

AI for Advanced Image Analysis

A Practical Guide for Microscopy Analysis
with ZEISS Software



Seeing beyond

Foreword

As the CEO of Carl Zeiss Microscopy, a global leader in microscopy and imaging solutions, it gives me great pleasure to introduce this book on AI for image analysis. We at ZEISS believe that technology can be a powerful tool for driving innovation and advancing science and we are proud to be leading the charge in the field of microscopy and imaging solutions.

This book is not just a collection of technical information: it is a source of inspiration for anyone who wants to unlock the full potential of AI in microscopy. Using Machine Learning and Deep Learning, we can now achieve results that were once thought impossible. The examples and case studies included in this book are a testament to the transformative power of AI in image analysis.

At ZEISS, we are committed to pushing the boundaries of what is possible and we are proud to be at the forefront of this exciting new field of AI-powered image analysis. Whether you are a researcher, clinician, or engineer, I believe this book will be a valuable resource for unlocking the full potential of AI in microscopy for you.

Dr. Michael Albiez

Member of the Management Board IQR & Head of SBU RMSZEISS
President & CEO Carl Zeiss Microscopy GmbH

As the head of sales and service for Carl Zeiss Microscopy, I am excited to introduce this book on the power of AI for image analysis. Our teams work tirelessly with our customers to provide the tools and support needed to achieve their goals, and AI technology is a game-changer that can supercharge their success. AI and Machine Learning are transforming the field of image analysis, and this book provides a comprehensive guide to these powerful new technologies. It covers the basics of AI and provides practical examples of how to apply these concepts to microscopy image analysis.

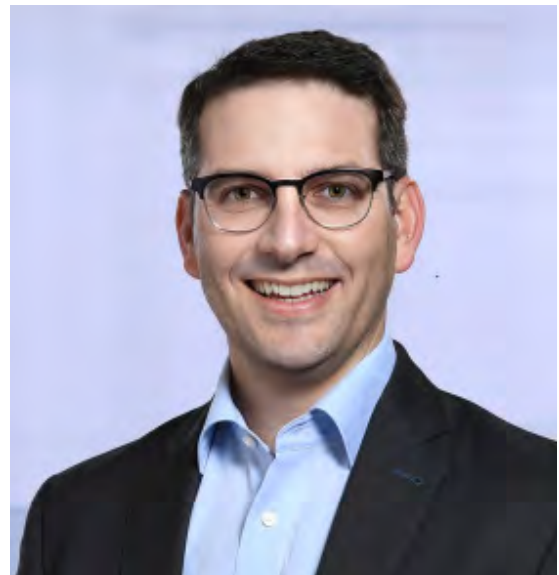
At ZEISS, we believe that AI can make our customers' lives easier by reducing manual time overhead in their workflows, both in terms of microscope hardware and software. We are proud to be pioneers in this exciting field and hope that our book will inspire and empower others in the microscopy community to take advantage of the incredible benefits of AI

Martin Fischer

Head of Global Sales & Service
ZEISS Research Microscopy Solutions



“We at ZEISS believe that technology can be a powerful tool for driving innovation and advancing science and we are proud to be leading the charge in the field of microscopy and imaging solutions.”



“AI and Machine Learning are transforming the field of image analysis, and this book provides a comprehensive guide to these powerful new technologies.”

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Cover image: The cover picture displays a cross-sectional view of an intestinal gut organoid captured at 20X magnification on ZEISS Celldiscoverer 7 and segmented using ZEISS arivis Pro image analysis software. The image highlights cell layer nuclei in red and luminal nuclei in yellow. For more information, please see *Chapter 5, section 3*.

The chapters in this eBook employ the new product names for arivis products, which have been rebranded by ZEISS following the acquisition. Specifically, arivis Vision4D is now known as ZEISS arivis Pro, arivis VisionHub as ZEISS arivis Hub, APEER cloud platform as ZEISS arivis Cloud, and the AI tools from APEER as arivis AI toolkit. It's worth noting that ZEISS offers all its data-agnostic image analysis tools under the ZEISS arivis product category. No matter the source, size or complexity of the image, ZEISS arivis family of integrated software products will help take your analysis results to new heights.

What is AI and why does it matter?

Why you need AI in your research

In 1955, John McCarthy, Assistant Professor of Mathematics at Dartmouth College, coined the term 'Artificial Intelligence' to represent the field of thinking machines, including cybernetics, automata theory, and complex information processing [1]. Today, Artificial Intelligence (AI) refers to the collection of techniques that mimic human intelligence in performing tasks.

AI has become ubiquitous in the 2020s, helping us in many aspects of our lives, from acting as personal assistants and delivering customized information on social media, to driving automobiles and trading stocks. In recent years, it has become popular to use AI capabilities for diverse image-processing applications. In research, AI has the potential to solve many challenges by enabling faster, more accurate analysis of large amounts of data. AI can significantly impact biotechnology, where it can optimize the drug discovery and development process, reducing the time and cost of bringing new therapies to market. AI can also benefit diverse image analysis applications, such as analyzing medical images to help

diagnose diseases and predict which treatments will likely be most effective for an individual patient.

While AI technology is rapidly developing, certain challenges hinder the adoption of AI in biomedical applications. Developing AI systems can be expensive for biotech startups, especially when hiring skilled personnel to develop and maintain AI systems. There are also ethical concerns around the use of AI for biomedical applications. Despite these objections, AI has seen rapid adoption in the past decade, primarily driven by its ability to solve challenges quickly. The exponential growth in AI-related publications reflects the technology adoption by the scientific community (see *Figure 1*).

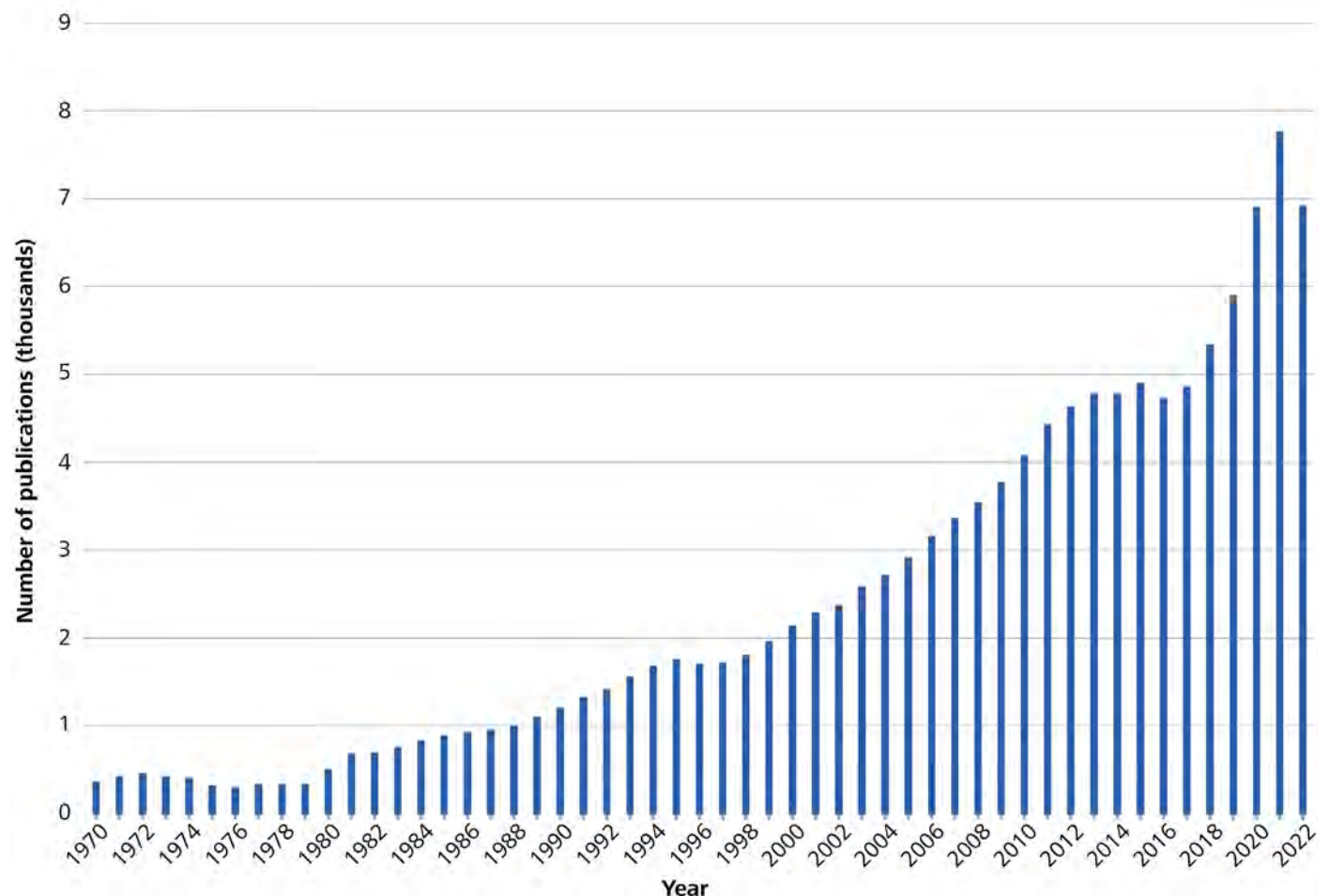


Figure 1: There has been a nearly exponential growth in the number of biomedical publications related to AI, including Machine Learning and Deep Learning, since the year 2000. (Data sourced from [PubMed](#) January 2023).

AI, Machine Learning, and Deep Learning: What is the difference?

Artificial intelligence, Machine Learning, and Deep Learning are related but distinct terminology (see *Figure 2*).

Artificial intelligence is the broadest term and describes techniques that mimic human intelligence in performing tasks. AI-related biomedical publications in the past decade primarily focused on solving challenges using Machine Learning and Deep Learning techniques.

Machine Learning is a subfield of AI that focuses on learning from data and improving processing efficiency and accuracy over time with experience. There are several Machine Learning algorithms available, encompassing various learning approaches such as supervised, unsupervised, and reinforcement learning.

Deep Learning is a Machine Learning technique that trains artificial neural networks on a large dataset, allowing them to learn and make independent, intelligent decisions. These networks have gained popularity due to their ability to learn and improve accuracy over time without explicit programming. They are well suited to solving image analysis challenges that require algorithms to identify complex patterns and features in the data. It is worth mentioning that, for the purposes of this book, a distinction is made between Deep Learning and non-Deep Learning-based algorithms. The latter algorithms are referred to as 'conventional' Machine Learning techniques.

Conventional Machine Learning vs. Deep Learning for image analysis

Conventional Machine Learning can learn from a small amount of data, but an expert engineer needs to handpick features to feed into a classification algorithm such as Random Forest [2] or Support Vector Machines [3] (SVM). Features can be obtained from training images through the use of digital image filters such as Sobel,

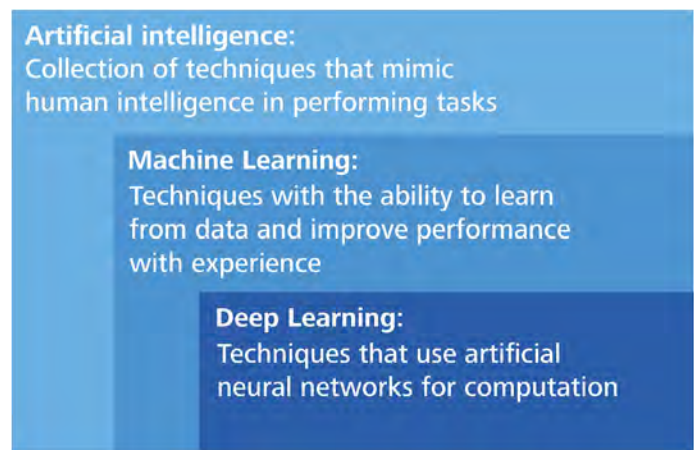


Figure 2: Deep Learning is a powerful subset of Machine Learning, which in turn is a subset of the broader field of artificial intelligence.

Entropy, and Gabor [4]. Alternatively, Deep Learning networks trained on extensive datasets can be utilized as a method for feature extraction instead of manual feature crafting. These approaches are ideal for scenarios where future data is not anticipated to vary much from the data used to train the model. For example, a small region of interest (ROI) from a large image can be used to train a model, which can then process the entire large image (see *Figure 3*). Similarly, users can take random 2D slices from a 3D volume to train a model to process the whole 3D dataset.

A conventional Machine Learning model may not work well on datasets distinct from the training data because the handful of parameters used by Machine Learning cannot be tuned to anticipate the variability in future data. Additionally, a handful of parameters is insufficient to capture the complexity in certain data making the model fail at solving complex challenges.

For example, conventional Machine Learning fails at segmenting organelles in an electron micrograph of a cell where the objects of interest (e.g., mitochondria) show up against a busy background (see *Figure 4*).

Deep Learning does not require hand-tuning of features by an

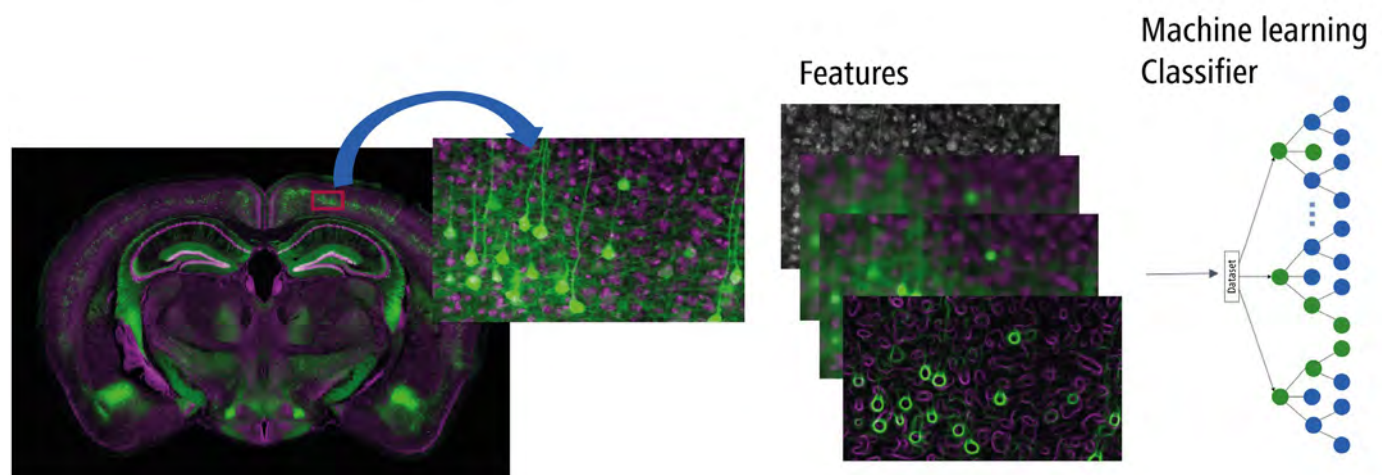


Figure 3: Training a model on a small ROI to create the Machine Learning-driven classifier. The figure shows a mouse brain cross-section imaged at 10x using ZEISS LSM980 with Airyscan. Sample courtesy of Prof. Jochen Herms, LMU München, Germany.

expert. It optimizes millions of parameters during training without humans explicitly engineering the features. These algorithms can learn multiple levels of detail and significance in the data, allowing them to identify high-level features important for the task.

This ability to learn by tuning millions of parameters using a vast amount of data makes Deep Learning algorithms generalizable to handle data with large variations, such as microscopy data that can vary because of sample preparation, lighting, background, objective, etc. This large number of features also enables Deep Learning to solve complex challenges, such as segmenting organelles against a busy background (see *Figure 5*). However, it is essential to note that Deep Learning algorithms learn from the given data. If the training data does not contain sufficient examples of the variations, the model may not perform well on those variations.

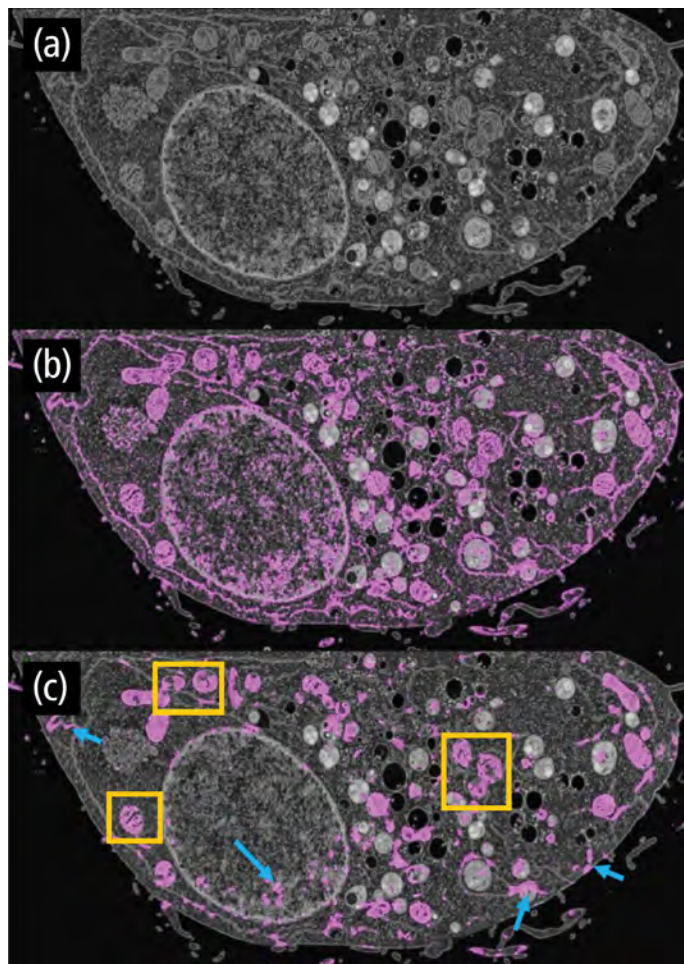


Figure 4: (a) A slice from a FIB-SEM volume of a HeLa cell that was high-pressure frozen. The sample is courtesy of Anna Steyer and Yannick Schwab of EMBL. (b) The segmentation result from conventional Machine Learning. A Random Forest algorithm was trained using features derived by applying the first convolutional layer in the pre-trained VGG16 model. The model was trained using the AI toolkit in ZEISS ZEN software. (c) This figure depicts the same outcome from (b), with the exception that the output has been cleaned using a conditional random field to remove isolated pixels. Although the segmentation was able to detect a majority of pixels from mitochondria, it failed to identify a significant number of pixels within these objects, thereby making it challenging to differentiate them entirely from the background. Furthermore, a large number of non-mitochondria pixels were erroneously labeled as mitochondria.

Microscopy image analysis automation powered by AI

A survey of PubMed publications since 2020 shows that AI technology has the potential to solve a wide range of challenges in biomedical research, including drug discovery [5], radiology [6], and medical image analysis [7].

Microscopy image analysis as a subfield saw rapid growth in AI-based applications, primarily driven by the goal to automate image analysis pipelines. Researchers have tried to automate microscopy analysis to remove human bias and improve throughput since the beginning of digital image analysis in the 1960s [8].

This book focuses on AI applications for microscopy image analysis, including various case studies and the no-code tools from ZEISS that make AI algorithms accessible to everyone. AI can be daunting, especially for users with little or no programming experience. The no-code interfaces are user-friendly and allow users with no coding experience to create automated image analysis pipelines. They also allow users to build custom workflows without technical expertise. Labscope, ZEN, and arivis are software platforms from ZEISS that provide no-code interfaces that enable AI-powered automated image analysis for scientific challenges.

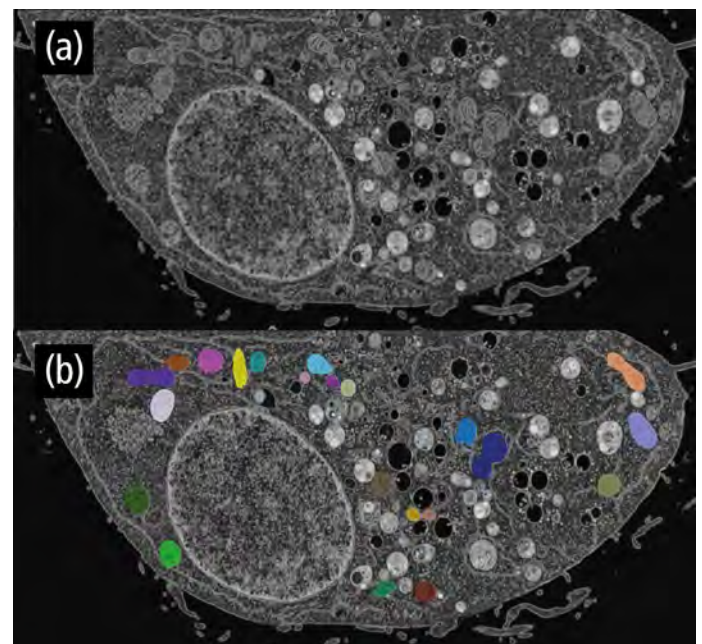


Figure 5: (a) This picture displays the same slice from a high-pressure frozen HeLa cell in a FIB-SEM volume as seen in *Figure 4a*. The sample is courtesy of Anna Steyer and Yannick Schwab of EMBL. (b) This image depicts the result of Deep Learning segmentation. The U-net based Deep Learning algorithm was trained on ZEISS arivis Cloud platform, using the arivis AI toolkit. The segmentation results from Deep Learning outperformed those obtained through conventional Machine Learning. It is important to note that the pixels utilized for training the conventional Machine Learning (as seen in *Figure 4*) and the Deep Learning (as seen in this figure) were not the same. Both approaches followed best practices, as advised by the respective software packages.

No-code products from ZEISS

ZEISS offers a range of no-code products to allow users to benefit from AI-powered image analysis solutions. These tools are accessible to a range of users, from routine labs and digital classrooms conducting small-scale experiments, to biotech and academic researchers conducting experiments with large, multi-dimensional datasets.

Products for routine lab tasks

Many routine lab imaging tasks, such as cell counting, can benefit from AI-powered automation. **Labscope** is an easy-to-use imaging app for routine labs and university or school biology classrooms. The app provides ready-to-use AI-powered solutions, including fast and effective cell counting, allowing its users to perform analysis on any microscope with a camera.

Products for automated image acquisition and segmentation

In biotech and academic research, users often automate the image acquisition process to ensure reproducibility and faster throughput. **ZEN** software suite makes high-quality image acquisition easy on research-grade ZEISS microscopes. ZEN also provides an 'AI toolkit' for image analysis that allows for smart microscopy; for example, using AI to automatically analyze a low-magnification survey image to detect regions of interest for high-magnification experiments. This allows for automated imaging of multiple large samples without any human intervention.

Automated imaging allows the collection of large amounts of data in a short period, which can be helpful for applications such as studying the effects of a particular treatment on multiple cells or organisms. But the image analysis throughput must keep up with image acquisition to maximize the benefit. ZEN's AI toolkit can be utilized to enhance application-specific automated image analysis solutions. Some sample applications within ZEN include 2D cell counting, cell confluency, gene and protein expression, as well as automated spot detection. As the data size, dimensions, and complexity increase, the analysis can be scaled up using the arivis software ecosystem.

Data-agnostic image analysis tools

arivis represents an ecosystem of software solutions designed for data-agnostic image analysis, allowing the analysis of images in many formats from different microscope vendors (and other imaging hardware, such as MRI and CT). The primary arivis solutions include ZEISS arivis Pro, ZEISS arivis Hub, and ZEISS arivis Cloud.

ZEISS arivis Pro is a visualization-centric multi-dimensional image analysis platform that provides interactive tools and the ability to develop automated analysis pipelines for virtually unlimited-size data with just a few clicks.

ZEISS arivis Hub enables the design and execution of large-scale experiments via parallelized processing using multiple computational workers on local workstations, servers, or cloud servers.

ZEISS arivis Cloud provides the infrastructure for cloud storage and computation of image analysis pipelines. Its AI toolkit, arivis AI, enables users to benefit from Deep Learning without needing to know how to code. These Deep Learning trained models can be incorporated into arivis and ZEN image analysis pipelines.

Figure 6 provides an overview of the ZEISS microscopy software ecosystem.



Learn more about ZEISS arivis Cloud

Train and share Deep Learning models on the cloud for AI-driven image analysis.

<https://www.zeiss.com/microscopy/en/products/software/arivis-cloud-ai.html>

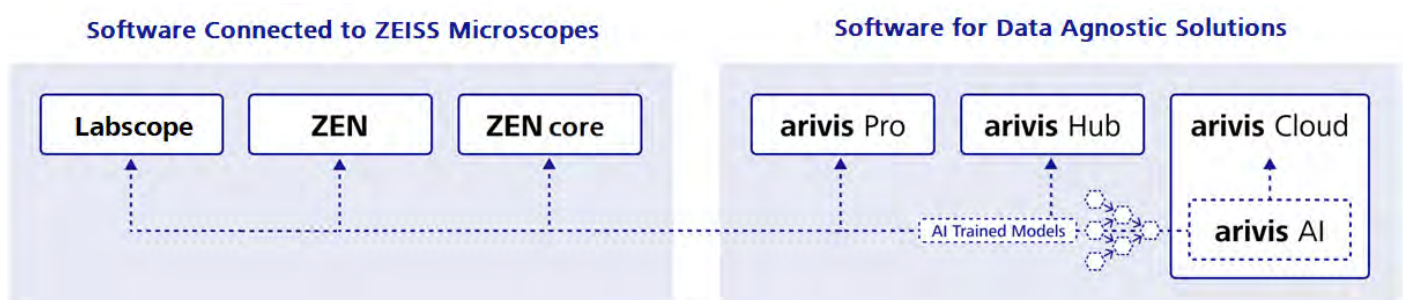


Figure 6: ZEISS microscopy software ecosystem.

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How to train custom AI models for image segmentation

What is image segmentation?

Image segmentation is the process of dividing an image into various sections corresponding to different regions of similarity, referred to as regions of interest (ROI) in scientific terminology. These regions represent the original image in a way that is easier to analyze.

In microscopy image analysis, segmentation is a key step in many applications. For example, automated counting, sizing, and tracking of biological cells enable high-throughput screening in drug discovery experiments (see *Figure 1*).

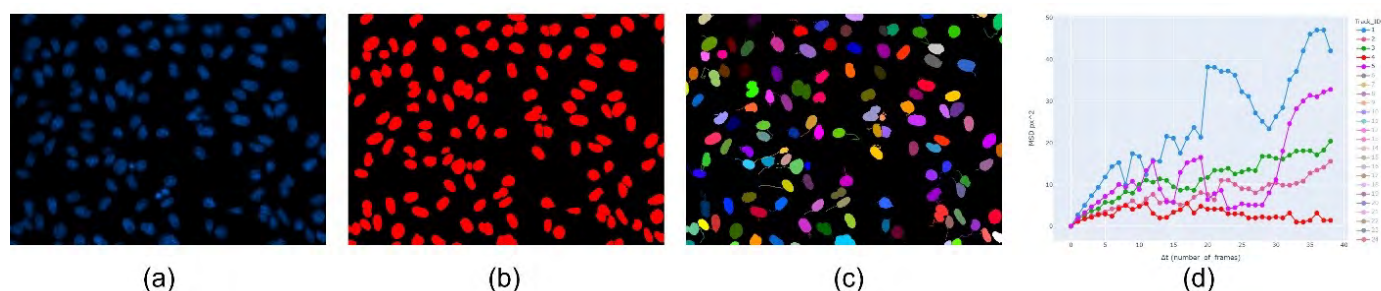


Figure 1: Segmentation in a microscopy experiment tracking cell nuclei. (a) Image showing the DAPI-stained cell nuclei in blue. (b) The nuclei from (a) were segmented by employing global thresholding and then separated using the Watershed algorithm. The segmented nuclei are depicted in red. (c) The nuclei were segmented and tracked throughout the time series, with each nucleus and its corresponding track displayed in randomly assigned colors. (d) A plot showing the mean squared displacement of selected nuclei.

Algorithms for image segmentation

Image segmentation has evolved significantly over the last five decades, from traditional techniques in the 1970s and 1980s to using Deep Learning in recent years. Traditional methods, such as thresholding, edge detection, and region growing, relied on manually tuning parameters making the results irreproducible and subject to human bias.

Otsu's segmentation method

A key method, called Otsu's method, provides a way to perform automatic segmentation using the histogram threshold approach [1]. Otsu's algorithm returns a single intensity threshold

value that separates pixels into either foreground or background classes.

Otsu's algorithm is a global thresholding method and assumes the image is homogeneous and follows a bimodal distribution.

Therefore, this approach may not be ideal for noisy images or showing multiple regions with similar mean grey levels but varying textures. However, its simplicity and computationally fast nature made it the preferred choice for simple segmentation tasks such as nuclei segmentation in fluorescence microscopy images (see *Figure 2*).

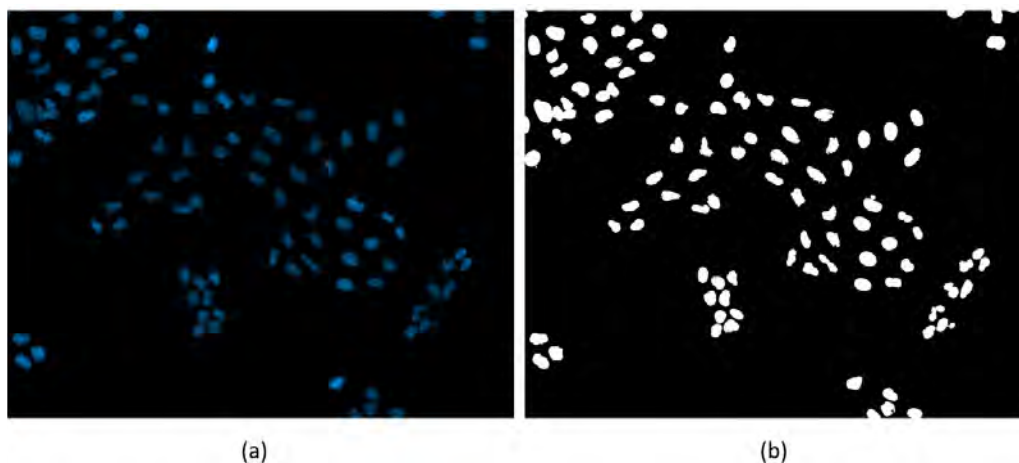


Figure 2: Otsu-based segmentation of a fluorescence micrograph. (a) Fluorescence micrograph of a sample stained with DAPI showing nuclei in blue. (b) Otsu segmentation shows the nuclei regions in white.

The Watershed algorithm

Otsu segmentation only divides the image into background and foreground, but it cannot distinguish between objects that touch one another. Additional image processing techniques, like the Watershed algorithm [2], are often used to separate touching objects. The Watershed algorithm separates objects by creating boundaries between regions 'flooded' from different markers, hence its name (see Figure 3).

However, a disadvantage of the Watershed method is that it may break down a single object into several pieces, depending on its shape.

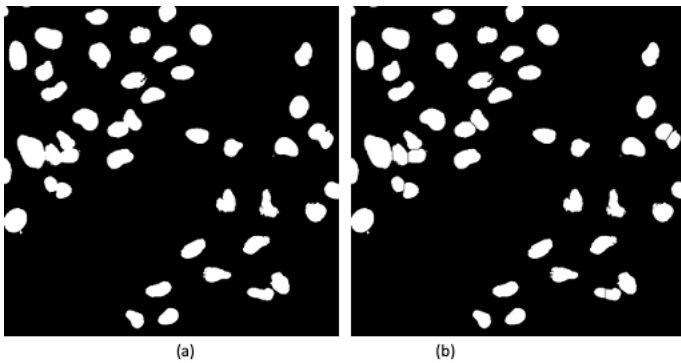


Figure 3: (a) Otsu-segmented binary image. (b) Otsu-segmented binary image followed by the Watershed separation of objects. The separation between grouped objects is evident in this image.

Machine Learning segmentation techniques

The 2000s saw the introduction of conventional Machine Learning techniques for image segmentation, including decision trees, random forests, and Support Vector Machines (SVM). These methods improved traditional techniques by incorporating contextual information and learning from data, making it possible to automate the segmentation of images with complex or varied intensity values and textures. Conventional Machine Learning works by training a classifier (e.g., a SVM) on various attributes associated with the training data. For images, these attributes can be defined via features extracted from them.

Digital image filters can be engineered to extract features representing various intensities and textural information in images. For example, the Sobel filter [3] calculates the image intensity gradient at any point and generates an image emphasizing edges. Similarly, the Gabor filter [4] combines sinusoidal and Gaussian functions to describe and show different textures. Adjusting filter parameters can create countless Gabor kernels that serve as feature extractors. For instance, a kernel with theta set to $\pi/2$ acts as a band-pass filter that emphasizes horizontal features in the image. Likewise, a kernel with theta set to π accentuates vertical features.

Figure 4 shows the application of these kernels on a cross-section of a NAND flash memory chip, illustrating that modification of the theta value can emphasize features oriented in a specific direction.

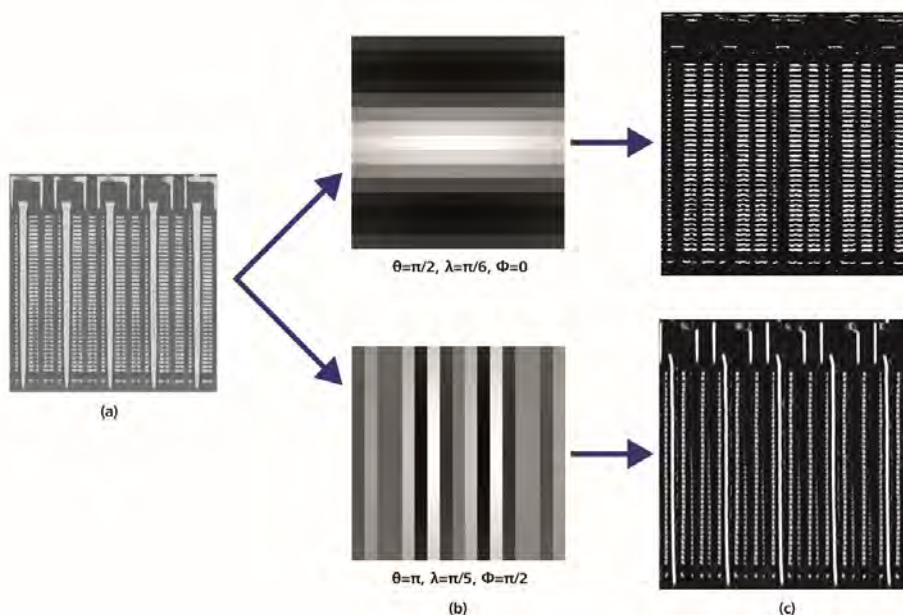


Figure 4: Using the Gabor filter to extract features from a micrograph of NAND flash memory. (a) A cross-section of a NAND flash memory chip imaged using ZEISS Crossbeam 550 FIB-SEM. (b) Digital filter kernels generated from adjusting Gabor parameters. (c) The features that are produced when the appropriate Gabor kernels are applied. One kernel emphasizes the input image's horizontal details (top), and the other highlights the vertical details (bottom).

Instead of handcrafting the features, Deep Learning networks trained on large datasets can also extract features from an image. For example, the VGG16 network [5] trained on the ImageNet [6] dataset can extract many features from images of a NAND flash memory chip (see Figure 5). These features can be used as input information for conventional Machine Learning algorithms capable of learning how to classify pixels (segmentation) or entire images (classification).

Although it is possible to use conventional Machine Learning techniques for a broad range of image segmentation, their effectiveness decreases as the images become more complex in shape and texture. Furthermore, these algorithms tend to perform poorly on images that vary in intensity compared to the training images, making them poorly generalizable to other datasets.

Deep Learning algorithms for image segmentation

Deep Learning algorithms demonstrate greater generalizability than conventional Machine Learning algorithms.

A convolutional neural network (CNN) is a Deep Learning algorithm explicitly designed for image processing tasks. One of the most popular CNN architectures is U-net, introduced in 2015 by Olaf Ronneberger *et al.* [7]. It is widely used for biomedical image segmentation. The U-net architecture is particularly good at image segmentation because it can learn both local and global features of images.

While Deep Learning is a powerful technique, it requires a lot of

labeled data and computational resources for training. But once trained, Deep Learning models can be used for extended periods due to their excellent generalizability. ZEISS provides software solutions to assist researchers in addressing the difficulties of analyzing massive amounts of data with limited resources, enabling them to achieve reproducible results at a quicker pace.

“ZEISS provides software solutions to assist researchers in addressing the difficulties of analyzing massive amounts of data with limited resources”

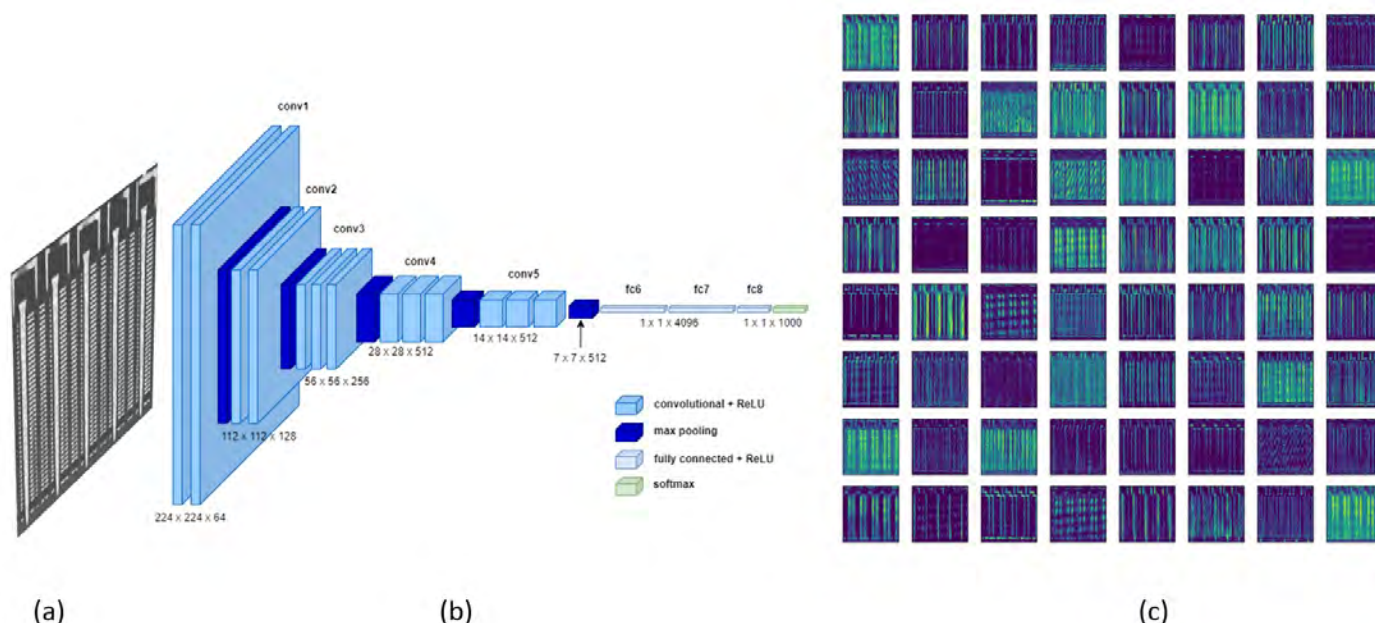


Figure 5: The use of a pre-trained Deep Learning model as a feature extractor. (a) A cross-section of a NAND flash memory chip imaged using ZEISS Crossbeam 550 FIB-SEM. (b) The VGG16 neural network was pre-trained on the ImageNet dataset. (c) Features obtained from the input image using the second convolutional block of the pre-trained VGG16 network. See reference 5 for technical details.

The ZEISS software ecosystem

Each method discussed has its strengths and weaknesses, and the choice of method depends on the application and the type of image being analyzed. The ZEISS software ecosystem offers a variety of powerful tools to train and integrate Machine Learning and Deep Learning models into image processing and analysis pipelines. The building blocks of the ecosystem are:

- **Labscope:** An easy-to-use imaging app for routine labs, universities, and schools.
- **ZEN and ZEN Core:** Universal software interfaces for image acquisition and analysis on advanced microscopes from ZEISS.
- **ZEISS arivis Cloud:** Infrastructure for cloud storage and computation of image analysis pipelines.
- **arivis AI:** A toolkit for data-driven training of Deep Learning models for image segmentation.
- **ZEISS arivis Pro:** Visualization-centric multi-dimensional image analysis software.
- **arivis VR:** A module for virtual reality visualization and collaboration during image analysis.
- **ZEISS arivis Hub:** Design and execution of large-scale experiments via parallelized processing using multiple computational workers.

Please note that 'arivis' refers to the data-agnostic image analysis

platform from ZEISS.

ZEN and arivis provide the tools for training Machine Learning and Deep Learning models that can then integrate into image analysis pipelines throughout the ecosystem. The rest of this chapter provides an overview of training custom Machine Learning models using ZEN and Deep Learning models using arivis AI, respectively.

Training Machine Learning models in ZEN

Note that training a Machine Learning model is similar in both ZEN and ZEN Core software packages. For simplicity, both products are referred to as ZEN in this chapter. ZEN offers sophisticated image analysis features (some AI-powered) and facilitates image acquisition through intelligent automation and feedback microscopy processes.

One part of the ZEN AI toolkit is a user-friendly interface that enables non-experts to create personalized Machine Learning models. This process starts with defining the ground truth by painting pixels for each segmentation class using a digital paintbrush. This process is commonly referred to as 'image annotation.' Clicking the 'Train and Segment' button trains the model in real-time (see *Figure 6*).

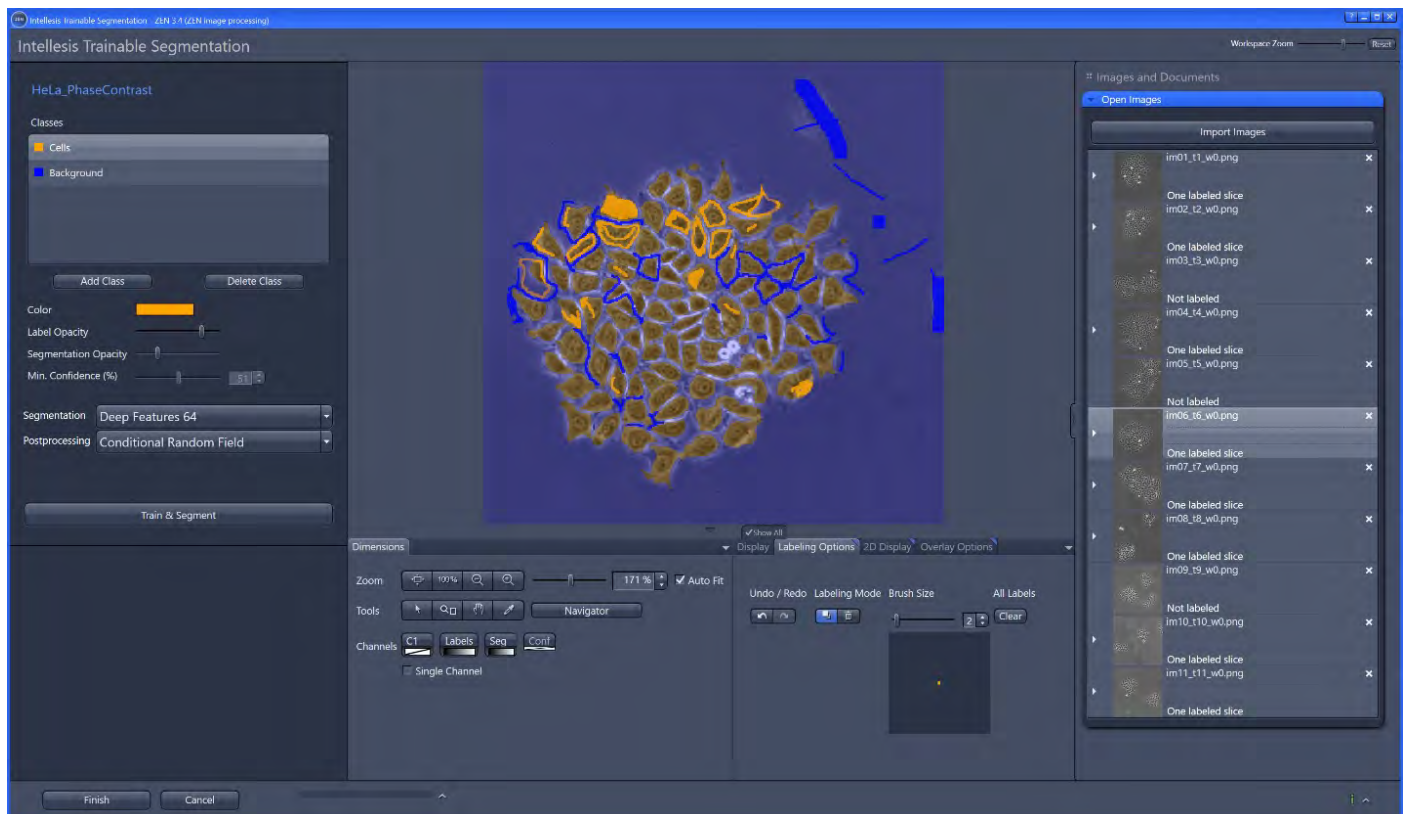


Figure 6: Conventional Machine Learning model training interface in ZEN.

Multichannel feature extraction is automatically performed based on the user-selected feature extractors. Users can add additional labels through further painting if the initial segmentation is unsuccessful in certain areas and can repeat this process until they are satisfied with the results.

The ZEN-trained Machine Learning model can be used as part of the image analysis pipelines within the ZEN software. It is also possible to import external Machine Learning and Deep Learning models into ZEN to use in analysis pipelines. External models can be imported using the [czmodel](#) open-source Python package.

Furthermore, Deep Learning models trained on ZEISS arivis Cloud can be seamlessly imported to ZEN. Thus, the AI toolkit in ZEN enables image analysis using custom-trained Machine Learning models and imported models for image analysis (see *Figure 7*). Previously, it was pointed out that conventional Machine Learning

experiences a decline in efficiency as the complexity of features in images increases, making these models unreliable.

This is where Deep Learning demonstrates its superiority, especially when a large amount of training data is accessible. The following section delves into the different elements of Deep Learning and outlines the procedure for training customized models for image segmentation using the arivis AI toolkit.

“The ZEISS software ecosystem offers a variety of powerful tools to train and integrate Machine Learning and Deep Learning models into image processing and analysis pipelines”

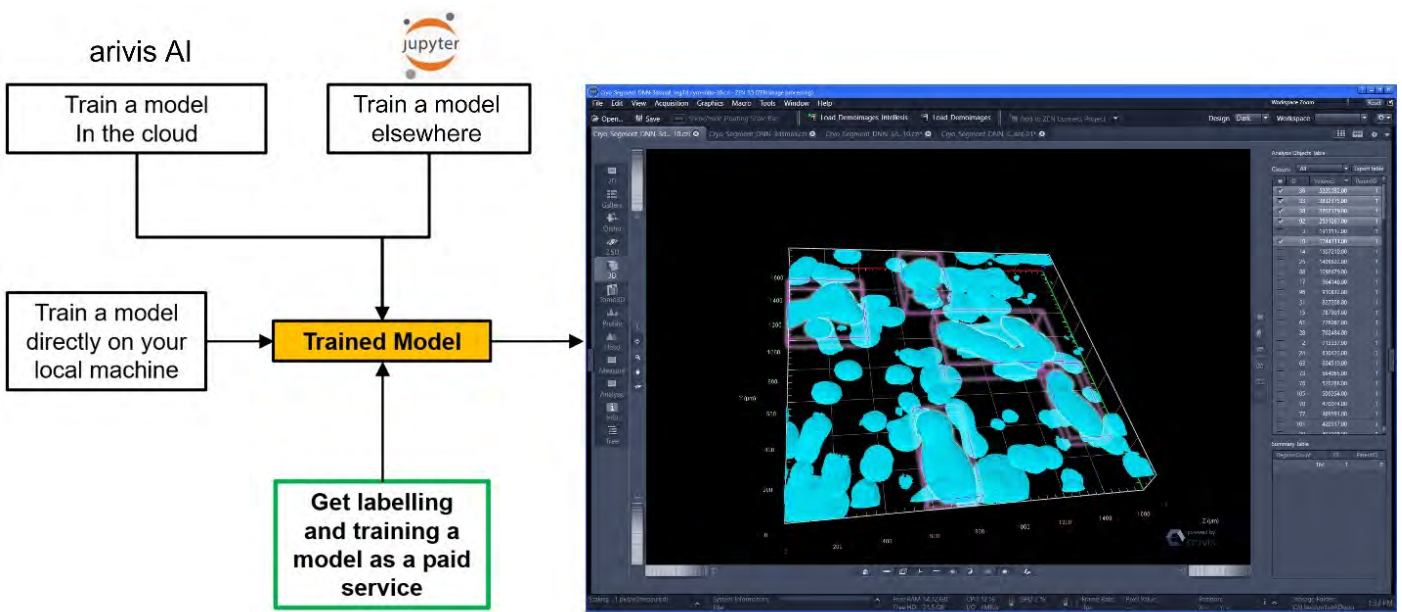


Figure 7: Flowchart depicting model sources compatible with ZEN.



Learn more about ZEN AI tools

https://github.com/zeiss-microscopy/OAD/tree/master/Machine_Learning

Training Deep Learning models using the arivis AI toolkit

arivis AI is an advanced Deep Learning toolkit that runs on ZEISS arivis Cloud. It helps users annotate images and train Deep Learning models for image segmentation. Users can utilize the resulting models on both ZEISS arivis and ZEN platforms. arivis AI offers a user-friendly interface that allows users to establish the ground truth by painting pixels and training a personalized model by clicking the 'Train' button. The following link leads to a video tutorial that explains the process of custom Deep Learning model training for image segmentation using arivis AI.

arivis AI employs the widely recognized U-net architecture (see *Figure 8*) for image segmentation but with encoder and decoder modifications to enhance speed and accuracy.

Several other improvements have been made in the Deep Learning training and segmentation process to make it user-friendly and accessible to individuals of any skill level. Examples include:

- [Using pre-trained weights.](#)
- [Allowing for partial annotations.](#)
- [Automatic definition of boundary annotations.](#)
- [Using image augmentation techniques.](#)
- [Selecting the segmentation tasks 'Semantic Segmentation' and 'Instance Segmentation'.](#)
- [Implementation of smooth tiling.](#)



Tutorial

Training Deep Learning models using arivis AI.

https://zeiss.widen.net/s/vdwc9lnxsp/en_how_to_arivis_tutorial_for_ai_ebook_0423ww

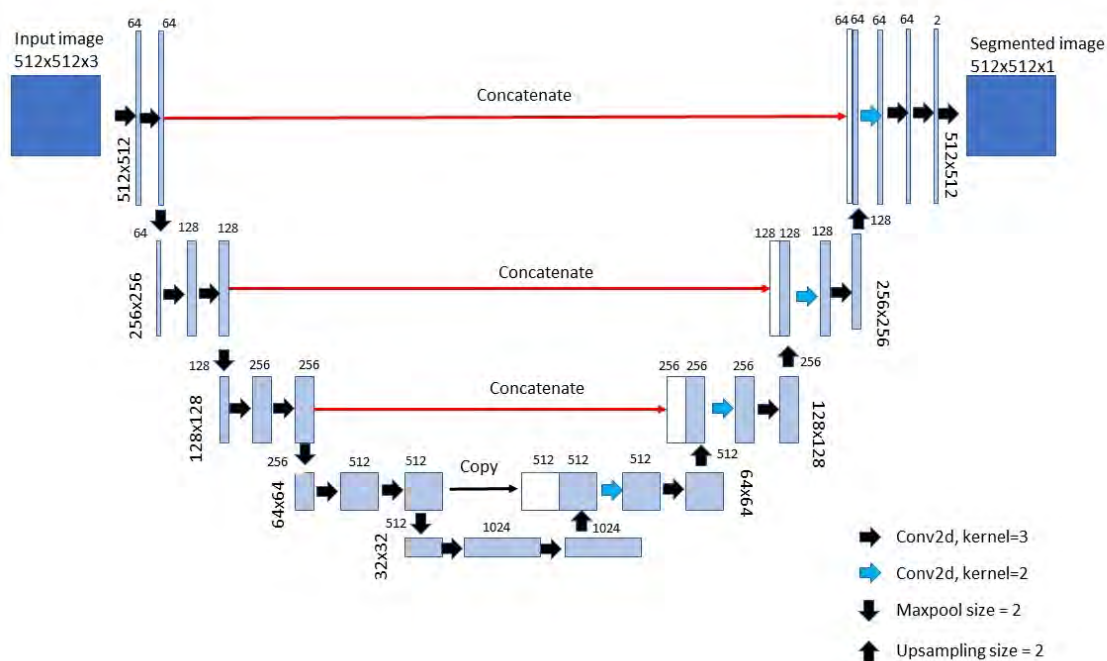


Figure 8: The illustration shows a re-created U-net architecture based on the original paper [7], which takes in an RGB input image with dimensions of 512 x 512 and produces a segmented image with the same dimensions for a chosen class.

Pre-trained weights

Unlike traditional Machine Learning, Deep Learning requires a large amount of data for training. However, arivis AI is equipped with preloaded, pre-trained weights, allowing faster model training with less data. With arivis AI, it is recommended to start with as little as 50 annotations. The users can then add additional labels based on the outcome of the initial segmentation. Thus, tweaking their trained model but investing only the necessary effort.

Partial annotations

Traditional training methods for Deep Learning-based semantic or instance segmentation algorithms often require extensive labeling. Every pixel in each training image must be annotated, including

the overly represented areas, which can be a time-consuming and inefficient process for users.

arivis AI introduces a more efficient method called 'partial annotations' for segmentation as part of its Deep Learning workflow, allowing users to concentrate on under-represented regions in training images, making the process more efficient. This is particularly useful for biotech applications where images are usually large (see *Figure 9*). Automatic boundary annotation further optimizes the usefulness of partial annotations.

Automatic boundary annotation

Segmenting the central pixels of objects is easier than segmenting the edge because the boundary between objects and the background is often uncertain. Thus, it is crucial that users properly annotate them during the training phase. arivis AI makes it convenient for the user to define these boundaries by automatically cutting out annotated objects from the surrounding background (see Figure 9).

Image augmentation

Image augmentation improves the generalizability of a trained model by giving the algorithm various variations of the training data, such as rotated, zoomed, and stretched images. This helps improve model accuracy when it analyzes new data because they might resemble the transformed images used during training. arivis AI performs various image augmentation operations in the background (see Figure 10).

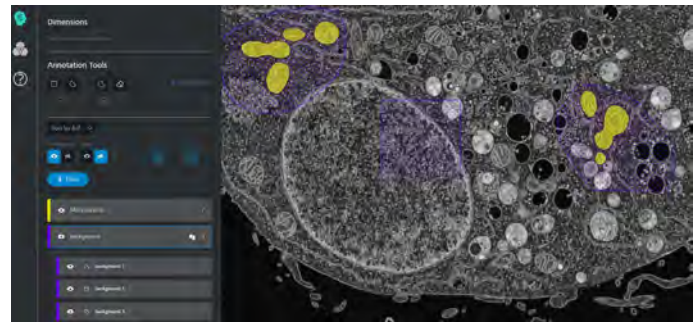


Figure 9: The training interface in arivis AI, highlighting selected mitochondria in yellow and background with purple dots. The image shows a slice from a FIB-SEM volume of a HeLa cell that was high-pressure frozen. The sample is courtesy of Anna Steyer and Yannick Schwab of EMBL.

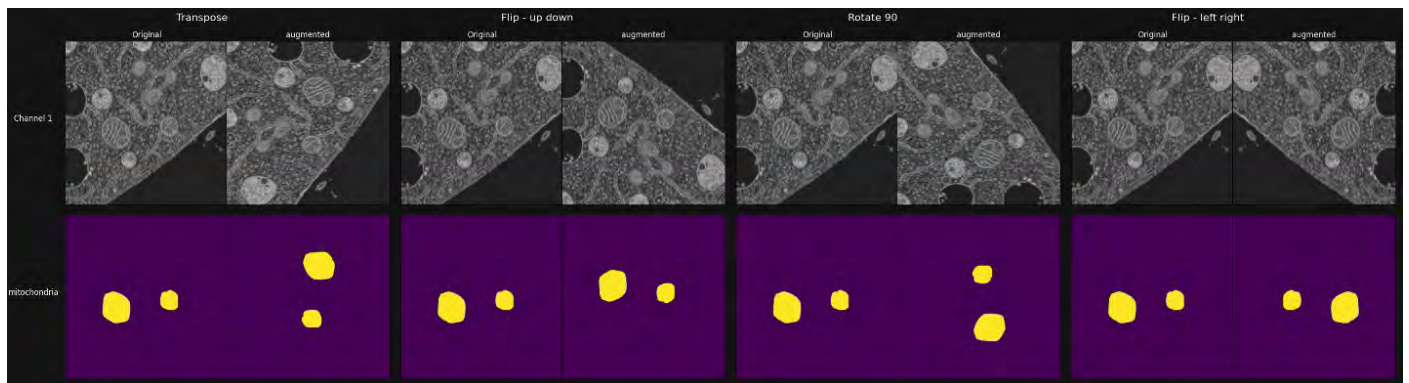


Figure 10: Augmented images and the respective masks produced while training a model in arivis AI. The image shows a slice from a FIB-SEM volume of a HeLa cell that was high-pressure frozen. The sample is courtesy of Anna Steyer and Yannick Schwab of EMBL.

Choosing a segmentation task

In arivis AI, users can select the segmentation approach appropriate to their desired application. There are two segmentation options:

1. Semantic segmentation (pixel-based).
2. Instance segmentation (object-based).

For example, when classifying regions of tissue, semantic

segmentation enables users to assign each pixel to a specific tissue class. When segmenting nuclei, instance segmentation is necessary as it allows the user to extract morphological parameters from every nucleus. arivis AI offers both options, giving the user the freedom to achieve their image segmentation goals (see Figure 11).

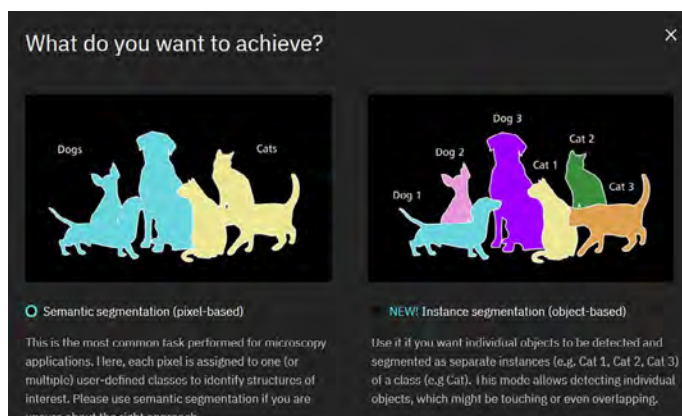


Figure 11: The arivis AI interface showing the segmentation choices available to the user.

“The arivis AI toolkit offers a range of features that simplify Deep Learning training”

Smooth tiling

Deep Learning-based segmentation uses a lot of device memory. To address this, it is common practice to divide large images into smaller patches and combine them back into the large image. However, simply arranging the patches back into a large image can result in edge artifacts where the continuity of objects may be disrupted (see *Figure 12*).

To avoid this, arivis AI uses predictions from overlapping tiles that get blended by weighing pixels closer to the tile center more heavily. This method is called 'smooth tiling'. The logic behind it is that pixels closer to the tile center represent more image context and are considered more reliable.

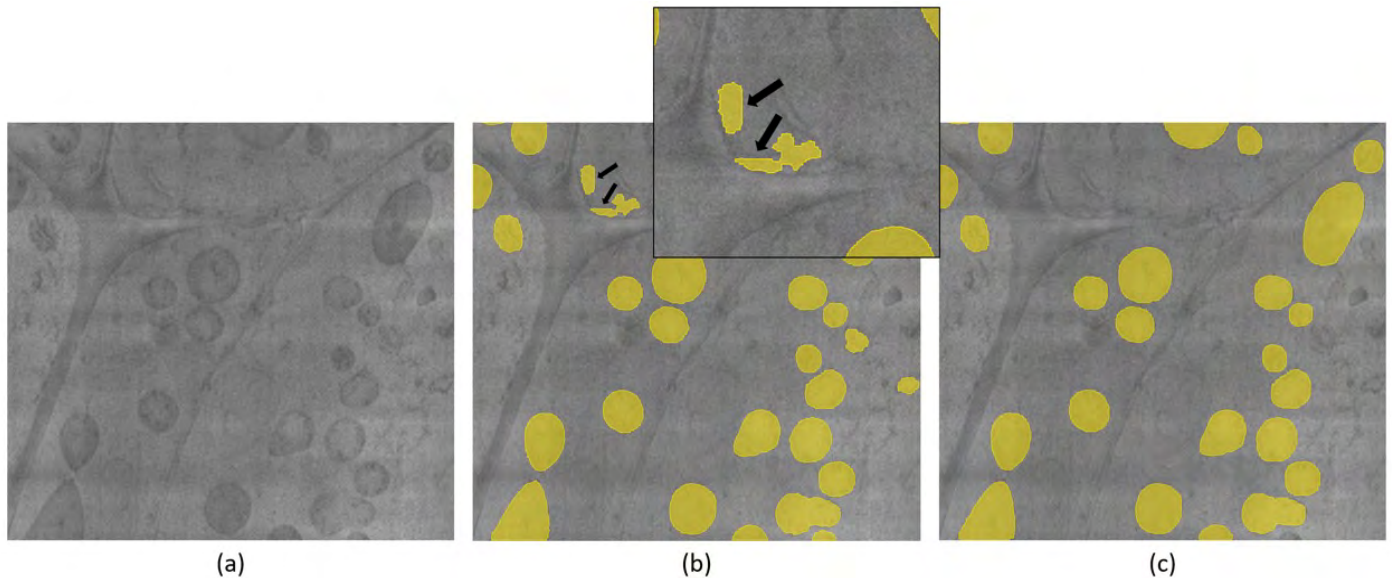


Figure 12: (a) Cryo-electron micrograph of a cell showing mitochondria. A Deep Learning model has been trained using arivis AI to segment faint mitochondria from the background. (b) The segmentation result without smooth blending produces noticeable artifacts along the patch edges, leading to incorrect classification of edge pixels as mitochondria. The straight edge is also clearly visible, as indicated by the black arrows. (c) The seamless integration of patches using 'smooth tiling' creates a segmented image without any visible anomalies. Sample courtesy of Dr. York-Dieter Stierhof from Eberhard Karl University of Tübingen.

Tips for achieving a reliable Deep Learning model

arivis AI offers a range of features that simplify Deep Learning training. However, the user can make many decisions to streamline the process. Here are some suggestions.

Standardizing the imaging conditions

The segmentation task's complexity is impacted by variations in imaging conditions. Standardizing the imaging parameters facilitates the algorithm's learning of the task, as fewer annotations are required. Adhering to the following guidelines ensures optimal training of arivis AI.

- The microscope illumination settings should remain consistent between images to maintain similar-intensity histograms between them.
- The magnification and binning should be the same for objects that have similar pixel sizes.
- It is recommended that the size of individual regions or objects that are being segmented be kept below 512×512 pixels.

Standardizing the experimental conditions

In addition to standardizing the imaging parameters, users can optimize other experimental parameters. The goal is to collect images where the objects or regions of interest are as consistent as possible. The measures below help users achieve this, where applicable:

- Maintaining a consistent size, location, and orientation for the regions of interest.
- Using the same sample preparation for microscopy images.
- Keeping the background homogeneous.
- And keeping the object density low (*i.e.*, the number of objects per unit area).

Avoiding complexity when defining classes

Try to avoid defining multiple classes to segment similar objects with minor differences. For example, instead of training an algorithm to segment small and large objects, it may be easier to train a model to segment all objects and use the size information to separate them into distinct classes after the initial segmentation. While segmenting objects into specific classes during the training process may seem easier and obvious, post-processing is usually more efficient because it enables a more generic model that can handle a wide range of objects to adapt to different applications.

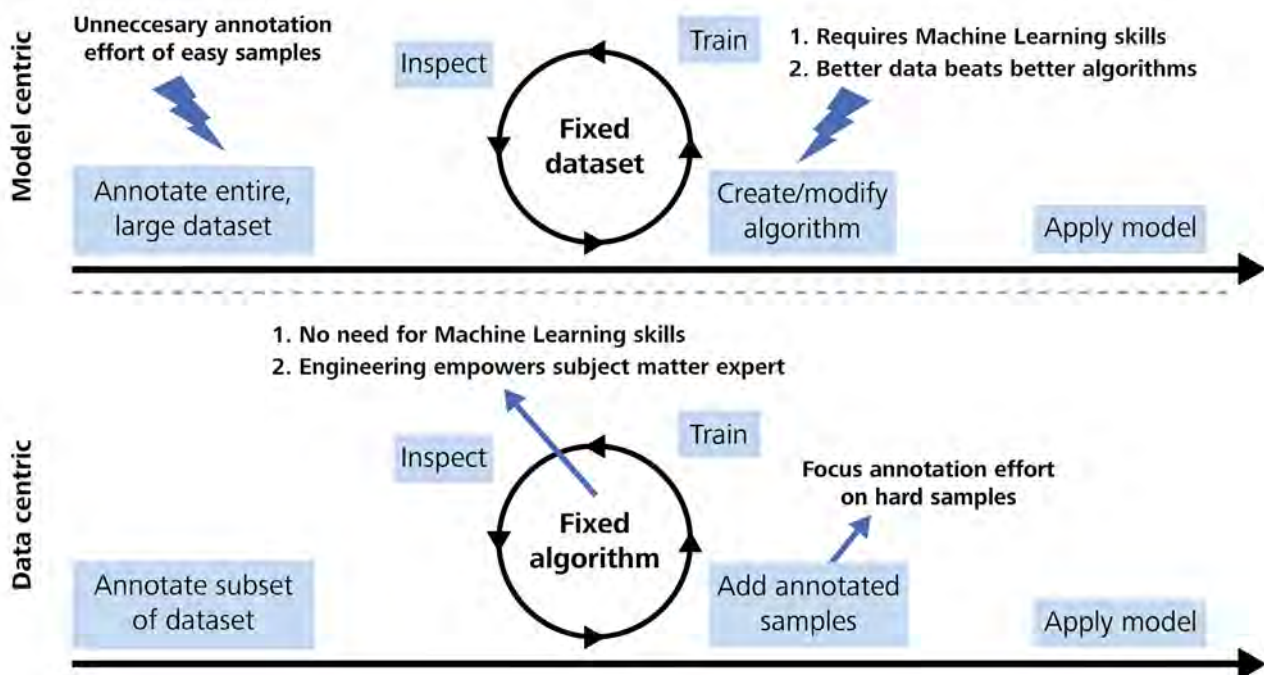


Figure 13: Model-centric versus data-centric model development for microscopy applications.

Starting simple and increasing the complexity as needed

Achieving robust segmentation of many classes across different imaging conditions is the ultimate goal of developing a Deep Learning segmentation model. However, it is challenging for the algorithm to learn all the complexity when provided only a few annotated objects within the large parameter space.

A data-centric approach [8] quickly develops a robust Deep Learning segmentation model. arivis AI provides the necessary tools to construct the perfect training dataset using the data-centric strategy (see *Figure 13*). Such datasets include a precise number of annotations in crucial areas to attain the level of segmentation robustness that the user desires.

It is recommended to approach complex tasks by starting with a small, straightforward portion and gradually increase the complexity in order to build the complete annotated dataset.

The recommended approach for segmentation is as follows:

Recommended approach for segmentation

1. Begin by selecting a single class to segment.
2. Approximately 50 objects or regions in similar images should be annotated (for example, from a single experiment).
3. After training, the accuracy of the algorithm at segmenting the first class should be evaluated.
4. To improve the algorithm's robustness, images with more variability (such as from different experiments) should be added and steps 2 and 3 should be repeated.
5. Once the first class has been successfully segmented across all images, additional classes should be annotated and trained by repeating these five steps.

This approach allows the user to concentrate on annotating challenging features rather than wasting time on easy ones. Gradually increasing the complexity helps the user develop intuition about which image features are difficult for the algorithm to learn.

In microscopy, variations in datasets arise because of different sample preparation procedures and various experimental conditions. Examples include the illumination source, magnification, and duration of observation. Therefore, to create generalized models, it is essential that the final annotated dataset reflects the diversity expected in future data.

Using AI-trained models in applications

Models trained with arivis AI can be incorporated into image analysis workflows across various ZEISS software packages including ZEN and arivis. The models can be used for image segmentation on ZEISS arivis Cloud, which is especially effective for applications where no further image analysis is needed beyond the initial segmentation and measurement. Users get a report that details over 18 morphological measurements extracted from the segmented objects, including the positions of the bounding boxes around each object. Users can also create customized workflows to define application-specific measurements.

Some applications will require advanced post-segmentation image analysis. arivis AI models can be downloaded and used with the ZEN, ZEISS arivis Pro, and ZEISS arivis Hub image analysis pipelines. These products offer push-button solutions for most standard applications. Real-time analysis of images captured using ZEISS microscopes is achievable using AI-powered image analysis pipelines in ZEN. Large multi-dimensional datasets can be imported into ZEISS arivis Pro (see *Figure 14*) for automated analysis regardless of whether they were collected using ZEISS or non-ZEISS microscopes. Automated analysis can be performed on these datasets and scaled up for faster processing with ZEISS arivis Hub. The following chapter provides an overview of how to use AI models for routine image analysis.

Additional tips to enhance image segmentation efficiency

1. It is advised not to annotate areas where the algorithm has already demonstrated mastery.
2. The recent training segmentations should be examined to determine areas where the algorithm struggles, and these areas should be given priority for annotating.
3. If the algorithm encounters difficulties in separating objects, adding a one-pixel border around the background between them may be helpful.
4. Recognizing rare classes can be a challenge for the algorithm. To improve its understanding of these classes, finding additional training images that include examples of these cases is recommended.

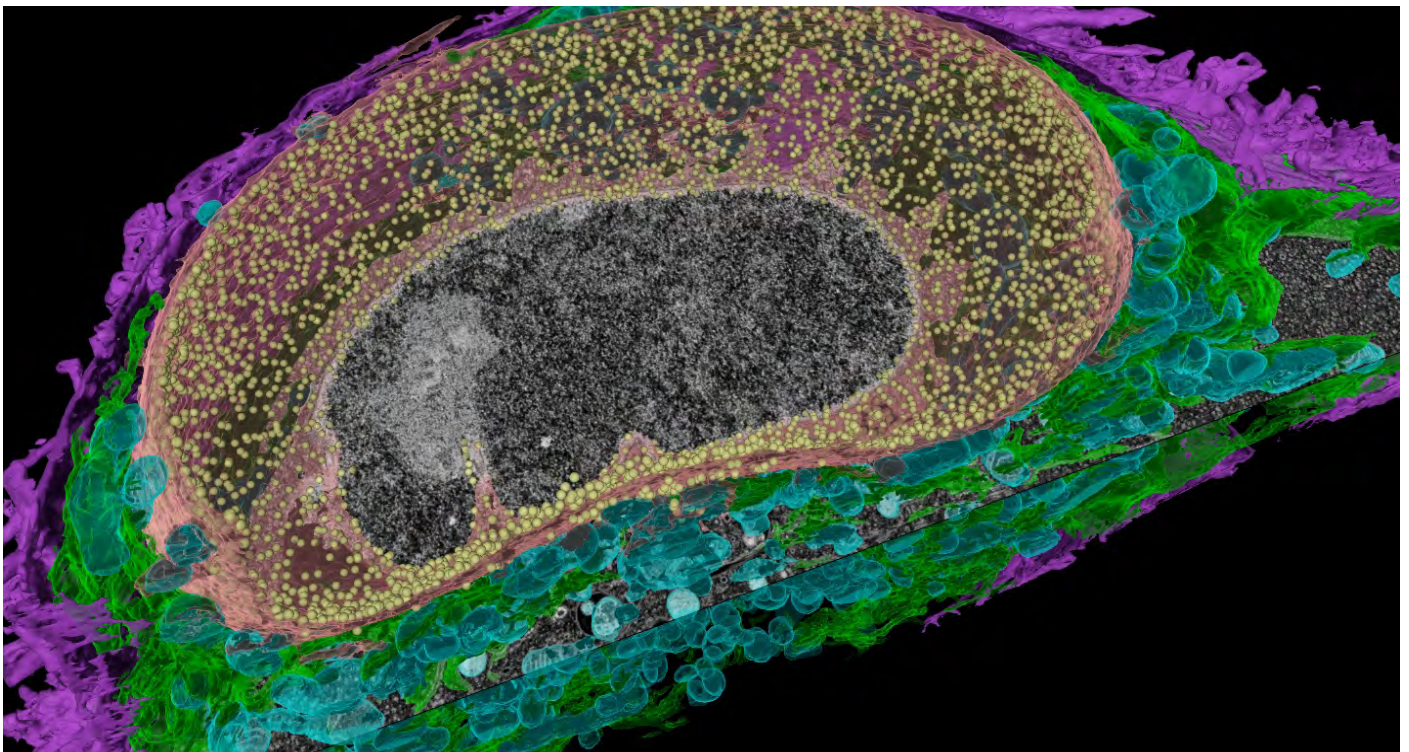


Figure 14: FIB-SEM image of a high-pressure frozen HeLa cell. Sample courtesy of Anna Steyer and Yannick Schwab. [Visit the arivis web page to learn more.](#)

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How to use AI models for routine image analysis

Life science encompasses diverse disciplines—from systematic zoology to human anatomy and protein interactions at the molecular level. Equally diverse is the application of microscopy in these branches of science. Microscopes are capable of much more than resolving smaller and smaller structures. The microscope is perhaps the best multitool in the laboratory, with uses in medical diagnostics, biotechnology, and the pharmaceutical sector.

Analysis and monitoring are two critical applications of microscopes. For example, tissue and blood samples are routinely analyzed for atypical cells and cell morphologies, and eukaryotic

cells in cell cultures are checked for their health and physiological behavior (see *Figure 1*). Furthermore, these applications are routine and repetitive, and the resulting images can answer crucial questions, such as:

- Are my cells healthy?
- Is there a detectable pathogen?
- Was the gene successfully inserted into my cells?



Figure 1: Cell cultures need regular monitoring to check their health and behavior.

While reliability and reproducibility are always critical, time is also important because microscopy experiments can produce a lot of data, all of which needs to be analyzed with care and validity.

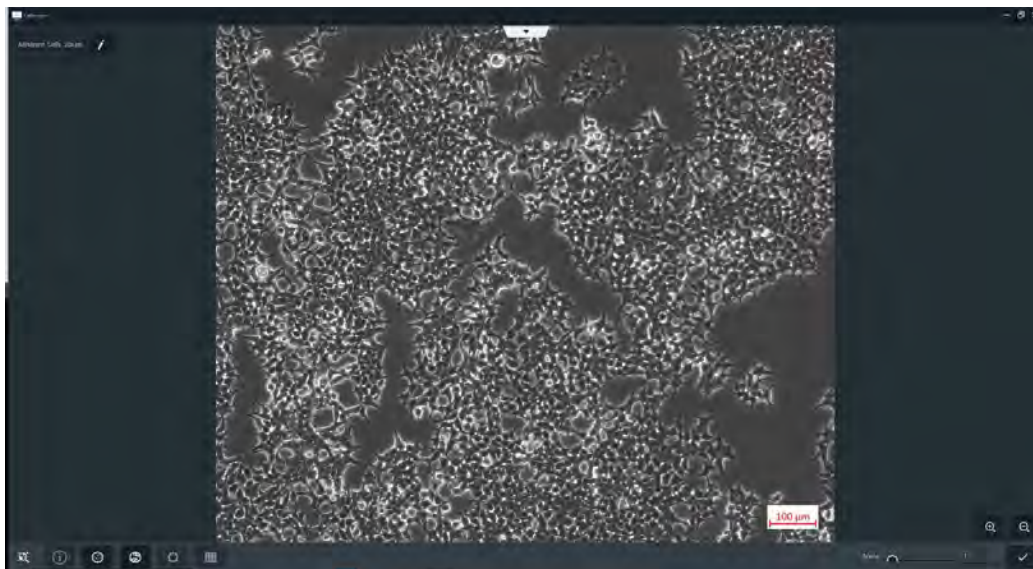
The potential role of AI tools in routine image analysis

AI tools can assist with repetitive and time-consuming microscopy tasks to save time and eliminate human error (see *Figure 2*). Artificial neural networks can identify processes, patterns, and states in organisms, tissues, and cells that humans may find difficult to detect even with advanced microscopy techniques.

These AI tools can also link vast amounts of data and learn from accumulated experience to refine specified processes. Manual work

that may have taken hours, days, or weeks can now be performed automatically with ease, and results are delivered in real time. Plus, the ability of AI to detect and analyze properties that would be difficult for humans to detect enables the fascinating prospect of revolutionary discoveries.

Like the human brain, AI algorithms constantly learn and improve. Features are detected, interpreted, and compared, and decisions and predictions are made. The accuracy of predictions and decisions improves with larger datasets, and with every new input or inquiry, the network learns to adapt to new structures.



“While AI tools for lab applications are sophisticated, their wider use may be limited”

Figure 2: Counting cells and determining their confluency manually can become cumbersome.

Overcoming limitations of AI tools

While AI tools for lab applications are sophisticated, their wider use may be limited because they can be difficult to adapt to new applications, require enormous amounts of computing power, or require advanced IT skills.

Ideally, AI tools should be accessible to as many people as possible, adaptable to different areas of interest, and work on inexpensive hardware.

The AI modules for the ZEISS Labscope imaging app offer these advantages and assist with performing time-consuming yet important lab tasks.

By combining Deep Learning methods with large training datasets, the modules can adapt to various cell types and morphologies on which they were not initially trained and can handle images of varying quality.

The versatility and the ability to collect reliable and reproducible data with minimal input and expertise required from the user make the Labscope AI modules from ZEISS an essential product for microscopists in life science, medicine, and biotechnology.

The role of AI tools for determining cell confluency

Cell confluency refers to the extent to which a layer of cells in a culture dish or flask has grown and spread to cover the surface area. It describes how densely packed the cells are and is typically expressed as a percentage of the total surface area covered by cells.

In general, cells are seeded into a culture dish or flask at a low density and allowed to grow until they reach a desired level of confluency (see *Figure 3*). At low confluency, cells are often actively dividing and may be used for experiments that require actively

proliferating cells. At higher confluency, cells may become more quiescent and may exhibit different behaviors or responses to stimuli.

Cell confluency is a fundamental parameter in cell culture experiments, as it can impact cell behavior and experimental outcomes. Monitoring cell confluency is routine for every cell culture, as it determines when cultures need to be transferred to a new cell culture vessel. This step may dictate whether an experiment can be carried out or not and thus has a significant impact on the laboratory workflow.

Challenges of measuring cell confluency

Traditionally, cell confluency is assessed by looking at the layer of cells under a microscope and estimating the degree of surface area coverage. However, relying on individual estimates of cell confluency has several disadvantages in cell culture experiments.

These include:

- Lack of reproducibility.
- Inaccuracy.
- Lack of standardization between laboratories.

These issues can be caused and exacerbated by different individuals making confluency measurements, and variability in how the cells were seeded.



Figure 3: Cells can be seeded in Petri dishes, flasks or even cell factories.

AI tools can improve reliability and reproducibility of confluency measurements

AI tools like ZEISS Labscope AI Cell Confluency address these issues, enabling reproducible and accurate measurements with the click of a button.

The AI-trained algorithm recognizes cells in culture vessels based on transmitted light microscopy images, regardless of cell type and magnification of the image, and provides a specific value for confluency in the respective frame. The algorithm also provides an average of all acquired data points in the culture vessel (see *Figure 4*). Also, users can retrospectively analyze already stored image data for confluency.

The ability to examine any number of sections of the culture vessel enables a statistical determination of cell density. Furthermore, the accumulated confluence data can be easily exported and further analyzed in statistical analysis software.

Given these advantages, the Labscope AI Cell Confluency module significantly enhances the efficiency and accuracy of cell confluency measurements, ultimately improving the reliability of experimental outcomes.

How AI can help with cell counting

Cell counting is another essential task in cell biology laboratories, enabling the determination of the number of cells in a culture vessel or experiment setup. This information is crucial for planning experiments and ensuring the available number of cells is sufficient.

Challenges associated with traditional cell counting

The traditional method for cell counting is to detach cells from the surface of the culture vessel using trypsin, transfer them to a counting chamber, and count them using phase contrast microscopy and a manual hand counter.

However, manual cell counting is a time-consuming and labor-intensive process, especially when large numbers of samples need to be counted. This can slow research progress and increase the likelihood of errors due to fatigue. It also relies on the observer's ability to visually distinguish between cells and debris, and to accurately count the cells in each grid, which can introduce significant subjectivity into the results, as different observers may count cells differently.

In addition, manually counting cells can increase the risk of contamination and impact cell viability. The results can be hard to reproduce since they differ across different observers, labs, and experiments. In cases when there are not enough cells for an experiment after manual counting, valuable time is lost both by the measurement itself and while the cells settle down and reattach to the culture vessel so they can continue to grow.

AI tools can help simplify cell counting

The AI Cell Counting module for Labscope overcomes these challenges by recognizing and counting cells in a field of view at the touch of a button. The AI algorithm can detect and differentiate cells regardless of their type or morphology. Moreover, the algorithm's reliability and reproducibility provide consistent and accurate results.

Like the Cell Confluency module, users can process and analyze existing images. In addition to the number for the cell count, a graphical representation of the detection process allows users to check the algorithm's functionality at any time. Results can be exported in common file formats for further processing in statistical tools such as Microsoft Excel.

“The AI-trained algorithm recognizes cells in culture vessels based on transmitted light microscopy images, regardless of cell type and magnification of the image”

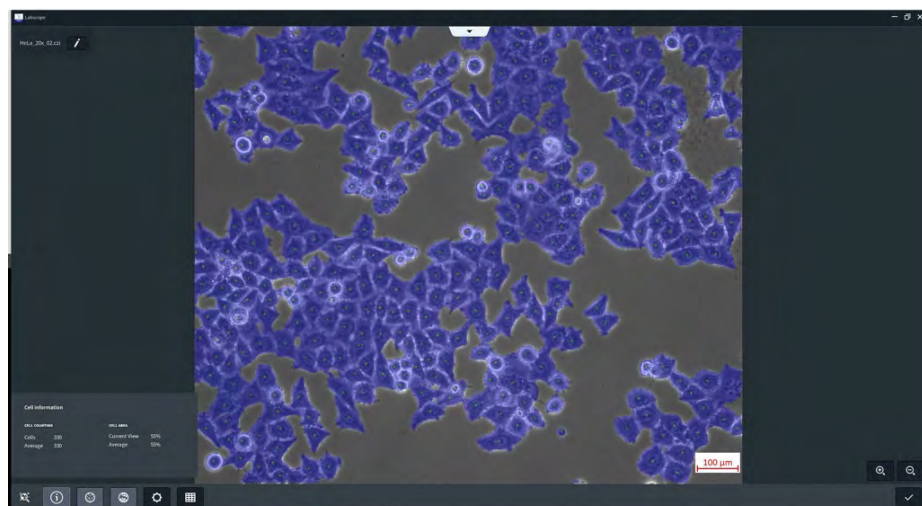


Figure 4: Screenshot showing ZEISS Labscope AI Cell Confluency measurement for HeLa cells. The module shows the confluency for the current field of view (55%) and the average of the already acquired field of views (55%).



Learn more about Labscope

Easy-to-use imaging app for connected microscopes, share your discoveries.

<https://www.zeiss.com/microscopy/en/products/software/zeiss-labscope.html>

The benefits of AI in routine image analysis

Using AI in daily laboratory work promises to optimize routine workflows and improve productivity. AI combined with microscopy will continue to be one of the game changers in everyday laboratory life. Routine microscopes like ZEISS Axiovert 5 digital, are already compatible with the AI modules for Labscope and offer all the advantages of automatic cell counting and automatic confluency measurement (see *Figure 5*). While the human factor remains essential in ensuring the accuracy and reliability of results, AI enriches microscopy examinations with tools for reducing errors and providing greater efficiency by eliminating the need to perform repetitive and time-consuming tasks.



Learn more about microscopy solutions for cell culture

Using cell contrast in current times.

<https://www.zeiss.com/microscopy/en/applications/laboratory-routine/microscopy-solutions-for-cell-culture.html>



Figure 5: ZEISS Axiovert 5 digital is an all-in-one cell imaging system based on AI.

The ZEISS arivis Scientific Image Analysis Platform

The ZEISS arivis Scientific Image Analysis Platform is the ultimate solution for handling multi-modal, multi-dimensional microscopy data with ease. This comprehensive family of software products, toolkits, and modules scales, parallelizes, integrates, and connects all image analysis pipelines, ensuring that image data proficiency and efficiency are enhanced at all levels and throughout the organization. You can concentrate on your research as ZEISS arivis takes care of your central imaging databases, from file storage format to user- and project-specific computations to standardized reporting. The platform also enables scaling of image analysis and applying automated AI-driven image analysis workflows, expanding your analysis capabilities.

ZEISS arivis Pro

If you have modern microscope systems, such as high-speed confocal, light sheet, super-resolution, electron microscopy, or X-ray instruments, which produce vast amounts of imaging data, then ZEISS arivis Pro (formerly Vision4D) is the software for you. This modular software can handle multi-channel 2D, 3D, and 4D images of almost unlimited size, regardless of local system resources. With ZEISS arivis Pro, you can process, analyze, and store your imaging datasets without constraints and get your results in next to no time!

With the ZEISS arivis Pro Analysis pipeline, even novices can start their image analysis journey today, without being an image analysis expert or programmer. This robust and flexible click-and-play solution allows you to process and quantify any multi-dimensional microscope image data easily. Whether you're a beginner or an expert, you can use predefined workflows for common use cases or combine different operators for denoising, segmentation, filtering, and other analysis tasks in a clearly structured pipeline with an interactive preview. The analysis strategy and iterative approach of ZEISS arivis Pro allow image processing and segmentation of a small field of view, a 3D/4D subset, or the complete dataset.

Once you've analyzed your data, you can easily review the results using synchronized split-view windows simultaneously in 2D and 3D views. This feature is particularly helpful for densely packed structures and tracking experiments. The software's integrated Machine Learning and Deep Learning functionality allow even difficult samples to be easily segmented without deep knowledge of AI analysis methods.

“3D image data is easily quantified using the ZEISS arivis Pro Analysis pipeline”

Benefits of ZEISS arivis Pro

- Instant analysis and visualization of images on any workstation or notebook, regardless of size or complexity.
- Advanced and user-friendly image analysis tools with interactive preview options.
- Integrated AI and Machine Learning for fast and reliable image processing, segmentation, and object classification.
- Local training and the ability to import pre-trained Deep Learning models, with access to ZEISS arivis Cloud and the arivis AI toolkit.
- Flexible and scalable workflows that connect seamlessly with ZEN, ZEISS arivis Cloud, MATLAB, and other open-source platforms such as Stardist and Cellpose.
- Distance measurements, classification, and compartmentalization.
- Robust 3D/4D image analysis pipeline for cell segmentation, tracking, tracing, annotation, quantitative measurement, and statistical analysis.
- Easy creation and export of high-resolution 3D/4D images and movies for fast publication and perfect presentation of your research data.
- Integration with arivis Pro VR for immersive and productive visualization and analysis in virtual reality (VR).
- Seamless connection to ZEISS arivis Hub, the scalable solution for data management, storage, and processing.



Learn more about ZEISS arivis Pro

Automated end-to-end image analysis pipelines for multi-dimensional images; no matter the source, size or complexity.

<https://www.zeiss.com/microscopy/en/products/software/arivis-pro.html>

Automatic neuron tracing for ZEISS arivis Pro

Automatic neuron tracing includes two established scientific methods to choose from, giving neuroscientists faster-than-ever results even with large microscopy imaging data.

With integrated algorithms, the module saves you precious time and effort when visualizing and tracing neuroscience datasets while at the same time ensuring flexibility for a wide range of sample data and imaging modalities (see *Figure 1*).

ZEISS arivis Storyboard capability helps you export impressive cinema-quality animations and visualizations with resolutions of up to 4k60 (4K at 60 fps).

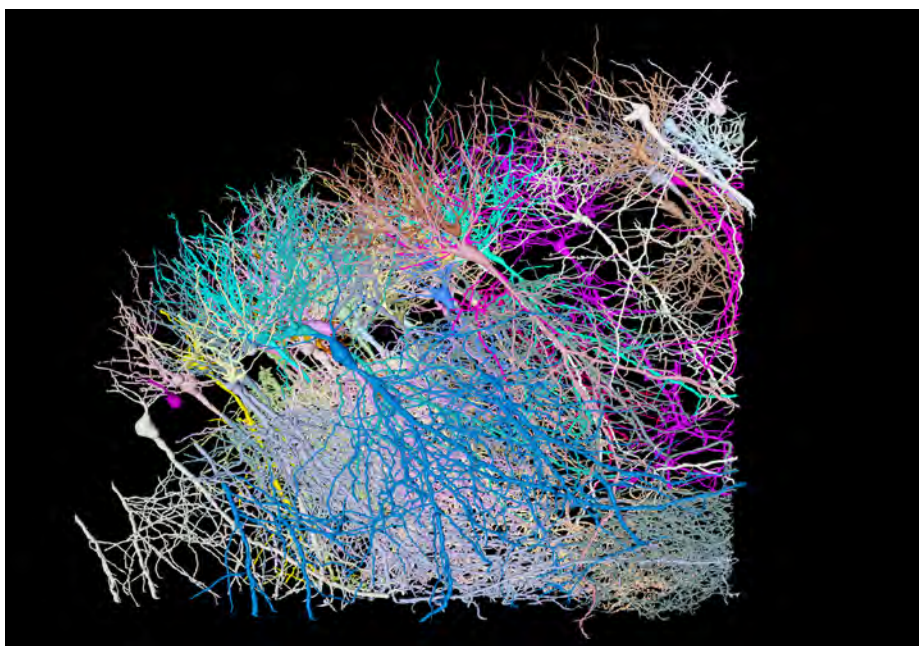


Figure 1: Automatic 3D neuron tracing of neuronal tissue (mouse brain) with Vision4D 4.0 (now ZEISS arivis Pro) and ZEISS LSM 980 confocal microscope. Original 3D tissue volume dataset (45GB CZI, confocal fluorescence microscopy, single channel) provided by Dr. Steffen Burgold, ZEISS RMS Customer Center Oberkochen, Germany.

Deep Learning training with the AI toolkit for ZEISS arivis Pro

The arivis AI toolkit is available for local AI-driven model training in ZEISS arivis Pro. It boasts a user-friendly graphical interface and a Deep Learning Trainer, which helps even inexperienced users to intuitively use advanced AI technology for their experiments, creating and training a Deep Learning model with a few clicks (see *Figure 2*).

You can annotate/label specific regions with ease thanks to the integrated drawing tool, which allows for 'sparse annotations' that require less time and effort than annotating the whole image. Monitoring Deep Learning training is simple and straightforward and can be done directly within ZEISS arivis Pro. Once trained, the model can be integrated into analysis pipelines or exported in the open ONNX format for sharing with colleagues and peers.

If you need to leverage the power of cloud computing or collaborate on analysis projects, the ZEISS arivis Cloud provides additional access for these purposes.



Visit ZEISS arivis Pro

<https://www.arivis.com/products/pro>



Talk with an expert

<https://www.arivis.com/contact/talk-with-an-expert>

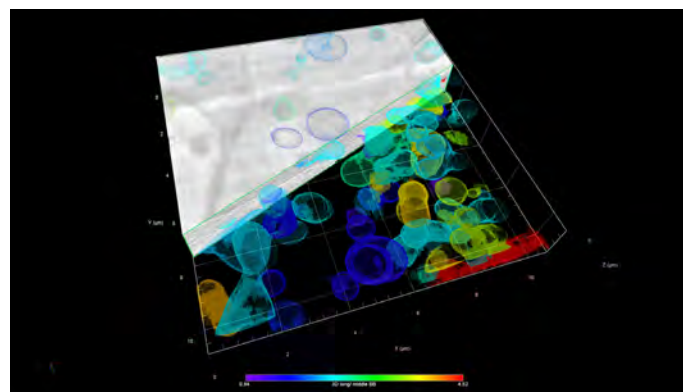


Figure 2: Sample of *Arabidopsis thaliana* cryo-volume electron microscopy (cryo-vEM) provided by York-Dieter Stierhof, Tübingen University. Volume imaging after cryo-fixation using high-pressure freezing with a ZEISS Crossbeam FIB-SEM (focused ion beam scanning electron microscope). Model creation and inference with the new local Deep Learning Trainer on ZEISS arivis Pro and then filtered the results based on the position in the volume (shown as a spectrum). The clipping plane demonstrates the successful segmentation of the objects in the volume as obtained with the Deep Learning Trainer.

arivis Pro VR

The arivis Pro VR (formerly known as VisionVR) is an advanced toolkit that can be used as an add-on for ZEISS arivis Pro or as a stand-alone viewer to display real image data in virtual reality. With patented direct volume rendering techniques, it eliminates the need for complicated manual data conversion or cumbersome surface model creation. With the arivis Pro VR toolkit, you have the power to manipulate your digital image data directly with your hands, including moving, rotating, scaling, and shaping. This freedom from the keyboard and mouse, coupled with real-world depth perception, allows you to intuitively mark, measure, classify, track, edit, and segment with precision. This efficient and interactive toolkit enables accurate proofreading, editing, tracking, and de novo segmentation of multi-dimensional images from various supported imaging instruments and systems. In the VR environment, you get a new perspective on your sample (see *Figure 3*).



Figure 3: The arivis Pro VR toolkit includes support for OpenXR™ runtimes from all major VR headset manufacturers, which makes it not only the most compatible but also future-proof for next-generation hardware and emerging standards. Immerse yourself in your data!

For a most natural and comfortable viewing experience, the arivis Pro VR toolkit provides frame rates of at least 75 frames per second per eye and reacts to head movements in under 20 ms. This high-quality performance allows you to fully immerse yourself in the data without experiencing motion sickness. arivis Pro VR uses patented volume rendering techniques to achieve this performance, even with arbitrarily large datasets (see *Figure 4*). With arivis Pro VR, you're positioned in a virtual theater to feel grounded and can 'walk-around' to gain spatial context. If you get lost in the virtual environment, simply push a button or use a voice command to reset your view.

Advanced features enable users to control their environment, changing its visibility, transparency, and background color. And with full support of the OpenXR™ standard, arivis Pro VR is the most future-proof VR toolkit for scientific image analysis, working seamlessly with all major VR headset manufacturers.

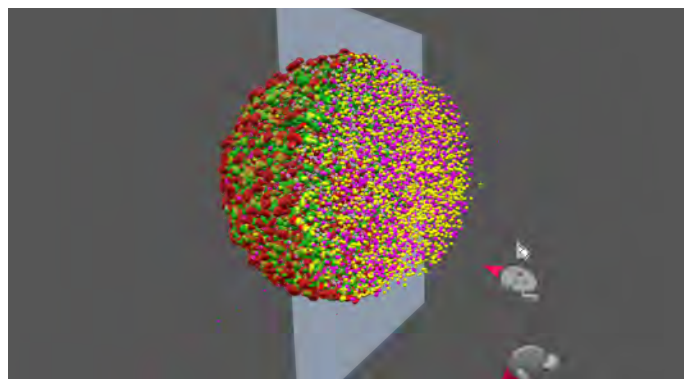


Figure 4: Machine Learning for object classification directly inside the VR environment. Organoid imaging with ZEISS Lightsheet 7 by ZEISS Customer Center Oberkochen.

Collaborative VR environment

With arivis Pro VR Collaboration mode, you can showcase your results to your colleagues in an engaging and interactive manner, regardless of your location. By sharing the same VR environment, you and your colleagues attending the VR session can collectively explore your sample. Using the same perspective, you can draw attention to areas of interest using Clipping and Laser Pointers to highlight structures and make new discoveries. This immersive and collaborative approach can revolutionize how you and your team analyze data.

Interactive measurement in VR

arivis Pro VR offers two versatile tools for precise measurement and counting. The Counting tool enables you to quickly place colored markers in your image, which arivis Pro VR will automatically count for you. Meanwhile, with the Measure tool, you can accurately measure distances, angles, or arbitrary path lengths. Save your measurements and counts as objects to further analyze in VR or on the desktop.

Edit and proofread tracks

The tracking function in arivis Pro VR enables you to import, visualize, and edit 3D tracks from any automatic analysis operation you created on your desktop in ZEISS arivis Pro. With the ability to interact with these tracks in a VR environment using your hands, you can cut, merge, or prolong them to refine the results from the automatic tracking algorithm. This feature is particularly helpful for images where two objects are close together, as it can help differentiate between them when the tracking algorithms struggle to do so.



Learn more about arivis Pro VR

A fully integrated software that displays real image data in an immersive VR environment.

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ZEISS arivis Hub

ZEISS arivis Hub, formerly known as VisionHub, empowers you to design and execute large-scale experiments that generate results from images, whether datasets are already stored or are being actively produced (see *Figure 5*). A virtual team of computational workers processes huge amounts of microscopy data by smartly utilizing processing cores in servers or workstations, coming to life whenever you need them. You can create customized and optimized workflows (with one or multiple pipelines) that extract valuable information from your image stores, and run them at scale to produce results faster, covering all levels of analysis. You can also choose from a range of standardized image analysis assays, harness AI for automated, reliable, repeatable, and speedy analysis, and easily share your findings with your peers. You can craft analysis pipelines in ZEISS arivis Pro and upload them to ZEISS arivis Hub, where others can vet and use them. Computing experts can leverage and parallelize their own innovative processing algorithms using convenient application program interfaces (APIs).

Benefits of the ZEISS arivis Hub

- Register and browse image collections, experiments, and results.
- Manage access for users and groups.
- Import datasets into the arivis framework and archive originals.
- Create experiments that produce results at scale.
- Leverage computation onsite and offsite in servers or workstations.
- Boost your throughput without worrying about budget with a scalable pricing model.
- Implement quality checks into experiments for increased efficiency and validity.



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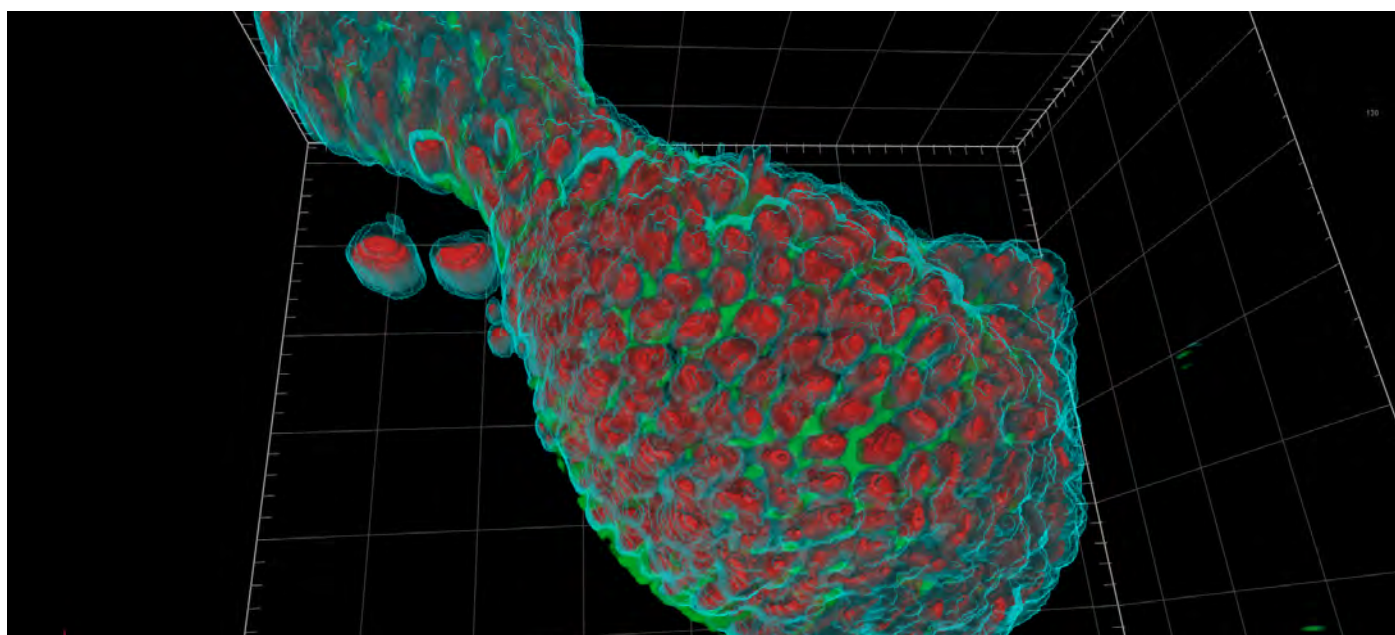


Figure 5: Intestinal organoid, well plate confocal Airyscan imaging with ZEISS Celldiscoverer 7. 3D visualization with ZEISS arivis Pro, high-content 3D analysis pipeline on ZEISS arivis Hub.

Table 1: Benefits of ZEISS arivis Hub

Server or Cloud	Scalability	Cost efficiency
Eliminate computing limitations	Run experiments and jobs in parallel	Screen compounds more rapidly
Leverage underutilized resources	Burst through bottlenecks	Using imaging to better diagnose defects
Get results faster	Distribute computing resources and access them from anywhere	Instantly share information produced from images

High-throughput and enterprise-level imaging science produce results across vast numbers of images and experiments. However, in many campuses and organizations, these results are isolated, making it challenging or extremely costly to perform powerful meta-analyses. The ZEISS arivis image analysis platform can be configured to expose spatially resolved results, summaries of results, and raw data contained by results objects to meta-analysis algorithms—including AI.

ZEISS arivis Cloud

ZEISS software experts developed a cloud-based solution to help researchers automate their image analysis, collaborate easily and thus solve their image analysis challenges. At the heart of ZEISS arivis Cloud is a Deep Learning toolkit (currently referred to as arivis AI toolkit) that makes it simple for researchers to train models and analyze complex images without needing to code. ZEISS arivis Cloud makes Deep Learning accessible to everyone, and empowers biotech and pharma scientists to accelerate research and development and commercialization using advanced technologies.

With the arivis AI toolkit on ZEISS arivis Cloud, researchers can quickly and easily train their own AI models for image segmentation and analysis. Its push-button functionality makes it easy for anyone, even beginners. By using partial annotations and tweaking their AI-driven models, scientists can save considerable time by avoiding repetitive manual steps, while reducing human bias and gaining advanced insights from their experiments.

ZEISS arivis Cloud allows for maximizing your computation resources while easily sharing results. You can create a customized application with a few clicks, based on your already-trained AI model. The model is easily exported for integration and further

analysis on other ZEISS software such as ZEN or ZEISS arivis Pro. ZEISS arivis Cloud enables scientists to leverage the experience of peers by referring to the user community and utilizing workflows and solutions already developed (see *Figure 6*). From routine applications to deep levels of advanced analysis, creating an automated application that is based on an AI-trained model is a simple and intuitive process. Coding is no longer a necessity to reach high-end analysis results that are reliable, reproducible, and which considerably shorten your time to market.

Benefits of the arivis AI toolkit

- Saves time and streamlines analysis by reducing manual steps.
- Reduces human errors and human bias for more accurate results.
- Improves throughput for enhanced efficiencies.
- Maintains quality and performance for reliable and consistent analysis.
- Makes coding an option, not a necessity.



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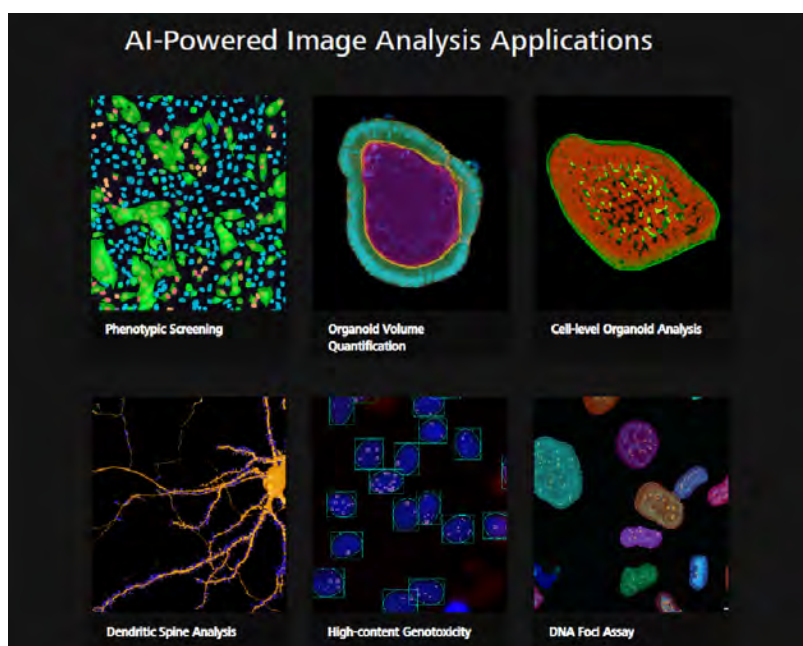


Figure 6: Examples of solutions that are powered by AI-trained models in ZEISS arivis Cloud. The image analysis can take place directly on the cloud, or the model exported for integration into a pipeline on local software for further analysis.



Learn more about ZEISS arivis Cloud

See how the cloud can help you collaborate, automate and advance our research with AI.

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Case studies

To truly understand and appreciate the power of AI for image analysis, practical applications are key. This chapter shares various case studies demonstrating the diverse and practical ways in which AI can aid image analysis. Through these examples, you'll see the potential impact and benefits that AI can bring to your imaging.

Analysis of FIB-SEM volume electron microscopy data

Focused ion beam scanning electron microscopy (FIB-SEM) is a powerful imaging tool that achieves resolutions of under 10 nm and produces highly detailed 3D image volumes. FIB-SEM highlights the entirety of the cell, generating images dense with cellular features, structural edges, and varying pixel combinations. The complexity of these images makes it difficult to use standard image processing segmentation algorithms to detect many cellular structures of interest. Therefore, quantitative analysis of FIB-SEM data often relies on the tedious and time-consuming manual drawing of features of interest on 2D slices of a 3D image volume.

AI-assisted volume EM (vEM) analysis using Deep Learning approaches offer a way to move beyond reliance on manual annotation for segmenting cellular structures [1]. Such an approach was used to develop a cell-profiling workflow using neural network training and image analysis tools that are readily accessible to researchers and do not require coding.

The first step was training the Deep Learning model. Using the arivis AI toolkit on the ZEISS arivis Cloud platform, subsets of organelles (mitochondria and nucleus) within a FIB-SEM image of a HeLa cell (see Figure 1) were manually drawn and used to train neural network models to identify these large organelles successfully (see Figure 2). These arivis AI-trained Deep Learning

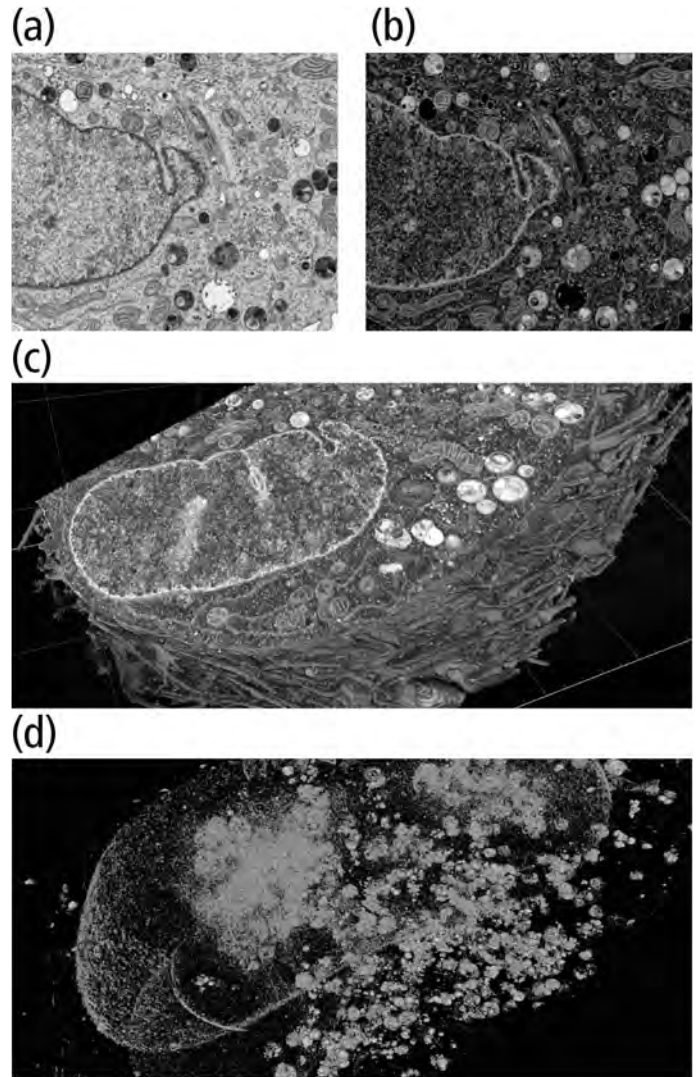


Figure 1: Overview of HeLa cell image set. The image set was collected using a ZEISS Auriga Crossbeam FIB-SEM. (a) A nm-resolution image volume of the HeLa cell. (b) Pixel intensities were inverted to achieve positive signals in a dark background. (c and d) 3D volumetric renderings of the image volume, which do not make sense without a positive signal in black background.

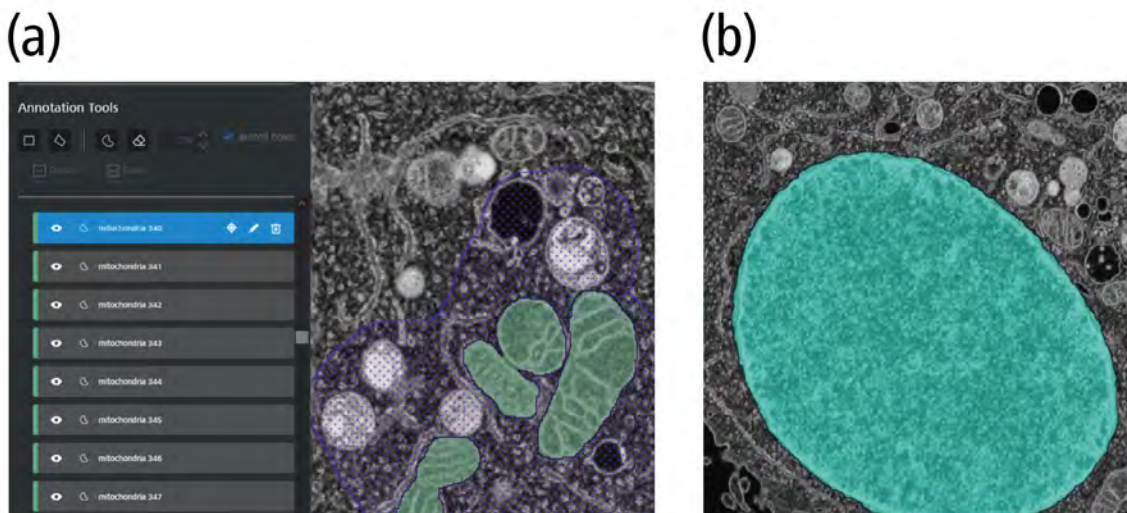


Figure 2: Generation of Deep Learning models for organelles using the arivis AI toolkit on the ZEISS arivis Cloud platform. (a) Mitochondria and (b) the nucleus were painted as individual classes for training.

models were initially used to infer mitochondria and the nucleus in ZEISS arivis Pro before analysis pipelines were built to filter and improve the initial inferences into usable 3D segments.

Segmentation and measurements of organelles

The neural network models developed from the arivis AI training allowed the automated measurement of organelle volume (see *Figure 3*). ZEISS arivis Pro computes the volume for all 3D objects, making it easy to calculate the percentage of total cell volume occupied by each organelle (see *Figure 3c*). The profiling results were consistent with previous measurements, showing that mitochondrial volume is ~10% of the cytoplasm volume within HeLa cells [2].

Mitochondrial characterization and spatial classification

Once the organelles were segmented, their distribution and surface-to-volume ratios were characterized (*Figure 4*). Analysis pipelines in ZEISS arivis Pro computed the distances of mitochondria to cellular structures. While the distances of each mitochondrion's center of geometry were not significantly correlated to the nuclear membrane (*Figure 4c*) or the plasma membrane (*Figure 4d*), the minimum distance of each mitochondrial center of geometry to either membrane did show a significant correlation (*Figure 4e*).

This method can be used with any cell structures that have been segmented and can measure distances between object surfaces or centers of geometry. It is also possible to scale this method using the ZEISS arivis Hub to allow the analysis of multiple cell image sets in parallel and produce automated, high-quality profiles.

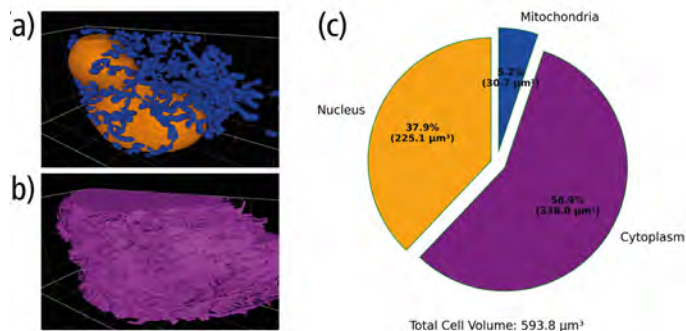


Figure 3: Segmentation results from a Deep Learning trained model can predict the percent of cell volume for organelles.

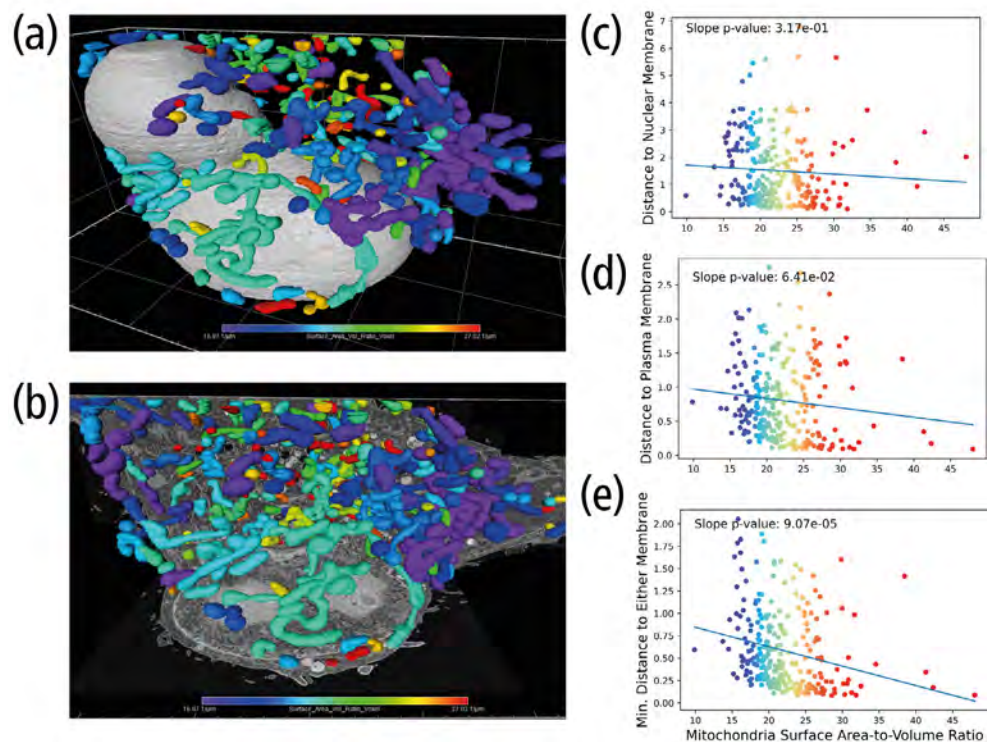


Figure 4: Mitochondrial surface area-to-volume ratios are negatively correlated with the distance to membranes.

Initial 3D segmentation of nuclear pore complex regions

3D segmentation of nuclear pore complex (NPCs) regions was limited by the image resolution (100–150 voxels per pore) and the 3D structure of each pore uniquely oriented to the curvature of the nuclear membrane. Extremely tedious annotation of the NPCs in all possible orientations would be required to segment and measure the nuclear pores. Instead, the relatively large (~400–2000 voxels) pockets under the pores were analyzed.

The under-NPC objects were used to derive objects representing the actual pores to create ground truths for a new 3D-aware Deep Learning neural network that can segment the NPCs directly (see Figure 5).

Once the segmentation of the NPCs was complete, the image stack and the corresponding NPC mask were rotated 30°, 60°, and 90° on the X and Y axes, and the resulting stacks were resampled to provide the 3D-aware augmented images of the 2D Deep Learning algorithm on the ZEISS arivis Cloud platform.

The trained model was used to segment the nuclear pores on the entire nucleus to characterize their spatial distributions (see Figure 6). Approximately 80% of the total NPCs in the nucleus were successfully segmented.

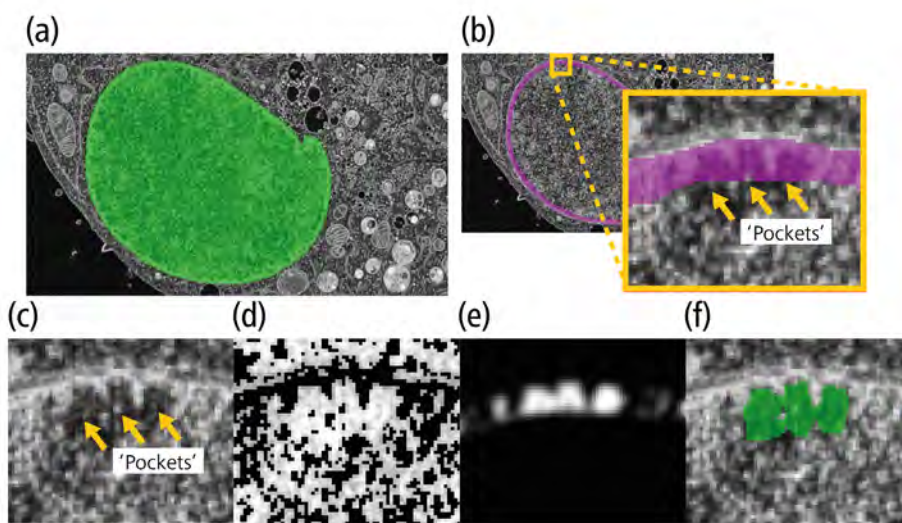


Figure 5: NPCs have variable density distribution across areas of the nucleus. Several processing steps were done to create masks of NPCs from the pocket objects. Taking the pocket objects (a), a binary masked image was generated (b), followed by a closing operation of the pockets to the nuclear membrane (c). Next, the nuclear membrane and pockets were used to mask the white space shown in panel c (d). These objects were then dilated (e). Masking using these objects enhances the visualization of NPCs (f).

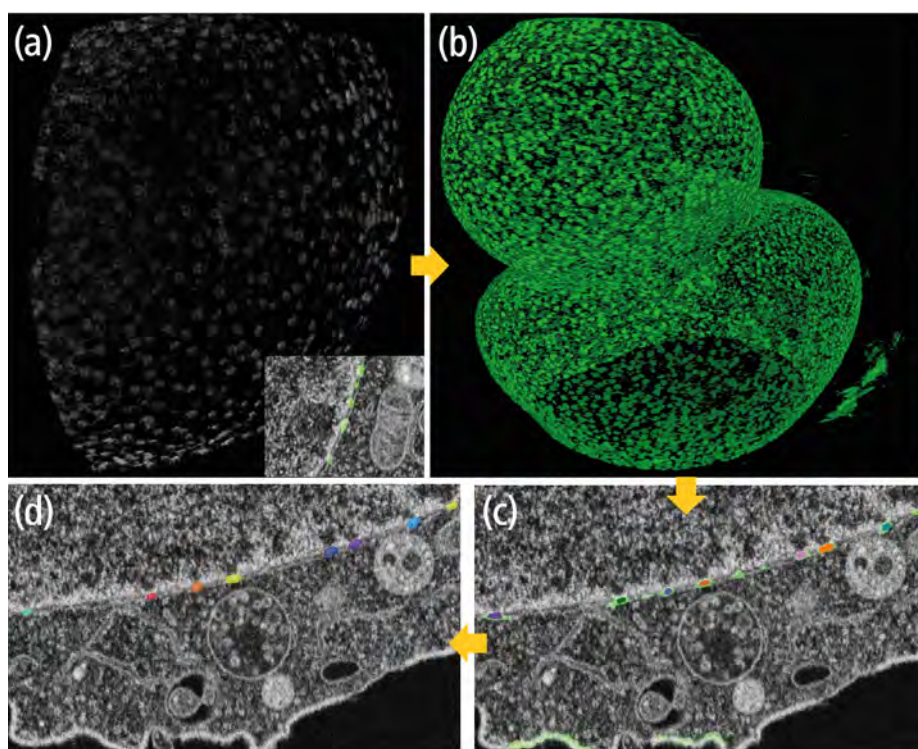


Figure 6: Training a 3D-aware neural network for nuclear pore segmentation. Several processing steps were done to create masks of NPCs from the pocket objects. Taking the pocket objects, a binary masked image was generated, followed by the 3D-aware resampling in preparation for arivis AI training (a). The resulting CZANN model was used to create the probability map in ZEISS arivis Pro with the Deep Learning Reconstruction operator (b). This 3D stack was filtered using the 'Preserve bright particles' operator, and the objects were segmented using the Watershed algorithm with a strict threshold (c). In the following step, the smaller subset of the particles was expanded by region-growing, while the largest particles were split and filtered with the segment feature filter (d).

Distribution/density analysis of nuclear pores

The segmented NPCs were used to view and quantify the 3D distribution of NPCs throughout the nuclear membrane using two approaches: (1) the ZEISS arivis Pro Distances operator and (2) the ZEISS arivis Pro Python application program interface (API) (see *Figure 7*). Both the ZEISS arivis Pro Distance operator and the kernel density Python script were capable of consistently identifying clusters of pores. Further characterization of the NPC distribution across the nuclear membrane found that NPC density is higher within the smaller nucleus section with higher curvature (see *Figure 7d*). In contrast, the larger section with a lower curvature degree has more low-density regions for nuclear pores.

The benefits of Deep Learning for analysis of FIB-SEM imaging

The combination of traditional and Deep Learning algorithms with prior biological knowledge can produce powerful workflows, as demonstrated in this chapter. By generating objects in the vicinity of NPCs, we can more accurately identify nuclear pores in 3D regions, which may not be clearly visible through 2D analysis alone. These 3D objects, representing nuclear pores, can then serve as ground truths for neural network training in Deep Learning. Overall, this approach can lead to more precise and comprehensive analyses of cellular structures.

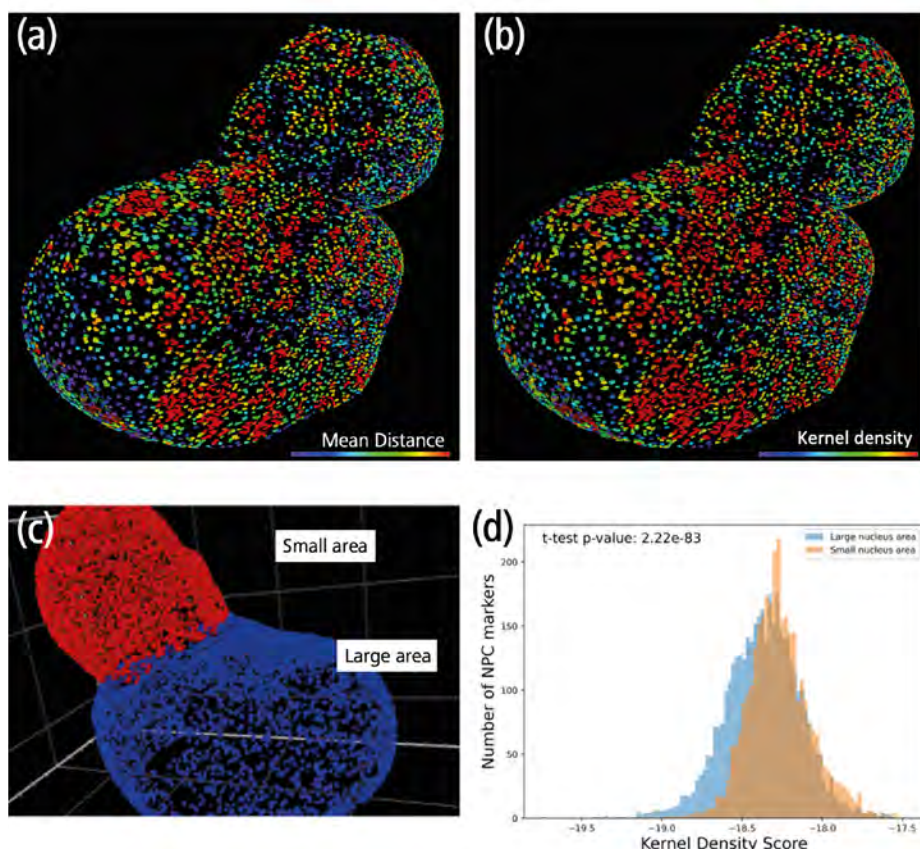


Figure 7: NPCs have variable density distribution across areas of the nucleus. (a) The average distance of each nuclear pore object to the nearest eight nuclear pore objects was measured using the Distance operator in ZEISS arivis Pro. The nuclear pore objects were then color-coded according to these distance measurements to represent the density of nuclear pores across the nuclear membrane. (b) As an alternative method of analyzing the distribution of the pore objects, the densities of NPCs were determined by taking the 3D centroid of each NPC object and calculating a Gaussian kernel density, with a kernel radius of 0.1 μm , using a custom Python script. (c) The density distribution of NPCs is significantly different across separate areas of the nucleus. Sectioning the nucleus into two sections, a larger and a smaller section, based on the nuclear cleavage furrow, reveals significant differences in kernel density scores. (d) A two-tailed *t*-test was performed to calculate the significance of differences between the kernel density scores in these two sections of the nucleus.

Notes

Original datasets imaged with a ZEISS FIB-SEM instrument and provided by Anna Steyer and Yannick Schwab, EMBL Heidelberg, Germany. Datasets first published in: Hennies J, Lleti JMS, Schieber NL, Templin RM, 1. Steyer AM, Schwab Y. AMST: Alignment to Median Smoothed Template for Focused Ion Beam Scanning Electron Microscopy Image Stacks. *Sci Rep.* (2020) 10(1):2004. doi: 10.1038/s41598-020-58736-7.

Analysis of mitochondria using Deep Learning

To understand the effects of hypoxic conditions on mitochondria in brain tissue, researchers from the Barrow Neurological Institute, Phoenix Children's Hospital used the ZEISS arivis Pro pre-trained Deep Learning model to segment all the mitochondria objects on the hippocampal tissue section. Exposure to hypoxic conditions means the mitochondria in these tissue samples have varying morphology: some appear normal, and some have 'swollen' morphology. Creating one Deep Learning model to recognize all mitochondria phenotypes in a single step posed an additional challenge.

Training the Deep Learning model

30 TEM serial sections were used with 309 mitochondria objects, annotated manually with the ZEISS arivis Pro 3.6 drawing tool to create ground truths for training the Deep Learning model (see *Figure 8*). The U-net model, with architecture very similar to the original publication [3], was used.

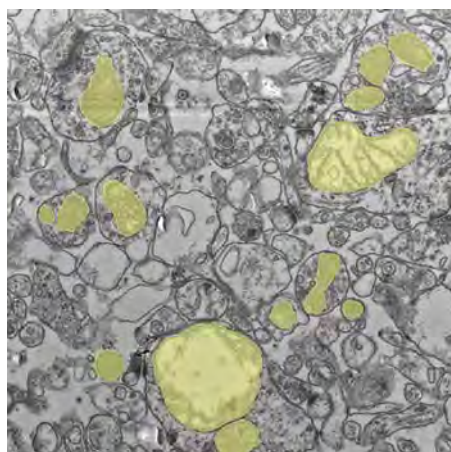


Figure 8: Manual annotation of control and swollen mitochondria phenotypes (in yellow) of TEM images of hippocampus tissue sections to create ground truths for training the Deep Learning model. Original imaging data was kindly provided by Dr. Wendy Bautista, MD PhD, Barrow Neurological Institute, Phoenix Children's Hospital.

Organoid analysis

Organoids are artificial three-dimensional model systems that can imitate the cellular composition and tissue architecture of organs while being easier to maintain and manipulate experimentally, making them ideal tools for developmental biology research.

Intestinal (gut) organoids are indispensable tools for studying both normal gut development and the mechanisms that lead to morbidities (e.g., inflammatory bowel disease). The Wnt pathway is a well-known signaling pathway regulating intestine development and maintenance. The functions and effects of Wnt are very intricate and context-dependent, with Wnt contributing

Using Deep Learning to segment and classify mitochondria

The Deep Learning model was applied to the whole dataset in ZEISS arivis Pro 3.6 for automated segmentation (see *Figure 9a*). ZEISS arivis Pro has an extensive list of quantitative features that characterize each object. In addition, it is possible to create custom features or import them from external sources. A custom object feature that computes the ratio of the mean intensity of each object to its volume was created to classify the objects into the 'control' and 'swollen' groups. For visualization purposes, each object was color-coded according to the value of the mitochondria phenotype custom feature (see *Figure 9b*).

Comparing the Deep Learning segmentation with the manual segmentation (see *Figure 9*) shows the accuracy of the Deep Learning model for segmenting mitochondria and how this segmentation, combined with the ability to create custom object features, can be used to classify individual mitochondrial phenotypes, simplifying the investigation of the effects of hypoxic conditions on mitochondria in brain tissue.

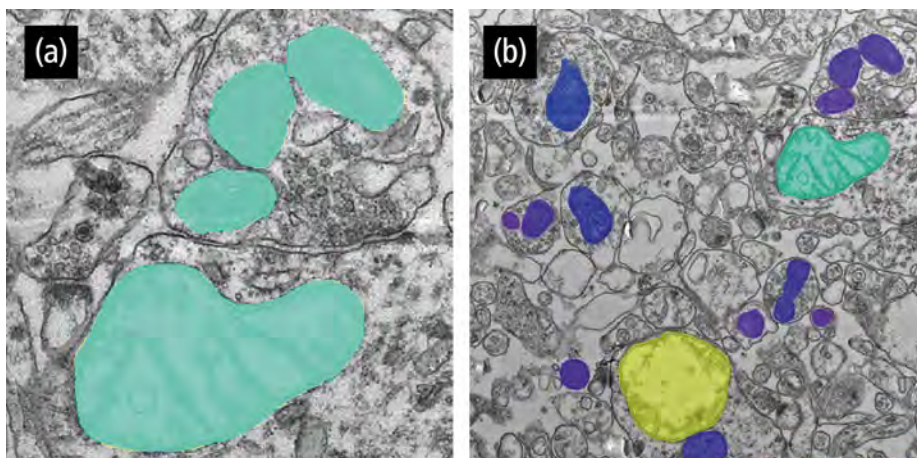


Figure 9: Deep Learning segmentation and classification of mitochondria objects. Left image shows manually segmented mitochondria (yellow objects) and the Deep Learning inference results (cyan objects) overlaid to illustrate the accuracy of the predictions. Right image shows the spectrum of the mitochondria phenotypes, which is reflected in the color of the corresponding objects [purple (normal) to red (extremely swollen)]. The phenotype is quantified as the mean intensity of the object divided by its volume and stored in the custom feature value. Original imaging data was kindly provided by Dr. Wendy Bautista, MD PhD, Barrow Neurological Institute, Phoenix Children's Hospital.

to maintaining healthy tissue stem cells and the transition and differentiation of stem cells into mature enterocytes (intestinal tissue cells). However, excessive Wnt activity (e.g., by genetic mutations) contributes to intestinal cancer.

Investigation of Wnt inhibition on organoid formation

To study the effect of Wnt inhibition, intestinal stem cells equipped with fluorescent proteins Histone2B-RFP and Mem9-GFP to mark cell nuclei and membranes were allowed to grow to organoids for 5 days in the presence or absence of Wnt signaling pathway inhibitor IWP-2. Organoids were then fixed and antibody-stained for aldolase B, a marker for differentiated enterocytes, and counterstained with

DAPI (for nucleus detection).

Image acquisition was performed using a confocal ZEISS CellDiscoverer 7 that combines widefield and confocal imaging modes. Single organoids were acquired at 20X magnification with image stacks spanning the complete organoid depth.

The ZEISS ZEN (blue edition) module 'Guided Acquisition' was used to acquire many individual organoids. This is an automated imaging workflow consisting of three parts. A large overview scan with a low magnification (*Figure 10a*), an image analysis pipeline to identify areas of interest, in this case, individual organoids on the overview image (*Figure 10b*), and a detailed scan of all identified positions (*Figure 10c*).

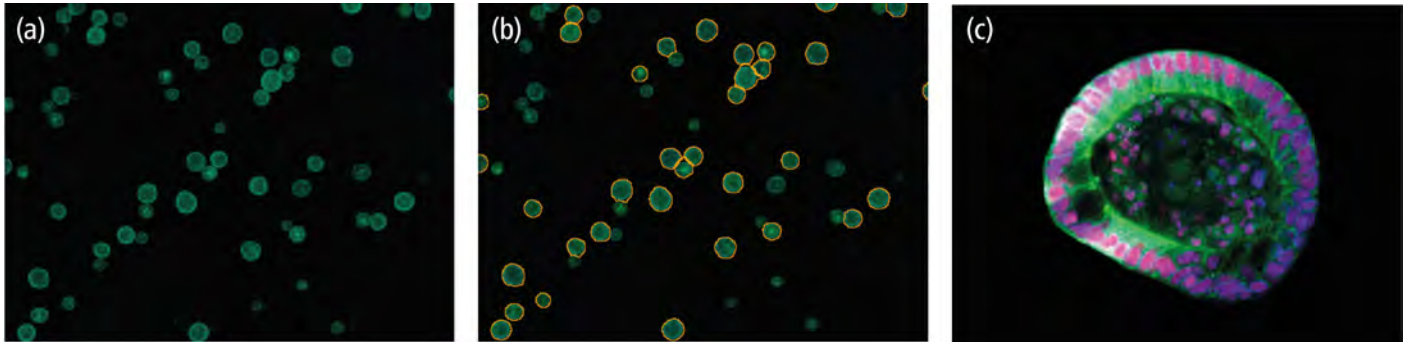


Figure 10: Imaging of Organoids. (a) Overview scan of organoids (widefield). (b) Identification of areas of interest. (c) Detailed confocal scan using Airyscan detector. The overview scan was performed with a 2.5x magnification in camera-based widefield mode. For detailed scans (20x magnification), image stacks spanning the complete organoid depth were captured in confocal mode using the Airyscan detector.

ZEISS arivis Pro

The images were analyzed using ZEISS arivis Pro with Machine Learning segmentation performed to segment the outer organoid cell layer. Next, the organoid lumen was determined by filling inclusions in the organoid cell layer segmentation. Nuclei were segmented with the blob finder function from H2B-RFP and DAPI channels. Nuclei within the organoid cell layer and the organoid lumen were separated into two object groups based on object distances to the organoid lumen. The cell bodies were segmented via regions growing from nuclei objects within the organoid cell layer. Finally, all object groups were stratified for single organoids to

enable better statistical analysis.

The validity and quality of the different segmentations applied during the analysis were checked. The organoid cell layer and organoid lumen were segmented with the Machine Learning segmenter. Employing Machine Learning leads to superior segmentation results compared to conventional threshold-based segmentation, allowing discrimination between cells in the cell layer (included in the objects) and lumen (excluded from the objects) based on complex image texture (see *Figure 11a*).

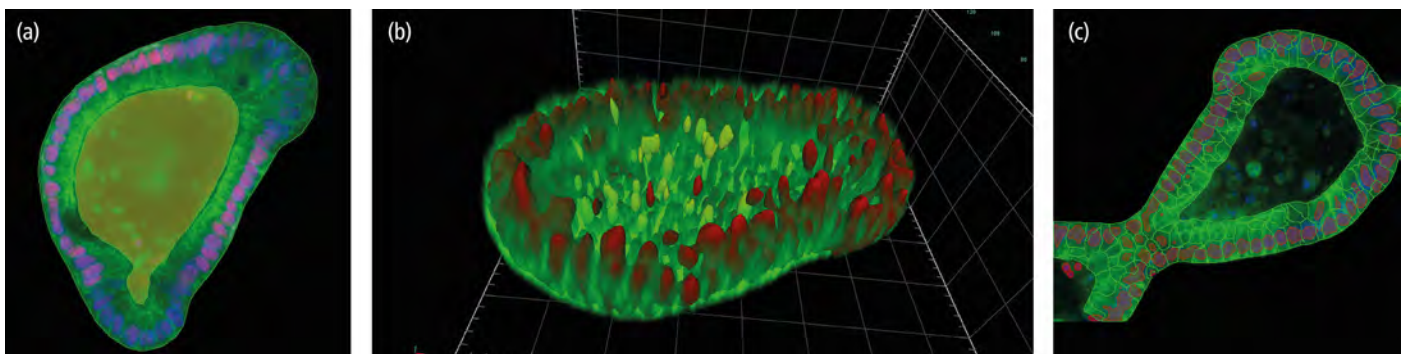


Figure 11: Organoid cell layer and lumen segmentation. (a) The cell layer overlay is shown in green, and the lumen overlay in yellow. (b) Nuclei in organoid cell layer and lumen. Cell layer nuclei are shown in red, and luminal nuclei in yellow. (c) Cell bodies in the organoid cell layer. Cell layer nuclei are shown in red, and cell layer cell bodies are shown in green.

Cell nuclei were segmented with blob finder segmentation, allowing high-quality separation of nuclei despite them being densely packed in 3D and despite intensity variations. By setting up relationships between the organoid cell layer and lumen object, nuclei were then further separated into cell layer nuclei and luminal nuclei (see *Figure 11b*). Cell bodies were segmented by region, growing from cell layer nuclei. By object filtering, they were restricted to the organoid cell layer (see *Figure 11c*).

Wnt inhibition affects the morphology of organoids

Analysis of organoid morphology showed a trend for larger volumes and particularly a larger spread of volumes in the control group, suggesting that Wnt inhibition interferes with the proper growth of the spheroids (see *Figure 12*). However, none of these trends were significant in a statistical *t*-test.

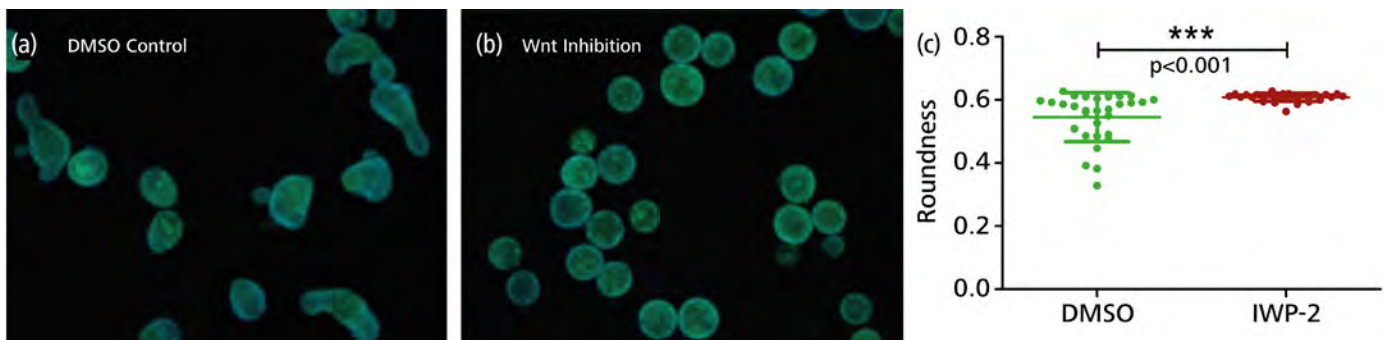


Figure 12: Wnt inhibition impacts morphology of organoids. Overview images of organoids treated without (a) and with (b) Wnt inhibitor. The images show that Wnt inhibition changes the morphology of the organoids, including size and shape. Control-treated organoids are larger and have an irregular shape. (c) The roundness of full organoids. Single data points, mean, and standard deviation are depicted. p -value from statistical t -test is shown.

The control-treated organoids formed more amorphous shapes, while organoids treated with Wnt inhibitor remained spherical. ZEISS arivis Pro offers several morphological parameters to analyze such observations. Statistical analysis of 'roundness' showed a significant drop in control-treated samples (see Figure 12c). Thus, Wnt inhibition indeed interferes with the formation of amorphous organoid shapes.

Cell numbers in different organoid compartments

The number of cells in the different organoid compartments were analyzed based on nucleus object counts. There was a significant increase in cell numbers for control-treated organoids compared to organoids exposed to Wnt inhibition ($p < 0.05$ each in statistical t -tests), indicating that Wnt inhibition interferes with proper organoid outgrowth.

Aldolase B is a marker for enterocyte differentiation and mainly localizes to the cytosol, making the cell body objects the best suited for analysis (see Figure 13a). Using ZEISS arivis Pro to extract channel intensities from different hierarchical layers, aldolase B expression was measured for the complete organoid (see Figure 13b), and the single-cell mean aldolase B intensities measured independently on every cell (see Figure 13c). In both cases, there is a strong and significant increase ($p < 0.001$ in statistical t -tests) in organoids that were mock-treated compared to organoids treated with Wnt inhibitor, adding further evidence that Wnt inhibition interferes with organoid maturation.

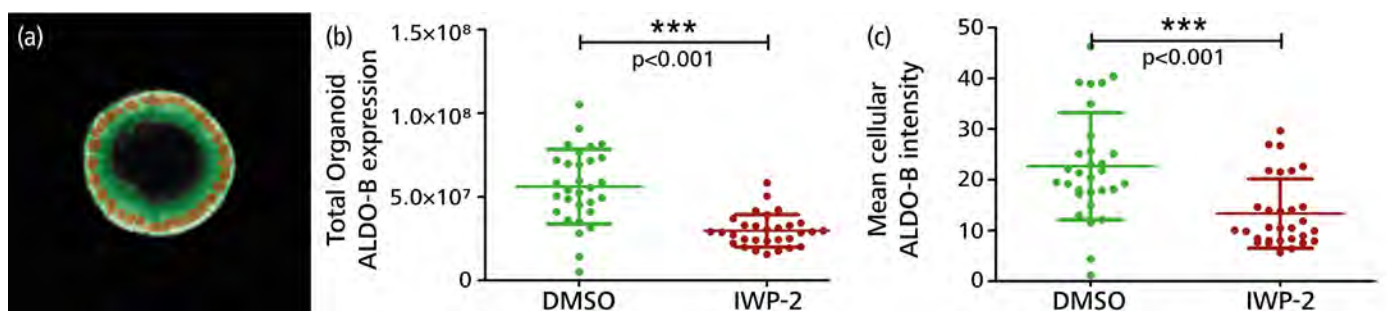


Figure 13: Localization of aldolase B expression in the organoids. (a) Aldolase B expression (gray) is localized to entire cell bodies (green) rather than the nuclei (red). (b) Total organoid aldolase B expression. Single data points, mean, and standard deviation are depicted. p -value from statistical t -test is shown. (c) Average cellular mean aldolase B intensity. Single data points, mean, and standard deviation are depicted. p -value from statistical t -test is shown.

Determining aldolase B-positive cells as an alternative read out

More realistically, cells are either 'positive' or 'negative' for aldolase B, as can be observed in a typical organoid cross section (see Figure 14). Therefore, a more suitable analysis strategy stratifies cells into aldolase B-positive and -negative groups, then evaluates the fraction of positive cells within an organoid.

Using a mean pixel intensity of 15 as a threshold for aldolase B-positive cells, positive and negative cells were generated that match well with the visual impression of aldolase B distribution in the example cross section (see Figure 14). Results are shown as total positive cells per organoid (see Figure 14b) and as the percentage of positive cells per organoid (see Figure 14c). Again, control-treated organoids had significantly more aldolase B-positive cells, indicating better organoid maturation.

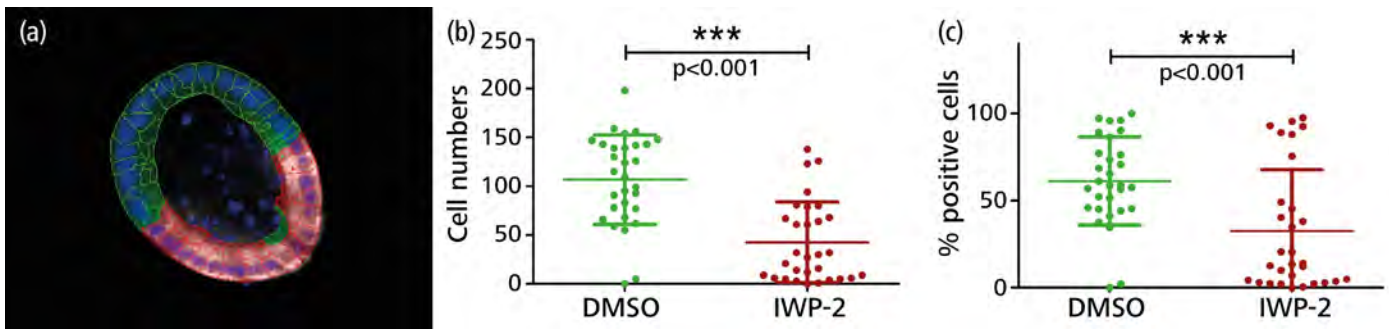


Figure 14: Determining aldolase positivity. (a) Localization of aldolase B expression in the organoids. Aldolase B expression (grey) is localized to the entire cell bodies (green) rather than the nuclei (red). (b) Number of aldolase B-positive cells per organoid. Single data points, mean, and standard deviation are depicted. p -value from statistical t -test is shown. (c) Percentage of aldolase B-positive cells per organoid. Single data points, mean, and standard deviation are depicted. p -value from statistical t -test is shown.

Summary

This study highlights how combining a ZEISS CellDiscoverer 7 and ZEISS arivis Pro for image analysis allows easy analysis of organoids and can help uncover biological insights, such as the role of Wnt signaling in intestinal organogenesis. Only 30 organoids per sample were analyzed, which is insufficient for a professional study and statistically relevant conclusions. This kind of 'real-world' use case helps users to learn about image analysis strategies they can use for their data.

Microscopy and Deep Learning for neurological disease research

Microscopy is one of the primary methods used to understand neurological diseases, such as Parkinson's disease, by studying neural circuits. By examining the cellular mechanisms that drive synapse formation and regulate synapse composition, researchers can identify patterns and rules necessary for establishing neural circuits. Mouse models are often used to investigate the generation and function of these circuits, which are relevant to various human diseases.

This analysis involves examining dendritic spines and neuronal projections to understand neural circuits. The sample used for

this study was provided by R. Thomas and D. L. Benson from Icahn School of Medicine at Mount Sinai, New York, USA. Primary neurons expressing tdTomato were isolated from the mouse brain and plated in a 96-well plate for microscope imaging. 3D z-stack images were captured using a ZEISS Cell Discoverer 7 microscope with LSM 900 and Airyscan 2, equipped with a 50x/1.2 water objective and 0.5x Tubelens. A 3D z-stack image from one of the wells clearly displays the reddish-yellow-colored neuronal projections and dendritic spines that need to be segmented (see Figure 15).

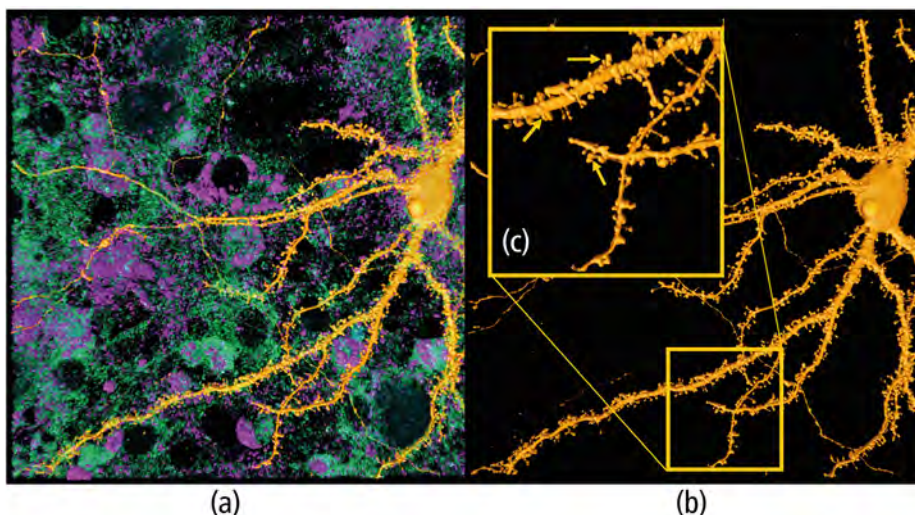


Figure 15: A four-channel microscopy image of a mouse brain with fluorescence of various labels. (a) Full image, and (b) a single-channel image of tdTomato that highlights the neuron structure requiring segmentation for dendritic spines and neuronal projections. (c) A zoomed-in view of a selected region from panel b, where yellow arrows indicate some dendritic spines, which are small protrusions from the neuronal projections.

Separating dendritic spines and neuronal projections with Deep Learning

A Deep Learning model must be trained to separate spines and neuronal projections. Deep Learning is superior to conventional Machine Learning when dealing with complex images, as is the case here, where spines and neuronal projections appear similar in images.

A Deep Learning-based semantic segmentation model was trained on ZEISS arivis Cloud using the arivis AI toolkit. The objective was to recognize two classes, namely dendritic spines and neuronal projections, in addition to the background. To create a ground truth for each of the three classes, twelve random slices were selected from the z-stack and partially annotated.

The annotation process involved using a digital paintbrush of different colors to mark respective pixels for each class. In this case, neuronal projections were painted in yellow, dendritic spines in green, and the background in dotted purple (see *Figure 16*).

To refine the trained model, initial results were visually inspected and annotations added to indicate areas where the model was unsuccessful. This iterative process is crucial in data-centric model training, where the expert's input is a vital part of the workflow. The iterative training process continued until the subject matter expert was content with the result. The model was then downloaded and integrated into an image analysis pipeline that involves segmentation followed by object analysis, utilizing the 3D toolkit in ZEN. *Figure 17* shows the segmented dendritic spines overlaid on the tdTomato fluorescence image.

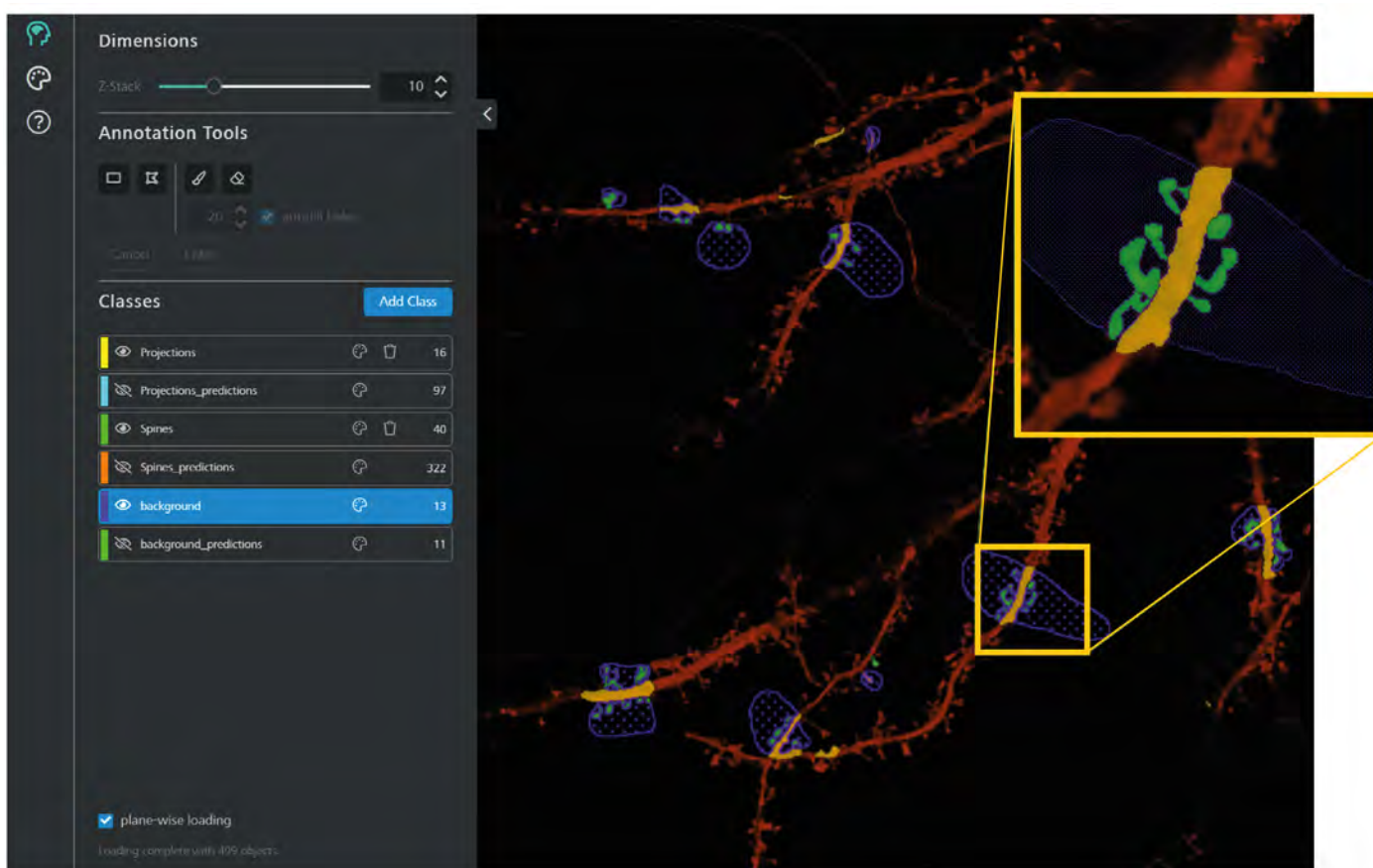


Figure 16: The arivis AI training interface on the ZEISS arivis Cloud with three defined classes for segmentation: projections (yellow), spines (green), and background (dotted purple). The inset image showcases a zoomed-in area with labeled classes representing each category's ground truth. It is important to note that the image is partially labeled, focusing on regions that provide useful information for the Deep Learning model.

How microscopy and Deep Learning can aid neurological research

Microscopy and Deep Learning are valuable tools in Parkinson's research, allowing researchers to study neural circuits and understand the cellular mechanisms that regulate synapse formation and composition. A Deep Learning-based semantic segmentation model was trained to separate dendritic spines and neuronal projections using 3D z-stack images captured from a ZEISS Cell Discoverer 7 microscope. An iterative process involving data-

centric model training was employed to refine the model before integrating it into an image analysis pipeline utilizing the 3D toolkit in ZEN.

The successful segmentation of dendritic spines using the trained model demonstrates the effectiveness of Deep Learning in complex image analysis and its potential to contribute to future neurological disease research.

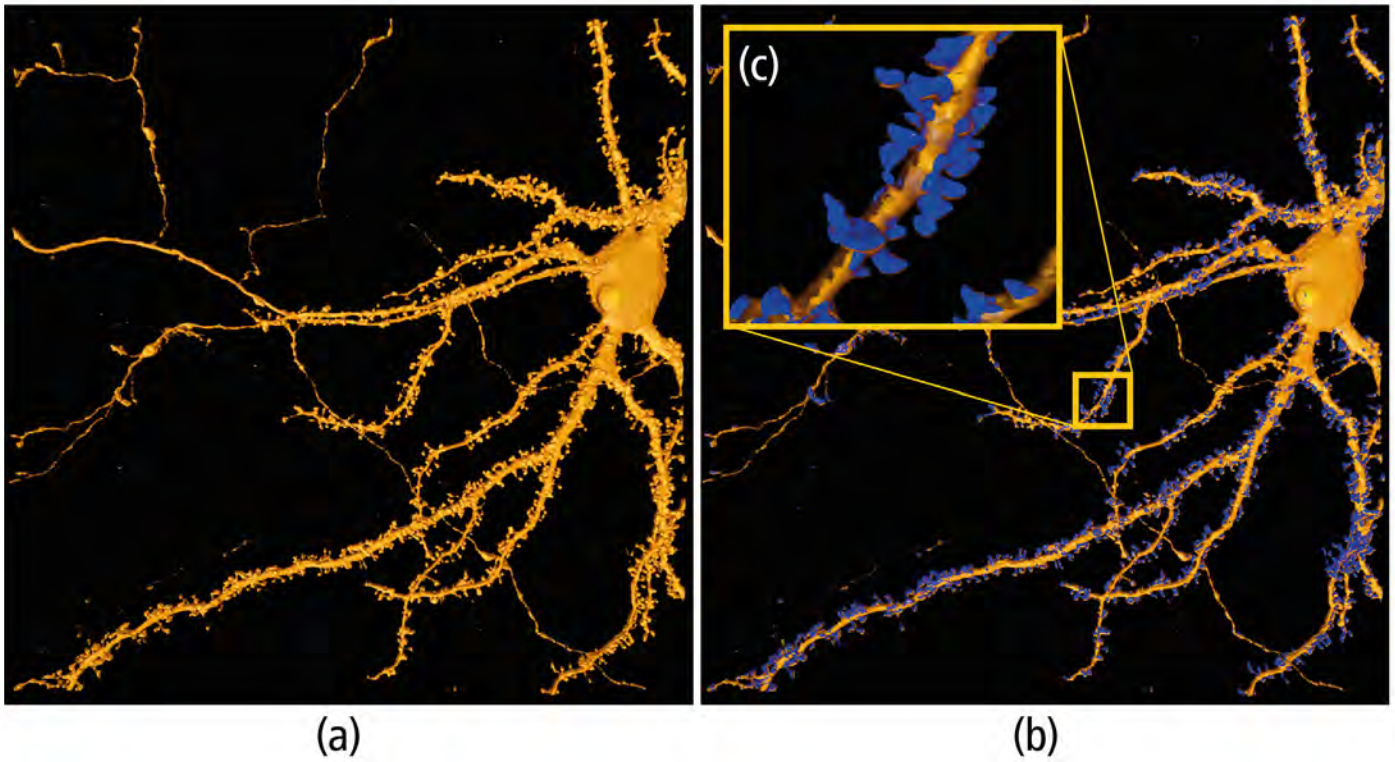


Figure 17: (a) Single-channel image of tdTomato highlighting neuron structure; same as Figure 15b. (b) Dendritic spines segmented in blue and overlaid on the image in panel a. (c) Inset image zooms in on a region from panel b to show clear segmentation of spines.

Improving microstructure analysis of aluminum oxide with Deep Learning

The importance of investigating the microstructure of aluminum oxide

Aluminum oxide (Al_2O_3) is a highly versatile material with excellent mechanical, electrical, and thermal properties. Its high resistance to wear, corrosion, and oxidation further contributes to its widespread use. The microstructure of aluminum oxide, which includes the size, shape, and distribution of its grains, inclusions, and grain boundaries, can significantly impact its physical and mechanical properties. For instance, the size and distribution of the grains can affect the strength, toughness, and hardness. The grain boundaries can influence its behavior under different conditions, such as temperature, stress, and corrosion. Thus, investigating the microstructure of aluminum oxide can help researchers and engineers optimize its properties for specific applications and understand its behavior under varying conditions.

Segmentation of aluminum oxide grains: Machine Learning vs. Deep Learning

The efficiency of conventional Machine Learning and Deep Learning approaches for image segmentation of aluminum oxide grains were compared using images collected from a polished aluminum oxide sample (courtesy of Bernthaler group at Hochschule Aalen). Images were captured using a ZEISS Crossbeam 550 focused ion beam scanning electron microscope with a pixel size of $0.03 \mu\text{m} \times 0.03 \mu\text{m}$ and 2048×1536 pixels in x and y dimensions. A backscattered electron detector provided the necessary contrast

between the aluminum oxide grains and grain boundaries, where grain boundaries appear darker than the grains. A single random image from the image stack was selected for training. The image was partially annotated on the ZEISS arivis Cloud platform, where pixels corresponding to the grains, grain boundaries, and inclusions were painted using a digital pen to define the ground truth (see Figure 18).

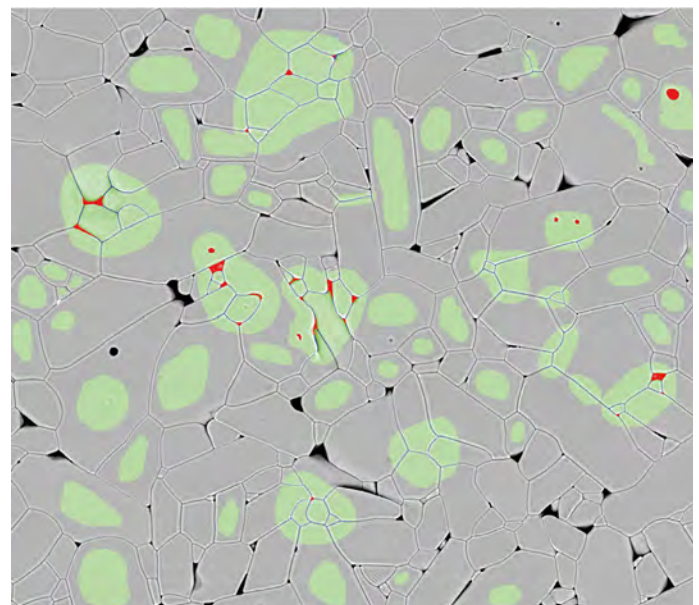



Figure 18: Aluminum oxide grains partially annotated on the ZEISS arivis Cloud platform for Machine Learning and Deep Learning training. The green areas indicate the aluminum oxide grains, the blue outlines correspond to the grain boundaries, and the red areas represent inclusions and pores.

The annotations were used to train a Deep Learning model using the arivis AI toolkit on the ZEISS arivis Cloud platform. arivis AI employs the widely recognized U-net architecture [4] for image segmentation but with encoder and decoder modifications to increase speed and accuracy.

Additionally, the annotations were exported to ZEN for use as ground truth labels for conventional Machine Learning training. Features from the training regions were extracted using the 'Deep Features 64' setting (see *Figure 19*). This setting extracts 64 features by applying 'layer 1' from the VGG19 network [5], pretrained on over 14 million images from the ImageNet database. It's important to note that no Deep Learning training occurs during the Machine

Learning training process. Instead, the pre-trained Deep Learning network is being used to extract features, which then serve as input to a conventional Machine Learning algorithm, Random Forest.



Discover further information on the features used in ZEN
https://github.com/zeiss-microscopy/OAD/blob/master/Machine_Learning/Feature_Extractors/feature_extractors.md

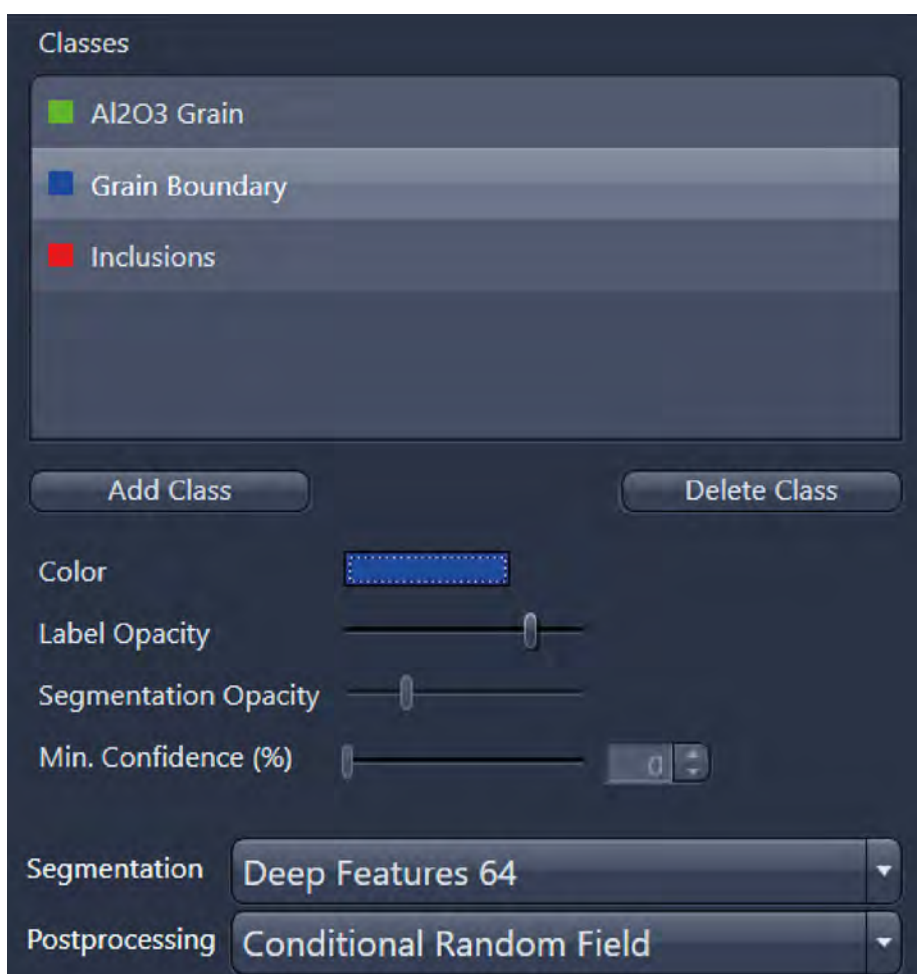


Figure 19: Conventional Machine Learning settings in ZEN for the aluminum oxide grain segmentation training. 'Deep Features 64' setting extracts 64 features from the training regions, and the 'Conditional Random Field' postprocessing refines the segmentation result by incorporating contextual information.

Deep Learning outperforms Machine Learning for grain segmentation

The results from both the Machine Learning and Deep Learning segmentation, respectively, for a random image in the dataset are shown in *Figure 20*. Similar to the training annotations, the segmentation result shows aluminum oxide grains in green, grain boundaries in blue, and inclusions in red. While the Machine Learning segmentation (*Figure 20b*) appears to be acceptable at first glance, many discontinuous grain boundaries are observed on closer inspection (*Figure 20c*). This is due to the inability of the pre-

engineered features to properly present the grain boundary features to the Machine Learning algorithm, despite being pre-trained on 14 million images. Any grain analysis using this approach will lead to an overestimated grain size distribution. Feature learning via Deep Learning training helps here, as it can learn the appropriate features needed to represent the grain boundaries accurately. Deep Learning successfully segmented the grain boundaries (*Figure 20d*), whereas conventional Machine Learning failed (*Figure 20c*).

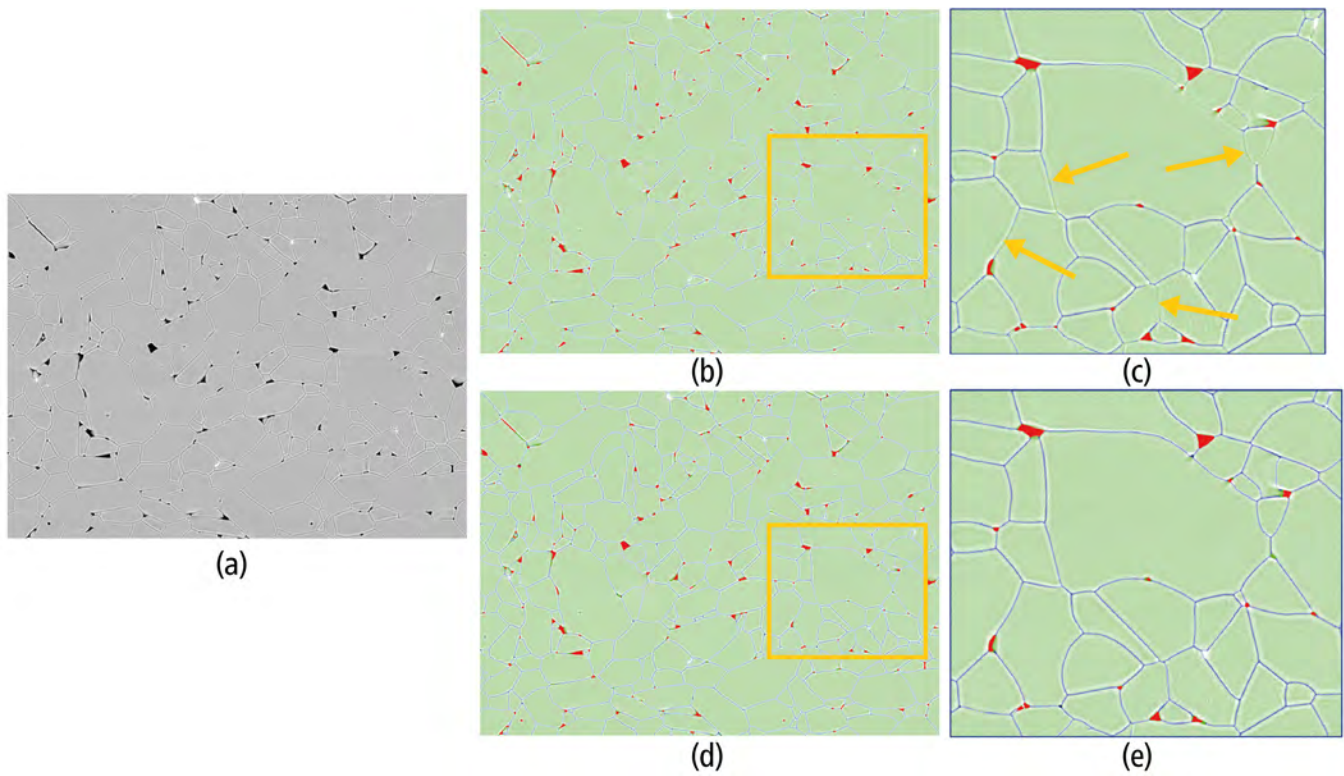


Figure 20: (a) Electron microscopy image of aluminum oxide microstructure. (b) Segmentation result of (a) obtained by applying a conventional Machine Learning model trained using the annotations from *Figure 18*. (c) A close-up of the area outlined by the square in (b). Although conventional Machine Learning methods produce results that appear satisfactory, upon closer examination, it becomes evident that numerous grain boundaries are not continuous. As a result, any attempt to measure grain size using this image would result in erroneous findings that are biased toward larger grain sizes. (d) Segmentation result of (a) obtained by applying a Deep Learning model trained using the annotations from *Figure 18*. (e) A close-up of the area outlined by the square in (d). Deep Learning segmentation resulted in continuous grain boundaries, which will yield more reliable grain size measurements.

Segmentation is often an intermediate step in a bigger analysis goal, such as grain size analysis. *Figure 21* shows the results from grain size analysis using the respective segmented images from Machine Learning and Deep Learning approaches. The analysis was performed using the ZEN software by assigning all enclosed regions within continuous grain boundaries to a specific grain.

The Deep Learning-based segmentation produces continuous grain boundaries that accurately represent the true grain structure in the aluminum oxide micrograph. However, the porous grain boundaries from the Machine Learning segmentation resulted in the bulk of the image being detected as a single grain (shown as the red region

in *Figure 21b*). Any subtle changes in image quality can result in significant differences in quantitative results if image segmentation is inconsistent. Deep Learning has better generalization ability and can forgive image variability to some extent, making it ideal for tasks where even subtle image variability is expected, and for applications that need highly reproducible results with minimal human intervention.

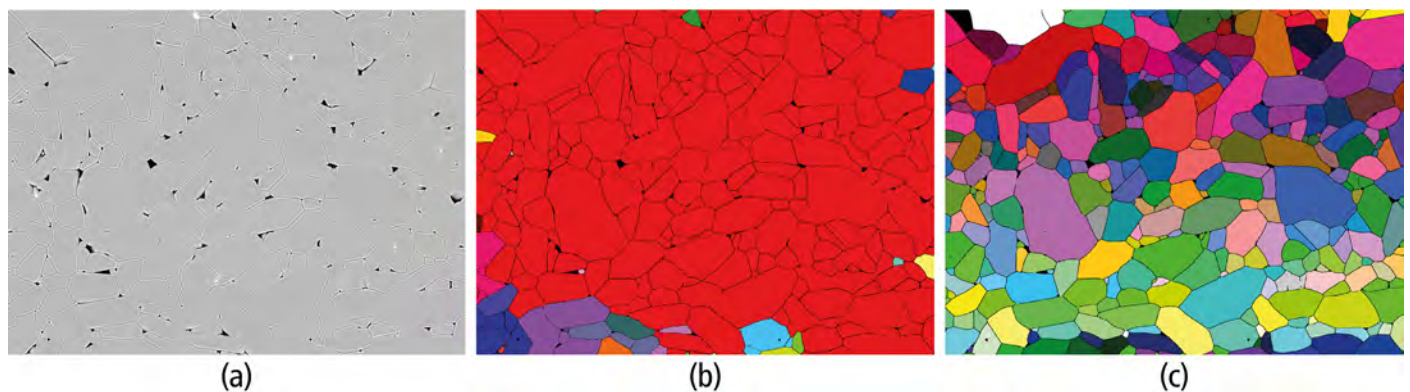


Figure 21: (a) Electron microscopy image of aluminum oxide microstructure, identical to that shown in *Figure 20a*. (b) Grain size analysis using the image segmented by conventional Machine Learning incorrectly assigns the bulk of the pixels to a single large grain, shown in red. (c) Analysis using the Deep Learning-segmented image demonstrates that the grains are correctly identified, offering more precise grain size distribution data when compared to Machine Learning.

Enhancing single-cell analysis with instance segmentation in phase contrast microscopy images

Cell tracking is a commonly used assay in biotech research, as it provides valuable insights into a wide range of diseases and conditions. For example, it can be used to monitor the behavior of cancer cells, including their proliferation, migration, and invasion, thus helping researchers to develop new cancer therapies and evaluate the effectiveness of existing treatments. While fluorescent labeling facilitates cell segmentation and tracking, researchers often choose to image cells in brightfield or phase contrast. This is because these imaging techniques can provide valuable information about cell morphology and structure, including the size, shape, and texture of the cell. Also, they do not require any additional preparation of the cells, such as labeling or staining, which means that the cells can be imaged directly in their natural state, without being altered by the labeling process. This is particularly important for studying certain cellular processes or phenomena, as adding fluorescent labels may interfere with or alter the behavior of the cells.

The challenges of segmenting brightfield micrographs

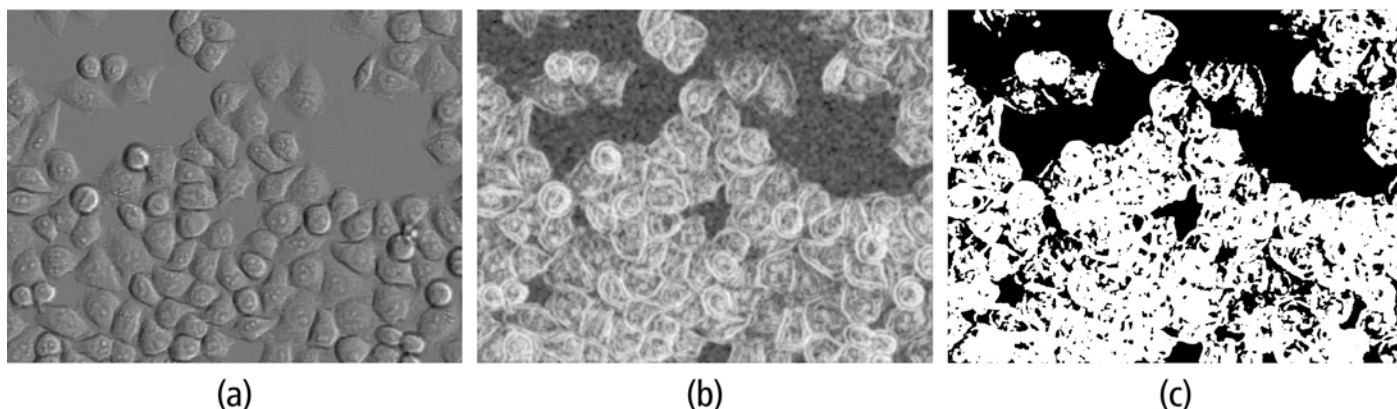


Figure 22: (a) Phase contrast image of HeLa cells captured at 10x magnification. (b) Entropy-filtered image revealing subtle variations in texture and tone from *panel (a)*. (c) Segmented regions containing cells against the background after applying a threshold to the image in (b). Note that while the cellular region is segmented, individual cells are not separated.

The benefits of object-based segmentation in biomedical applications

Both conventional Machine Learning and Deep Learning techniques (such as the use of U-net [4]) share a similar limitation: they cannot separate individual cells, which is essential for accurate tracking algorithms. While these methods may produce satisfactory results by defining an additional border class, a more reliable approach is to use object-based segmentation algorithms, also known as ‘instance segmentation’ in the AI community. This method is more effective in accurately segmenting individual cells, allowing for more precise tracking and analysis of their behavior.

Instance segmentation is a computer vision technique used for identifying and outlining individual objects within an image. Unlike semantic segmentation, which assigns a single label to each pixel in an image, instance segmentation identifies and separates objects

However, segmenting cells in brightfield and phase contrast images can be challenging, primarily because the average grey level of the cells is often equal to the average grey level of the background. This makes it impossible to segment cells using conventional threshold techniques. One solution is to apply digital filters to generate filtered images that can then be segmented using threshold techniques. For example, an entropy filter can highlight regions of high texture (see *Figure 22b*), which can help separate cells from the background.

However, this approach fails at properly separating cells from each other (see *Figure 22c*). Watershed-based separation is often used to address this issue, but it can lead to inconsistent results between frames, potentially making cell tracking discontinuous between frames.

based on their unique characteristics, such as shape, size, and color. It is particularly useful for biomedical applications, such as cell segmentation in brightfield and phase contrast microscopy images.

Instance segmentation of HeLa cells to track their movement, shape and size

In this case study, HeLa cells grown over time in a multi-well plate were imaged under phase contrast mode using a ZEISS Celldiscoverer 7 microscope with a Plan-Apochromat 20X/0.95 objective and 0.5x Tubelens yielding an effective magnification of 10x. To study the cells at a single-cell level, including tracking over time, they were segmented using the instance segmentation approach. The arivis AI toolkit on the ZEISS arivis Cloud platform was used to annotate the training images (see *Figure 23*).

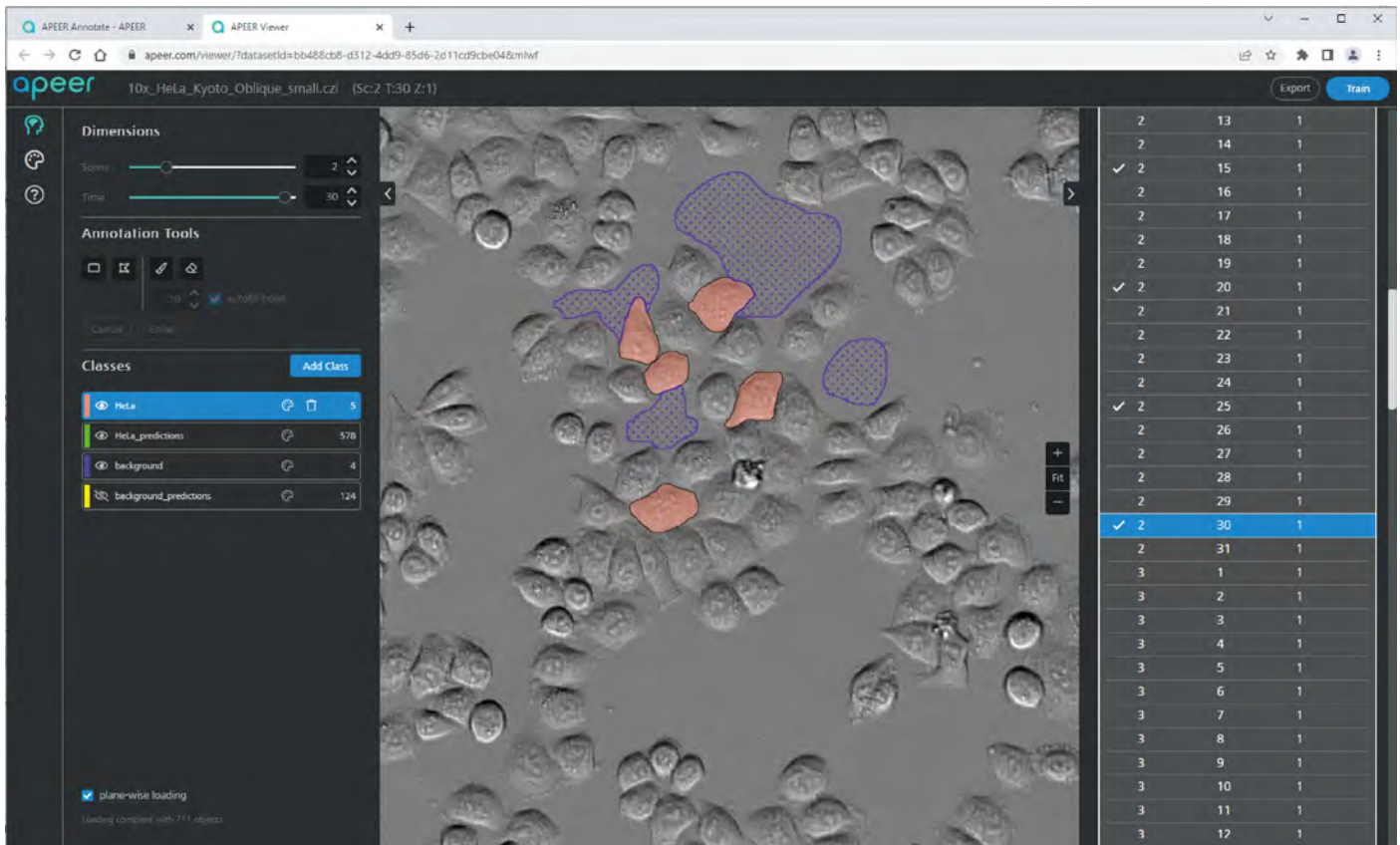
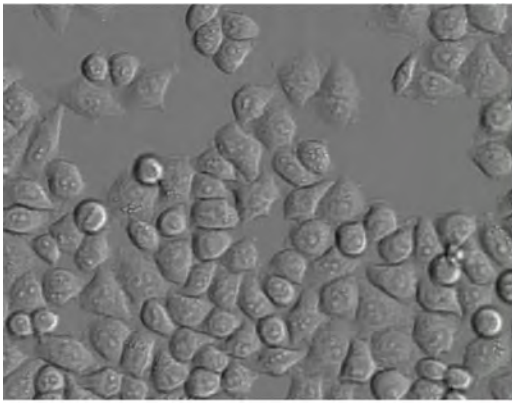


Figure 23: A screenshot of the annotation interface from arivis AI displaying a partially annotated training image of HeLa cells. The cells are clearly labeled in red, while the background is labeled in dotted purple.

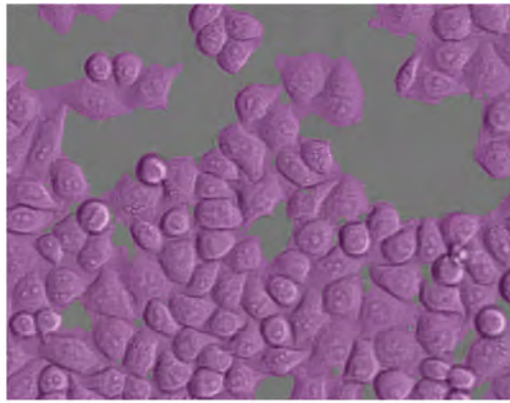
The arivis AI toolkit offers tools for both semantic segmentation and instance segmentation. A semantic model was initially trained only for demonstration purposes to illustrate the differences between the semantic and instance approaches. *Figure 24* shows the input image and its corresponding semantic segmented images. The segmentation successfully distinguished the cellular region and the background, but failed to separate the cells. Semantic segmentation is adequate if only the area fraction of the cellular region is required, but instance segmentation is the appropriate tool for tracking and extracting individual cellular information.

There are various Deep Learning-based algorithms available for instance (object-based) segmentation such as a modified version of U-net, but the most widely known algorithms are Mask R-CNN [6] and Mask2Former [7]. arivis AI uses a Mask2Former approach, which has been adapted to work with microscopy data and is capable of segmenting images with multiple input channels. The loss function is also customized for training with partial annotations, further improving the efficiency and accuracy of the training process. The annotations shown in *Figure 23*. were used to train the initial instance model, and further annotations were added based on the results to better segment regions where the model encountered difficulty, primarily the regions with high density of cells. This data-centric approach saves time by focusing on annotating challenging areas instead of wasting time on simple ones. *Figure 25* illustrates the results of instance segmentation on

the same input image as in *Figure 24*. The instance segmentation effectively separated individual cells, allowing for the tracking of cells in the time series image dataset.

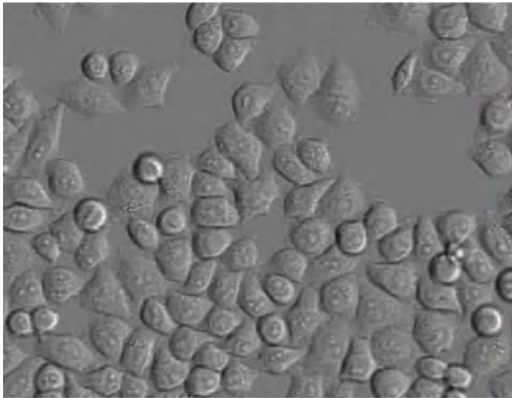


(a)



(b)

Figure 24: (a) Phase contrast image of HeLa cells captured at 10x magnification. (b) Semantic segmentation result using a U-Net-based Deep Learning architecture. The pink area represents the cellular region, which has been successfully segmented from the background. However, it should be noted that individual cells are not separated by this approach.



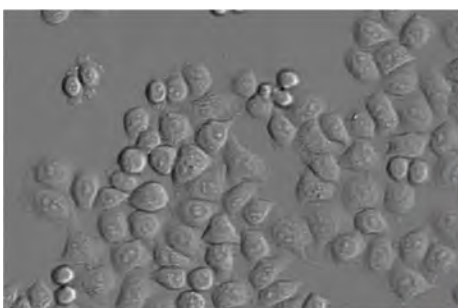
(a)



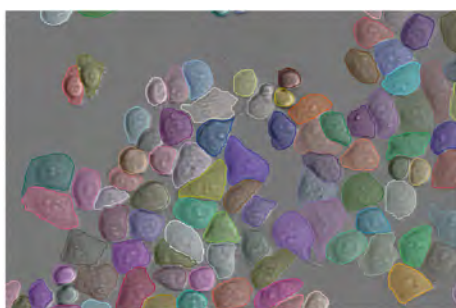
(b)

Figure 25: (a) Phase contrast image of HeLa cells captured at 10x magnification. (b) Result of instance segmentation using the Mask2Former Deep Learning method, clearly separating individual cells and enabling direct use of the result in applications such as cell tracking.

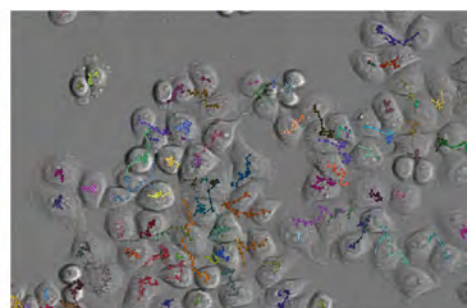
All images from the time series underwent segmentation using the trained model. The resulting masks were imported into ZEISS arivis Pro for further analysis, where cells were tracked and followed individually throughout the time course. Tracking was made easy by the well-separated, segmented masks generated through instance segmentation. Even cell division events were detectable in tracking, with daughter cells retaining their tracking identity. *Figure 26c* displays the first image in the time series with tracks overlaid to show the cell center positions at each time point.



(a)



(b)



(c)

Figure 26: (a) Phase contrast image of HeLa cells captured at 10x magnification. (b) Result of instance segmentation using the Mask2Former Deep Learning method. (c) Result of the tracking algorithm showing cell tracks overlaid on the original image from (a). The tracking analysis was performed using ZEISS arivis Pro.

While this particular use case focused on the use of instance segmentation for cell tracking, the instance segmentation approach can provide insights from images in many other ways. For example, the size and shape of cells can provide crucial information about their state and behavior, enabling the monitoring of the effects of various treatments on cells. As instance segmentation separates

individual cells, they can be sorted based on size (see *Figure 27b*) or shape (see *Figure 27c*) with ease.

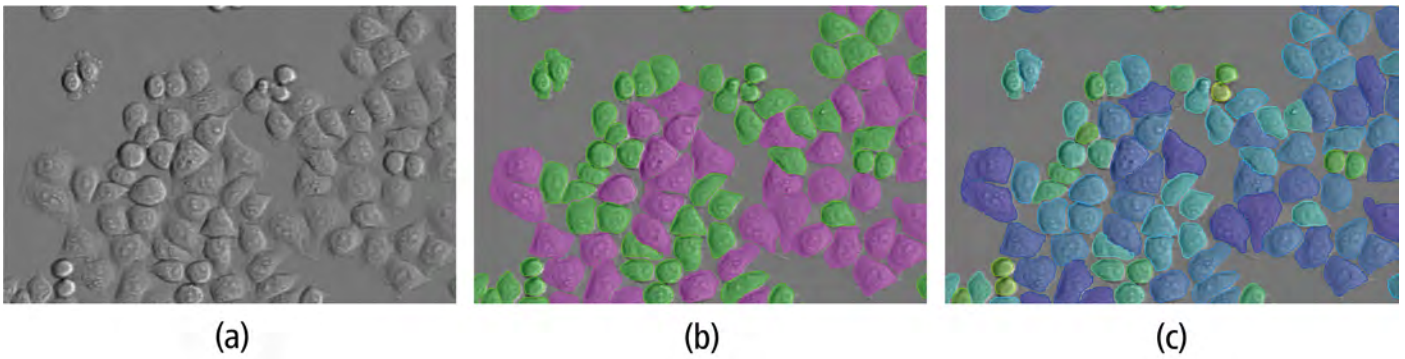


Figure 27: (a) Phase contrast image of HeLa cells captured at 10x magnification. (b) Cells are color-coded by size, with smaller cells in green and larger cells in pink. (c) Cells color-coded by shape, with rounded cells in green, less rounded cells in purple, and cells with medium sphericity in cyan.

In summary, instance segmentation enables the easy segmentation and separation of individual objects, allowing for various insights to be extracted through object tracking and sorting based on size and shape, among other methods. arivis AI's data-centric approach saves time and ensures efficient annotation of complex features for instance segmentation model training. The resulting trained model can then be used in end-to-end applications such as cell tracking.

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Summary

This book provided a comprehensive overview of the importance of AI, and how to use the technology for image analysis, and showed a diverse array of use cases.

The first chapter introduced the reader to the concept of AI and its significance in research, explaining how AI had become increasingly important in the field of image analysis. It introduced AI, Machine Learning, and Deep Learning, emphasizing that Deep Learning is the right technology for image analysis tasks with challenging images. The chapter also introduced the suite of software products by ZEISS that makes AI accessible to everyone.

The second chapter focused on image segmentation. It provided a historical overview of various image segmentation approaches, including Otsu thresholding, the Watershed algorithm, conventional Machine Learning, and Deep Learning. It also explained how conventional Machine Learning models could be trained in ZEN and Deep Learning models could be trained using the arivis AI toolkit. The chapter also highlighted several improvements to Deep Learning in the arivis AI toolkit, emphasizing the importance of data-centric model training. It also provided extra tips to enhance the efficiency of image segmentation.

In the third chapter, the book discussed how AI tools could be used in routine image analysis applications. Integration of AI was demonstrated using examples from microscopy, such as tissue and blood sample analysis for atypical cells and cell morphologies. Furthermore, it showed how AI tools help with repetitive and time-consuming tasks and eliminate human error. The chapter reviewed the ZEISS Labscope imaging app and showed how its AI modules benefit these applications.

The fourth chapter focused on using trained AI models and the image analysis pipelines in ZEISS arivis Pro. It explained how Deep Learning models trained using the arivis AI toolkit on ZEISS arivis Cloud could be imported into ZEISS arivis Pro to automate entire image analysis processes and push the boundaries of analysis complexity. The reader was informed about how these AI-driven models enable a new level of automation in image analysis, which

results in increased throughput, reduced human bias, and shorter time required to generate reproducible and reliable results.

Since the use of AI technology had become increasingly significant in science and industry, the fifth and final chapter of the book centered on a few pertinent case studies. These case studies showcased how AI-enabled analysis of microscope image datasets provided new and faster answers to research or engineering problems. One of the case studies demonstrated the potential application of AI tools in segmenting and measuring organelles, characterizing mitochondria, and classifying the spatial distribution of nuclear pores using a volumetric FIB-SEM dataset. Another case study demonstrated how AI image analysis assisted in understanding Wnt inhibition in organoid formation. Other case studies demonstrated similar benefits in a diverse range of scientific fields. For example, segmentation of neurons into dendritic spines and neuronal projections for neurological disease research; segmentation of the microstructure of aluminum oxide in assessing specimen viability for materials science applications; and accurate segmentation of cells to help distinguish them in phase contrast microscopy.

This book presented a comprehensive guide to the use of AI technology in image analysis.

Looking ahead, several emerging trends and future directions in the field of AI for image analysis are worth noting. One is the application of AI to volumetric image data, such as CT and MRI scans. AI techniques are being developed to automatically detect and segment abnormalities in these images, such as tumors or other lesions. Another promising area is the use of AI for real-time image analysis, where AI algorithms can analyze images as they are being acquired. This will give immediate feedback and enable real-time adjustments to experimental protocols. Additionally, as AI techniques become more sophisticated and are trained on larger and more diverse datasets, we can expect to see more accurate and reliable analysis of complex images, such as those with multiple overlapping objects.

For readers looking to apply AI technology to their own image analysis, here are a couple of additional tips and best practices to keep in mind.

- Carefully consider the problem and determine whether AI is the appropriate tool to use.
- Have a clear understanding of the data and ensure that there is sufficient high-quality data to train the models.
- Select the AI tools carefully, as different algorithms may be better suited to different types of data and analysis tasks. For example, the instance segmentation algorithm is better suited to segment individual cells separately, while the semantic segmentation algorithm is more appropriate when cells need to be segmented collectively from the background.
- Continually evaluate and validate the models and incorporate feedback from domain experts to ensure accurate and meaningful results.

In closing, we thank readers for their time and interest in this book. We hope it provided a useful introduction to the exciting possibilities of AI technology for image analysis and inspired readers to explore these techniques further in their own research and work. AI is a rapidly evolving field, and there is much to learn and discover. By continuing to learn and explore, we can unlock new insights and capabilities in image analysis that have the potential to revolutionize many fields, from healthcare to materials science and beyond.

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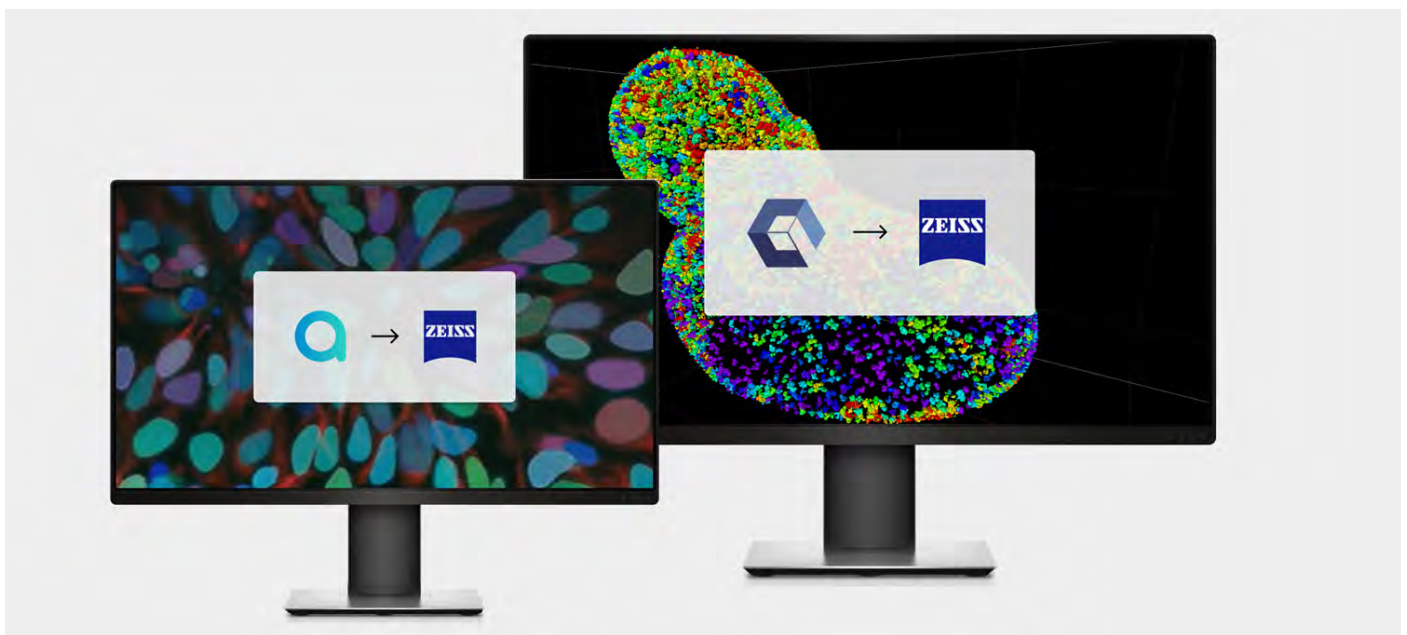
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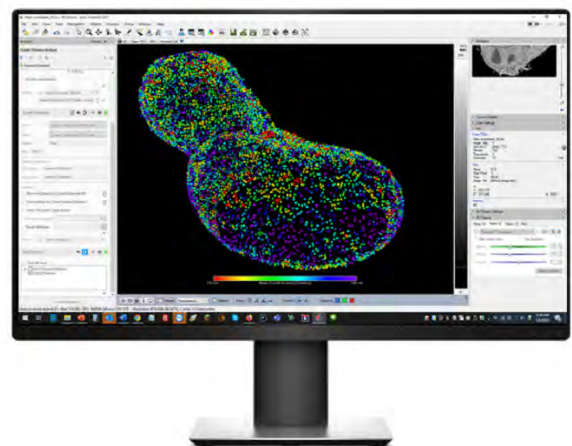
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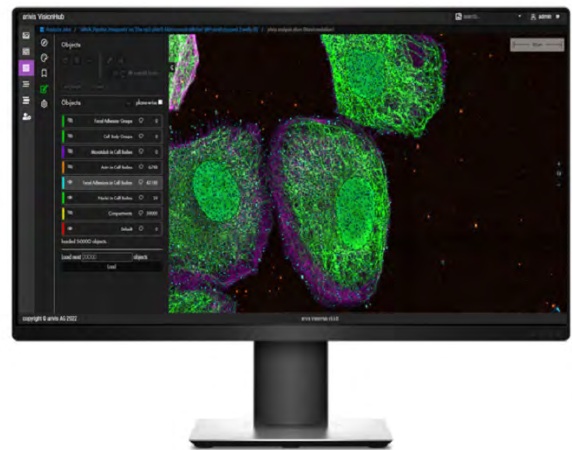
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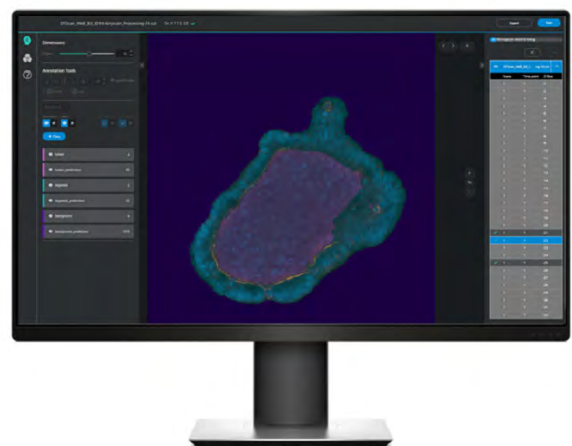
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- Dr. Benjamin Schwarz, Market Sector Manager, Carl Zeiss CMP GmbH

Chapter 4: How to use AI models for advanced image analysis

- Dr. Johannes Amon, Carl Zeiss Microscopy Software Center Rostock GmbH
- Maria Marosvoelgyi, Product Manager, Carl Zeiss Microscopy Software Center Rostock GmbH
- Christian Götze, CTO Carl Zeiss Microscopy Software Center Rostock GmbH
- Andreas Suchanek, CEO Carl Zeiss Microscopy Software Center Rostock GmbH

Chapter 5: Case studies

Analysis of FIB-SEM Volume Electron Microscopy Data

- Dr. Mariia Burdyniuk, Customer Success Specialist, Carl Zeiss Microscopy, LLC
- Dr. Christopher Zugates, Head of Customer Success, Carl Zeiss Microscopy, LLC

Analysis of Mitochondria Using Deep Learning

- Dr. Wendy Bautista, Physician Scientist, National Cancer Institute (NCI)
- Dr. Mones Abu Asab, Senior Ultrastructural Scientist, National Eye Institute, NIH

Organoid Analysis

- Dr. Philipp Seidel, Product Marketing Manager Life Sciences Software, Carl Zeiss Microscopy GmbH
- Dr. Volker Doering, Application Development Engineer, Life Sciences Automation, Carl Zeiss Microscopy GmbH

Microscopy and Deep Learning for Neurological Disease Research

- Dr. Sreenivas Bhattiprolu, Director, Digital Solutions, Carl Zeiss X-ray Microscopy, Inc.
- Dr. Kevin O'Keefe, Senior Software Sales Biotech Pharma, Carl Zeiss Microscopy, LLC
- Dr. Amita Gorur, Senior Applications Scientist, Carl Zeiss Microscopy, LLC
- Dr. Christopher Zugates, Head of Customer Success, Carl Zeiss Microscopy, LLC
- Dr. Andy Schaber, Product Application Sales Specialist, Carl Zeiss Microscopy, LLC

Improving Microstructure Analysis of Aluminum Oxide with Deep Learning

- Dr. Sreenivas Bhattiprolu, Director, Digital Solutions, Carl Zeiss X-ray Microscopy, Inc.
- Tim Schubert, Materials Scientist, Institut für Materialforschung (IMFAA)

Enhancing Single-Cell Analysis with Instance Segmentation in Phase Contrast Microscopy Images

- Dr. Sreenivas Bhattiprolu, Director, Digital Solutions, Carl Zeiss X-ray Microscopy, Inc.
- Dr. Sandra Lemke, Product Owner - AI and Applications, Carl Zeiss Microscopy GmbH
- Dr. Frank Vogler, Applications Specialist, Carl Zeiss Microscopy Deutschland GmbH
- Dr. Marion Lang, Product Manager, Carl Zeiss Microscopy GmbH

Chapter 6: Summary

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