

From Eye to Insight



Aivia 16

Release Notes

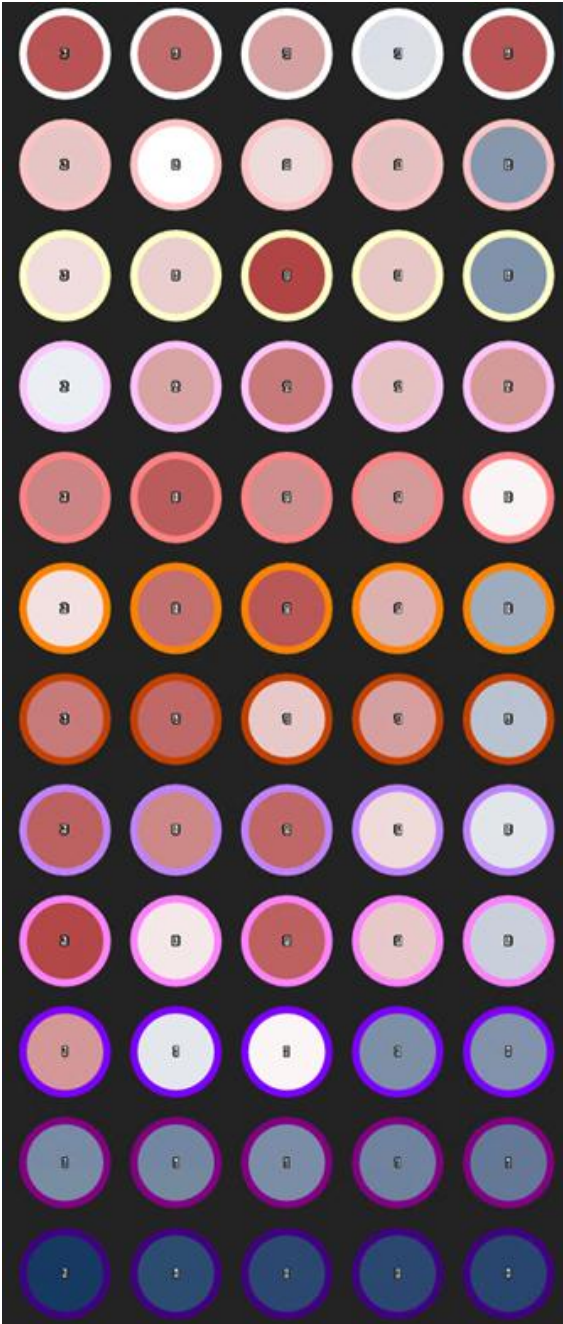


Image credit: multi-well heat map of live-dead assay cell count ratio with Aivia 16 by Dr. Won Yung Choi, Ph.D. of Leica Microsystems

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Release Documentation for Aivia 16

This document describes the version 16 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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IMPORTANT NOTE:

Please **DO NOT RUN**
AIVIA AND MICA LAS X
ON THE SAME WORKSTATION
AT THE SAME TIME
although each can be run
separately

Date: March 14, 2026, applying to Aivia 16 release, r43753

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Won Yung Choi, Ph.D. Product Manager & Scientific Innovation Manager AI (wonyung.choi@leica-microsystems.com)

Aivia Demo License (link)..... 30
For Internal Leica Associates Only**Error! Bookmark not defined.**

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 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 Z4G5PC

- Operating System: Win11 Pro64
Processor: Intel Xeon W3-2435, 8 Cores
Main Memory: 64GB (2X32GB) DDR5 4800 ECC REG RAM
Graphics Board: NVIDIA RTX A4000 16GB 4DP GFX
System Drive: 512GB PCIe 2280 TLC M.2 SSD
Temp Drive: 4TB M.2 SSD RAID0 (2x 2TB PCIe 2280 TLC M.2)
Data Drive: 4TB M.2 SSD (1x 4TB PCIe 2280 M.2)

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 11 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 16 is a major release containing 239 features, usability improvements, security updates, and 201 bug fixes since Aivia 15 release on April 11, 2025. Powered by AI, Aivia 16 offers a number of new features including multi-well analysis, aggregate summary charts, dose response plot, multi-well heatmap, and updated Segment by Example 2.0 for multiplexed cell detection and multi-compartment intracellular analysis to go from eye to insight faster.

Aivia 16 Key Features Summary

All Aivia Packages:

- Updated Segment by Example now only needs 3 painted examples to deploy a pre-trained deep learning algorithm for cell segmentation without the user needing to learn to code or train deep learning models
- Multiple multi-well plate phenotype export for Leica LMD (Laser Microdissection Microscope)
- Track smoothing for improved visualization
- Scatterplot quadrant-based automatic gating
- Save and reapply gating strategies for consistency
- Batchable Gate by Rules

For Aivia Accelerate (NEW Package):

- Multi-well plate analysis using the updated Experiment Explorer
- 5 new assays for guided workflows from the Launchpad (Counting, Morphological, Live-Dead, Apoptosis, and Custom to deploy any existing recipes)
- Summary chart with aggregated measurements for well per condition and for the entire plate
- Dose response curve
- Self-Training 2-Class Classifier for automatic phenotyping of cells into two classes

For Elevate Neuro:

- All the new features of Aivia Go and Accelerate

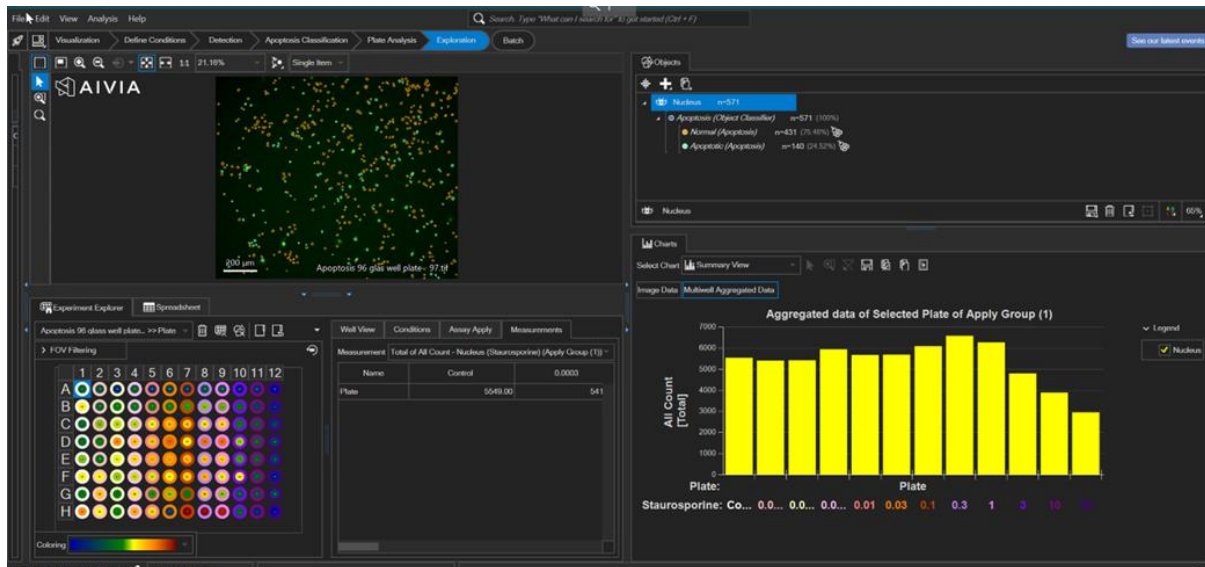
For Elevate Cell Bio and Apex packages only:

- All the new features of Aivia Go and Accelerate
- Multiplexed cell detection with multiple channel inputs using Segment by Example (SBE 2.0) in 2D/3D
- Multi-Compartment Cell Analysis (nucleus, cytoplasm, vesicles) using Segment by Example (SBE 2.0) in 2D

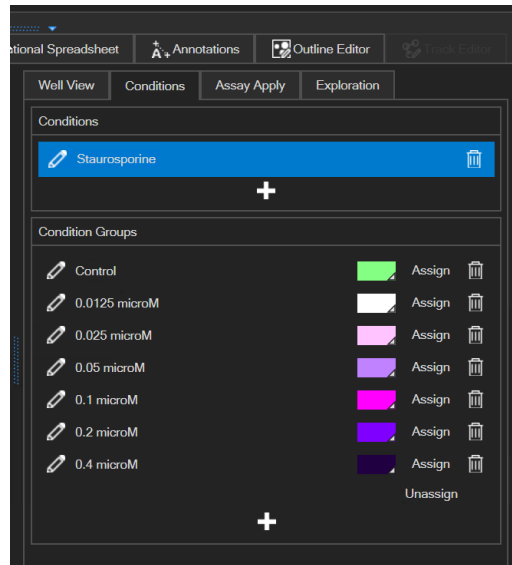
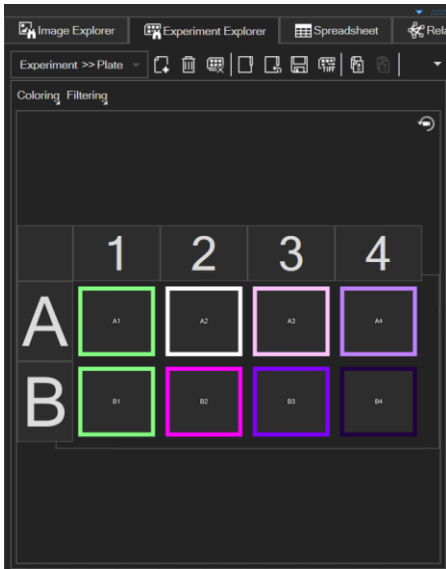
Aivia 16 Features

Updated Multi-well Analysis

Import multi-well files (.lif, .xlef) from Leica's Mica, DMi8 and STELLARIS into Aivia's Experiment Explorer for visualization, condition setting (e.g. drug and dosage), and aggregation of data into summary charts per well, condition and plate.



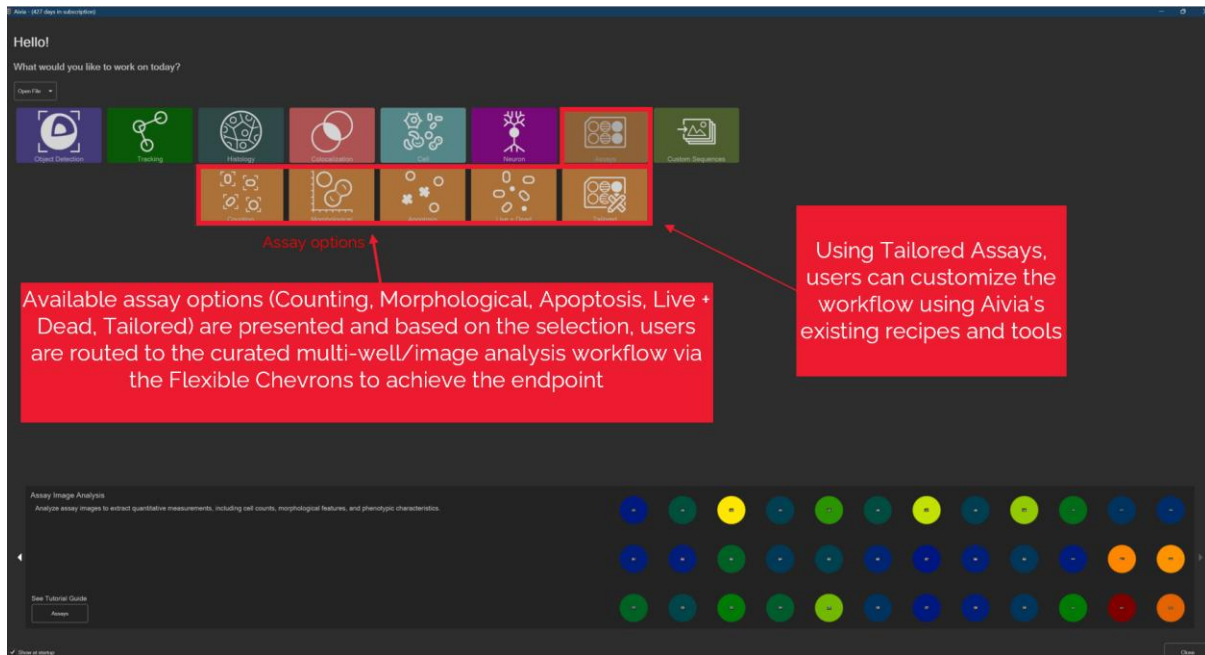
New Experiment Explorer interface with aggregated summary chart of summary count. Live-dead assay by Dr. Oksana Sirenko of Molecular Devices, imaged on a Mica by Dr. Seung-Yi Lee of Leica Microsystems and analyzed in Aivia 16 by Dr. Won Yung Choi of Leica Microsystems.



Users can select a single well, one or more columns/rows/entire plate to assign the conditions and condition groups. Users can define the drug conditions for the wells for automatic assignment of all the FOVs in the well to the condition

Launchpad for Assay for Multi-well Files and Individual Images

Aivia 16 has 5 assay options that can be started from the Launchpad: Counting, Morphological, Live-Dead, Apoptosis, and Tailored which can be used to build custom assays using any of the available recipes from Aivia.



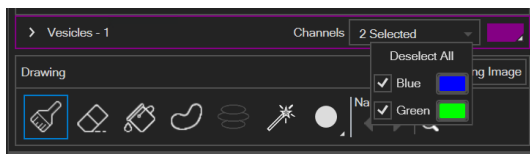
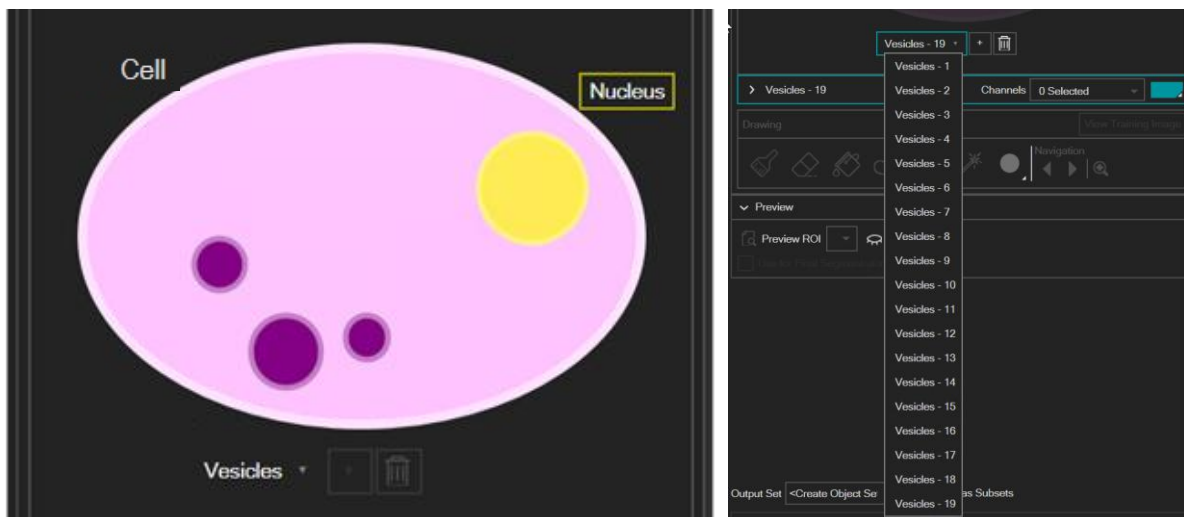
Launchpad sets up a sequence of flexible chevrons to guide users towards the analysis endpoint.

Segment by Example (SBE 2.0) for Multiplexed and Multi-compartment Cell Segmentation

Segment by Example (SBE) has been expanded to detect multiple types of cells in one Segmenter and generate phenotypes at the detection step (e.g. live dead cells) enabling detection of cells with different morphology/input channels/intensity in one go. SBE can detect cells with multiple channel inputs, making it ideally suited for assays where multiple channel inputs can be used to detect multiple cell types or highly-multiplexed cells in spatial biology applications. SBE now only requires only 3 example cells (small, medium and large) to estimate the parameters for the pre-trained generalist deep-learning algorithm. Furthermore, for 2D images, SBE can perform multi-compartment cell analysis (nucleus, cytoplasm and vesicles) for built-in spatial relation analysis between different compartments. Additionally, SBE can now detect as many vesicles as required by the application endpoint.

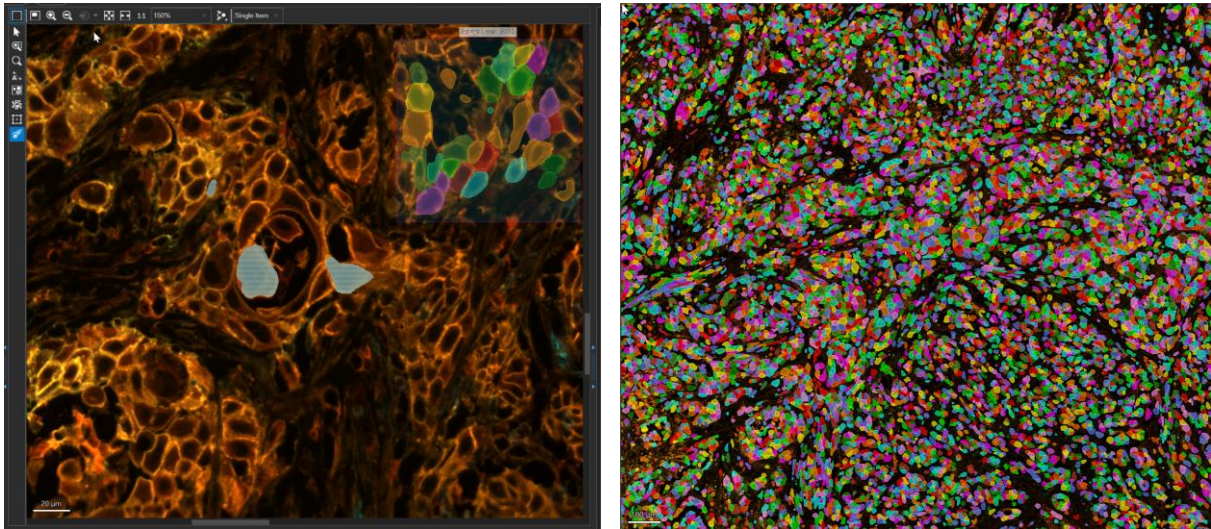
With a single interface, SBE functionally replaces the following eight classic Aivia recipes:

- Nuclei Count
- Cell Count
- Cell Count- Cellpose
- Multiplexed Cell Detection (now with no nuclear stain required)
- 3D Multiplexed Cell Detection (now with optional nuclear stain)
- Cell Analysis
- Cell Analysis- Cellpose
- 3D Object Analysis – Cellpose



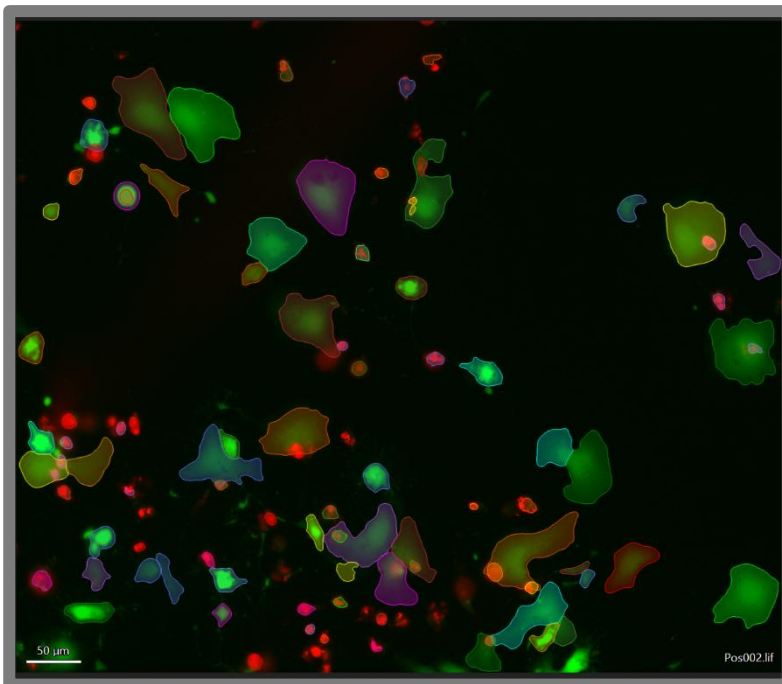
3 painted examples per structure are sufficient to detect cells, nucleus and vesicles with as many vesicle types as users wish to handle.

Top left: new multi-compartment cell analysis interface for SBE. Top right: new vesicle detection interface with the option to add additional vesicles. Bottom: different painting modes available in SBE and the option to select multiple channel inputs

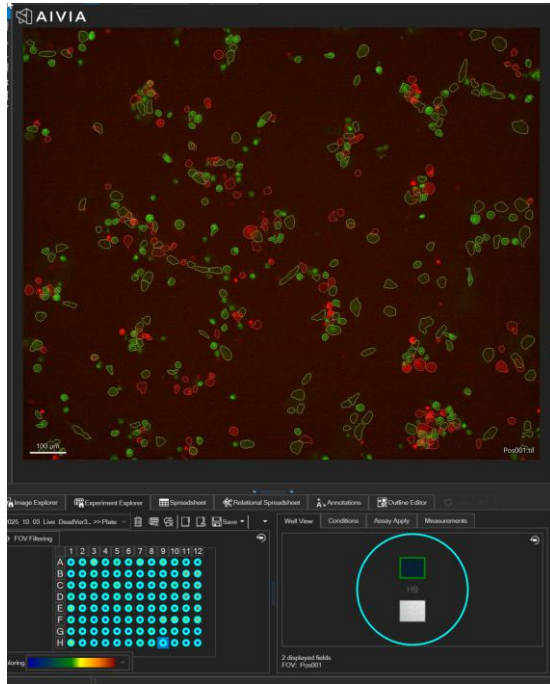
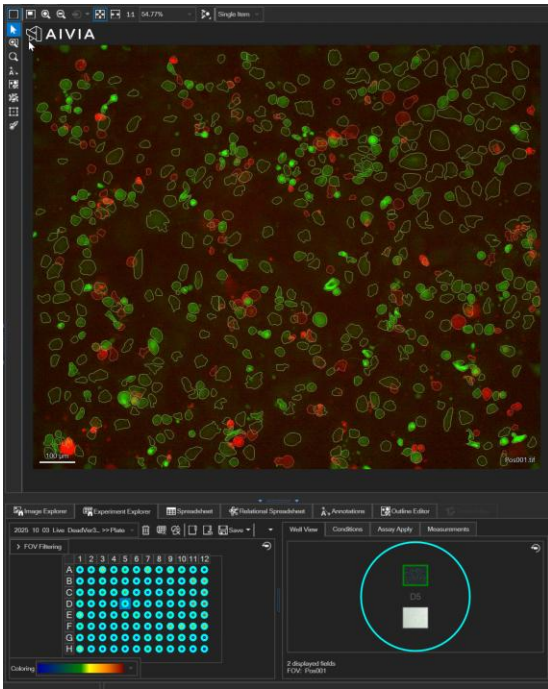


Left: Examples of 3 painted cells (small, medium and large in light blue grey) for cell detection using multiple-channel inputs, with the preview overlay at the top right. Right: the final result with 23,681 cells detected.

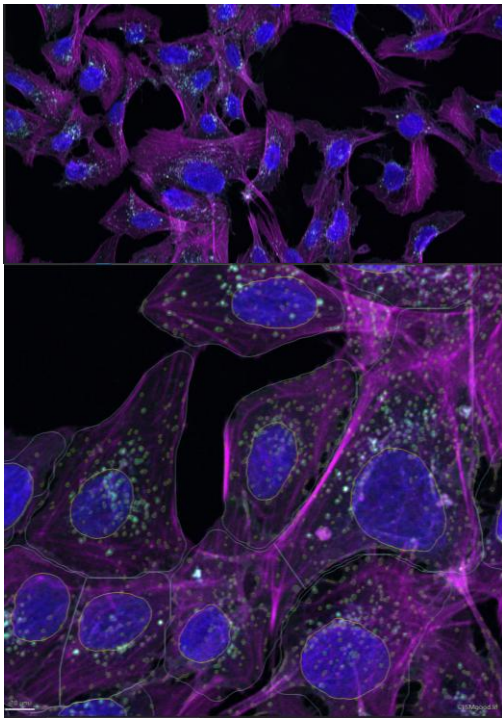
Image credit: Pancreatic tumor section with 30 protein markers imaged using Cell DIVE by Dr. Melinda Hill of Leica Microsystems.



The same SBE Segmenter (with 3 cells painted in green and 3 in red) can handle a wide range of morphological and intensity diversities in the same experiment.



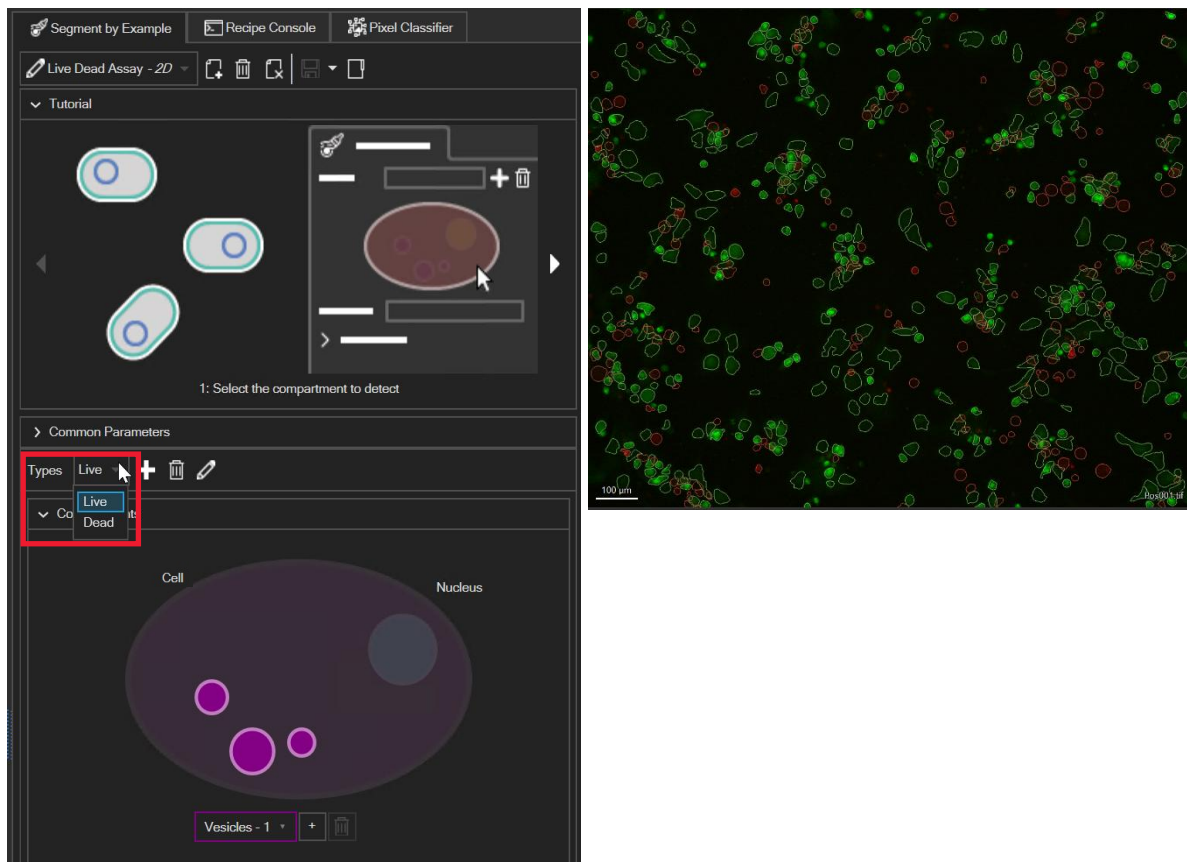
The same SBE settings estimated from 3 painted examples for each phenotype can be used to detect cells in both examples above (small and large with morphological and intensity variation) to handle multiple drug/dosage conditions on a multi-well plate.



SBE Output Example for nucleus, cytoplasm and vesicles. Image credit: Dr. Oliver Schlicker of Leica Microsystems.

Live-Dead Assay

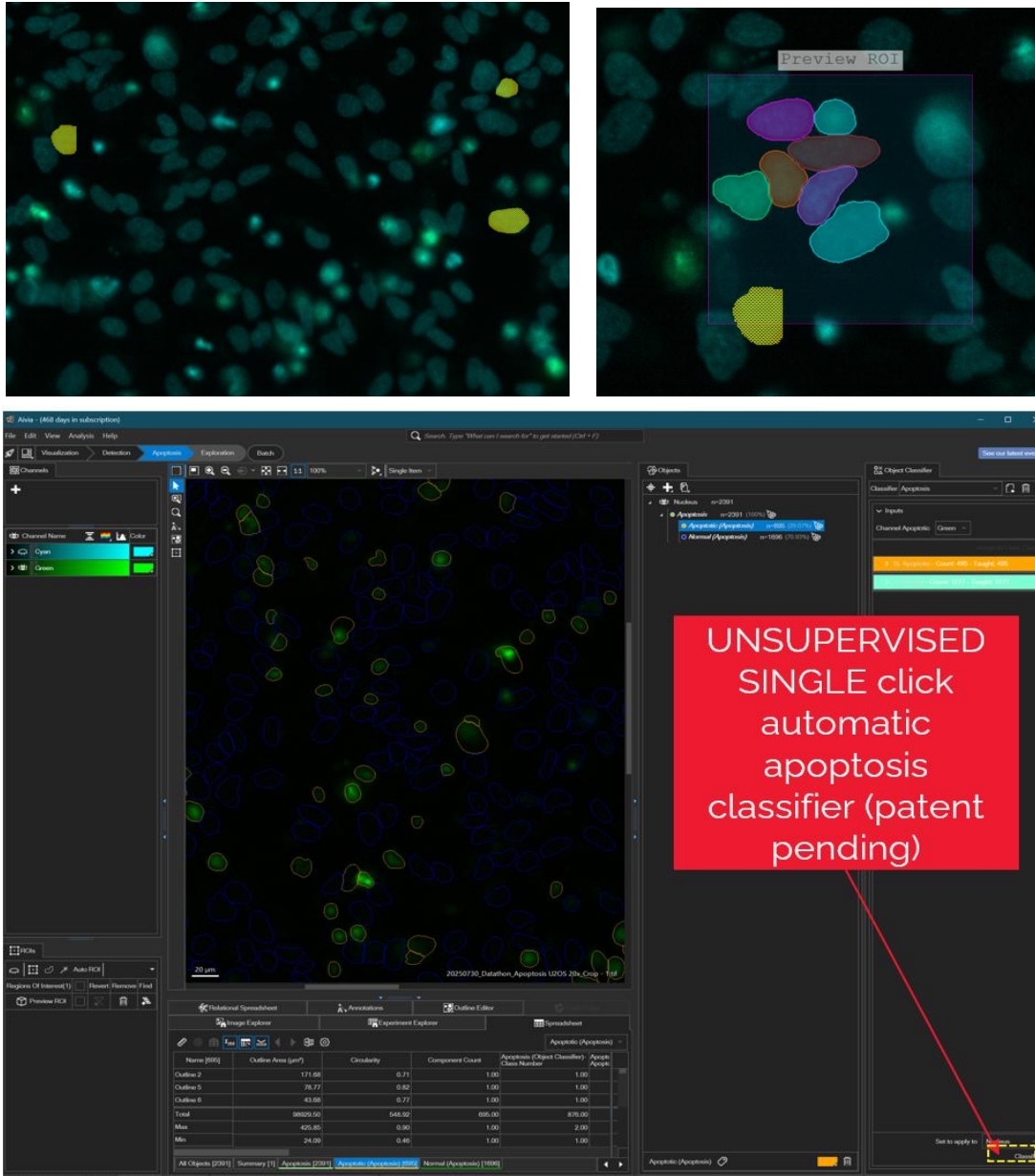
Segment by Example makes it possible to phenotype at the detection step with the new option to define *types* such as live or dead cells which can take in different channel inputs and/or are of different sizes and intensity.



Left: SBE can be used to generate different classes of cells using the new Types feature to detect live/dead cells or any other number of cell classes that have different channel inputs and are of different sizes/intensity. Right: Live and dead cells detected using SBE. Image credit: live dead assay by Dr. Oksana Sirenko of Molecular Devices acquired on a Mica by Dr. Seung-Yi Lee of Leica Microsystems, analyzed by Dr. Won Yung Choi of Leica Microsystems.

Apoptosis Assay

Apoptosis assay enables detection of apoptotic and normal cells by painting only 3 examples to segment, then with a single click classify cells into apoptotic or normal cells using the latest patent-pending Self-training 2-Class classifier.

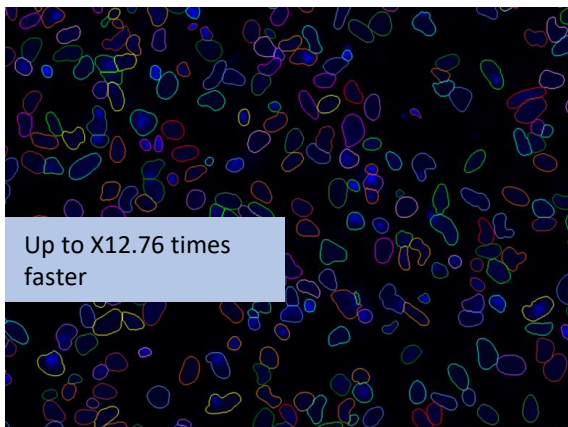


Users only need to paint 3 nuclei to get a preview of the segmentation of the nucleus, then segment the whole image with another click with no classifier training required. Image credit: apoptosis assay by Dr. Oksana Sirenko of Molecular Devices imaged using Mica by Hoyin Lai of Leica Microsystems, analyzed by Dr. Won Yung Choi of Leica Microsystems.

Counting Assay

For high-throughput cell counting applications, a new deep-learning model has been trained using a combination of publicly available datasets, datasets provided by Molecular Devices (a fellow Danaher sister company), and datasets generated using Leica devices internally by Leica R&D. This fast cell detection model can detect and count cells up to 12.76 times faster than our previous fastest model.

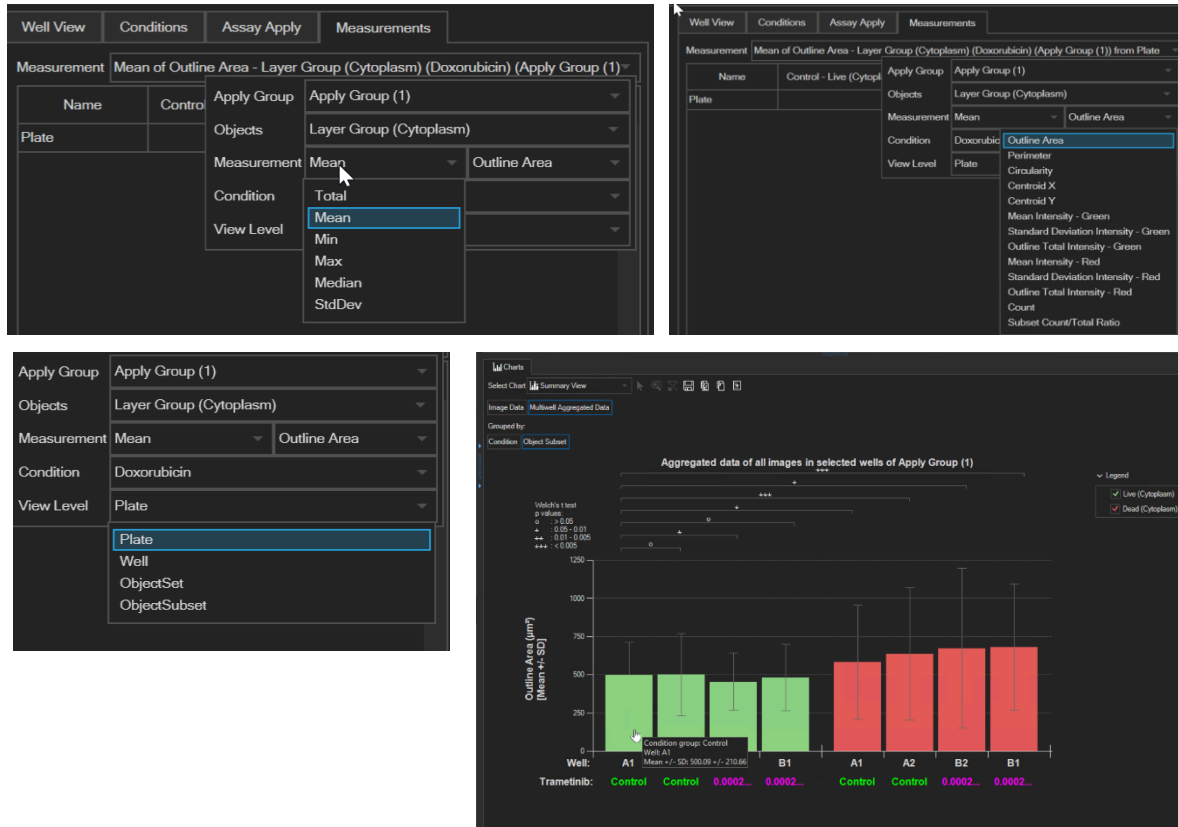
	Aivia's fast model	Cellpose pretrained model
Network architecture	UNet with residual channel attention	UNet-based
Model size (number of parameters)	3.36MB (882,082)	25.22MB (6,610,387)
Number of input channels	1	2 (cell and optional nucleus channels)
Number of output channels	2 (flow maps)	3 (probability and flow maps)



Aivia 16 features our fastest ever cell detection method that is developed by Leica Microsystems to achieve fast and accurate cell detection and counts.

Aggregate Summary Charts

Aivia 16 enables plate-level insight generation by aggregating measurements per well or plate level by condition (e.g. drug type) or condition groups (e.g. dosage).



Experiment Explorer summary measurement interface. Measurement aggregation options and available measurements per level in the new Experiment. Welch's t-test results are auto-calculated for aggregate measurements.

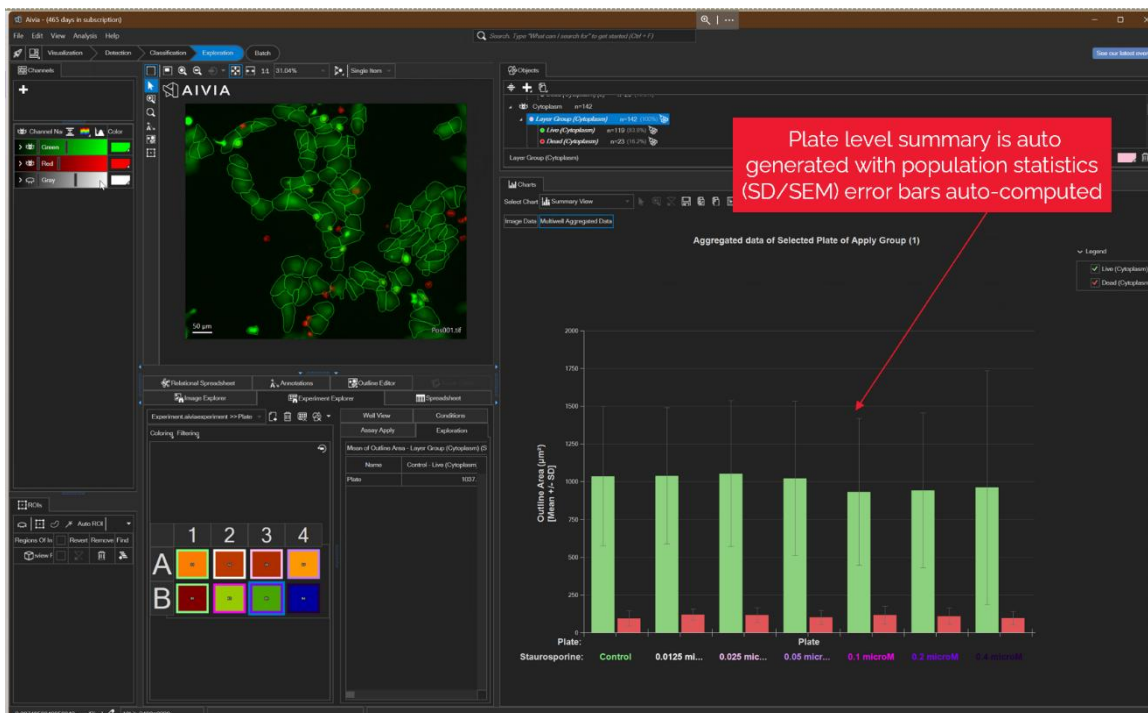
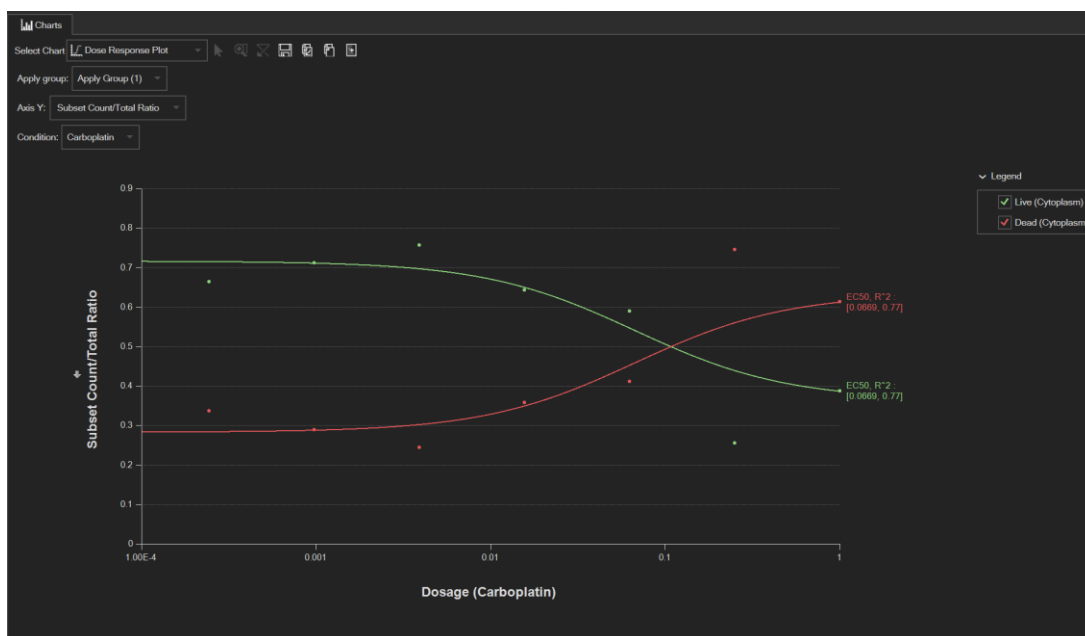
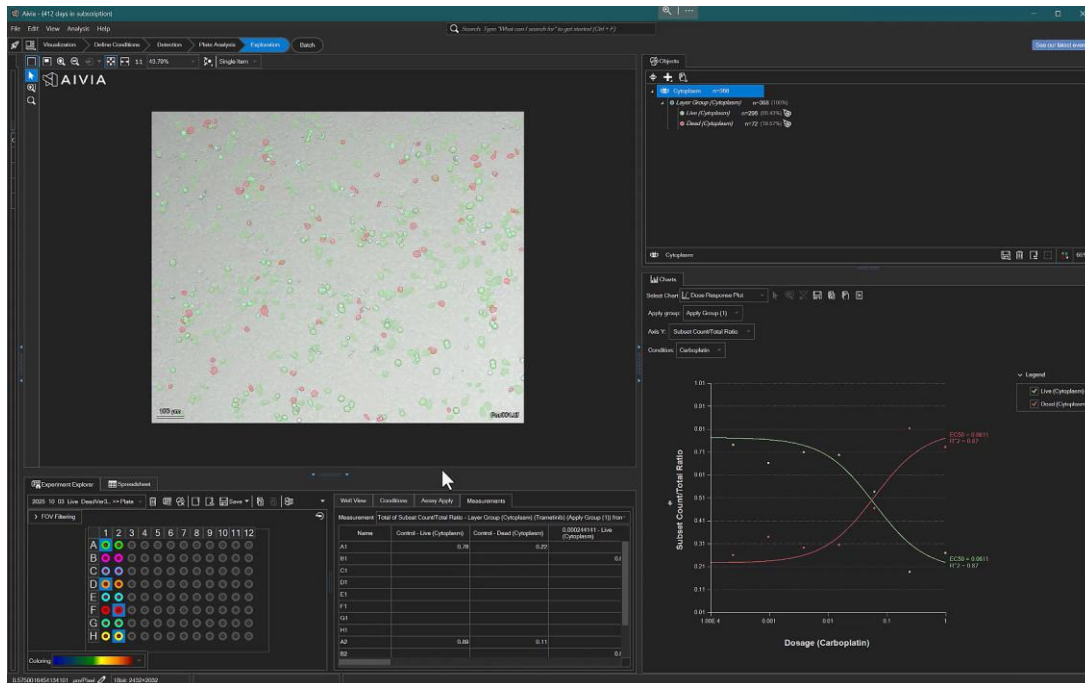


Plate level summary enables selection of population statistics such as standard deviation (SD) and standard error of the mean (SEM) on the charts.

Dose Response Chart

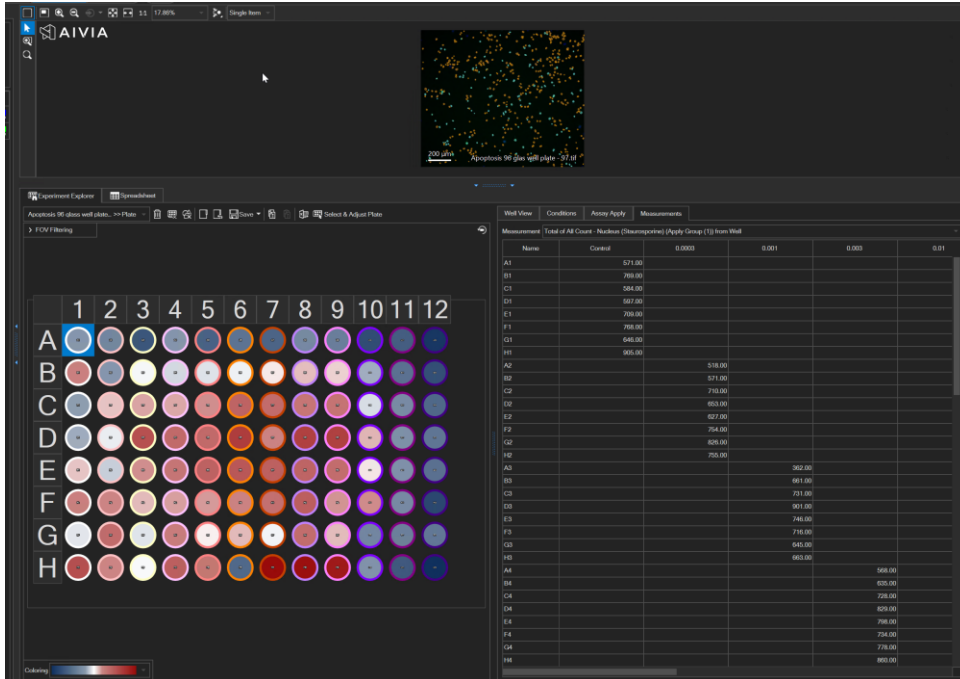
For multi-well assays, plot dose response charts with automatic curve fitting options (Michaelis-Menten and Hill), calculation of EC50 (for half-maximal effective concentration as a measure of drug potency) and R2 (coefficient of determination, an indicator of how well a regression model fits the data).



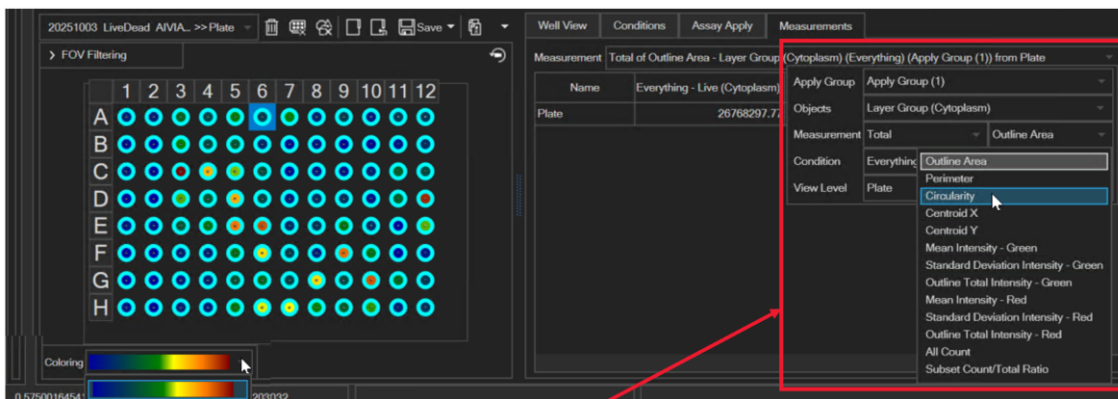
An example dose response chart with Staurosporine live dead assay by Dr. Oksana Sirenko of Molecular Devices, imaged on a Mica by Dr. Seung-Yi Lee of Leica Microsystems and analyzed using Aivia 16 by Dr. Won Yung Choi of Leica Microsystems.

Multi-well Heatmap

For multi-well plate analysis, users can visualize any well-level measurements using the integrated heatmap functionality.



Multi-well plate heatmap with Staurosporine live dead assay by Dr. Oksana Sirenko of Molecular Devices, imaged on a Mica by Dr. Seung-Yi Lee of Leica Microsystems and analyzed using Aivia 16 by Dr. Won Yung Choi of Leica Microsystems.



Available measurements for the Plate-Viewer Heatmap. Different coloring can be applied and the color of the rings can be defined in the conditions tab

Different LUTs can be applied to the plate heatmap for visualizing different aggregated measurements.

Multi-well Data Export

All the measurements for individual detected objects and aggregated summary measurements at the well and plate level per condition can be exported as .xls or .csv for easy import into Excel or statistical software such as SPSS. Conditions and condition groups already have numerical values assigned for easy insight generation upon import for analysis such as multivariate analysis or paired t-tests.

Exported files can be imported to Excel for creating charts and pivot tables.

.csv export is formatted for easy import into SPSS with all the condition groups with numerical values for statistical analysis. Aviva exported .csv files can be immediately used for statistical tests such as independent samples t-test without any variable remapping.

Chart export into presentations/documents with white background

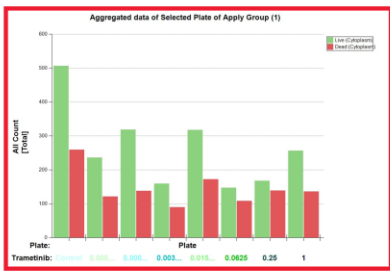
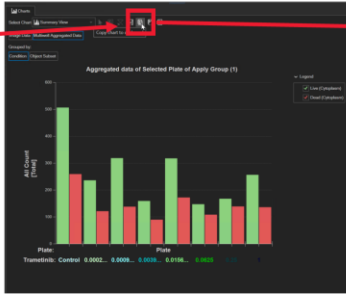
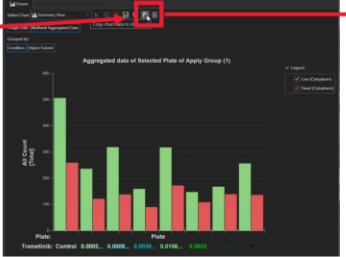


Chart DATA export into Excel for easy to view summary of the data

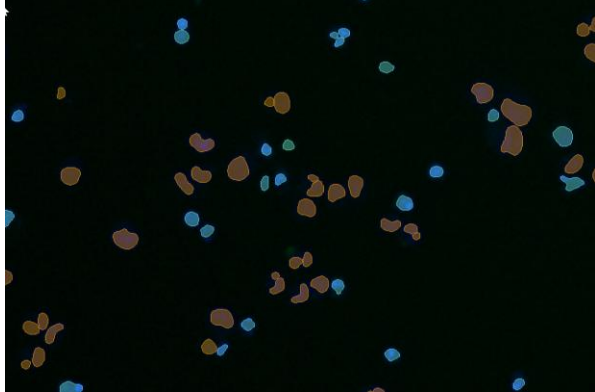


	A	B	C
1	Column1	Column2	
2		Counts	
3		[Total]	
4	Control Live (Cytoplasm)	507	
5	Control Dead (Cytoplasm)	260	
6	0.000244141 Live (Cytoplasm)	237	
7	0.000976563 Dead (Cytoplasm)	122	
8	0.000976563 Live (Cytoplasm)	319	
9	0.000976563 Dead (Cytoplasm)	138	
10	0.00390625 Live (Cytoplasm)	160	
11	0.00390625 Dead (Cytoplasm)	90	
12	0.015625 Live (Cytoplasm)	318	
13	0.015625 Dead (Cytoplasm)	173	
14	0.0625 Live (Cytoplasm)	148	
15	0.0625 Dead (Cytoplasm)	109	
16	0.25 Live (Cytoplasm)	168	
17	0.25 Dead (Cytoplasm)	139	
18	1 Live (Cytoplasm)	257	
19	1 Dead (Cytoplasm)	137	

Summary charts can be easily exported for publication or easy summary view of the data.

Self-Training 2-Class Classifier

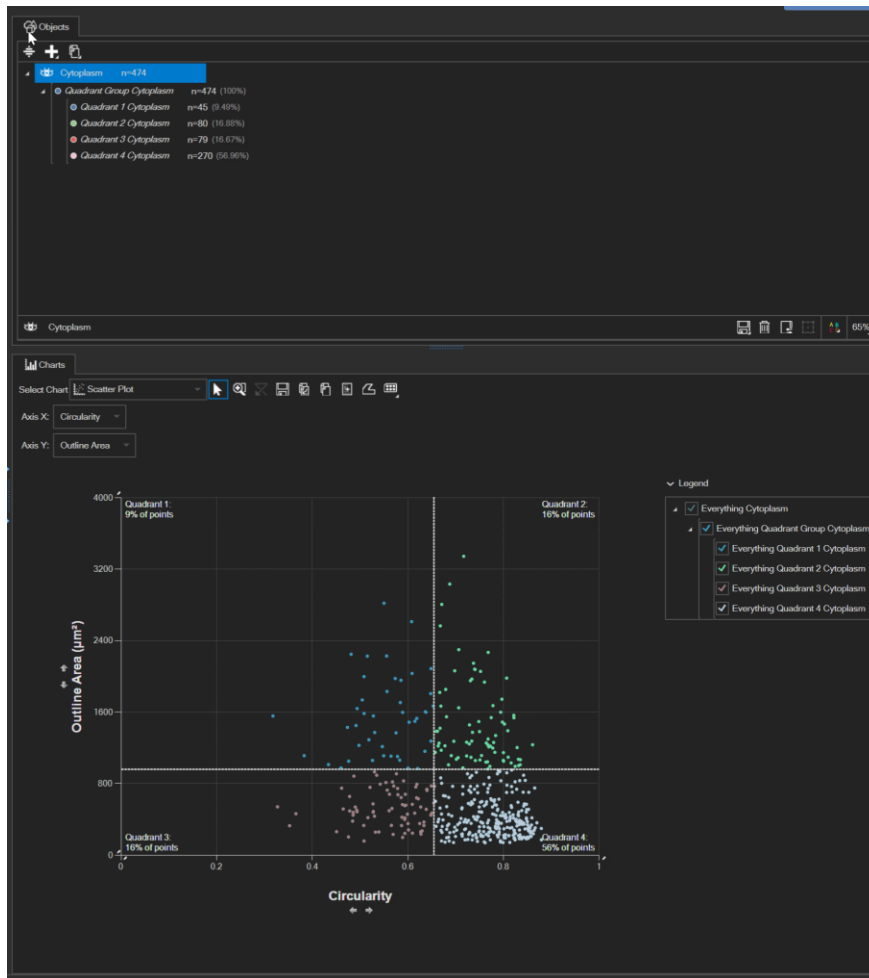
Our patent-pending Self-Training 2-Class Classifier can create and apply a random-forest classifier for 2 phenotypes based on one or two measurements chosen by the user without requiring user training of example cells. With a single click, all detected cells can be auto-assigned to two classes for training and classification without users specifying examples for each class. Assigning an object to the other class can be achieved with a single click for retraining under the hood.



Automatically classified apoptotic cells (blue) vs normal cells (red). Image credit: apoptotic assay by Dr. Oksana Sirenko of Molecular Devices, acquired on Mica by Hoyin Lai of Leica Microsystems, analyzed using Aivia 16 by Dr. Won Yung Choi of Leica Microsystems.

Automatic Quadrant Gating and Phenotyping for Assays

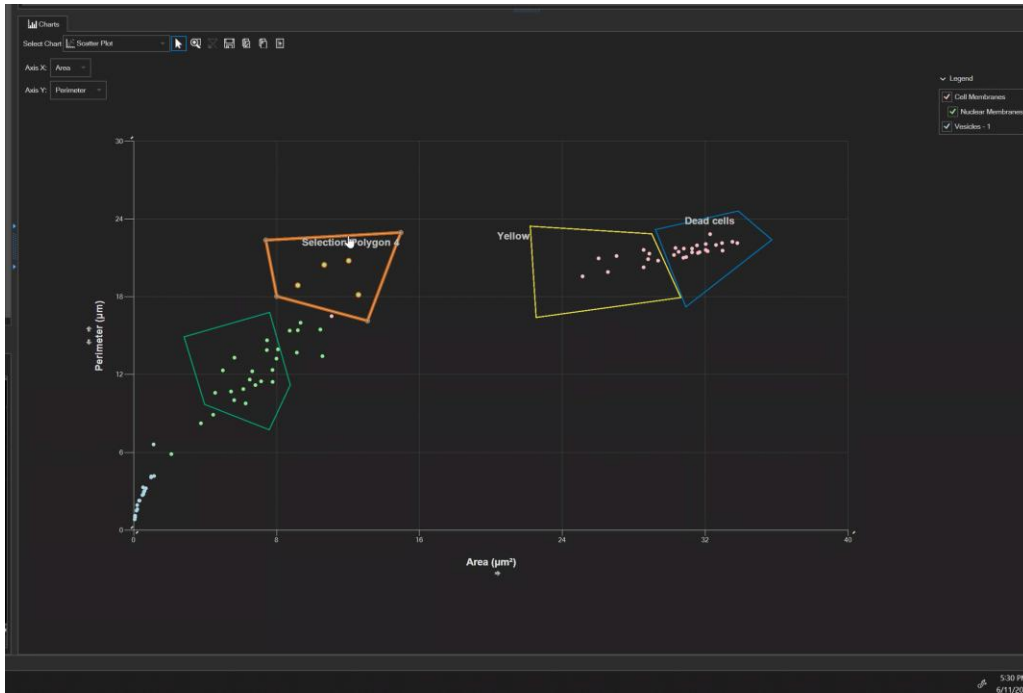
On a scatterplot, quadrants can be created by right clicking anywhere in the scatterplot. Quadrants show % of datapoints contained within each quadrant. Generate quadrant-based phenotypes with a single click.



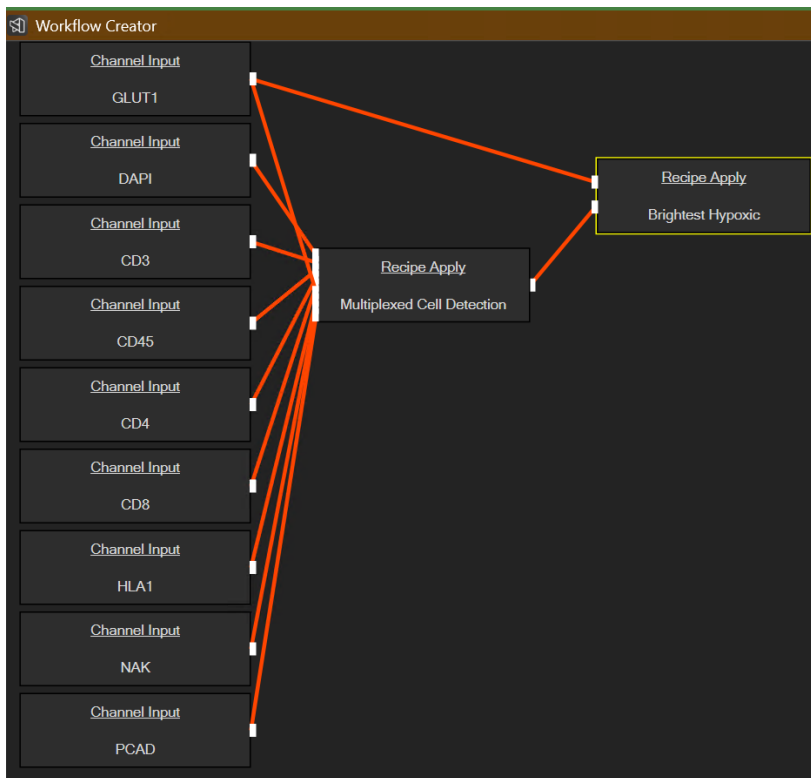
An example of quadrant-based gating along outline area and circularity dimensions. With a single click, four different phenotypes are generated from the dataset.

Uniform Gating Strategy via Scatterplot or Batchable Gate by Rules

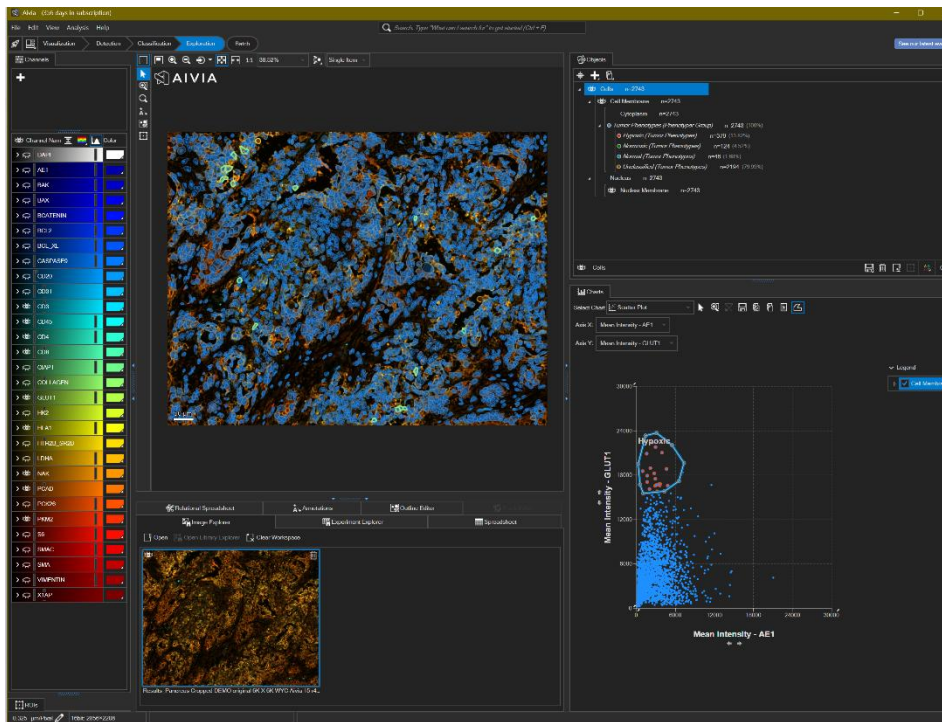
Manual polygon or rectangular gating in a scatterplot can be saved and applied on a new image for uniform gating strategy. Additionally, Gate by Rules, a flexible tool that enables users to create a series of gating rules based on measurements, can be batch-applied using the Batch workflow.



Polygon gating rules can be named, saved, and reapplied for consistent gating strategy across all the images in an experiment.



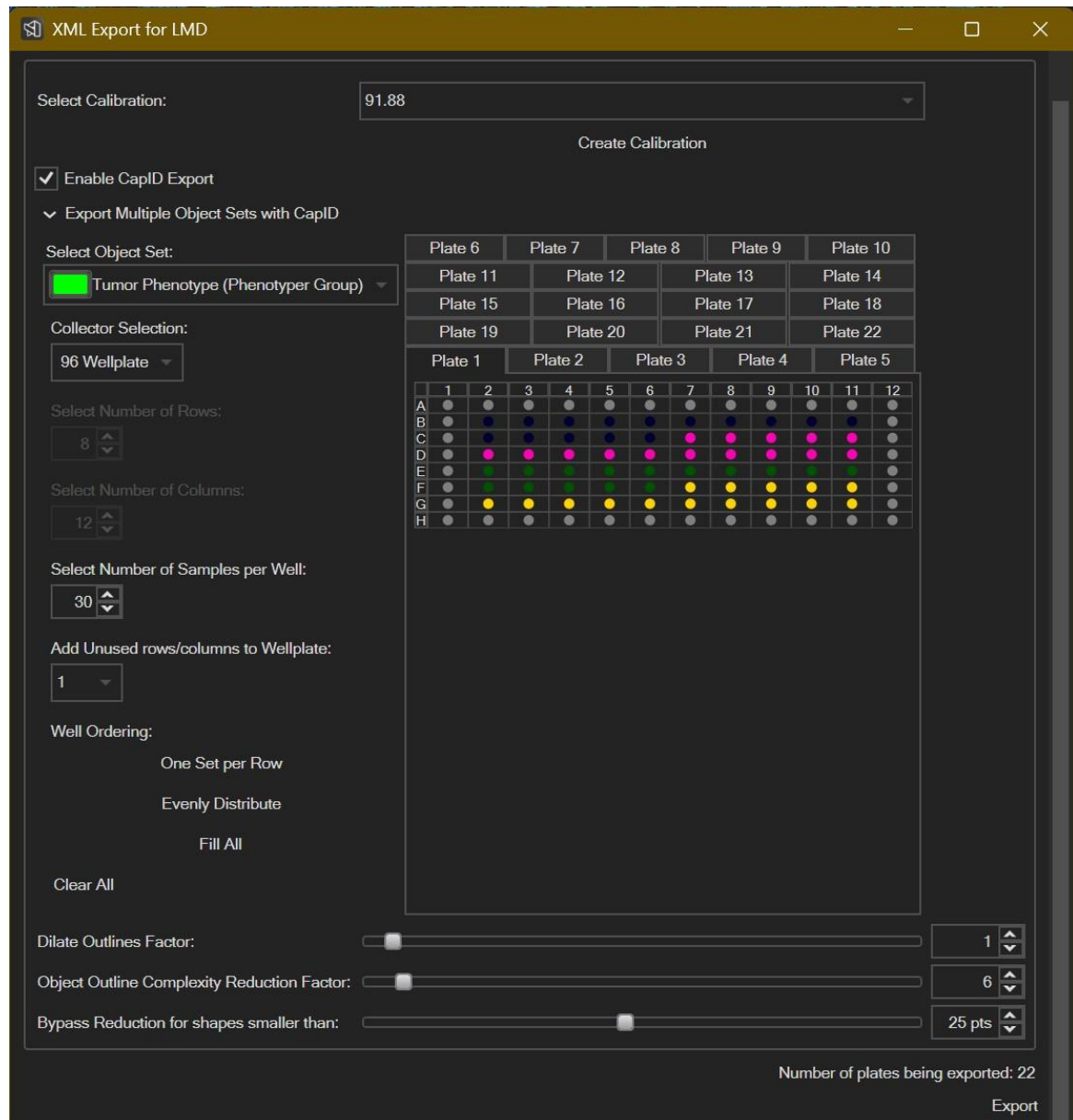
An example of Gate by Rules application (yellow box: phenotyping brightest hypoxic tumor cells) that is auto-created for batching using a single click of the Batch button for Workflow creation.



Interactive visualization of gated cells in the scatterplot in the lower right hand side shown in the image viewer in the middle (cells with orange outlines). Image courtesy of Dr. Melina Hill of Leica Microsystems imaged on Cell DIVE.

Multiple Multi-well Plate Export for LMD

For Leica laser microdissection microscopes (LMD), phenotypes generated using any classification method in Aivia can be now exported to multiple multi-well plates with automatic assignment of the samples into the wells.



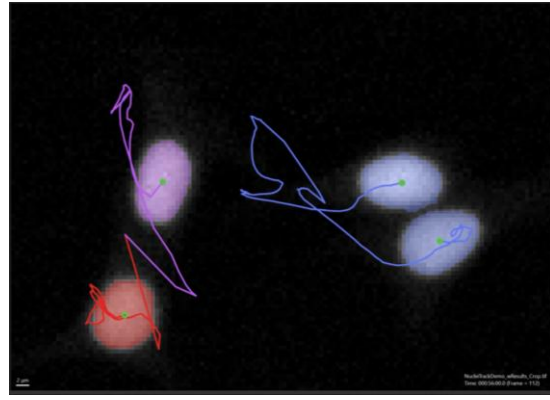
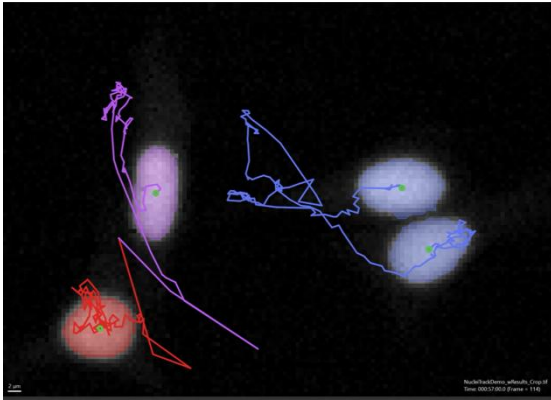
New multiple multi-plate sample export interface for the LMD system. Based on the user selection for the number of samples per well, all the cells to be collected are automatically assigned to different plates and vessels.

TauContrast Image Import Update

During the import of the TauContrast files, the calculation for the Average Arrival Time has been updated to match the LASX output, making it possible to compare results of Aivia analysis from different TauContrast images.

Track Smoothing

Multiple track smoothing options (Simple Moving Average, Exponential Moving Average, Gaussian Smoothing) are now offered for optimized visualization of tracked objects in time-lapse images. Track smoothing is purely for improved visualization and does not change the actual measurements.

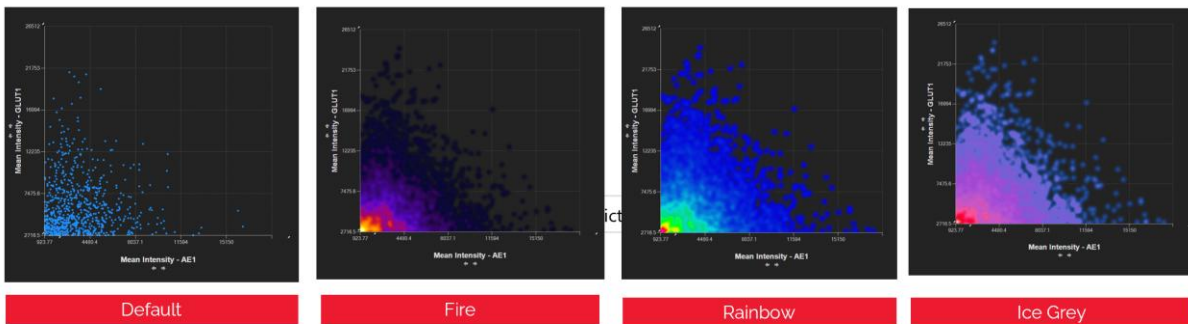


Left: track visualization in Aivia 15

Right: With simple moving average track smoothing in Aivia 16.

Density Heatmap for Scatterplot

Plot datapoints for two measurements using the scatterplot density heat map with different color schemes for easy visualization of data distribution for dense datasets.



Density heatmap options for easy visualization of densely packed datapoints in a scatterplot.

New Aivia Accelerate Package and Comparison of Features

	Go	NEW Aivia Accelerate	Elevate Cell Bio	Elevate Neuro	Apex
Large multiplexed image rendering (102 in 2D, 25 in 3D)					
Deep-learning based cell segmentation (SBE)					
Time lapse analysis					
Track smoothing			New in Aivia 16		
Pixel Classifier					
Object Classifier					
Workflow Creator batching					
Spatial relations analysis					
Charts including 2-sided violin charts, scatterplot, Pearson heatmap					
Large multiplexed image rendering (102 in 2D, 25 in 3D)					
Biologically relevant channels grouping					
Confidence mapping					
Animation					
Python plug-ins					
Bioimage.io models (26 total) drag and drop apply					
Spatial relation analysis (nearest neighbor, nearest 5, overlap)					
Phenotype export for LMD for multiple multi-wells			New in Aivia 16		
Gate by Rules batching			New in Aivia 16		
Automatic gating and phenotyping on scatterplot			New in Aivia 16		
Multiwell plate analysis (heatmap, export)			New in Aivia 16		
Assays (Live-dead, Apoptosis, Counting, Morphological and Tailored)			New in Aivia 16		
Summary Chart, dose response curve, binned scatterplot			New in Aivia 16		
Automatic classifier (Phenograph-Leiden, Self-Training 2-Class Classifier , k-means)			New in Aivia 16		
Automated Report Generation					
Phenotyper					
Dimensionality reduction (UMAP, PaCMAP, t-SNE)					
Marker-Cluster Dendrogram					
Spatial relations batching					
Automatic 2D ROI Detection					
Multiplexed cell detection by Segment by Example (SBE) in 2D/3D			New in Aivia 16		New in Aivia 16
Multi-compartment Cell Analysis by Segment by Example (nucleus, cytoplasm, vesicles) in 2D			New in Aivia 16		New in Aivia 16
Automatic neuron tracing and analysis (soma, dendrite, spines)					
Deep-learning based soma detection for dense neuronal images					
Floating License Manager					

Updated Wiki

The following Wiki pages have been updated for this release:

<ul style="list-style-type: none"> • SBE 2.0 (link)
<ul style="list-style-type: none"> • Experiment Explorer Assay Apply, multi-well heatmap & export measurements (link)
<ul style="list-style-type: none"> • Launchpad update (link)
<ul style="list-style-type: none"> • TauContrast Average Arrival Time (link)
<ul style="list-style-type: none"> • Self-Training 2-Class Classifier (link)
<ul style="list-style-type: none"> • Dose response plot (link)
<ul style="list-style-type: none"> • Gating by quadrants, selection or polygons in the scatterplot (link)
<ul style="list-style-type: none"> • Polygon gating in the binned scatterplot (link)
<ul style="list-style-type: none"> • Box or polygon gating in the dimensionality reduction plot (link)
<ul style="list-style-type: none"> • Density heatmap overlay for the scatterplot (link)
<ul style="list-style-type: none"> • Aggregated data summary view (link)
<ul style="list-style-type: none"> • Advanced measurement visual editor (link)
<ul style="list-style-type: none"> • Assay information (coming soon)
<ul style="list-style-type: none"> • SPSS/Excel import of data (coming soon)

Aivia Demo License (link)

