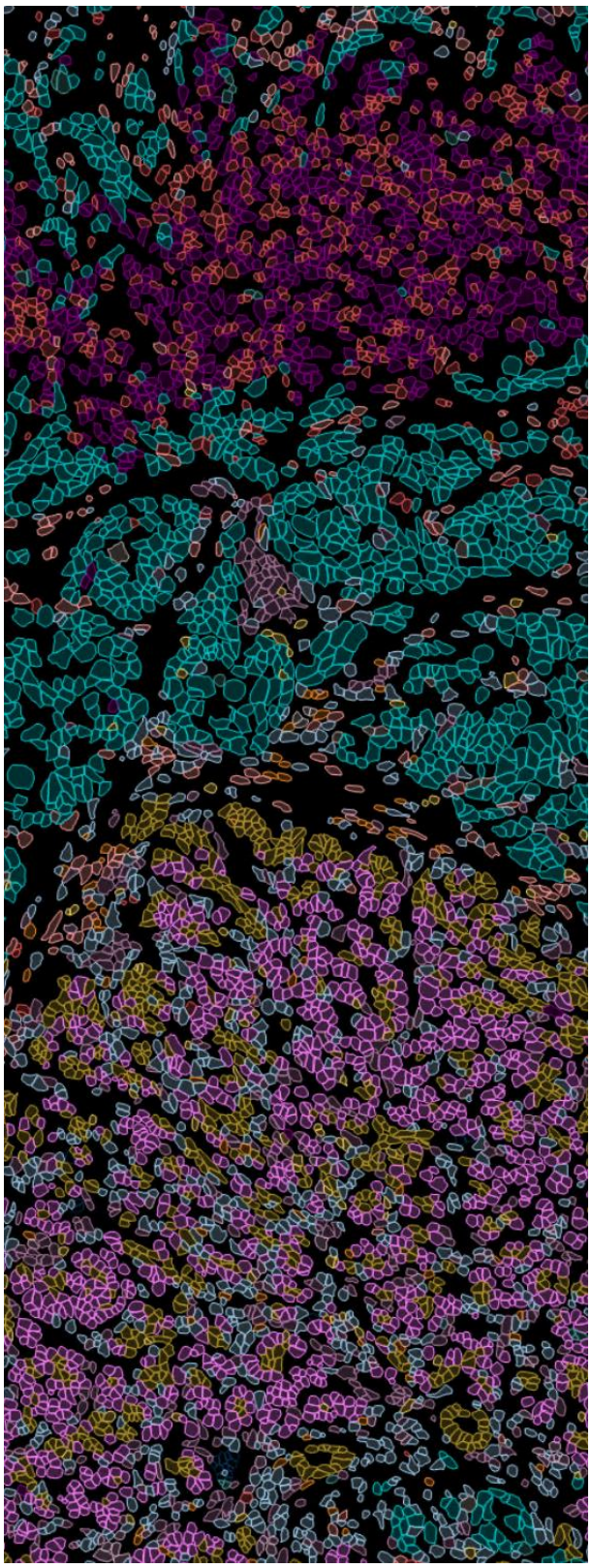
From Eye to Insight

Aivia 13

Release Notes



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

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Release Documentation for Aivia 13

This document describes the version 13 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.

Date: November 10th, 2023 applying to Aivia 13 r40236.

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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

NEW: For 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

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 13 Release

Aivia 13 is a major release containing 150 features, usability improvements, security updates and 134 bug fixes since Aivia 12.1 release on June 28th, 2023. Powered by AI, Aivia 13 offers easy to use spatial insight generation tools for large, multiplexed 2D images (85K x 57K in size with up to 100 channels) to go from eye to insight faster.

Key Features Summary

All Aivia Packages:

- Visualize large, multiplexed 2D images (85K X 57K in size with up to 100 channels) with millions of detected objects
- Automatic tissue detection for region of interest (ROI) delineation
- Improved existing recipes (Cell Count, Nuclei Count, Cell Count-Cellpose), Image Enhancement Functions (Cellpose Enhancement, Cellpose Object Detection), and Pixel Classifier for larger 2D images
- Large image-compatible Pearson Correlation Heatmap, Violin Plot and Scatterplot
- Multi-well Scatterplot for a single well, an experimental condition, or for all conditions for the entire plate

For Elevate and Apex packages only:

- 2 new recipes for cell detection: Cell Analysis-Cellpose and Multiplexed Cell Detection with an optimized version of the Cellpose generalist deep-learning algorithm
- Phenotyper for machine-learning based classification, and 2 automatic clustering options: k-means and PhenoGraph-Leiden unsupervised automatic clustering methods
- New spatial insights with Marker Cluster Dendrogram, Summary Plot, Binned Scatter Plot, and Dimensionality Reduction (UMAP, PacMAP, t-SNE)

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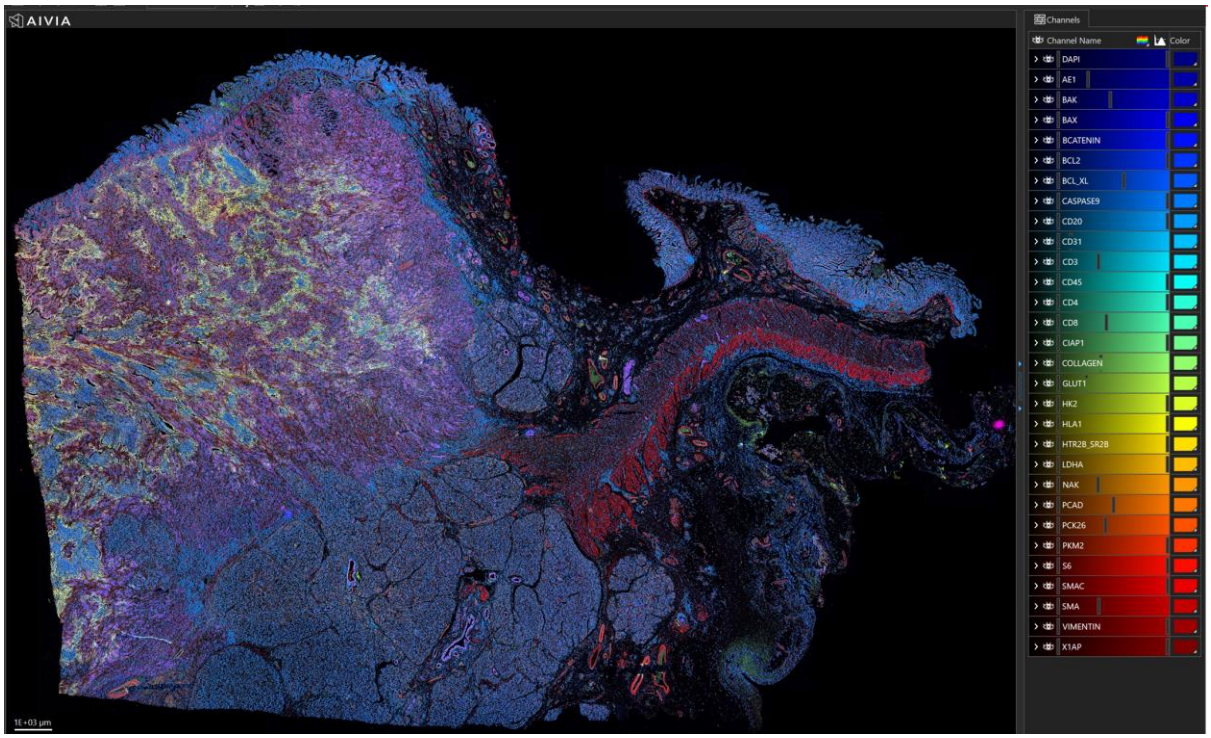
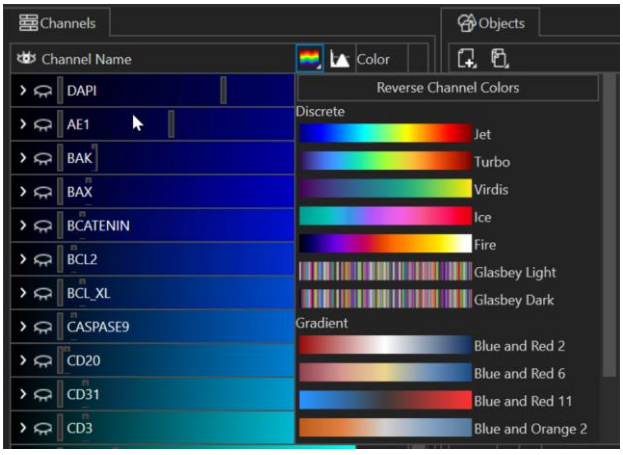
Visualize Large 2D Multiplexed Images

With 2D rendering engine overhaul, Aivia 13 enables visualizing large, multiplexed 2D images (85K x 57K in size with up to 100 channels) faster than our previous versions.



A 230GB, 30 channel multiplexed pancreatic ductal adenocarcinoma image acquired on Leica Cell DIVE by Dr. Melinda Hill of Leica Microsystems.



With updated color maps for different functionalities, Aivia 13 enables meaningful data interpretation when working with numerous colors for markers, detected objects, phenotypes and exploration via charting options. In the example above for viewing channels, users can apply discrete and gradient color maps that stretch over the total number of channels for ease of interpretation for channel visualization when working with multiplexed data with markers that are organized alphabetically (with DAPI being the first channel for Cell DIVE images).

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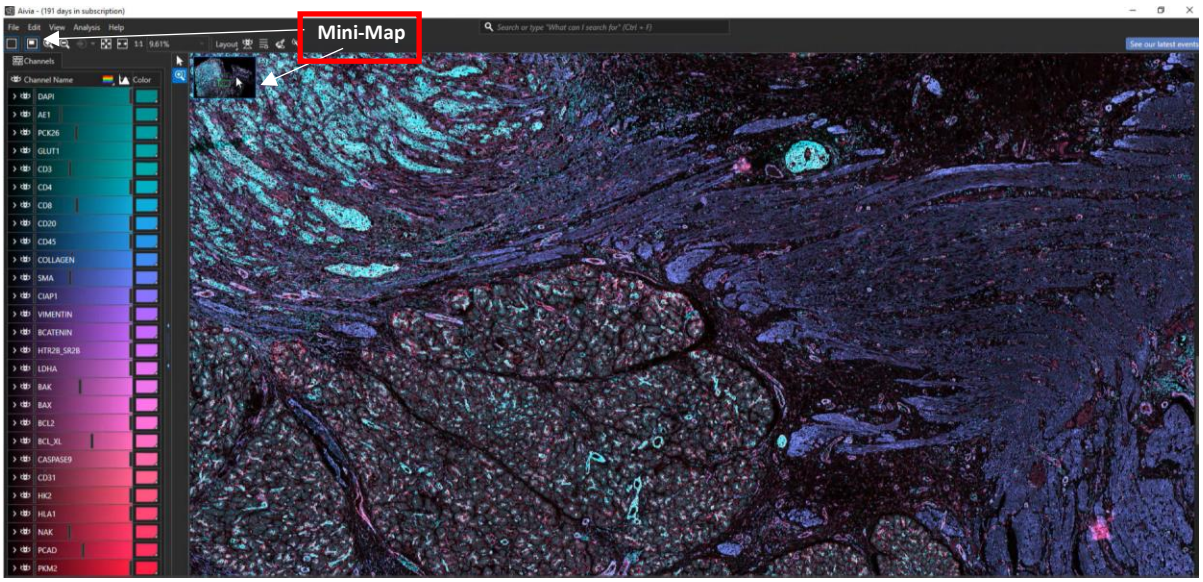
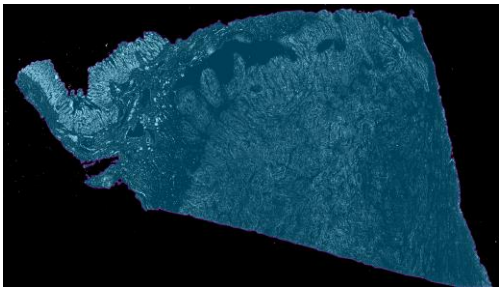
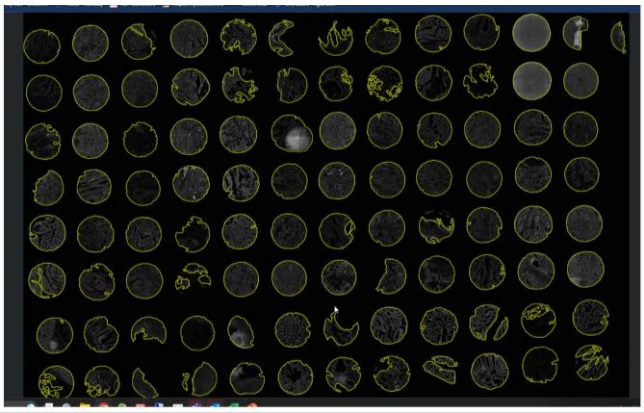


The new Mini-Map functionality enables navigating to a specific location of interest from anywhere on your image quickly.

Automatic Tissue Detection





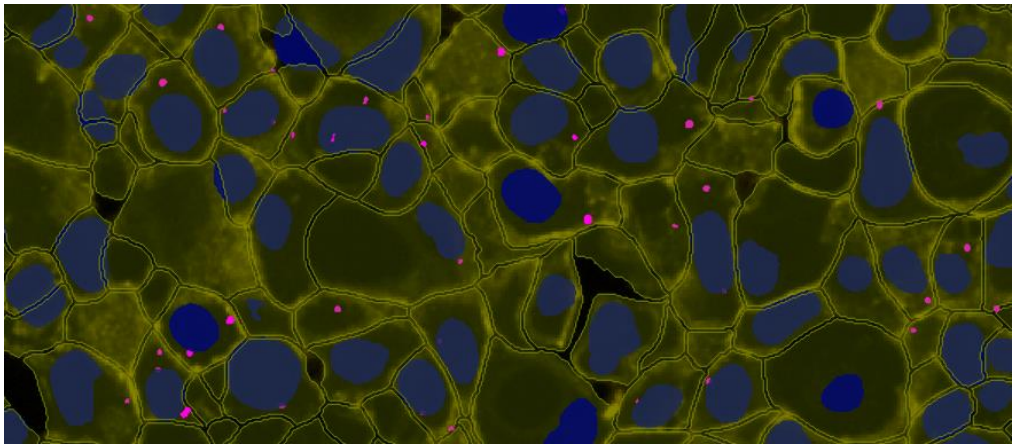
Automatic tissue detection enables users to automatically delineate regions of interest (ROI) based on any channel for efficient downstream analyses of large tissue sections (left) and Tissue Micro Array (TMA) cores (right).

AI-Driven Cell Analysis

Aivia 13 includes 2 new recipes for cell detection: Cell Analysis – Cellpose, and Multiplexed Cell Detection. Both of these recipes include a modified version of the Cellpose generalist deep-learning cell segmentation algorithm (Stringer et al, 2020)[1], optimized for larger 2D images.

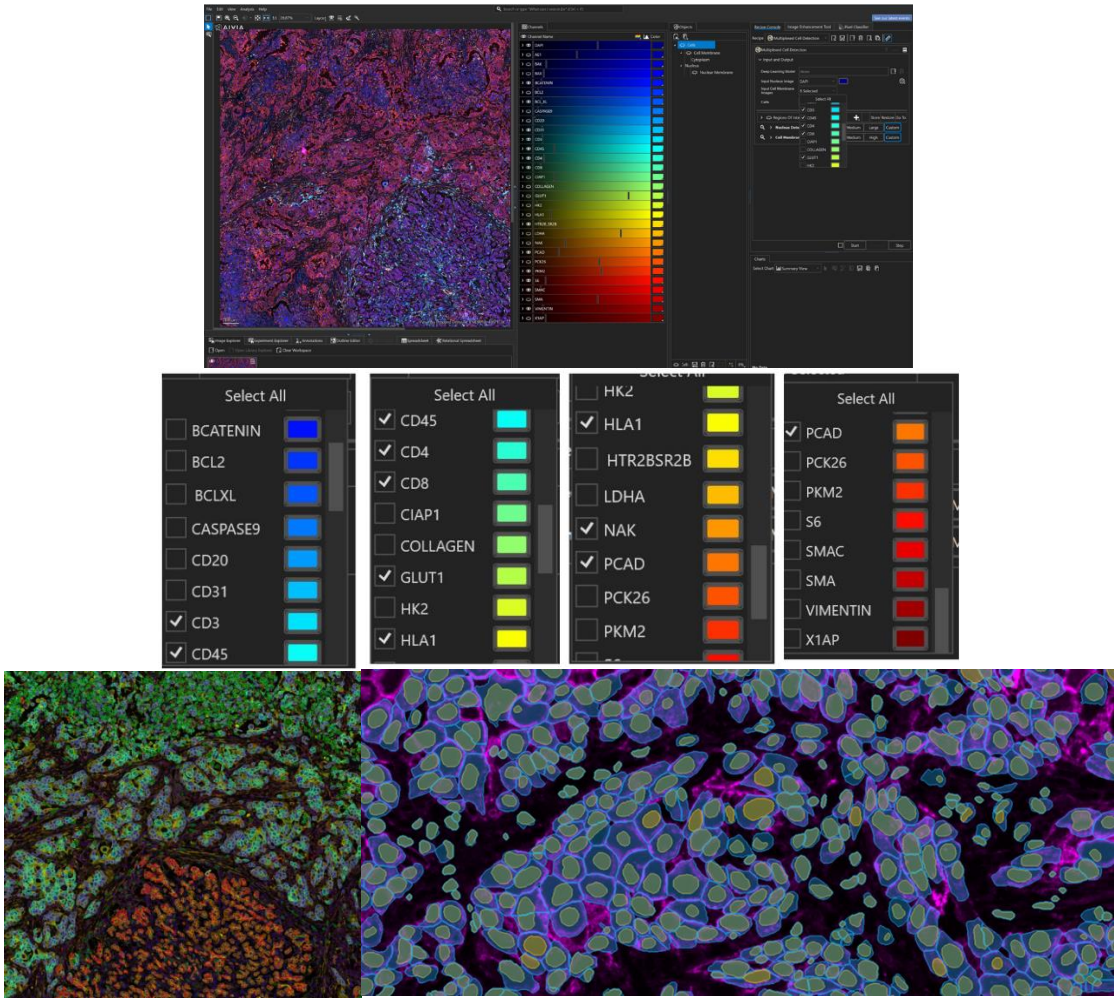
Cell Analysis-Cellpose Recipe

Using a modified version of Cellpose optimized for larger 2D images, Cell Analysis - Cellpose Recipe applies the generalist deep learning model for nucleus and membrane detection for accurate segmentation and partitioning of cells. With cellular imges with vesicles, intracellular distance relationship from vesicles to the nucleus and the membrane are automatically calculated.

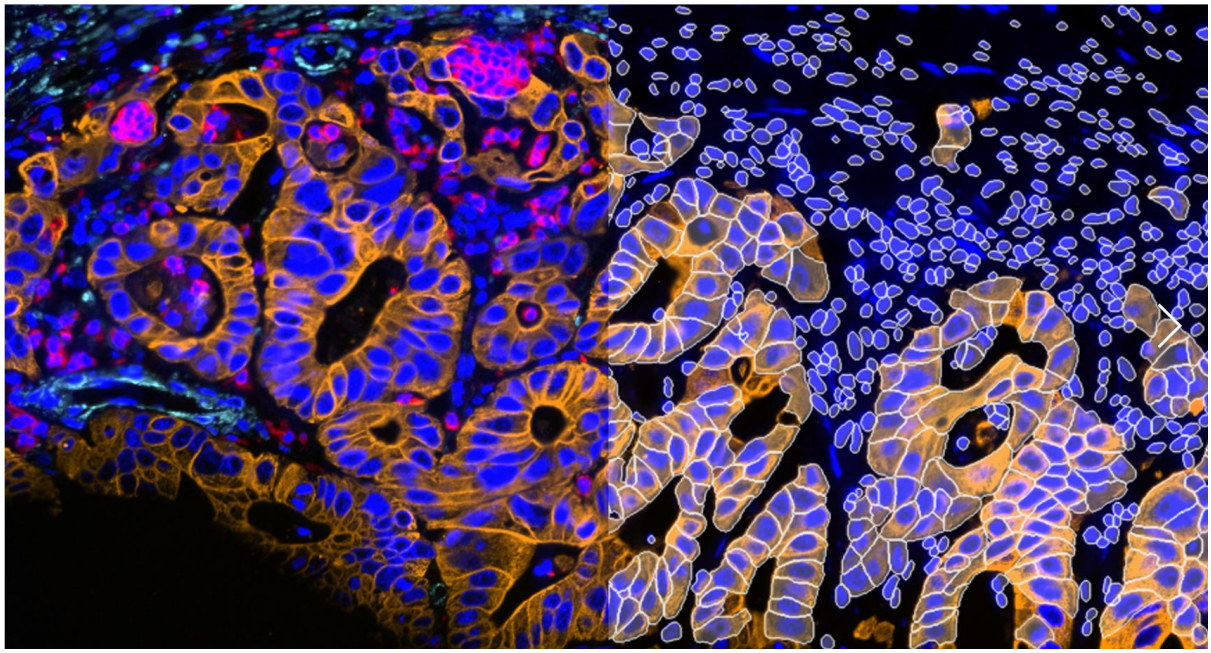


Multiplexed Cell Detection Recipe

With a modified version of Cellpose optimized for large, multiplexed 2D images (tested up to 85 k X 57 k, 30 channels), Multiplexed Cell Detection Recipe leverages multiple markers for membrane detection.



New Multiplexed Cell Detection Recipe user interface (top left) with the channel selector (middle 4 images), detected cells and an enlarged image to showcase segmentation and partitioning result (bottom images). 30 channel multiplexed pancreatic ductal adenocarcinoma image acquired on Leica Cell DIVE by Dr. Melinda Hill of Leica Microsystems.



Colon adenocarcinoma tissue with cells detected using Multiplexed Cell Detection Recipe (detected object outlined in white – right panel). Image courtesy of Dr. Melinda Hill, Leica Microsystems.

Improved Pixel Classifier and Existing Recipes for Larger 2D Images

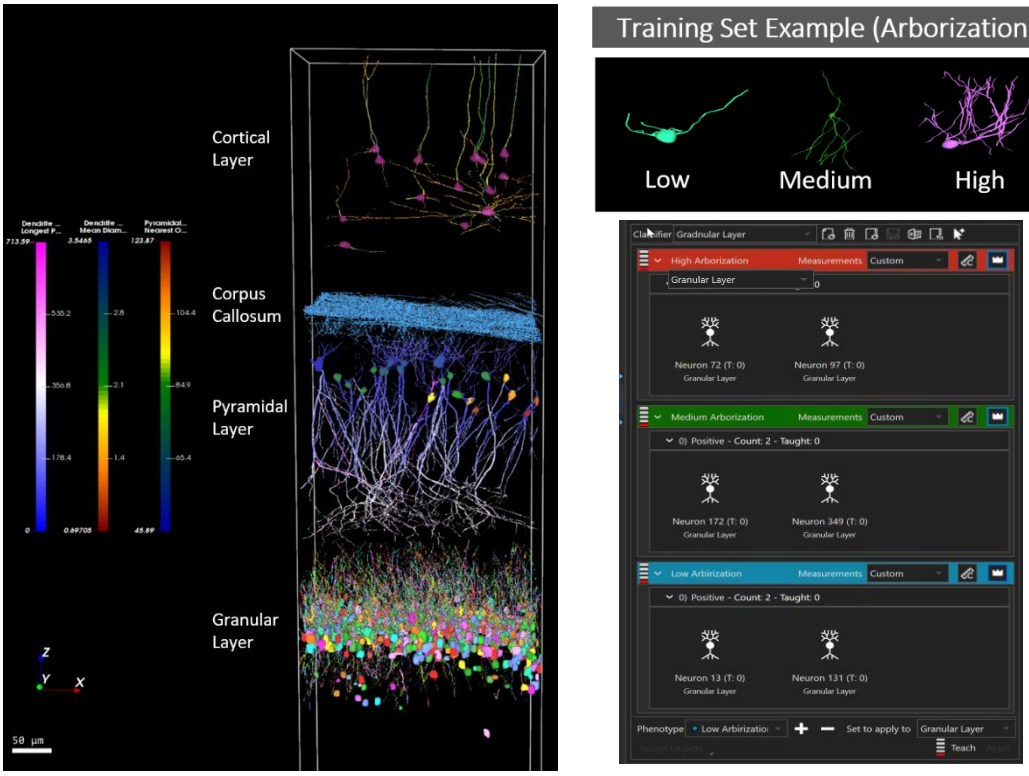
Pixel Classifier and the following 2D recipes were optimized for larger 2D image handling: Cell Count, Nuclei Count, Cell Count - Cellpose Recipes with significant reduction in processing speed by over 98% compared to Aivia 12. The following Image Enhancement Functions are similarly optimized for larger 2D images: Cellpose Enhancement, & Cellpose Object Detection.

Supervised and Unsupervised Phenotyping Powered by AI

Aivia 13 introduces 3 new phenotyping methods to generate deeper insights about the types of cells within the image.

AI-Driven Phenotyper

The Phenotyper leverages your expert knowledge about your phenotypes of interest to build a classifier. Select a few representative cells to train Aivia and quickly classify cells into different phenotypes using machine learning.



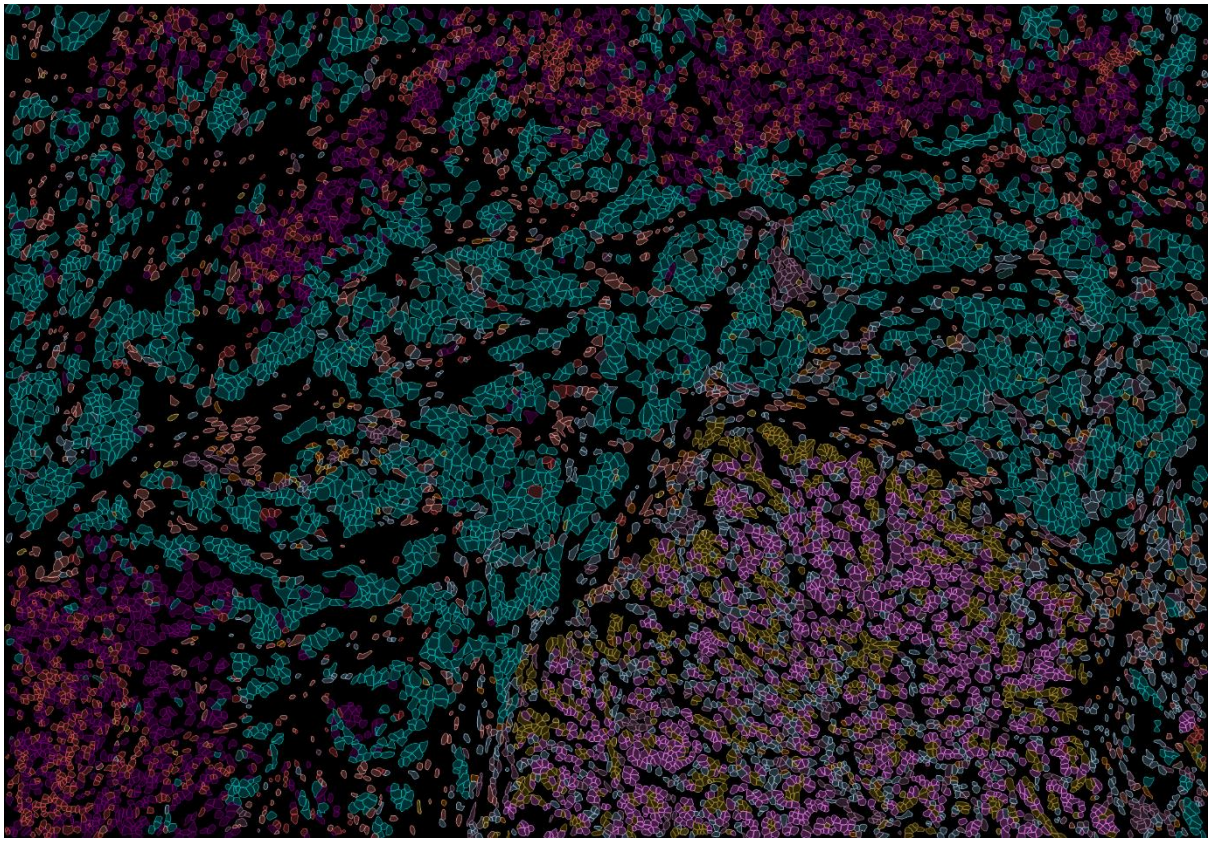
Granular neurons in the cleared mouse cortex were segmented using Cellpose for soma detection and dendrites were traced. The neurons in the granular layer were used for training the Phenotyper with two neuron examples in each class which could effectively classify 546 neurons in this image. Image courtesy of Dr. Hu Zhao of Texas A & M.

Automatic Clustering: k-means[2]

Using any intensity or morphological measurements from detected objects, Aivia automatically generates a user-specified number of clusters within the image via k-means clustering.

Automatic Clustering: PhenoGraph-Leiden[3]

Using the PhenoGraph-Leiden method, initially developed for high-dimensional data, Aivia performs unsupervised automatic clustering based on any intensity or morphological measurements from detected objects.

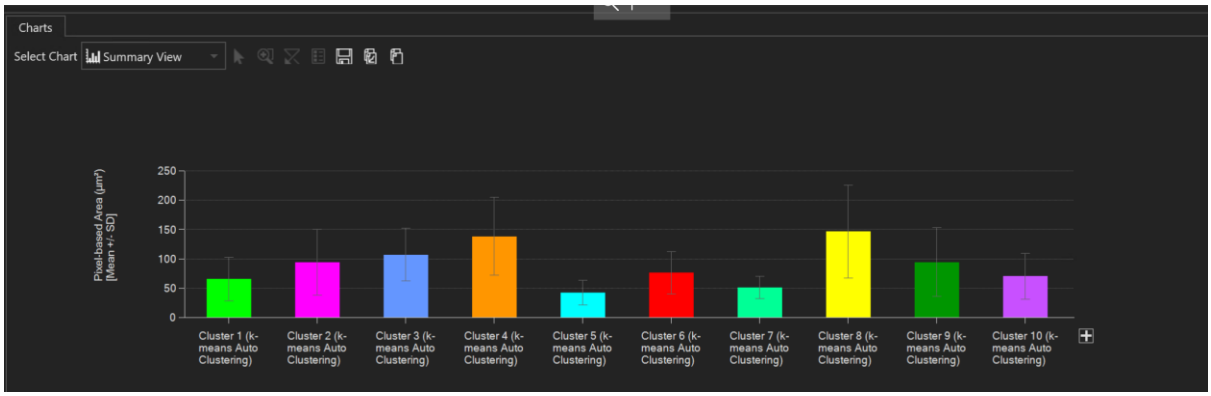


Phenotypes generated using the Phenograph-Leiden automated clustering method in pancreatic ductal adenocarcinoma tissue with cells detected using Multiplexed Cell Detection Recipe. Purple-colored cells correspond to hypoxic tumor cells, as confirmed by the intensity profile for markers such as GLUT1+. In contrast, the turquoise cells represent normoxic tumor cells based on the intensity profile for AE1+ and GLUT1- markers. Image courtesy of Dr. Melinda Hill, Leica Microsystems.

Insight Generation via Data Exploration

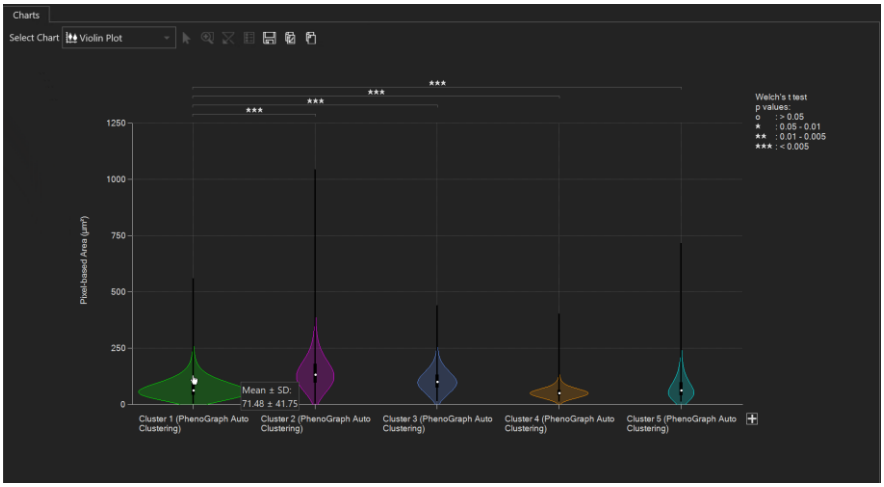
Summary Charts

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

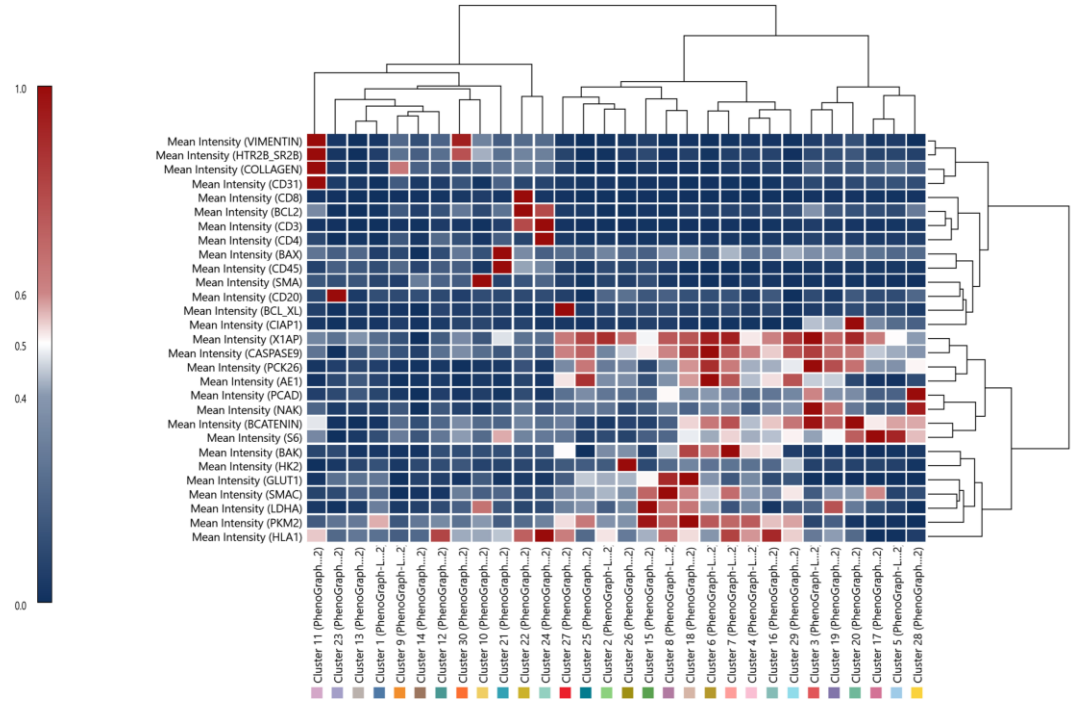
Violin plot for comparingcompares data distribution for millions of objects in different object groups or phenotypes



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.

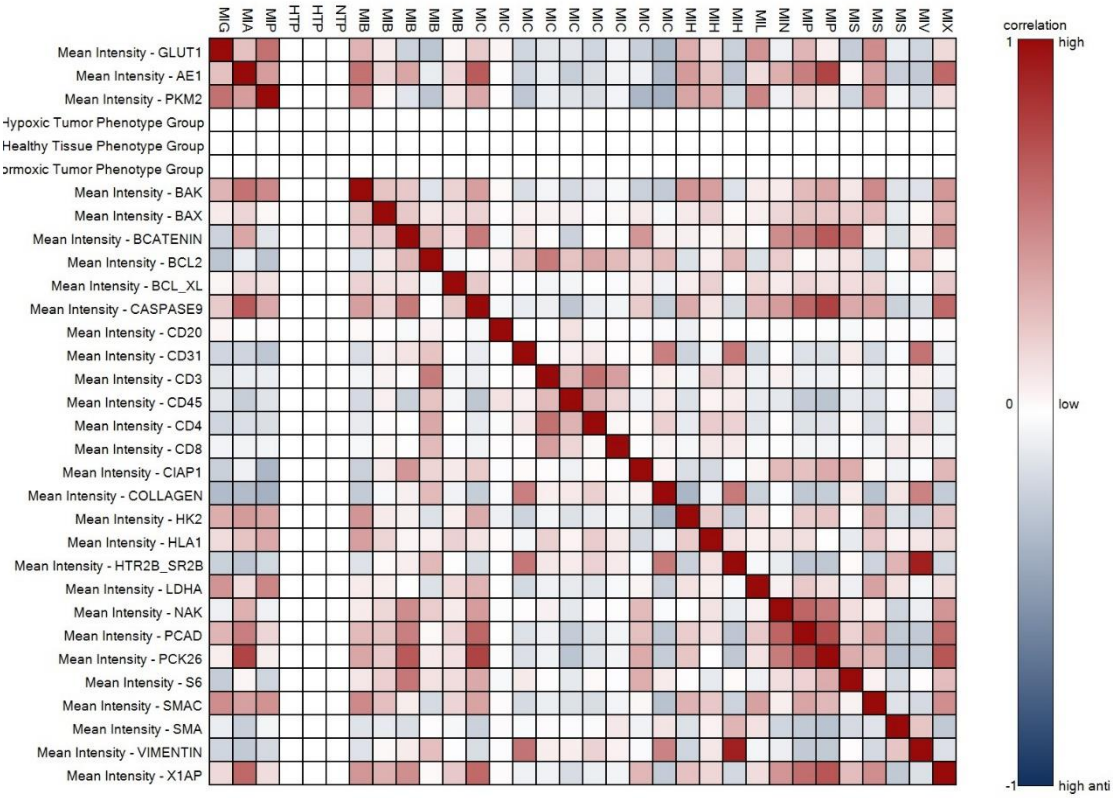


The dendrogram above shows a heat map normalized per measurement against phenotypes, and the dendrogram branch height reflects the measurement distance (how closely measurements are related) between clusters or user-specified measurements. Selecting any rectangular cell in the dendrogram highlights the cells

in the corresponding clusters on the image to enable interactive visualization and data exploration for insight generation.

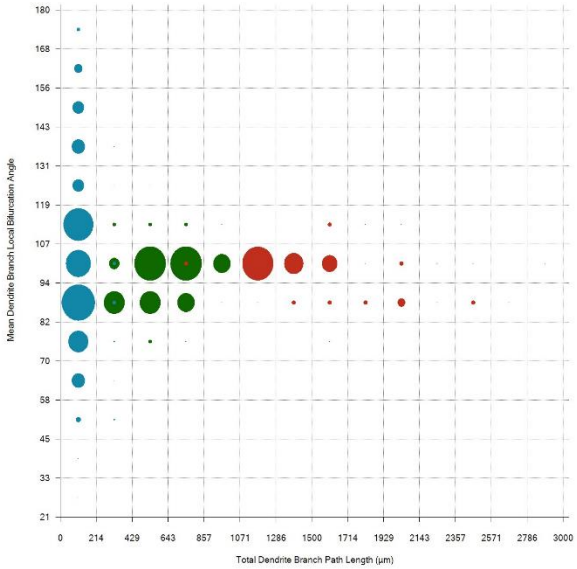
Pearson Correlation Heat Map

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.



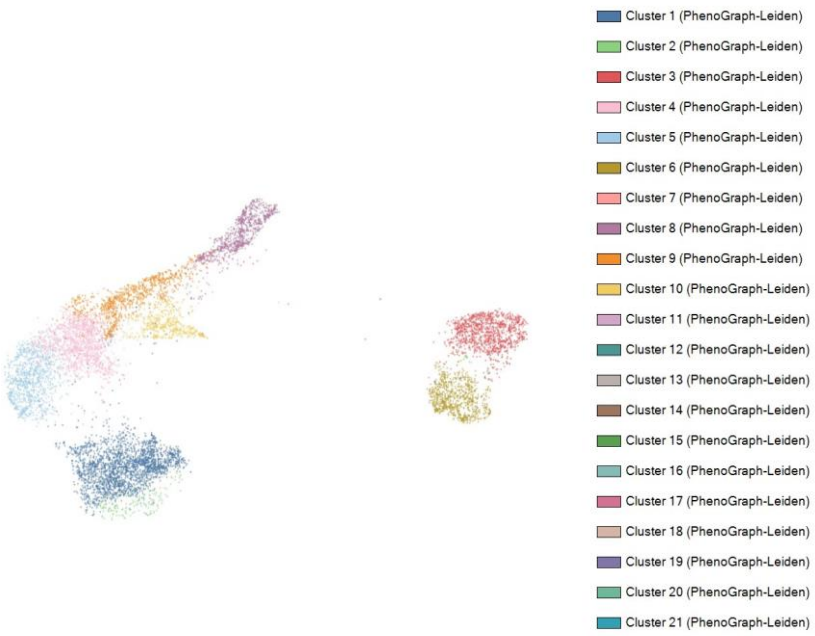
Binned Scatter Plot

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.

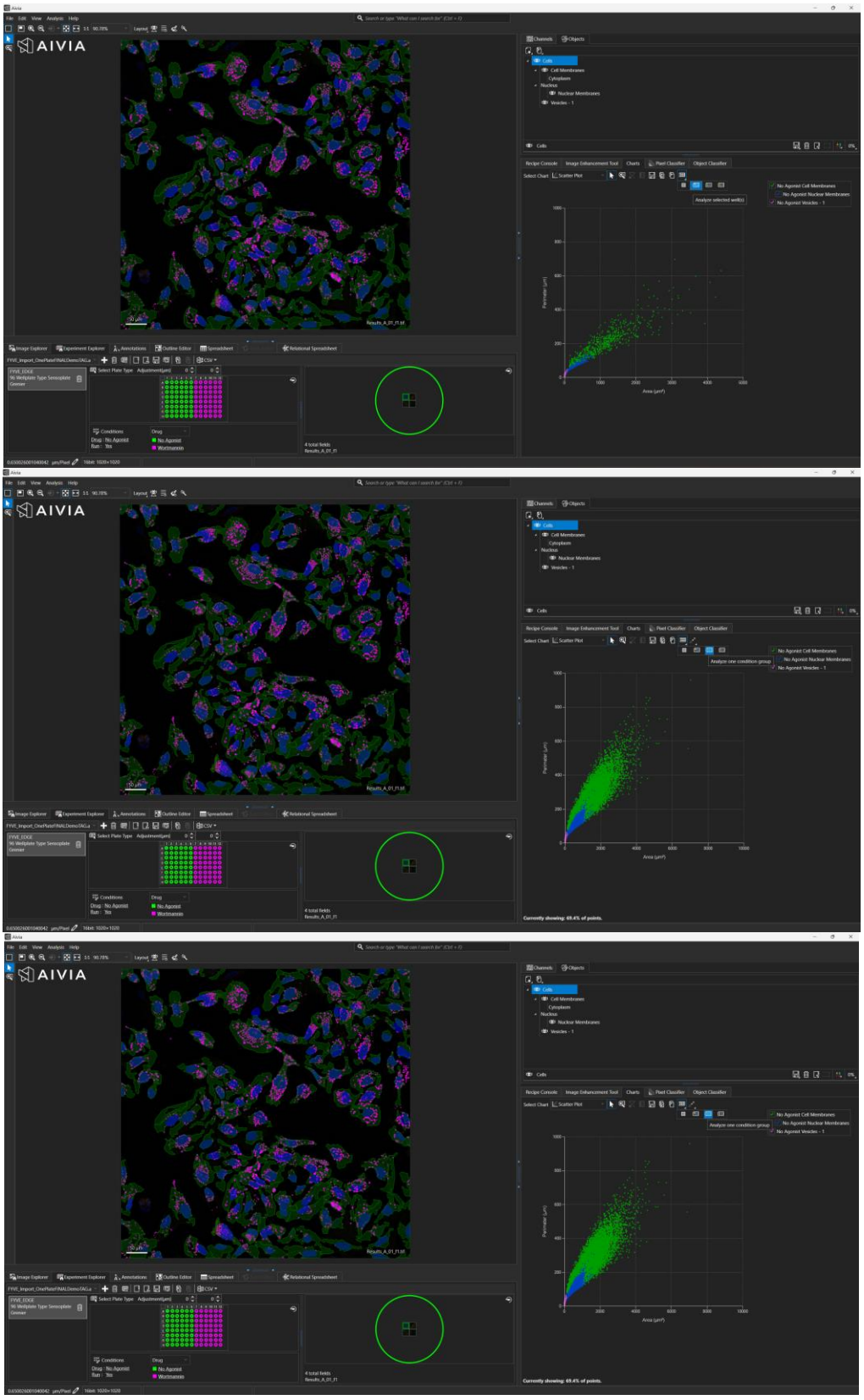


Dimensionality Reduction Plot

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.



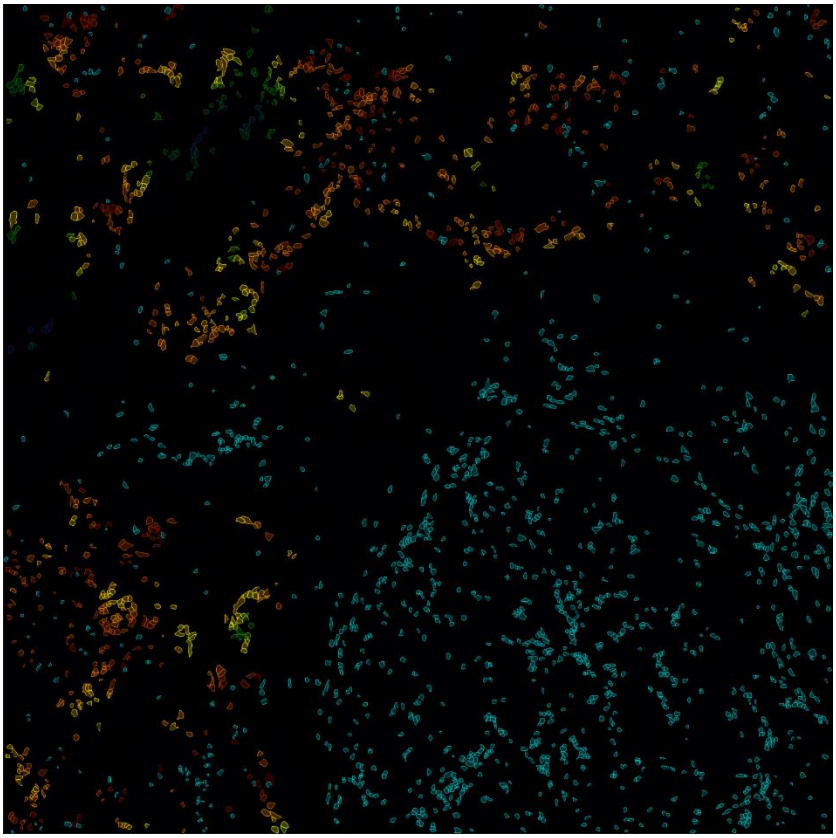
Multi-well Scatter Plot



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.

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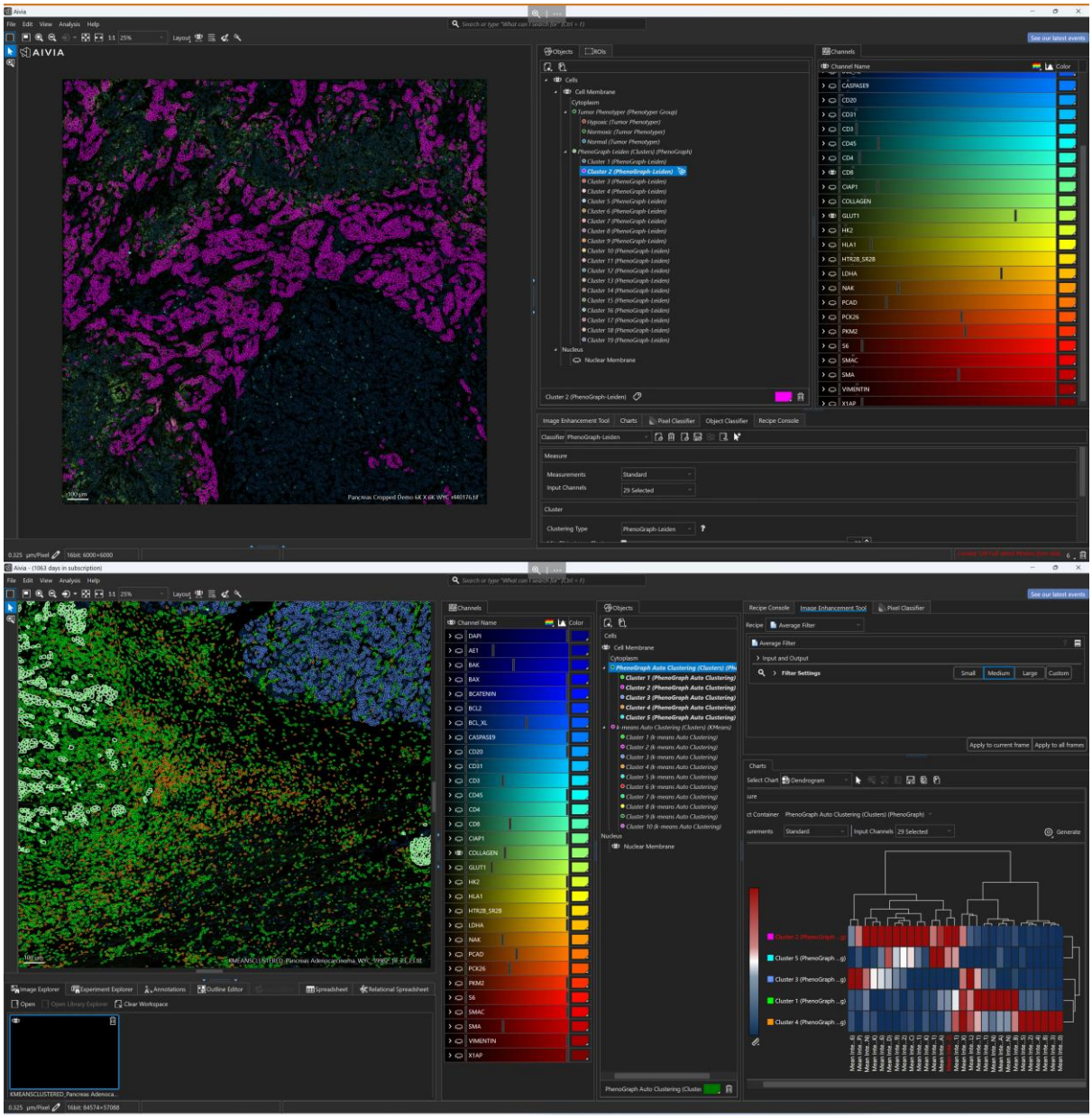
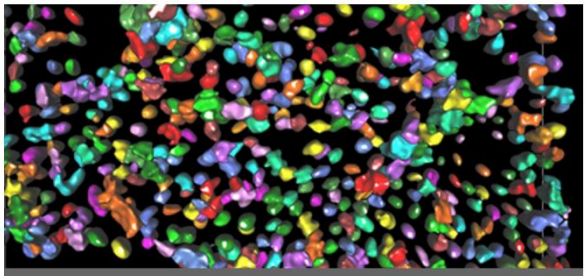


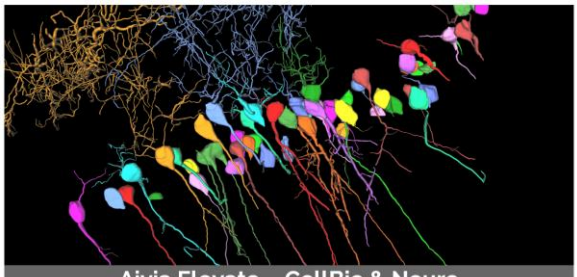
Using the new Spotlight function (top image), users can click on the phenotype of interest to visualize the selected phenotype with emphasis along with any other marker/structures of interest enabling insight generation in the spatial context. Users can further explore the relationship between clusters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

New Features for Subscribers



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 (coming soon)
- Binned Scatter Plot (link)
- Summary View (link)
- Regions of interest (link)

Aivia Demo License (link)

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