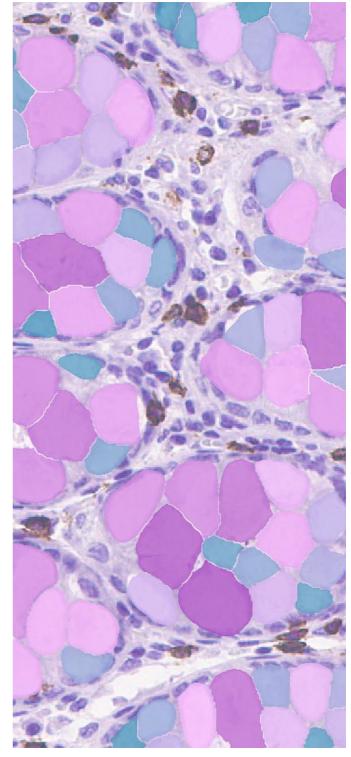
From Eye to Insight





Aivia 15

Release Notes

Image credit: ab109218 CD6 human colon image by Will Howat Ph.D. from Abcam, analysis with Aivia 15 by Won Yung Choi, Ph.D. of Leica Microsystems using Segment by Example

Leica Microsystems CMS GmbH Am Friedensplatz 3 D-68165 Mannheim, Germany

Release Documentation for Aivia 15

This document describes the version 15 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: April 23, 2025, applying to Aivia 15 release, r42239

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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 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 <u>contact us.</u>

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 15** is a major release containing 133 features, usability improvements, security updates, and 157 bug fixes since Aivia 14.1 release on September 15th, 2024. Powered by AI, Aivia 15 offers usability improvements including easy-to-deploy painting-based deep learning 2D/3D cell segmentation, and significant performance improvement for 3D segmentation to go from eye to insight faster.

Aivia 15 Key Features Summary

All Aivia Packages:

- Launchpad & Guided Sequence for the optimized workflow based on the userselected analysis endpoint and the image dimensions
- Custom analysis with Flexible Chevrons that can be modified, saved, reloaded and shared
- Segment by Example deep-learning based 2D/3D cell segmentation with a painting interface
- Faster 3D pipeline up to 69% and faster Leica file loading
- File import functionality for slide scanners (.svs, .ndpi, .mrxs, Phillips.tiff, .vms) and .qptiff
- Drag and drop import of 26 total Bioimage.io models converted to .aiviadl
- Phenotype export for Leica's LMD laser microdissection microscopes

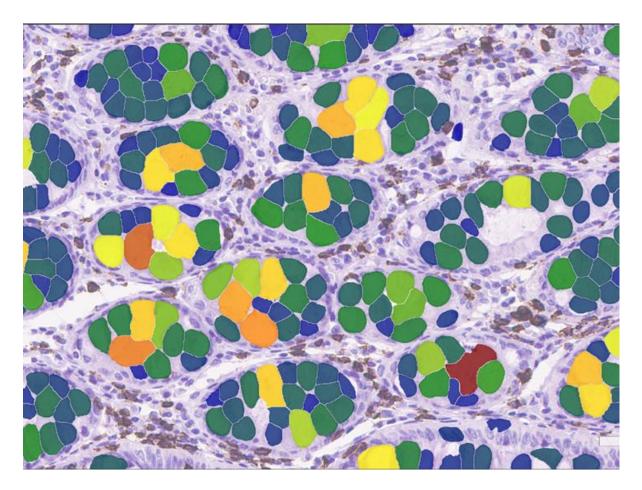
For Elevate and Apex packages only:

- Batching Spatial Relations with the Batch Workflow Creator
- Automated Report Generation for easy sharing of findings
- Heatmap binned scatterplot for insight generation from a large number of datapoints

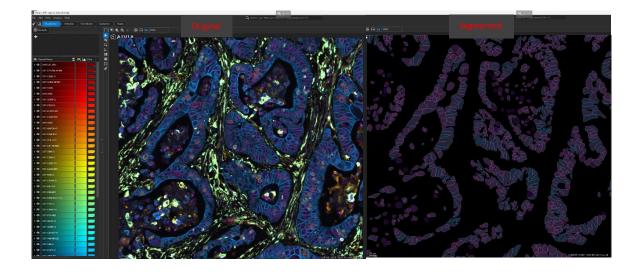
Aivia 15.0 Features (Released on April 23, 2025)

Deep-Learning Powered **Segment by Example** for 2D and 3D Cell Segmentation

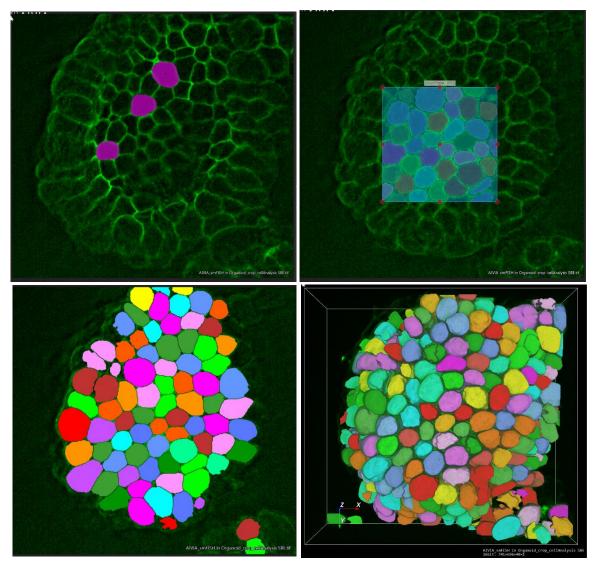
Using a simple painting interface, Segment by Example accurately detects 2D and 3D cells of interest based on a deep-learning algorithm (modified from Cellpose¹) to handle cellular variations without the need to code or train deep-learning models.



Paint on 2D or 3D cells to deploy a pre-trained deep learning model via Segment by Example for accurate segmentation. (Image credit: ab109218 CD6 human colon from Will Howat Ph.D. from Abcam, analysis with Aivia 15 Segment by Example by Won Yung Choi, Ph.D. of Leica Microsystems).



48 channel human colon channel image acquired on a Leica Cell DIVE system, analyzed using Aivia Segment by Example and colored by circularity measurements. (Image credit: Melinda Hill, Ph.D. of Leica Microsystems, analyzed in Aivia by Quyen Tran, Ph.D. of Leica Microsystems)

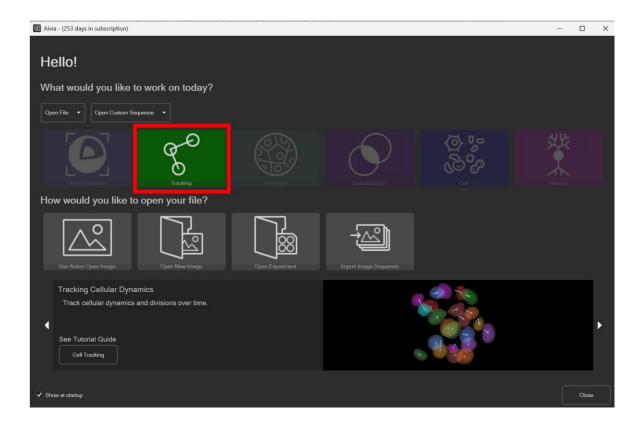


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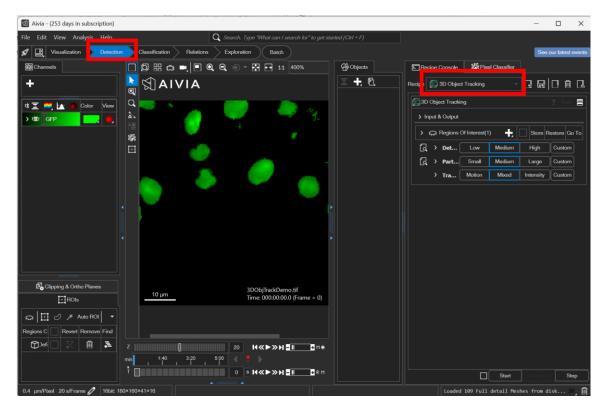
An example of a 3D intestine organoid with cells detected by Segment by Example by painting on a few cells on a single z-plane using only the membrane signal. **Top Left**: Example of painted cells. **Top Right**: Preview in an ROI. **Bottom Left**: Segmentation results viewed on a single 2D plane. **Bottom right**: Painting on 5 cells on a single Z-plane can produce complete 3D meshes using Segment by Example (Image credit: Intestine organoid by Andreas Moor, Ph.D. at ETH Zurich, analyzed using Segment by Example by Won Yung Choi, Ph.D. of Leica Microsystems)

Optimized Analysis with Aivia Launchpad

Aivia Launchpad simplifies quantitative analyses with pre-set Guided Sequences and tutorials. Aivia presents relevant analysis options based on the quantitative endpoint the user selects and image dimensions. The Flexible Chevrons can be used to create customized analysis sequences that can be modified, saved, reloaded, and shared.



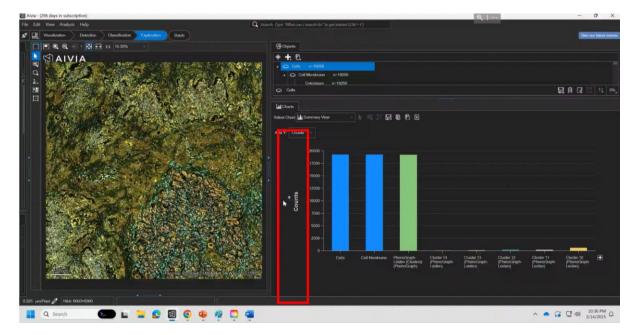
Aivia Launchpad helps users to get started on the target analysis with a Tutorial Guide and by launching the pre-set Guided Sequence for the analysis workflow.



Selecting "Tracking" as the endpoint on the Analysis Launchpad launches the appropriate recipe ("3D Object Tracking" recipe in this example) that is matched to the dimension of the image.

Data Exploration for Deeper Insights

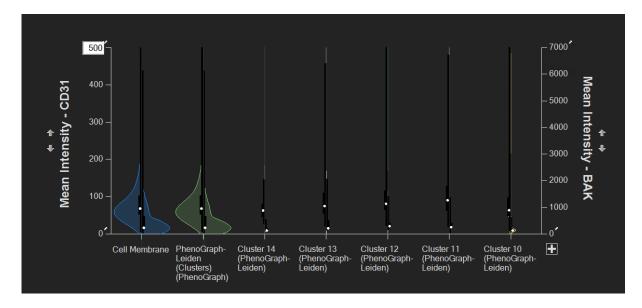
Use Measurement Selectors to quickly navigate charts through different measurements.



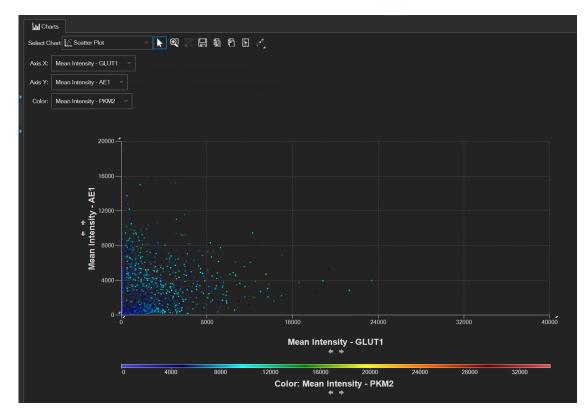
Page 10 of 23 (MC-0011015) Won Yung Choi, Ph.D. Product Owner & Manager – Data & Analysis (<u>wonyung.choi@leica-microsystems.com</u>) Generate rapid insights from different measurements by updating charts with Measurement Selectors (Image credit: pancreatic ductal cancer tissue section by Melinda Hill, Ph.D., Leica Microsystems., analyzed using Aivia by Won Yung Choi, Ph.D. of Leica Microsystems.)



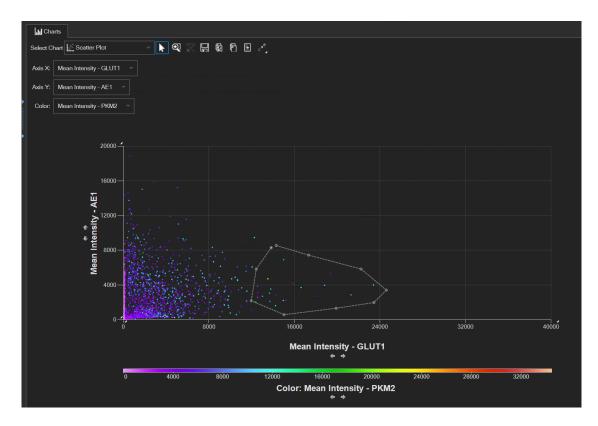
Measurement selector is available for both axes for the Scatter plot for easy navigation of different measurements.



Measurement selector is also available for the two-sided violin plot for easy exploration of measurements and the maximum value for each axis is user-editable.

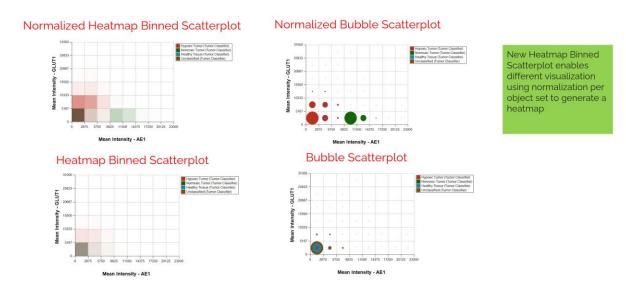


A heatmap view is also available for the scatterplot for any measurements.



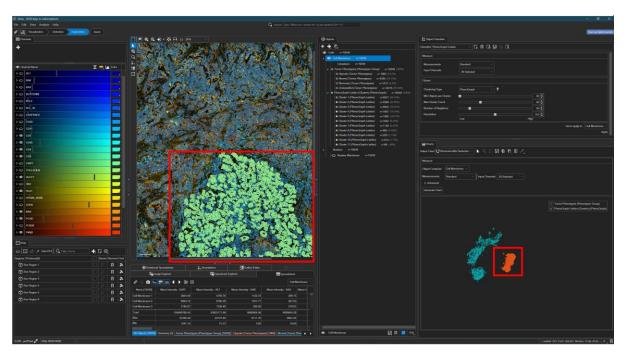
Gate any number of objects based on measurements using polygons or rectangles.

Gain insights from large datasets with our new heatmap binned scatterplot visualization.

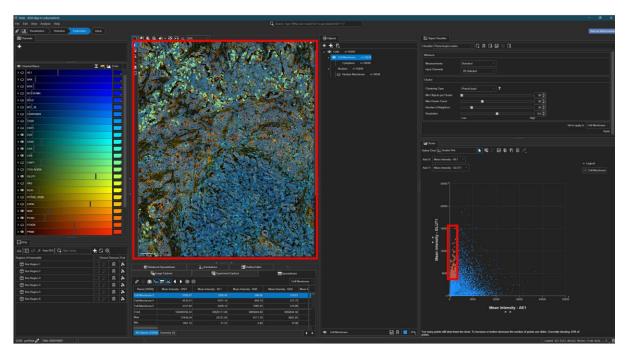


Examples of varied binned scatterplots that summarize the distribution pattern of a large number of datapoints (19,248 cells).

Phenotype by gating based on measurements or gating directly in charts (scatterplot/dimensionality reduction) to train classifiers.



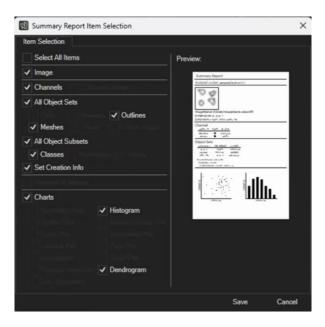
Gating for cells using UMAP dimensionality reduction for supervised classification using the Phenotyper.



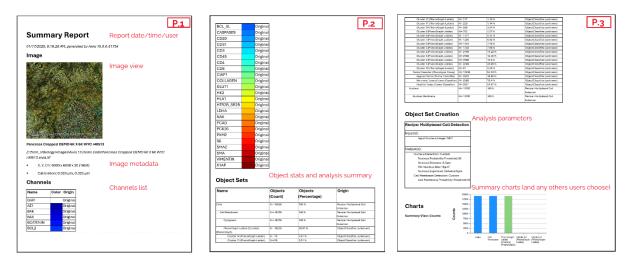
Gating for cells in a scatterplot based on measurements for supervised classification using the *Phenotyper*.

Automatic Summary Reports for Insight Sharing

Share your findings with automated summary reports, including metadata, segmentation settings, phenotyping results, and charts exportable in .html, .docx, and .md file formats



Summary reports can be generated automatically with options for users to edit before exporting.



Example of an automatically generated summary report, user has options to add or remove any content.

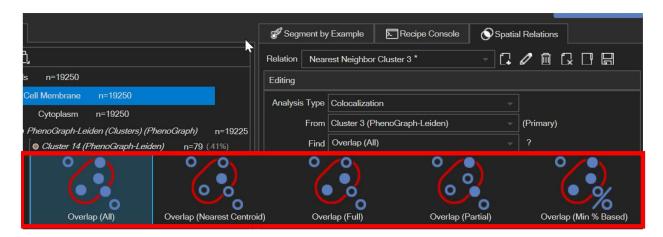


Add any chart to the summary report.

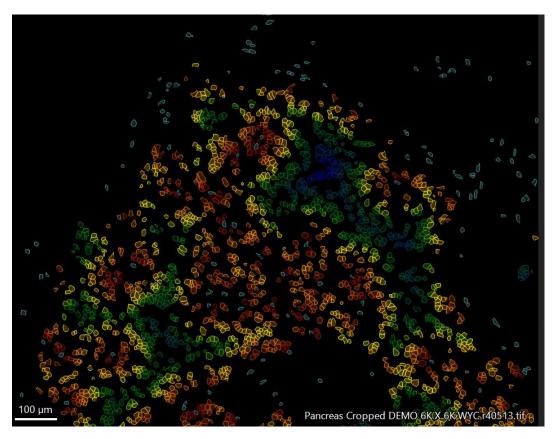
Performance Improvement

3D detection speed has improved up to 69%² with our fastest mesh creation and smoothing for all 3D analysis pipelines. Loading of Leica LASX files are up to 99% faster³.

Generate insights about object-based colocalization (partial, full and %-based overlap), and nearest neighbor analysis (nearest, nearest 5, and all in the search range) for any object sets or phenotypes.



Updated Spatial Relations tool enables colocalization and nearest neighbor analysis for any set of objects or phenotypes.

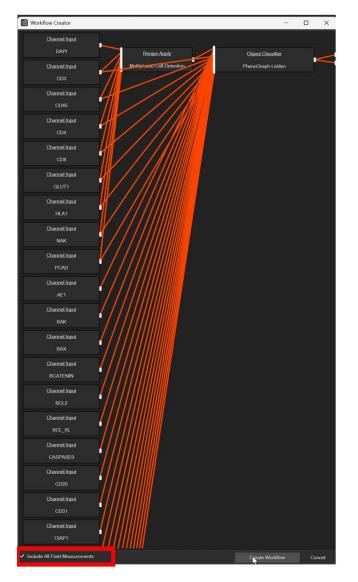


An example of a heat map of the nearest neighbor analysis between Cluster 3 (cyan cells) to Cluster 12 (rainbow heatmap) with the nearest cells in **red**, the furthest cells in **blue**

Easily create batch analyses for objects, phenotypes, spatial relations, classification, and channel modification with the Workflow Creator.

ysis	Theip		_		
\geq	Detection	Classification	Exploration	Batch	
			Objects	• n	
ſ	Nearest Ne	ighbor	Relations	• .	~
		Nearest Neighbor		0	K

Any channels, objects, and relations can be set for batching using the new Workflow Creator which includes all the steps needed to get to the endpoint. By selecting the Nearest Neighbor batching option, all the steps leading up to that analysis (pre-processing, segmentation of objects, classification, and spatial relation analysis) can be automatically set up for batching.



Workflow Creator displays all the input and outputs for each step for verification before starting the batch process. Optionally include all measurements related to the chosen objects or spatial relations.

Phenotype Export for Leica's Laser Microdissection Microscopes

Export cells based on measurements, object class, or classified phenotypes for laser dissection on Leica LMD microscopes, with automated assignment to collection tubes or multi-well plates.

Export Multiple Object Sets with CapID elect Object Set:		1	2	3	4	5	6	7	8	9	10	11	12
PhenoGraph-Leiden (Clusters) (PhenoGraph)	- A	•		•		•							
ollector Selection:	В	•	•	•	•	•	•	•	•	•	•	•	•
96 Wellplate	С												
	D	•	•	•	•	•	•	•	•	•	•	•	•
	E	•	•	•		•	•	•		•	•	•	•
	F							•		•		۲	•
	G	•	•	•	•	•	•	•	•	•	•	•	•
Select Number of Samples per Well:	H	•	•	•	•	•	•	•	•	•	•	•	•
1													
dd Unused rows/columns to Wellplate:													
0 -													
Vell Ordering:													
One Set per Row													

Automatically assign any cells or phenotypes into multi-wells or collection tubes for Leica Laser Microdissection microscopes

Bioimage.io Models Converted to .aiviadl for Drag-and-Drop Apply in Aivia

Supporting active collaboration with the scientific open-source community, we now offer drag-and-drop import of 26 Bioimage.io models (converted to .aiviadl) directly into Aivia's recipe console. The re-packaged models are available on Aivia's model library (https://www.aivia-software.com/model-library).

iber v	Name	Aivia compatible versions 🗸	Model name	Description
9	egmentation for bacteria -		bioimageio-001-Segmentation-bacteria-105281_zenodo_7261974.aiviadl	2D U-Net model segments the contour, foreground and background of Bacillus Subtili
1 T	ensorflow version			bacteria imaged with Widefield microscopy images.
	iegmentation for bacteria - Keras Pytorch version	15	bioimageio-001-Segmentation-bacteria-105281_zenodo_7261974_keras.aiviadl	2D U-Net model segments the contour, foreground and background of Bacillus Subtili bacteria imaged with Widefield microscopy images.
	Pancreatic cell segmentation -	12.1.13.0.14.0	bioimageio-002-Pancreatic-cell_segmentation-105281_zenodo_5914248.aiviadl	U-Net trained to segment phase contrast microscopy images of pancreatic stem cell
	ensorflow version			on a 2D polystyrene substrate.
F	ancreatic cell segmentation - Keras	15	bioimageio-002-Pancreatic-cell_segmentation-105281_zenodo_5914248_keras.aiviadl	U-Net trained to segment phase contrast microscopy images of pancreatic stem cell
	ytorch version			on a 2D polystyrene substrate.
3	luclei cell segmentation	15.0	bioimageio-003-HPA_nucleus-105281_zenodo_6200999.aiviadl	Nuclei segmentation model for segmenting images from the Human Protein Atlas
4	Body Cell segmentation	12.1, 13.0, 14.0, 15.0	bioimageio-004-HPA_cell_body-105281_zenodo_6200635.aiviadl	Cell Body segmentation model for segmenting images from the Human Protein Atlas
	leuron Segmentation in EM	13.0 to 15.0	bioimageio-005-CREMI_3D_EM-105281_zenodo_5874741.aiviadl	EM neuron segmentation for CREMI 3D challenge.
	D UNet Mouse Embryo Fixed	13.0 to 15.0	bioimageio-006-3D_MouseEmbryoFixed-105281-zenodo_6383429.aiviadl	A 3D U-Net trained to predict the nuclei and their boundaries in fixed confocal image
6	b offer house Emplye haed	1010 10 1010	bisingPero oco op_i logocciup for inco 100501 fellogo_000045900000	developing mouse embryo.
3	D Unet Mouse Embryo Live	13.0 to 15.0	bioimageio-007-3DMouseEmbryoSegmentation-105281_zenodo_6384845.aiviadl	A 3D U-Net trained to predict the cell boundaries in live light sheet images of develop mouse embryo.
8	lucleiSegmentationBoundary	13.0 to 15.0	bioimageio-008-NucleiBoundary_Segmentation-105281_zenodo_5764892	This model segments nuclei in fluorescence microscopy images. It predicts boundar maps and foreground probabilities for nucleus segmentation in different light microscopy modalities, mainly with DAPI staining.
	CovidIFCellSegmentationBoundaries	13.0 to 15.0	bioimageio-009-CovidIFCellSegmentationBoundary-105281_zenodo_5847355.aiviadl	This model segments cells in immunofluorescence microscopy images. It predicts
9	South CelloeginentationDoundaries	10.0 10 10.0	bonnagen 000 Contan Geneegmentationboandary 100201_tenoto_007/000.amaat	boundary maps and foreground probabilities and was trained on Vero E6 cells image
		13.0 to 15.0		with a high-throughput-microscope, as part of a Covid19 antibody test.
10	iveCellSegmentationBoundary	13.0 to 15.0	bioimageio-010-LiveCell_Segmentation-105281_zenodo_5869899.aiviadl	This model segments cells in phase-contrast microscopy images, which are often us in live-cell imaging. It predicts boundary maps and foreground probabilities. The boundaries can be processed e.g. with Multicut or Watershed to obtain an instance segmentation.
11 2	D Unet Arabidopsis Ovules	13.0 to 15.	bioimageio-011-2D_ArabidopsisOvule-105281-zenodo-7805067.aiviadl	A a variant of 2D U-Net trained to predict the cell boundaries in confocal stacks of Arabidopsis ovules.
	D UNet Arabidopsis Apical	13.0 to 15.	bioimageio-012-2D_ArabidopsisApicalStem-105281-zenodo-6334881.aiviadl	2D Unet trained on z-slices of confocal images of Arabidopsis thaliana apical stem c
	ID Unet Lateral Root Primordium	13.0 to 15.	bioimageio-013-3D_ArabidopsisLateralRoot-105281-zenodo-6334777.aiviadl	A 3D U-Net trained to predict the cell boundaries in lightsheet stacks of Arabidopsis Lateral Root Primordia.
	D Unet Arabidopsis Ovules	13.0 to 15.	bioimageio-014-3D_ArabidopsisOvuleNuclei-105281-zenodo-7772662.aiviadl	Unet trained on confocal images of Arabidopsis Ovules nuclei stain with BCEDiceLos
14	o one (nuo dopoio o tateo	1010 10 101		The network predicts 1 channel: nuclei probability maps.
3 15	D UNet Arabidopsis Ovules	13.0 to 15.	bioimageio-015-3D_ArabidopsisOvuleCells-105281-zenodo-6334583.aiviadl	A 3d U-Net trained to predict the cell boundaries in confocal stacks of Arabidopsis ovules.
	D Unet Arabidopsis Apical Stem	13.0 to 15.	bioimageio-016-3D ArabidopsisApicalStem-105281-zenodo-6346511.aiviadl	3D Unet trained on confocal images of Arabidopsis thaliana apical stem cell.
	fitochondriaEMSegmentation	15	bioimageio-017-Mitochondria_EM_Boundary-105281_zenodo_5874741.aiviadl	Segments Mitochondria in EM neuron images (3D).
F	PlatynereisEMnucleiSegmentationBo IndaryModel	15	bioimageio-018-Platynereis_Nuclei_EM_Boundary-105281_zenodo_6028097.aiviadl	Segments cell nuclei + boundaries Platynereis 3D
F	PlatynereisEMcellSegmentationBoun laryModel	15	bioimageio-019-Platynereis_Cells_EM_Boundary-105281_zenodo_6028280.aiviadl	Segments cell boundaries Platynereis 3D.
	1itochondriaEMSegmentation2D	15	bioimageio-020-Mitochondria_EM_Boundary-105281_zenodo_6406803.aiviadl	Segments mitochondria in serial sections of TEM (ssTEM).
	inhancerMitochondriaEM2D	15	bioimageio-021-EnhancerMitochondriaEM2D-105281_zenodo_6406756.aiviadl	Uses input probability from Random Forests to generate a more reliable prediction o mitochondria (2D sections)
E	nhancerMitochondrialEM3D	15	bioimageio-022-EnhancerMitochondriaEM3D-105281_zenodo_6811491.aiviadl	Uses input probability from Random Forests to generate a more reliable prediction o
22				mitochondria (volume, 3D)
	M3DBoundaryEnhancer	15	bioimageio-023-EnhancerBoundariesEM3D-105281_zenodo_6808325.aiviadl	Another enhancer, this time for any cell membrane boundaries.
	nhancerBoundaryEM2D	15	bioimageio-024-EnhancerBoundariesEM2D-105281_zenodo_8142283.aiviadl	Same, 2D this time
25 (CebraNET /itoNet	15 15	bioimageio-025-CebraNET-105281_zenodo_7274275.aiviadl bioimageio-026-MitoNet-stupendous-sheep.aiviadl	Generates membrane probability from SEM 3D cubes. This model generates probability of mitochondria

26 Bioimage.io models now available as .aiviadl models for drag-and-drop directly into Aivia's recipe console

Expanded File Format Support for Virtual Slide Scanners and .qptiff

Aivia can now import files from virtual slide scanners (Aperio .svs, Hamamatsu .ndpi/.vms, Mirax .mrxs, and Phillips .tiff) and Akoya .qptiff for spatial biology analysis and interactive exploration of multiplexed data.

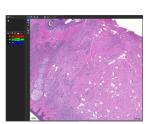
Aperio.svs

Hamamatsu .ndpi

Mirax .mrxs

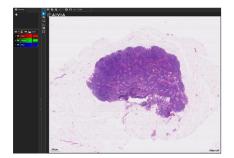






Phillips .tiff

Hamamatsu .vms

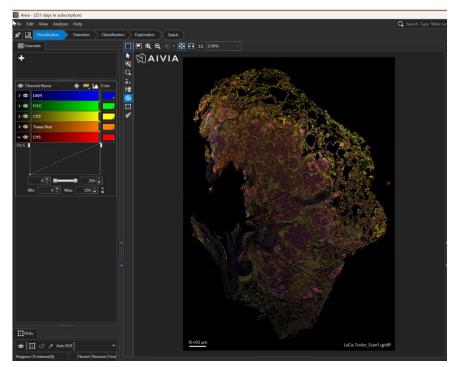


Virtual slide public files from Openslide.org



The following virtual slide scanner files now open in Aivia via openslide (up to 3 channels): Aperio .svs, Hamamatsu .ndpi, Mirax .mrxs, Phillips .tiff, Hamamatsu .vms to be used for downstream cell segmentation, phenotyping, and spatial analysis.

Virtual slide files can be imported into Aivia for downstream segmentation, classification and data exploration for insights.



LuCa-7color_Scan1.qptiff from openmicroscopy.org

Akoya's .qptiff files can be natively handled in Aivia for file opening, and for downstream spaital biology analysis including multiplexed cell detection.

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New Features for Subscribers in Version 15

NEW Aivia 15 Features	Aivia Go	Aivia Elevate	Aivia Apex
Launchpad & Guided Sequence	\checkmark	\checkmark	\checkmark
Custom analysis with flexible chevrons	\checkmark	\checkmark	\checkmark
Segment by Example (SBE)	\checkmark	\checkmark	\checkmark
Faster 3D pipeline	\checkmark	\checkmark	\checkmark
Faster Leica file loading	\checkmark	\checkmark	\checkmark
New file format import	\checkmark	\checkmark	\checkmark
Workflow Creator batching	\checkmark	\checkmark	\checkmark
Chart measurement selector	\checkmark	\checkmark	\checkmark
New Bioimage.io models (26 total)	\checkmark	\checkmark	\checkmark
Phenotype export for LMD multi-well	\checkmark	\checkmark	\checkmark
Spatial Relations batching	×	\checkmark	\checkmark
Automated report generation	×	\checkmark	\checkmark
Heatmap binned scatterplot	×	\checkmark	\checkmark
Pre-15 Distinguishing Features	Neither Neuron nor Cell recipes	Neuron or Cell recipe	Both Neuron and Cell recipes + Floating License Manager

Updated Wiki

The following Wiki pages have been updated for this release:

- Launchpad (link)
- Workflow Creator (code name Rubber Stamp) (link)
- Flexible Chevrons (link)
- Spatial Relations tab (link)
- Library Explorer for importing Leica library files (link)
- Summary report export (<u>link</u>)
- Charting improvements (update to existing pages) (link)
- LMD export (<u>link</u>)
- Segment by Example (link)
- BioZoo New Models (<u>link</u>)
- Golgi-stained neuron tracing (link)
- Workflow Processor (link)

References

1. Stringer C, Wang T, Michaelos M, Pachitariu M. Cellpose: a generalist algorithm for cellular segmentation. Nature Methods. 18: 100-106. (2021)

2. Speedup compared to Aivia 14

3. Compared to LASX 4.8.29041 when project files are smaller than 175 GB or have fewer than 190 images with the thumbnails off



Aivia Demo License (link)