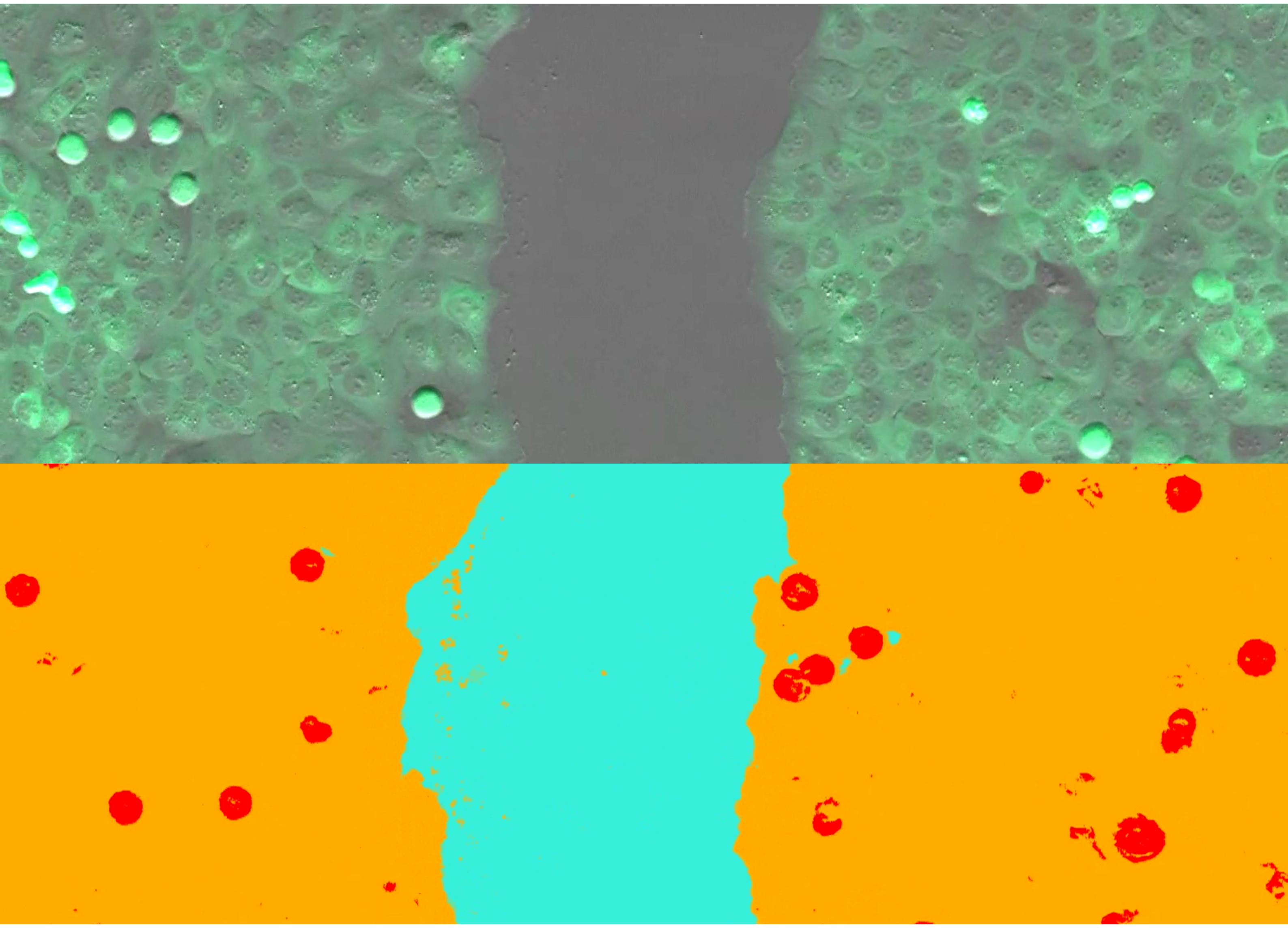
Application Note



ZEISS ZEN Intellesis

Machine Learning Approaches for Easy and Precise Image Segmentation

ZEISS ZEN Intellesis

Machine Learning Approaches for Easy and Precise Image Segmentation

Authors: Rosy Manser, Rebecca Elsässer, Volker Döring
Carl Zeiss Microscopy GmbH, Germany

Date: July 2018

Challenges in Image Analysis

Why do scientists use microscopy to advance their research? Is it because they like looking at pretty images? As enjoyable as these are, the real value from microscopy infrequently originates from images, but from the data that they provide. Thus, the acquisition of microscopic images is only the first step in the image (analysis) workflow of most scientists. Very often this imaging workflow consists of multiple steps: Image acquisition, reconstruction, processing, segmentation and then image analysis. Currently, the image segmentation step is one of the biggest challenges faced by today's microscopists.

Why is segmentation so important? Segmentation lays the foundation for subsequent image analysis steps, i.e. data extraction and quantification. In order to count, describe or quantify the parameters of a specific feature in the specimen, e.g. the number of mitotic cells (Figure 3), it is crucial to precisely identify and separate the features of interest from the rest of the image. This is exactly what segmentation enables: Based on different characteristics of the features of interest, it can accurately assign each pixel in an image into one of several classes, e.g. background, migrating cells, organoid (see Figure 5). Subsequently, these feature classes can be used to run image analysis algorithms in order to extract data, e.g. analysis of growth rates of an embryo (Figure 4), or improve the visualization of the data (Figure 2). For reliable data extraction, however, it is imperative to obtain reliable and accurate segmentation results. This is not trivial to achieve, and with conventional threshold-based methods this frequently requires an image processing specialist to create a segmentation workflow using a combination of digital filters and tools.

Machine learning for image segmentation

Image segmentation is often the starting point for subsequent processing steps. Generally speaking, two approaches to achieve segmentation exist: Classical threshold-based and machine learning methods. While it is possible to obtain very good results with threshold-based segmentation, a profound knowledge of image analysis is necessary. Machine learning on the other hand offers even non-experts the possibility to easily create robust and reproducible segmentation results. In addition, there are datasets where threshold-based methods struggle or fail to generate meaningful results (e.g. low contrast brightfield images). In these rather difficult cases, machine-learning methods are often still able to yield good image segmentation outcomes (Figure 5).

There are various options when it comes to machine learning in image analysis. For example different approaches to training a neural net and how the classification works. Two methods can be used to train a neural net – either supervised or unsupervised learning. In supervised learning, a teacher provides an input of classified data – in the context of image analysis this can be a dataset where some features are manually labeled by the user, this forms the so called "ground truth". In unsupervised learning, the algorithm tries to identify different features from many unlabeled images on its own. In both cases a neural net will define a set of rules by itself that enables it to later classify features in images that are different from the one used for training.

Pixel-based and object-based classification are different ways that a neural net analyses and classifies image content. Pixel based models are generally easier to train while object based models can achieve better and faster classification results at the cost of a more demanding initial training process.

Machine learning based image segmentation can be easy to use, since in principle only knowledge about the structures of interest is necessary without the need to know and adjust a vast set of frequently complex image processing parameters. Users can train a model simply by painting the different structures of interest within an example image. Based on the initial labeling a neural net is trained automatically and this model can be used repeatedly to segment different datasets.

The resulting analysis model is more powerful than classical threshold based segmentation, as it combines a variety of different filters (e.g. intensity, edge detection, texture, …) that make the segmentation results more robust and reproducible for quantitative data analysis. In addition, this method minimizes errors and any influence that may result from user bias.

The software module ZEN Intellesis makes all these advantages of machine learning accessible within the ZEN blue software platform. It enables easy and precise segmentation of multidimensional images including 3D datasets. Furthermore, it supports images from a vast variety of different imaging modalities, ranging from classical wide field, confocal and super-resolution to electron and X-ray microscopy.
ZEN Intellesis can be used with image formats readable by the ZEN imaging software including CZI, TXM, TIFF, JPG, PNG and more.

In summary, the segmentation module ZEN Intellesis can be trained to use machine learning to automatically identify objects within an image according to a predefined set of rules (the model). This enables any microscopy user to perform image segmentation even on complex data sets without programming experience or advanced knowledge of how to set up an image segmentation. The result – a correctly classified image – can then be seamlessly integrated into the ZEN image analysis workflow in order to extract data.

ZEN Intellesis at work

The following examples highlight the potential and advantages of machine learning based segmentation utilizing the new software module, ZEN Intellesis.

Neuroscience Applications

a. Separation of spines and dendrites in a GFP-labeled neuron

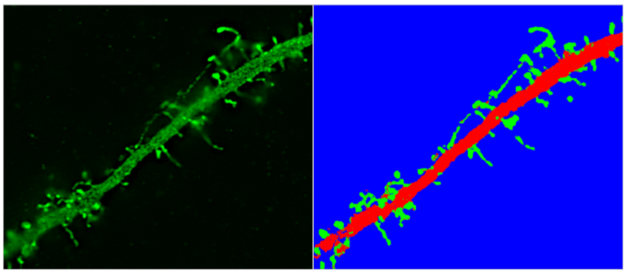


Figure 1 *GFP expressing neuron. Left: The raw image acquired with structured illumination shows spines on a dendrite. Right: Result of ZEN Intellesis Segmentation. A good separation of spines (green) from dendrite (red) and background (blue) is possible.*

This SIM image of GFP labelled neurons has been acquired with Elyra PS.1. Here, the challenge is to separate the spines from the dendrites in order to quantify the number of spines. Thresholding alone will simply not succeed, because the intensities of the dendrite and spines are very variable, and thus, not consistently different enough between the two features. As a result, additional factors need to be taken into account in order to separate them. Having created the classification matrix by labeling some areas of the spines and the dendrite in the training module, the resulting segmentation shows very nice separation and identification of both components of the neuron. Accurate counting and quantification of the spines is now feasible, which allows for reliable comparison of the same measurables in multiple different samples.

b. Drosophila brain sections

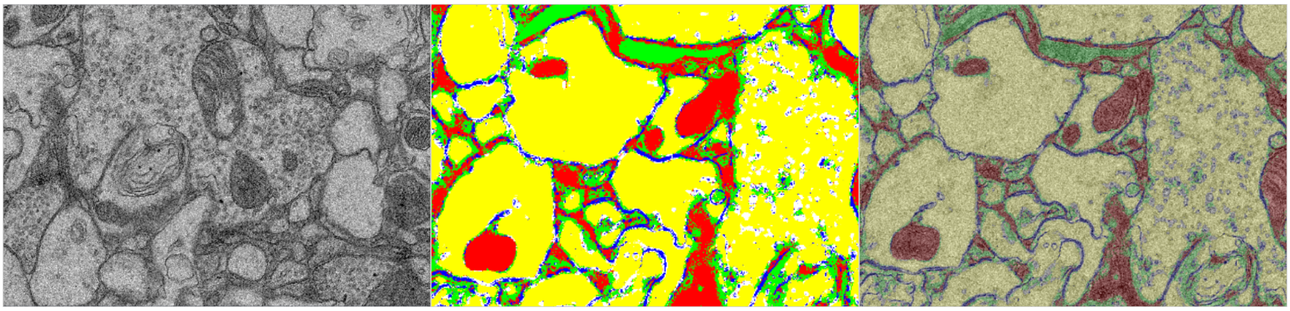


Figure 2 *Calyx region of a Drosophila brain. Left: EM image showing mitochondria, synapses and presynaptic vesicles. Middle: Subset of left image showing segmented areas for mitochondria (red), membranes (blue), cytoplasm (yellow), intercellular space (green) and vesicles (white), segmented with ZEN Intellesis. Right: Overlay of second image and raw data. Images courtesy of Max Planck Institute for Molecular Genetics, Berlin.*

When it comes to electron microscope data, and especially EM data, one of the biggest issues is the level of noise. This makes it challenging for classical segmentation approaches, because thresholding alone does not provide accurate results. This is an EM image of a section of the calyx region of a 30-day-old Drosophila. The goal of the experiment was to identify the different structural components of the sample.

5 different groups of features have been classified: vesicles are identified in white, membranes in blue, mitochondria in red, cytoplasm in yellow and intercellular space in green. All of the information from the painted pixels is used for the segmentation. After image segmentation utilizing ZEN Intellesis all five components can be distinguished and quantified, despite the relative high noise and small grey-scale differ-

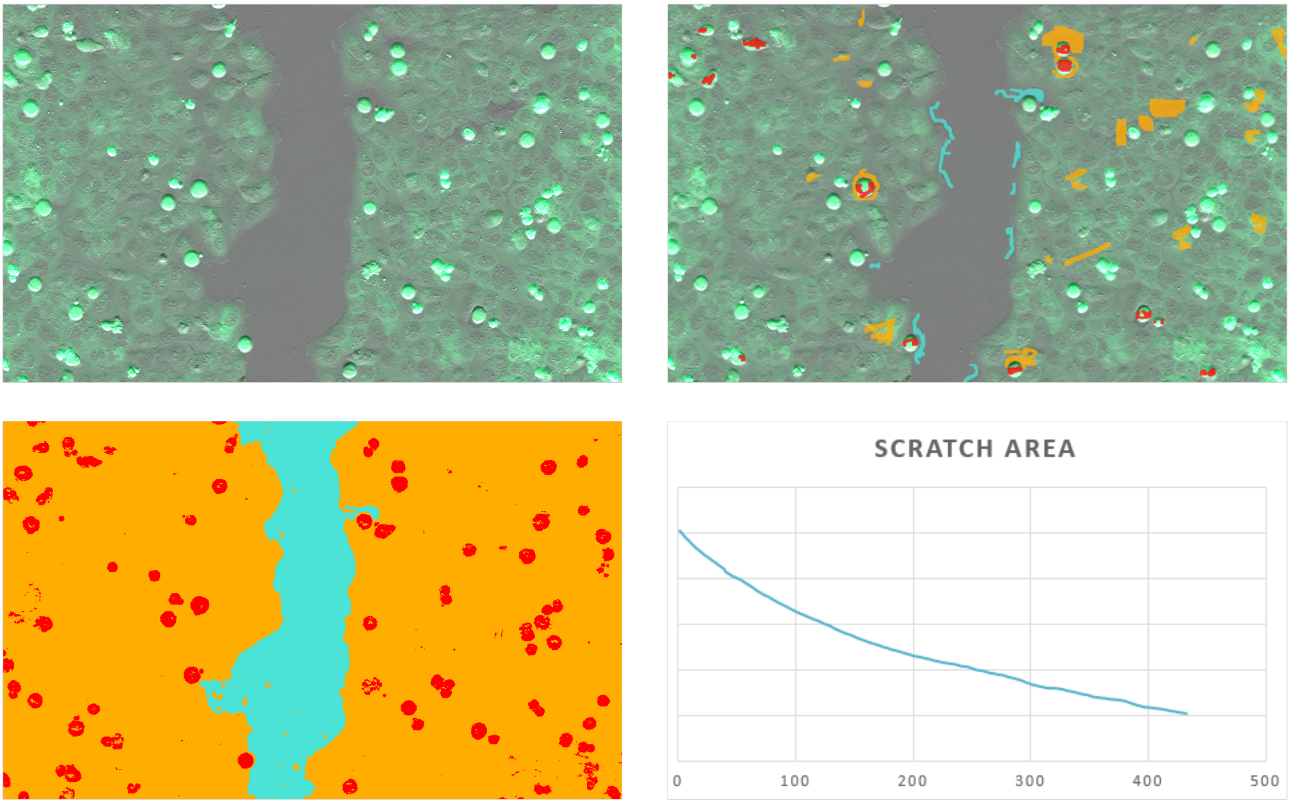


Figure 3 *Scratch Assay of GFP expressing HeLa cells. Top left: One frame of a recorded time-lapse movie. Top right: Labeled pixels used for training of ZEN Intellesis. Bottom left: Segmentation result of trained ZEN Intellesis model – scratch area (turquoise), cell layer (orange) and mitotic cells (red). Bottom right: Based on ZEN Intellesis segmentation results the size of the scratch area was measured over time using the ZEN Image Analysis module.*

ences within the image. The approach of ZEN Intellesis to consider a large number of different features for the segmentation (rather than just greyscale differences as for classical thresholding approaches) ensures an accurate result.

When the segmented data and the original image are overlaid, it is obvious that the visibility of different structures in EM images is significantly improved. This allows measurement and quantification of the various sample structures.

Cell Biology Research – Scratch assay

This is an example of a wound healing assay. Cells are grown to confluency and then a scratch creates a gap within the cell layer. The rate at which the cells bridge this gap and the number of mitotic cells can be used to assess the impact of different genes or pharmaceutical agents on cell proliferation.

The images show the raw data and the segmented data. Also the labeled areas for initial training are shown. The precise segmentation allows a reliable quantification and the extraction of numerical data from this dataset. Here, the total scratch area (as a measure of how well the cells proliferate) has been determined. Without the power of ZEN Intellesis to enable efficient and accurate segmentation of the original dataset, these quantifications would be extremely challenging and considerably less reliable in terms of numerical accuracy.

Developmental Biology – Mouse embryo growth assay

The development of a mouse embryo was imaged using Brightfield on a Celldiscoverer 7. The goal of the experiment was to assess embryo size and how it changed over a 16 hour time series. In this proof of principle experiment, it was possible to visualize embryo development over this duration for the first time due to the stable incubation environment of the Celldiscoverer 7.

The first step to automate the process of assessing the embryonic growth was to segment the embryos at each time point from the background. During the initial training of the ZEN Intellesis model, the background has been allocated in turquoise and the embryo in green simply by painting some areas within one example image. The resulting processed images clearly differentiate background from embryo in each of the time lapse images.

The segmented data is then analyzed to provide a measure of embryo size at each of the time points. All of that information can be compiled into numerical data using the ZEN Image Analysis module, which enables quantification of the embryo growth over time.

Using the ZEN Intellesis module for the automatic segmentation of the embryos from the background saves a huge amount of time and also increases the reliability of the data relative to segmenting the data by hand.

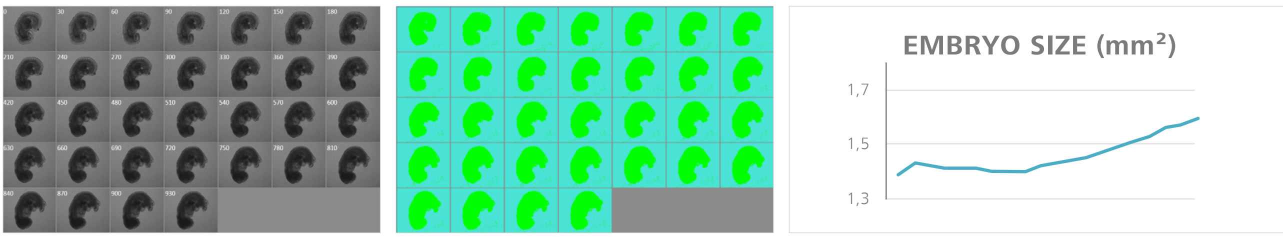


Figure 4 *Growth Assay of mouse embryo. Left: Image series of time-laps movie. Middle: Segmentation result of trained ZEN Intellesis model – embryo (green), background (turquoise). Right: Based on ZEN Intellesis segmentation results the Embryo size was measured over time using the ZEN Image Analysis module. Images courtesy of Max Planck Institute for Molecular Genetics, Berlin.*

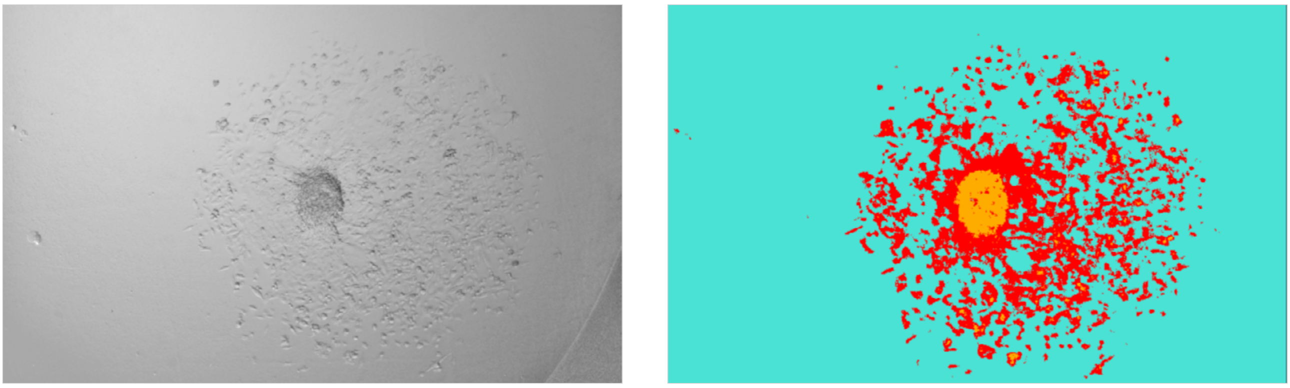


Figure 5 *Organoid and migrating cells in transmitted light. Left: Phase Gradient Contrast image from the Celldiscoverer 7. Right: Segmentation result of trained ZEN Intellesis model – organoid (orange), migrating cells (red), background (turquoise). Images courtesy of DKFZ, Heidelberg.*

Cancer Research Organoid growth

This is an organoid of immortalized mammalian cells that was imaged using the Celldiscoverer 7. The goal of this experiment was to explore the total size of the organoid, as well as the behavior of the cells that migrate from the organoid. By quantifying these parameters, it is possible to understand how different treatment conditions can affect the behavior of the cells in the organoid, which can then potentially be extrapolated to different cancer models.

The major challenge with segmenting this dataset arises from the edge of the well in the bottom right hand corner that shows a significant amount of gradient. For classical threshold based segmentation approaches, this poses a real issue because the relative intensity alone does not provide an accurate measure for segmenting the data. Employing all of the different image parameters and convolutions that ZEN Intellesis utilizes, the painted pixels provide a much more comprehensive and accurate feature set on which the segmentation can be based.

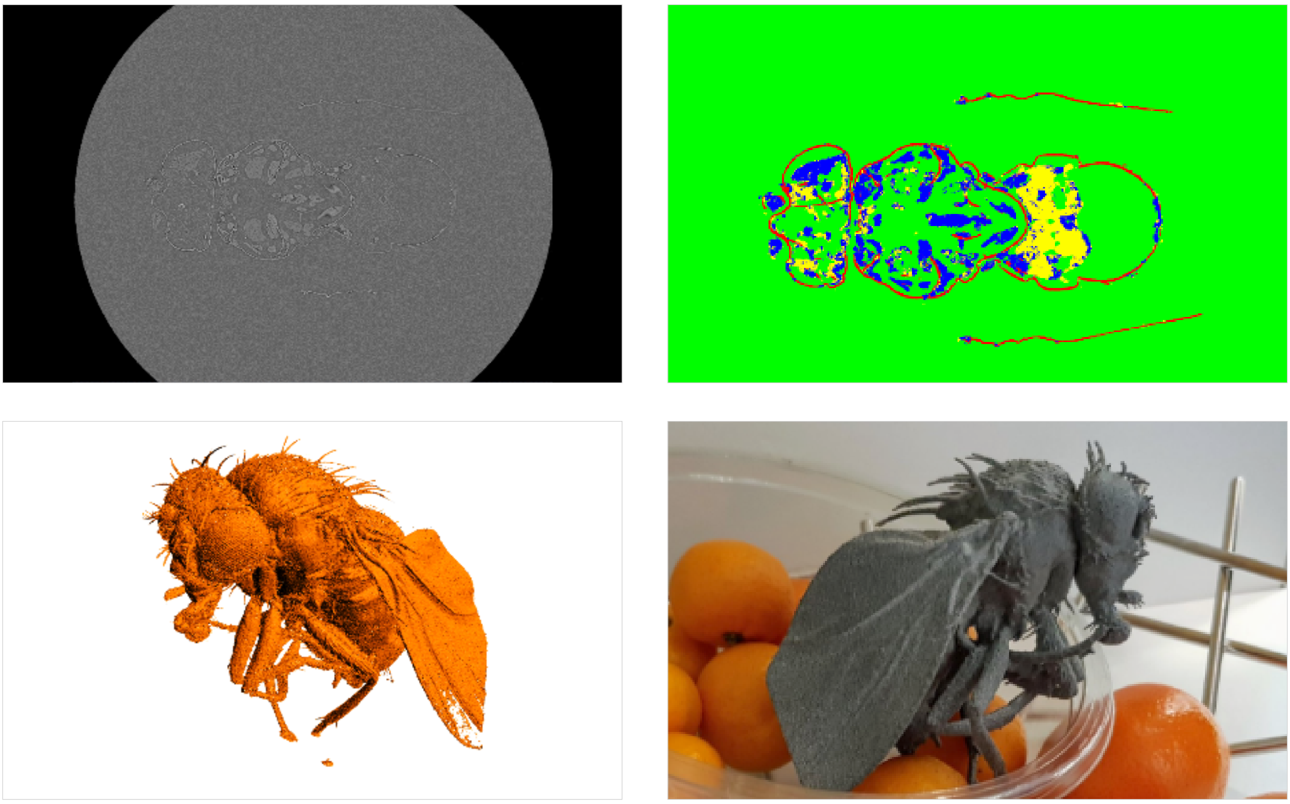


Figure 6 *X-Ray micrograph of a Drosophila. Upper left: One slide of a 1400 slide z-stack from Drosophila imaged with a 520 Versa. Upper right: Segmentation result of trained ZEN Intellesis model – exoskeleton (red), inner structures (blue and yellow), background (green). Lower left: rendered 3D model based on exoskeleton class (red in upper left image). Lower right: 3D printed model.*

The result is a very accurate segmentation of the organoid itself, the migrating cells and the background. This allows for precise quantification of the size of the organoid itself and also the spread and behavior of the migrating cells.

Handling 3D data sets – Visualization and 3D printing
This is an X-Ray dataset of an adult Drosophila melanogaster. This sample has been critical point dried and then imaged using the 520 Versa to create a 3D dataset of the whole fly. On the left side a single slice of the z-stack is shown. The goal of the experiment was to create a 3D rendering of the fly that could be used for comparative measurements by creating a physical model using a 3D printer.

In order to accurately segment the surface of the fly, several different structures were identified in the classification matrix including the exoskeleton (in red), and two different internal elements (in yellow and blue). The segmented image shows that by using this combination of features the exoskeleton can be cleanly separated from the rest of the dataset.

Having identified the exoskeleton, a 3D rendered model of the fly was generated. This model shows an incredible level of detail regarding the external structure of the fly. Using this rendered model data, a physical 3D model of the fly was printed with the help of a 3D printer. This fabulous tactile training tool can be used for teaching and public engagement in science. All of this is made feasible by the ZEN Intellesis module which provided the accurate segmentation necessary to identify all of the features of the fly´s exoskeleton.

What is Machine learning?

Machine learning is a subdivision of artificial intelligence. By machine learning IT systems can identify patterns and develop solutions based on existing databases and algorithms. Thus, computer programs based on machine learning can use algorithms to find solutions for new and unknown problems independently.

Machine learning is not a new concept. It was appreciated over 20 years ago that machine learning was extremely useful for image analysis, but the computational demands of the processing prevented it from being used in mainstream applications. Since computing power has significantly increased and the relative cost has significantly reduced in the last 20 years, machine learning is now used a great deal for image analysis. The applications for which it can be used are constantly growing. A few additional examples of areas where machine learning already is being used are:

- facial recognition
- advertising
- personal assistants
- autonomous ("self driving") vehicles
- news feeds
- optical character recognition

These provide an idea of how common this technology area is.

Carl Zeiss Microscopy GmbH
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/zen-intellesis