) and 'Tumor Marker' (1 Selected), each with a 'Positive - Count: 0 - Taught: 0' status.

Users can group biologically relevant markers into a channel group with a custom name

Automatically create phenotype groups based on channel groups

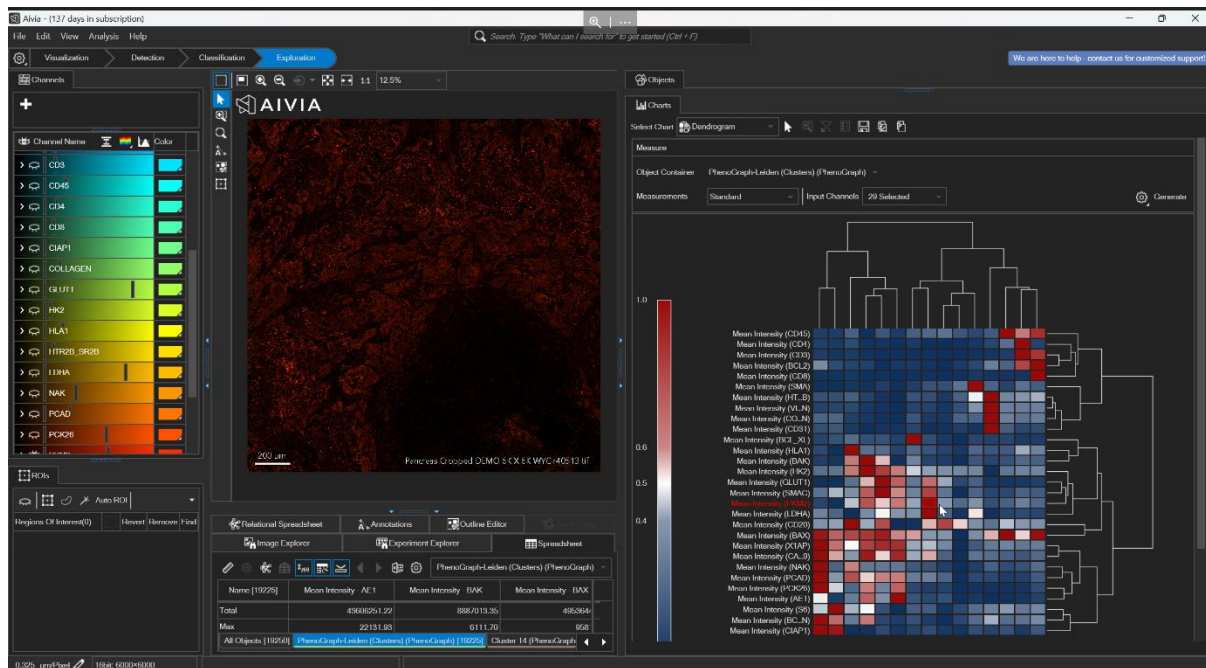
Phenotyper classifier classes are created automatically with biologically relevant channels and group names predefined that users can further modify if desired



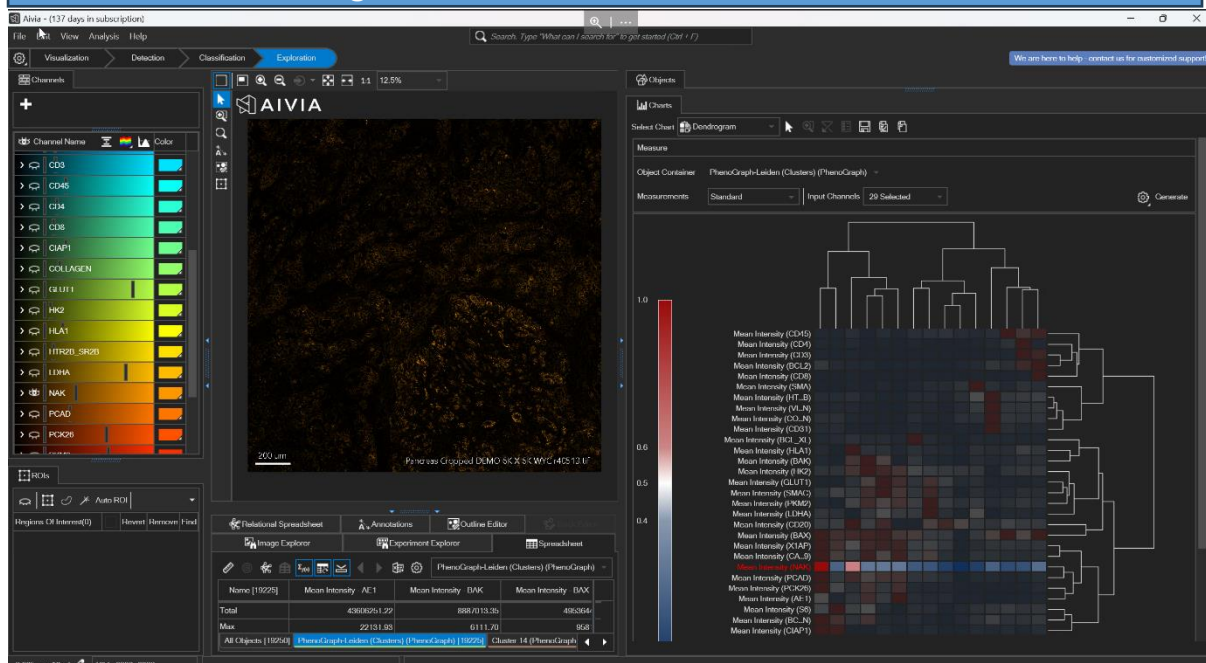
Selecting the Phenotypic Group tile highlights all marker channels that belong to the same Phenotypic Group.

Dendrogram Interactivity Features

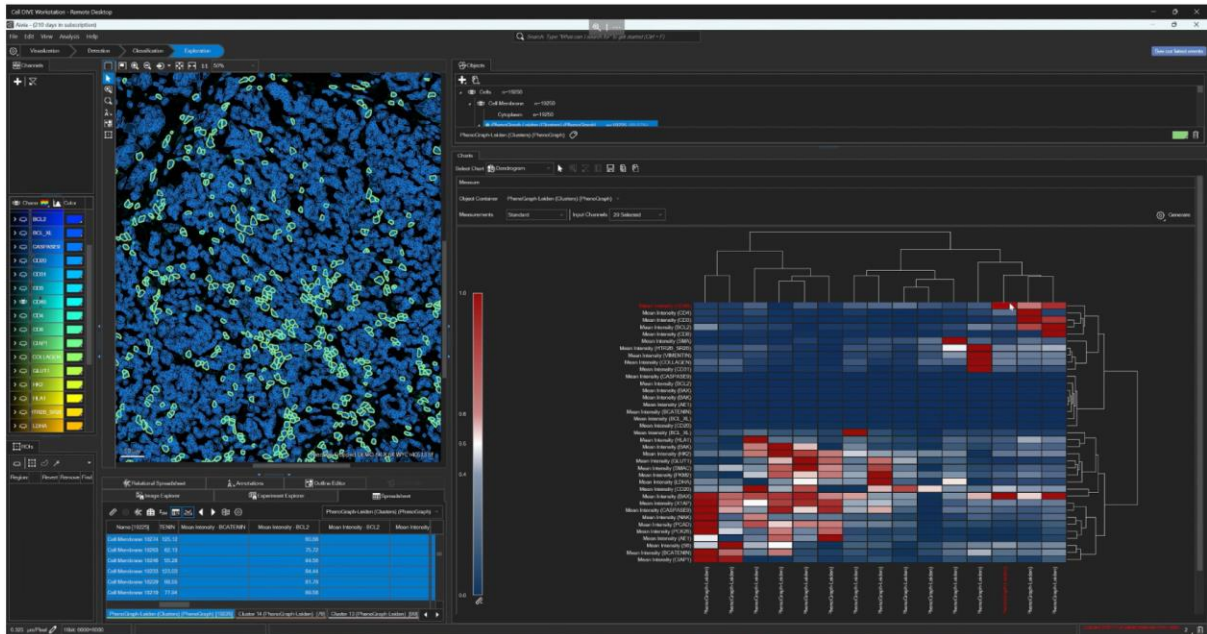
Marker-Cluster Dendrogram enables interactive exploration of the relationship between intensity or morphological measurements and phenotypes. In Aivia 14, a number of interactivity features are implemented to make this powerful data interpretation tool easier to use when working with many dimensions of data (measurements, clusters, heatmap of measurements, images with detected objects).



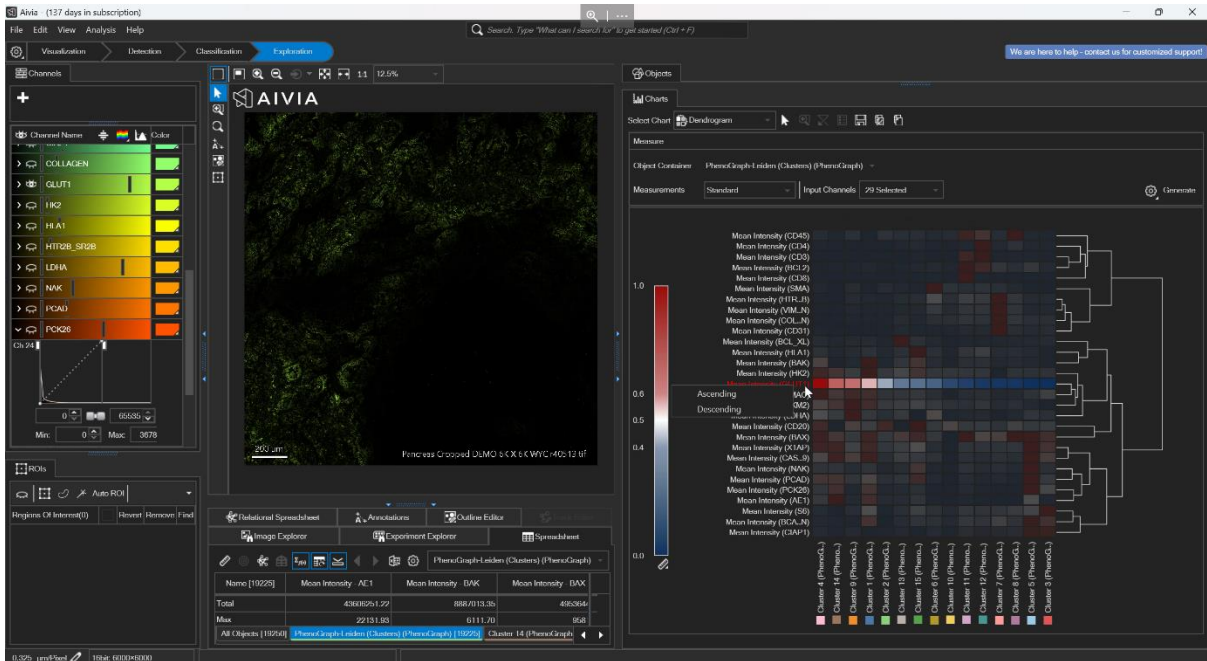
When a single tile is selected and the measurement is for intensity, the channel is turned on in the Image Viewer.



When an intensity measurement is selected, the channel turns on and the entire row is highlighted for easier interpretation of the heatmap

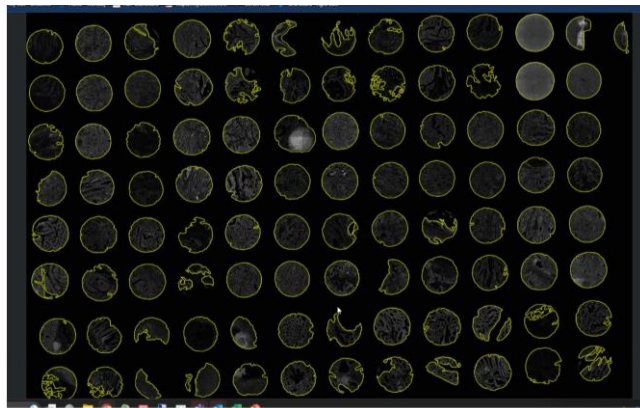
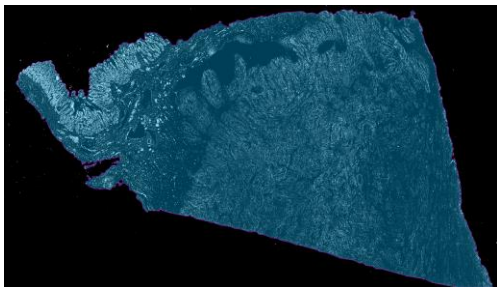


When the objects are turned on, clicking on a single cell in the Dendrogram enables users to see the selected Cluster and the channel turned on in the Image Viewer. Clicking on a single cluster highlights the selected Cluster in the image.



Measurements and Clusters can both be sorted in an ascending or descending orders (and be reset to default as needed) for ease of interpretation of data. When the objects are turned on, clicking on a single cell in the Dendrogram enables users to see the selected Cluster and the channel turned on in the Image Viewer. Clicking on a single cluster highlights the selected Cluster in the image.

Automatic ROI Detection with Multiple Channel Inputs

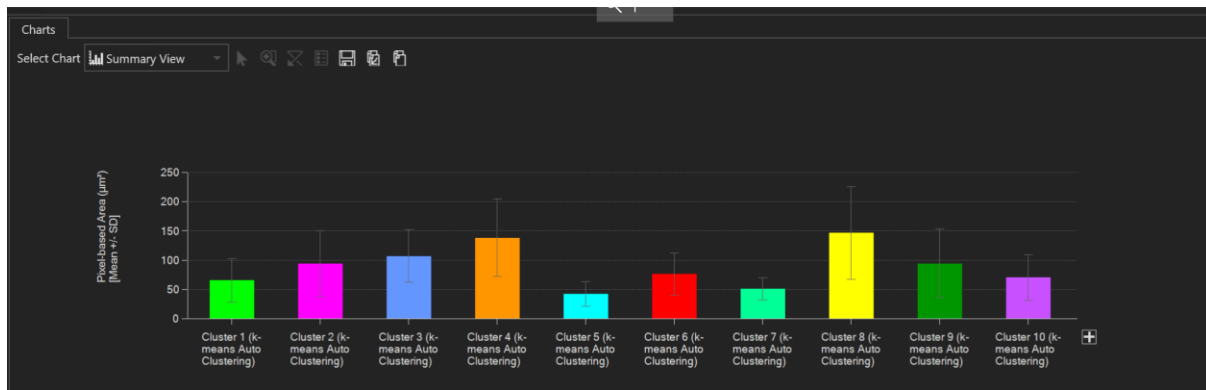


Automatic tissue detection enables users to automatically delineate regions of interest (ROI) based on any channel for efficient downstream analyses of large tissue sections (left) and Tissue Micro Array (TMA) cores (right)

Insight Generation via Data Exploration

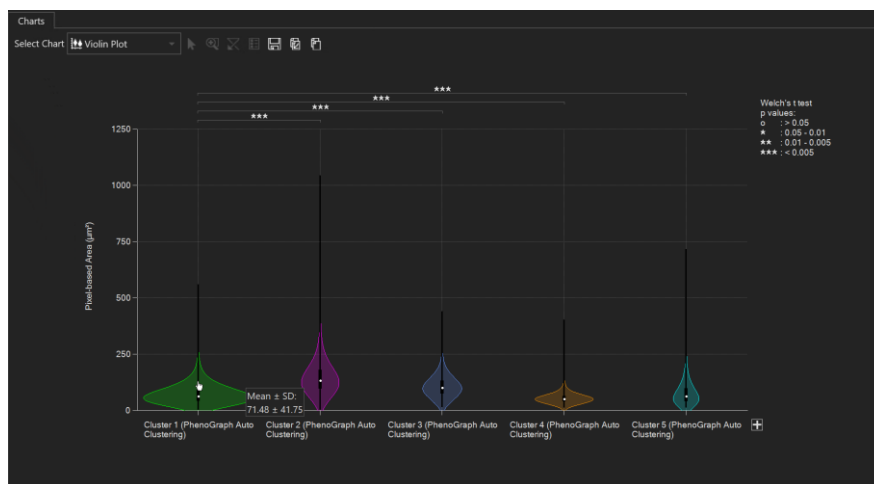
Summary Charts

For any object types (e.g. millions of cell membranes) and phenotypes, users can quickly generate summary charts with Standard Deviation or Standard Error of the Mean error bars.



Violin Plot Now Optimized for Millions of Objects

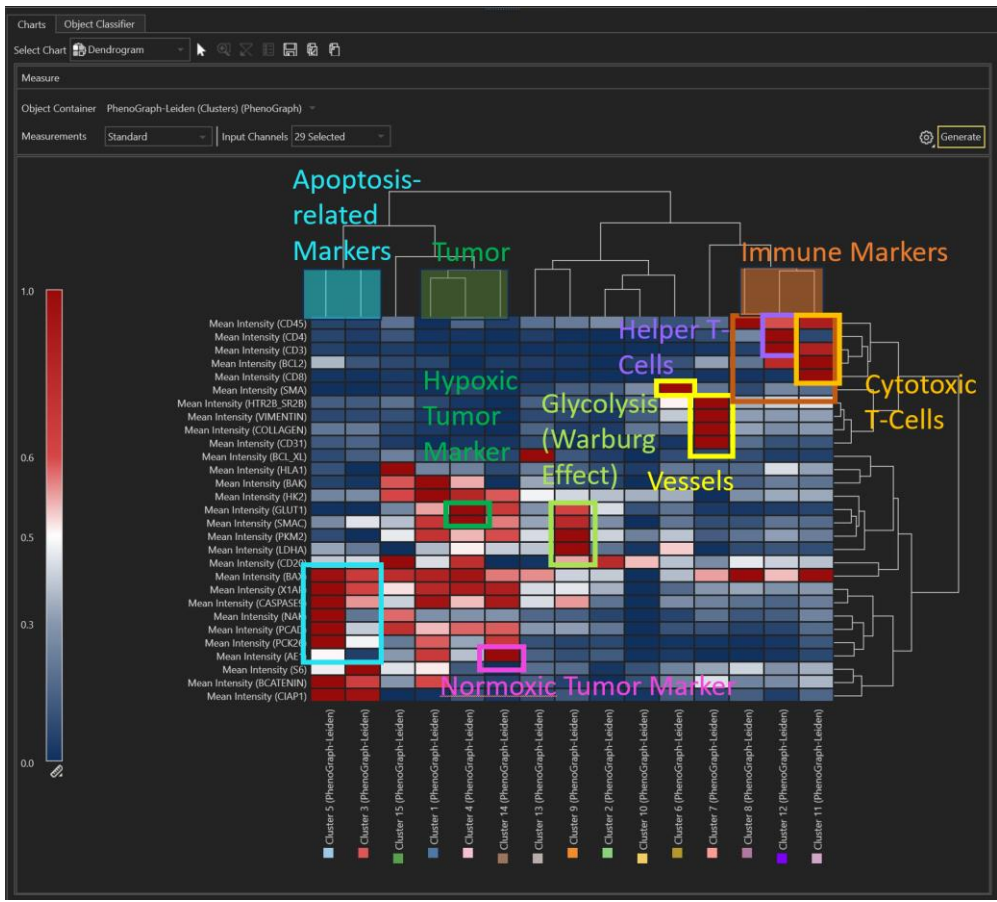
Violin plot for comparing compares data distribution for millions of objects in different object groups or phenotypes



Violin plot shown above enables users to compare different phenotypes with Welch's t-test values automatically generated and displayed.

Marker-Cluster Dendrogram

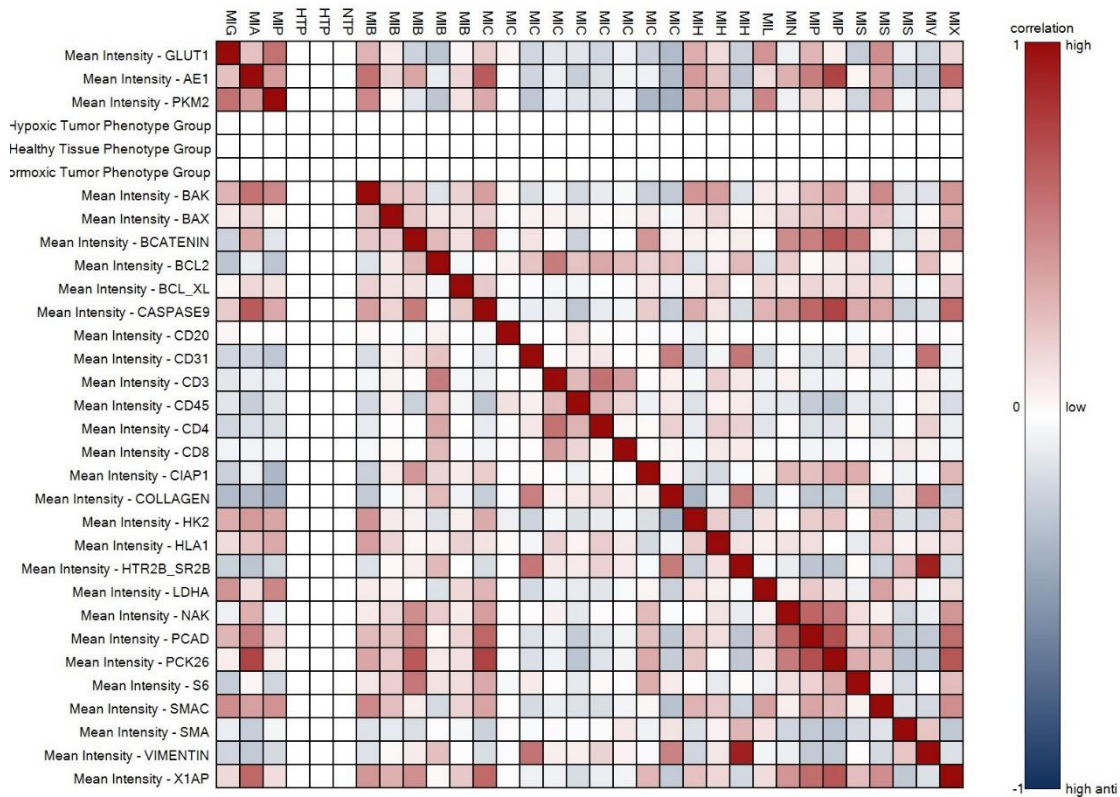
Marker-Cluster Dendrogram enables interactive exploration of the relationship between intensity or morphological measurements and phenotypes.



The dendrogram above shows a heat map normalized per measurement against phenotypes, and the dendrogram branch height reflects the measurement distance (how closely measurements are related) between clusters or user-specified measurements. Selecting any rectangular cell in the dendrogram highlights the cells in the corresponding clusters on the image to enable interactive visualization and data exploration for insight generation. Overlaid colored texts indicate clustering of known cancer-related markers in this example to demonstrate how different related markers group together or away from each other.

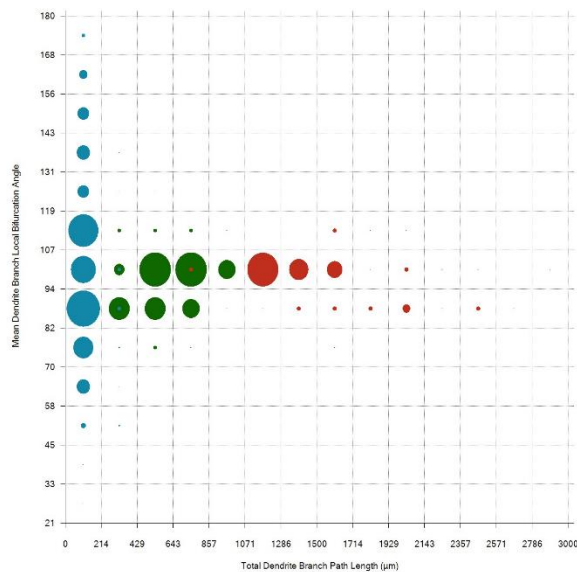
Pearson Correlation Heat Map

Pearson Correlation Heat Map, which displays the correlation between two measurements for an object or phenotype, is now capable of handling millions of objects in large 2D images.



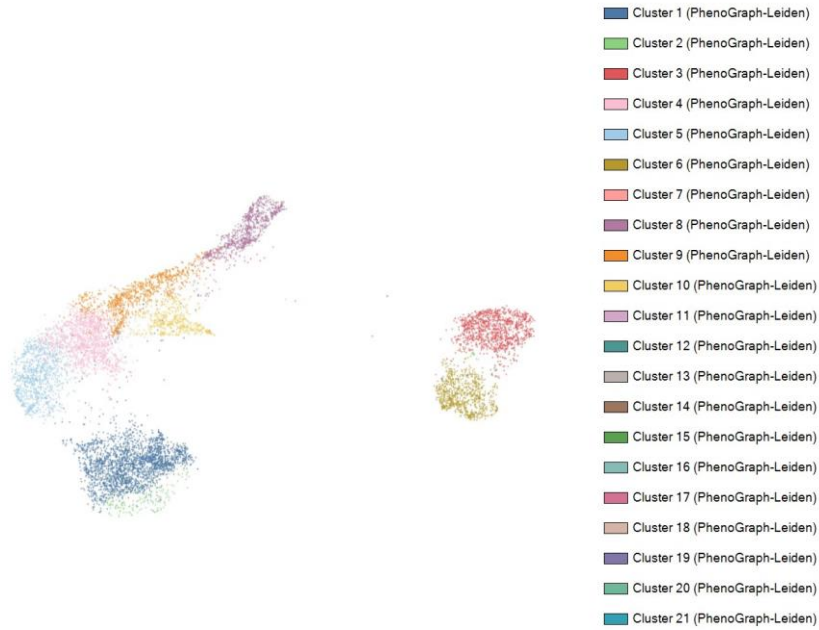
Binned Scatter Plot

Binned Scatter Plot shows data distribution of two measurements for different objects or phenotypes, with size of the circle corresponding to the count of objects within each bin.

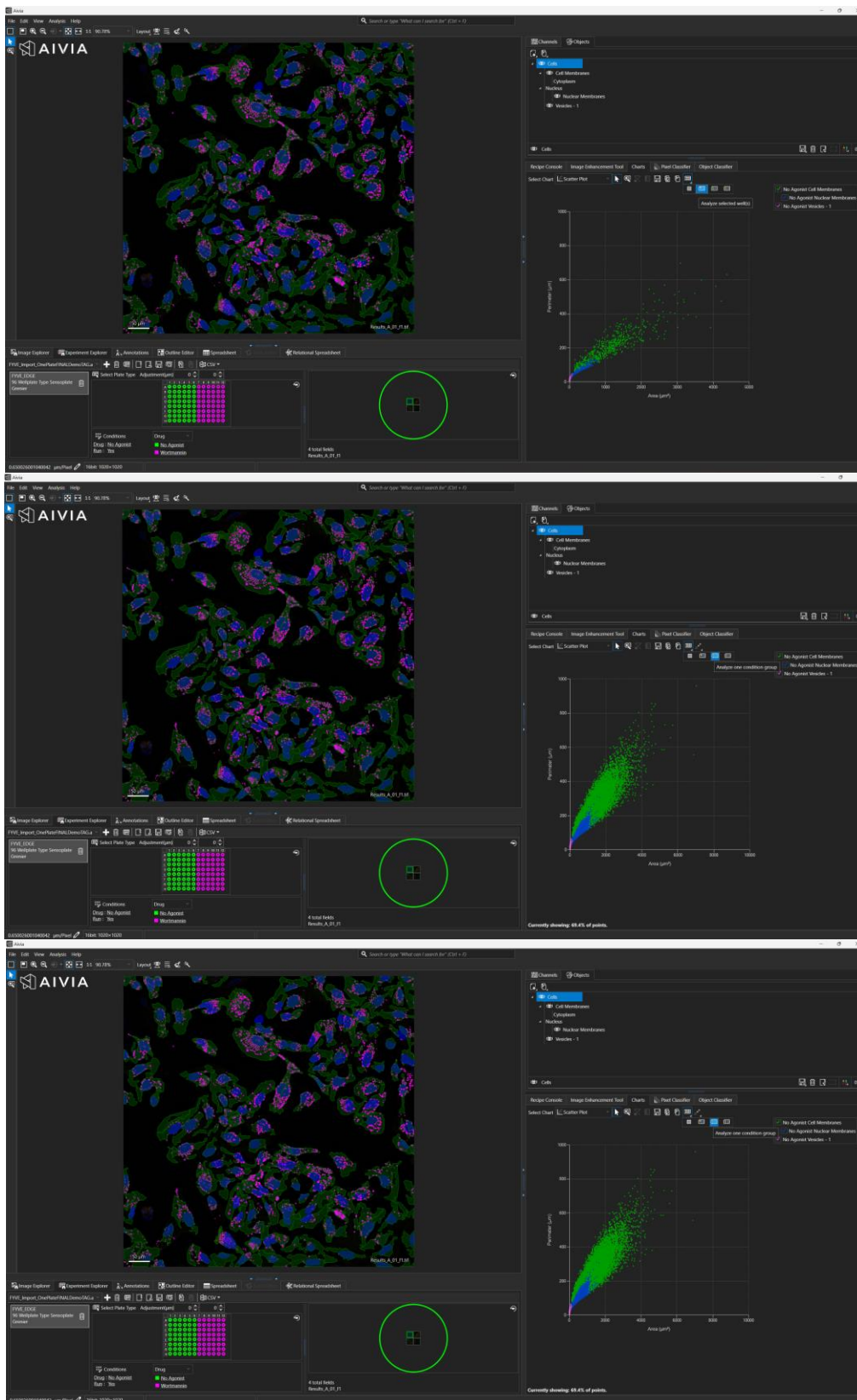


Dimensionality Reduction Plot

Dimensionality Reduction Plot is embedded within Aivia to transform high dimensional data into two dimensional space for simplifying data interpretation. Aivia offers three algorithm for dimensionality reduction: UMAP, PacMAP, and t-SNE.



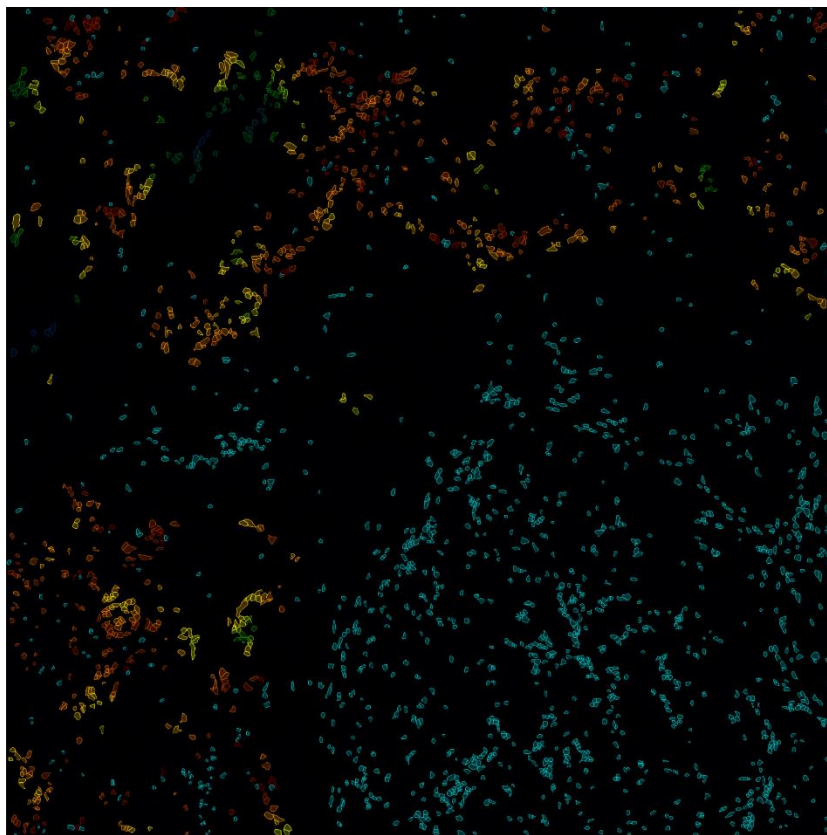
Multi-well Scatter Plot



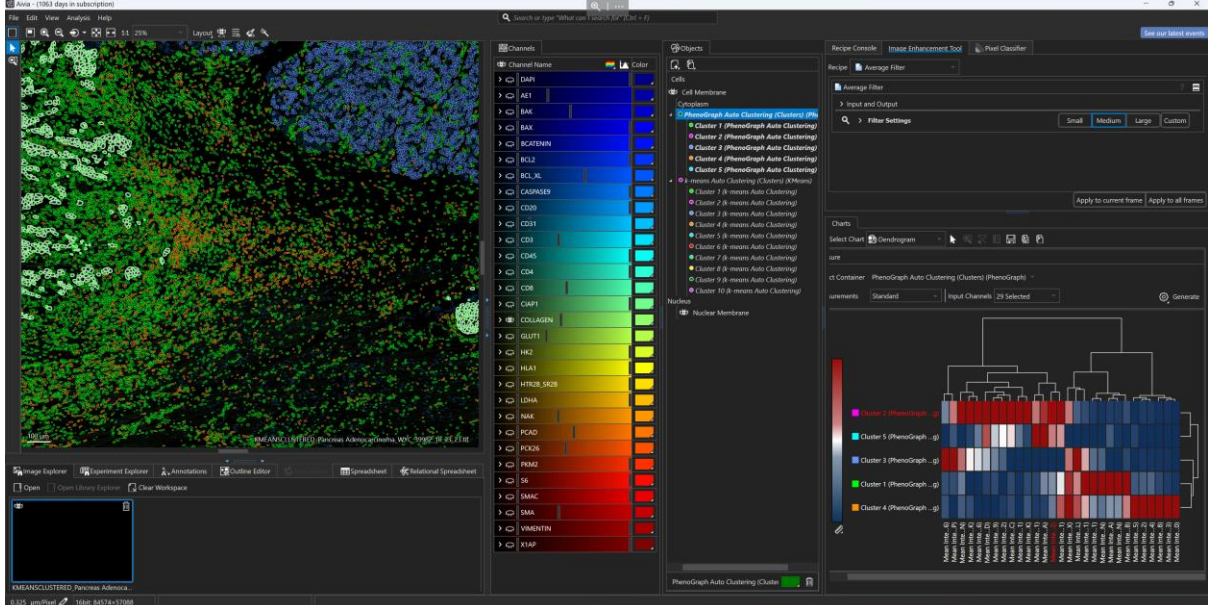
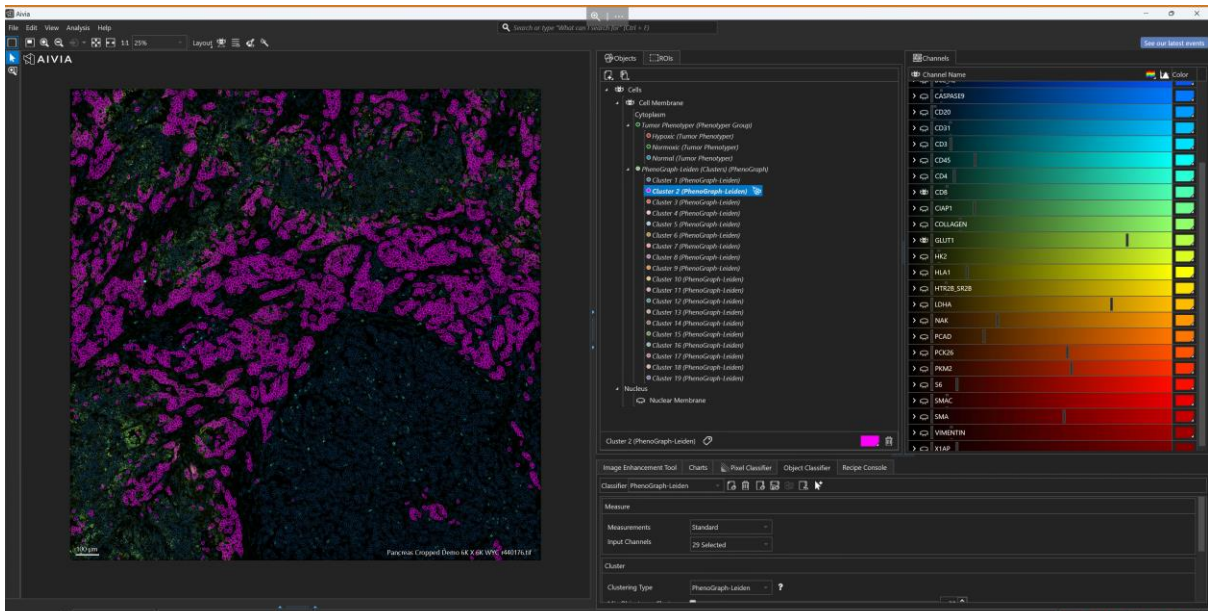
Multi-well Scatter Plot for all the field of views in a single well (top), for a single condition on the plate (middle) or all the wells for all the conditions on the plate (bottom). Image courtesy of Dr. Melinda Hill, Leica Microsystems.

Spatial Insights

Achieve accurate vertex-to-vertex distance measurements between individual objects (of any type, or morphological complexity) or between phenotypes using our Relation Tool. Understand the spatial relationship between cells or phenotypes by interactively selecting objects or phenotypes in charts and visualizing the selection using the new Spotlight feature that emphasizes phenotypes or objects for easy inspection.



High-expressing GLUT1+ cells are color coded (from red to blue) based on the distance to Immune cells (turquoise). Image courtesy of Dr. Melinda Hill, Leica Microsystems.



Using the new Spotlight function (top image), users can click on the phenotype of interest to visualize the selected phenotype with emphasis along with any other marker/structures of interest enabling insight generation in the spatial context. Users can further explore the relationship between clusters and markers by selecting on a particular cell in a dendrogram (bottom image) and visualize the selected phenotype in the image.

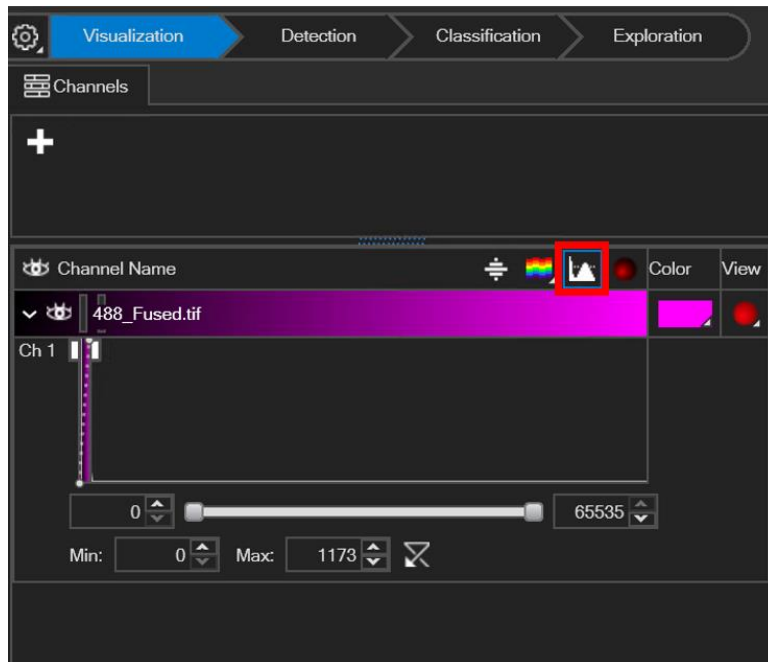
14.1 Improvements (Released on September 15, 2024)

Viventis File Import Support

Please follow the steps below for opening ome.tiff files exported from Viventis in Aivia 14.1:

- Always open the ome companion file in the folder if available

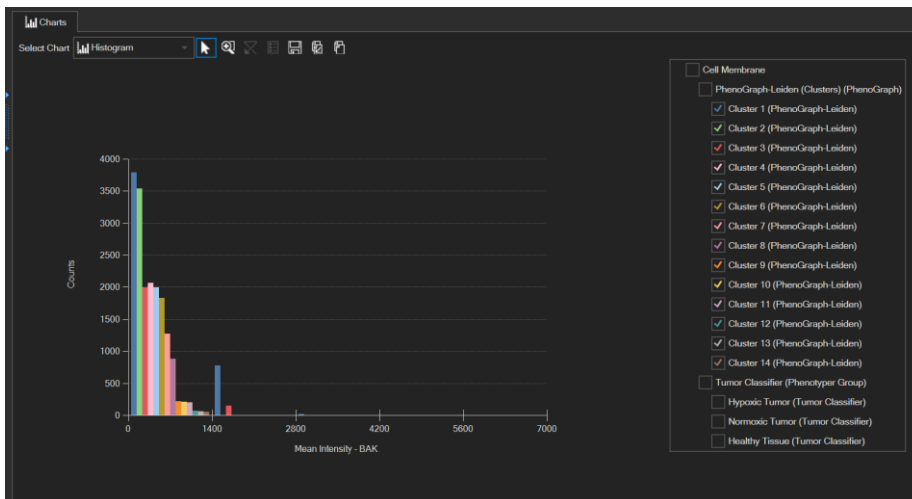
- For best performance, open in Aivia or in Community for conversion to Aivia.tiff before beginning any analysis
- Auto-LUT is auto-selected to optimize visualization of the imported images.



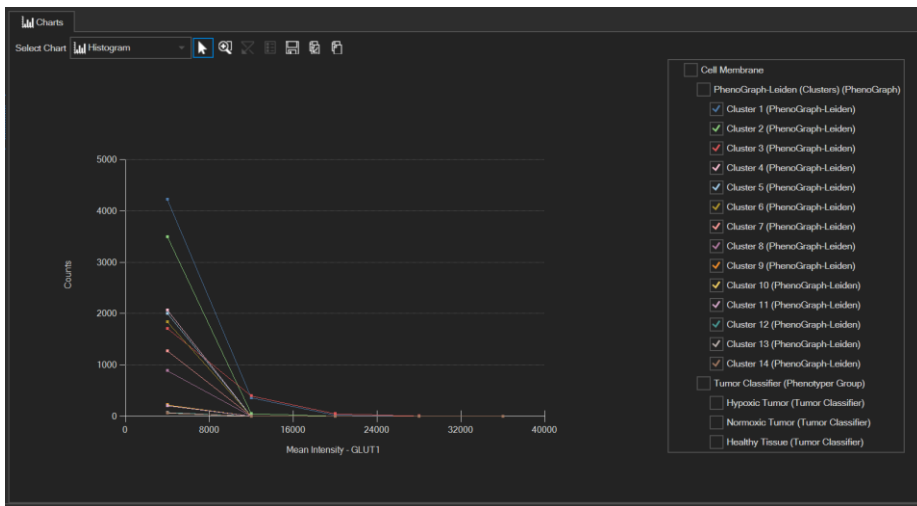
Charting Improvements

In Aivia 14.1, users can now plot histograms with phenotype selection with multiple visualization options:

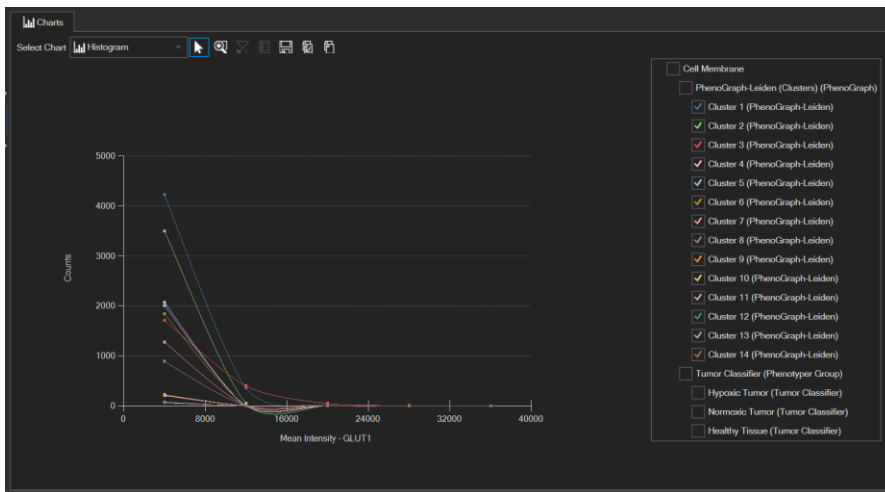
Phenotype charting in a histogram (bar graph)



Phenotype charting in a histogram (line graph)

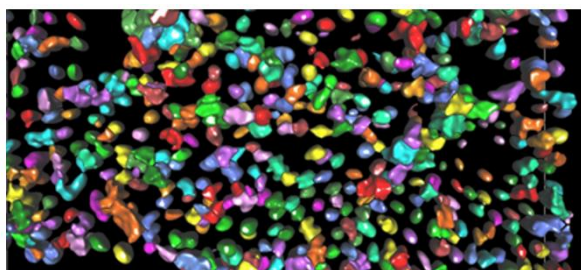


Phenotype charting in a histogram (spline)



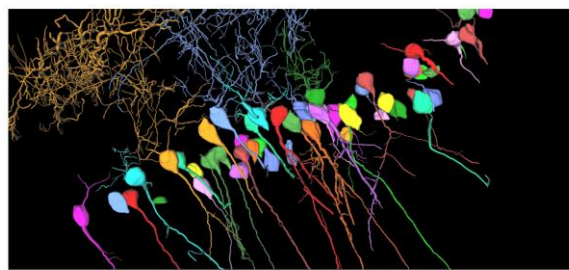
In addition, a number of mouse scroll-based functionalities are implemented (adjustment of max values for the X- and Y- axes, zooming on a specific region of the X-axis) by scrolling on the axes region of the graph.

New Features for Subscribers in Version 14



Aivia Go

- Visualization improvements for large 2D, multiplexed datasets
- Improvement to nuclei and object detection recipe as well as pixel classifier for large 2D dataset
- Auto ROI detection



Aivia Elevate – CellBio & Neuro
Aivia Apex

- Visualization improvements for large 2D, multiplexed datasets
- Improvement to nuclei and object detection recipe as well as pixel classifier for large 2D dataset
- Auto ROI detection

Only for Elevate and Apex packages

- 2 new cell detection recipe
- AI powered Phenotyper and automatic clustering
- New charts: dendrogram, summary view and dimensionality reduction, binned scatter plot

References

1. Stringer C, Wang T, Michaelos M, Pachitariu M. Cellpose: a generalist algorithm for cellular segmentation. *Nature Methods*. 18: 100-106. (2021)
2. MacQueen, J. Some methods for classification and analysis of multivariate observations. *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability, Volume 1: Statistics*. 281-297. (1967).
3. Traag, V.A., Waltman, L. & van Eck, N.J. From Louvain to Leiden: guaranteeing well-connected communities. *Sci Rep* 9, 5233 (2019).

Updated Wiki

The following Wiki pages have been updated for this release:

- Mutiplexed Cell Detection ([link](#))
- Phenotyper ([link](#))
- Automatic Object Classifier: k-means and PhenoGraph-Leiden ([link](#))
- How to auto-classify objects and visualize them ([link](#))
- Dendrogram ([link](#))
- Dimensionality Reduction ([link](#))
- Object visualization – Spotlight ([link](#))
- Binned Scatter Plot ([link](#))
- Summary View ([link](#))
- Regions of interest ([link](#))

[Aivia Demo License \(link\)](#)

