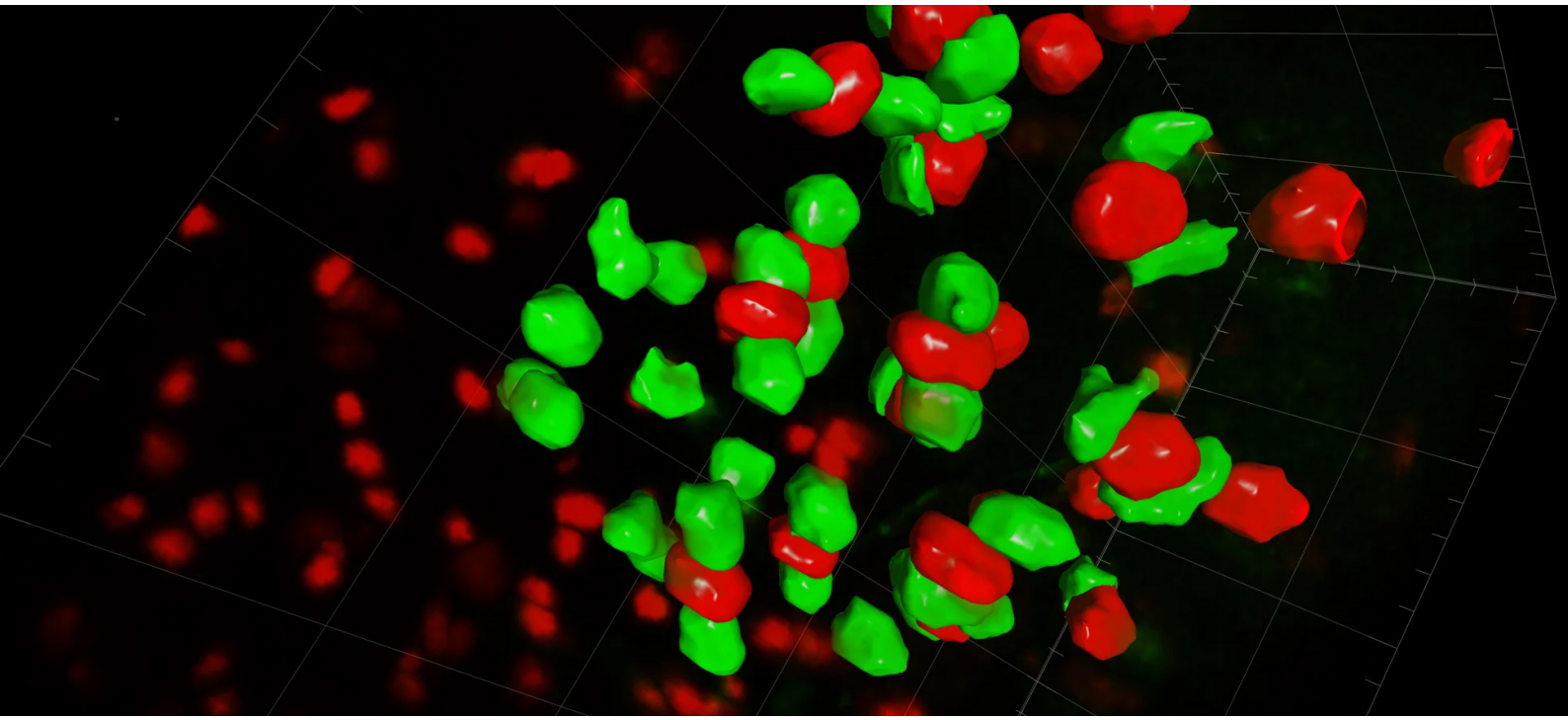
White Paper

Selected Life Science Research Studies with ZEISS arivis Pro



ZEISS

Seeing beyond

Case studies in this collection compiled by:

Johannes Amon Ph.D.

Carl Zeiss Microscopy SW Center Rostock GmbH

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Case Study 1:
Exploring Whole Organisms at Single Cell Resolution

Integrating subcellular information into the context of whole tissues is important for understanding cellular networks and their contribution to normal and diseased conditions. Challenged by the size and opacity of tissues, only recent advancement in imaging techniques paved the way to study intact organs at a subcellular resolution. However, using techniques such as Light-Sheet Microscopy is accompanied by the generation of large image data files, which complicates post-imaging data handling and processing drastically. The acute brain injury research group of Dr Ali Ertürk at the Institute of Stroke and Dementia Research (ISD) in Munich, is leading in breaking the limitations of volumetric imaging by using Light-Sheet Microscopy combined with innovative tissue clearing methods. Tackling the challenges of large imaging data sets, the lab benefitted from the unique feature of ZEISS arivis Pro (formerly Vision4D) as an imaging data platform for easy handling and processing of data sets unlimited in size. Based on the arivis ImageCore Technology, ZEISS arivis Pro is unreached in its performance of handling large imaging data sets even on standard hardware. This product combines a variety of data pre-processing features and interactive visualization tools with the ability to handle large data sets, giving the user the possibility to standardize complete workflows.

In this study, Dr Ali Ertürk and his team analyzed the mouse spinal cord using Light-Sheet Microscopy (LaVision Biotec, Ultramicroscope II) and uDISCO tissue clearing, Due to sample size, image acquisition was done via mosaic tile scanning resulting in three individual stacks with different z-orientations. This imaging procedure necessitated a demanding post-imaging processing in order to fuse the individual stacks into one complete data set of the spinal cord.

This process was further complicated by the size of one data set (300 GB per volume). Only the interactive Volume Fusion tool of ZEISS arivis Pro was able to fuse these large data sets to one resulting image 1.2 TB and open the possibility to study the spinal cord in its native structure.

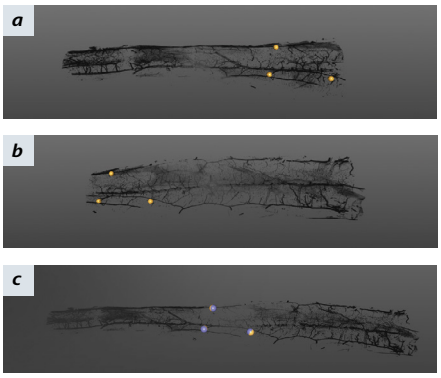

Figure 1: *Fusion of separate mouse spinal cord data sets. arivis Volume Fusion allows the fast combination of large data sets with different orientations (a, b) into one data set (c).*

About the author

Dr Ali Ertürk
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www.helmholtz-munich.de/bioengineering-center/ibio/pi/ali-ertuerk

The acute brain injury research group led by Dr Ali Ertürk is interested in understanding the cellular and molecular mechanisms leading to chronic neurodegeneration after acute brain damage including early onset dementia, epilepsy and neuropsychiatric disorders. To map the pathological brain, the lab utilizes cutting-edge techniques including high-resolution 3D imaging of the entire brain, a technique developed in the lab. Additionally, the lab screens for novel molecular players that are altered in chronically affected brain regions to develop effective ways to halt secondary neurological problems after acute brain injuries. As one of the main struggles in neuroscience is the difficulty to analyze long connections in the brain, the lab in parallel develops and applies new imaging tools to improve the capability to visualize and analyze complicated anatomical connections in the brain.

www.ncbi.nlm.nih.gov/pubmed/27548807

Case Study 2:

Understanding Gastrulation and Different Migration Phenotypes in an Early Mouse Embryo

This is a research area that can generate large image files as we try to capture bigger fields of view and higher spatial and temporal resolution. Isabelle Migeotte's group used two-photon microscopy and live imaging to follow and understand the gastrulation process in the mouse embryo. In particular, they focus on the differentiation and cell migration process of cells in the mesoderm layer.

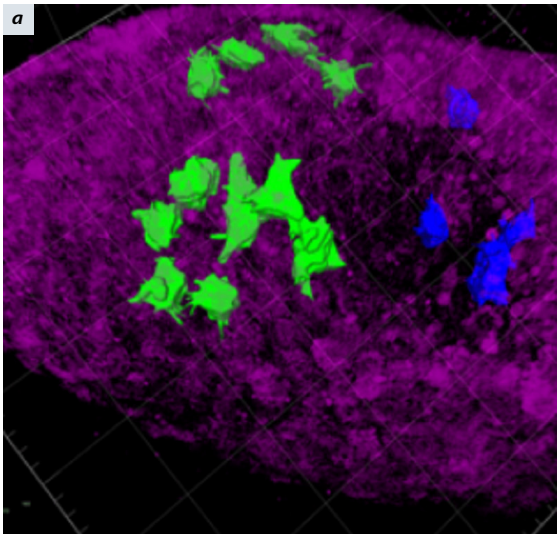

Figure 2a: *Morphologically, extra-embryonic cells are larger, longer and develop small protrusions (blue colored cells) versus the embryonic cells (green) which are smaller in volume with more protrusions. On average embryonic cells were approximately 2.000 µm³ in volume with a mean filopodia length of 8 µm. Extra-embryonic cells were double inside (4200 µm³) with shorter filopodia (6 µm).*

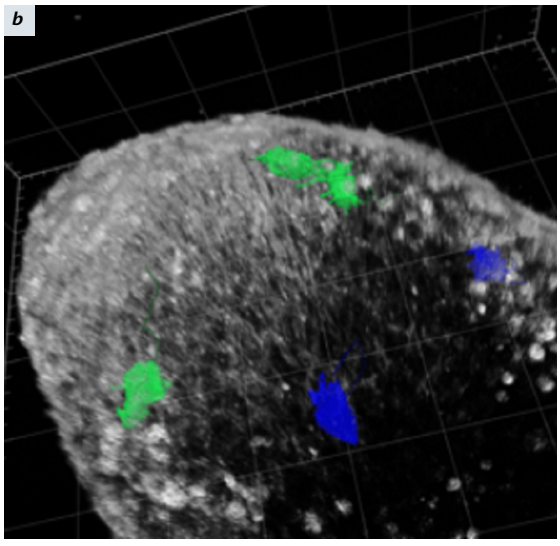

Figure 2b: *In terms of migration patterns, embryonic cells tend to have a more directional trajectory (green) versus a zigzag movement shown by extra-embryonic cells (blue). Researchers report that embryonic mesoderm cells moved at a mean speed of 0.65 µm/min and travel approximately a distance of 90 µm, with a track straightness value of 0.48 (a value of 1 representing a linear path). Extra-embryonic cells migrated slightly slower at an average speed of 0.45 µm/min, travelled shorter distances of around 70 µm with a track straightness of 0.3.*

Figures 2a and b: *Saykali et al. Distinct mesoderm migration phenotypes in extra-embryonic and embryonic regions of the early mouse embryo. Descriptions in text.*

About the authors

Bechara Saykali, Navrita Mathiah, Wallis Nahaboo, Marie-Lucie Racu, Latifa Hammou, Matthieu Defrance, Isabelle Migeotte

Isabelle Migeotte Lab
Université Libre de Bruxelles, Belgium

Distinct mesoderm migration phenotypes in extra-embryonic and embryonic regions of the early mouse embryo. eLife 2019;8:e42434.

www.elifesciences.org/articles/42434

Case Study 3:
Advanced 4D Tracking of Meiotic Spindle Dynamic

With its expertise on correlative light and electron microscopy (CLEM), the laboratory of Professor Dr Thomas Müller-Reichert at the TU Dresden is committed to study the details of meiotic cell divisions and the function of the spindle apparatus during this process using the model organism *C. elegans*. Whereas mitotic spindle dynamics have been studied in detail within *C. elegans*, the dynamics during male meiosis remain mainly elusive. Using the live animal, Gunar Fabig, Ph.D. student at the Müller-Reichert lab, conducted for the first time a quantitative analysis of spindle dynamics during male meiosis I and II in 4D. Combining the advantages of *C. elegans* (transparency, easy mounting, the possibility to fluorescently tag proteins, accessibility of the gonad) with spinning disc microscopy (fast image acquisition), Gunar was able to obtain 3D stacks of the full male gonad every 30 s for 45 to 60 min.

Analyzing the spindle pole dynamics of wild-type *C. elegans* in these data sets was quantitatively approached by measuring the spindle pole-to-pole distance over time. By doing so, the researchers were confronted with two major challenges. First, due to 4D imaging of live animals, every spindle was randomly located and orientated within the data set. Second, although mechanically immobilized, a slight movement of the roundworm could not be prevented. These conditions necessitated a demanding image analysis including 4D segmentation, 4D tracking and the possibility to correct tracking failures as a consequence of sample movements. Combining his imaging approach with ZEISS arivis Pro, allowed Gunar to tackle the challenges, extract the desired information in an efficient manner and develop a standardized analysis pipeline. He specifically benefitted from the possibility to analyze randomly oriented spindles by calculating the center of mass of the spindle poles and to edit wrong tracks very easily with the track editor. Using this interactive tool, individual tracks could be easily identified, selected and edited, which includes merging or splitting of tracks by a simple drag and drop function.

About the author

Gunar Fabig
Müller-Reichert Lab, Experimental Center, Medical Faculty Carl Gustav Carus, Dresden University of Technology (TUD)

"The segment tracking enabled us to analyze the spindle elongation over time very efficiently. So far, it is the only working solution for us to analyze our data to our full satisfaction."

Based on this fast, quantitative analysis of spindle dynamics in wild-type C. elegans *using ZEISS arivis Pro, the Müller-Reichert Lab is now able to further complete the big picture of the meiotic spindle and address outstanding questions. Altogether, this analysis will shed light onto the molecular mechanism, which allows the spindle apparatus to accurately segregate paired and unpaired chromosomes.*

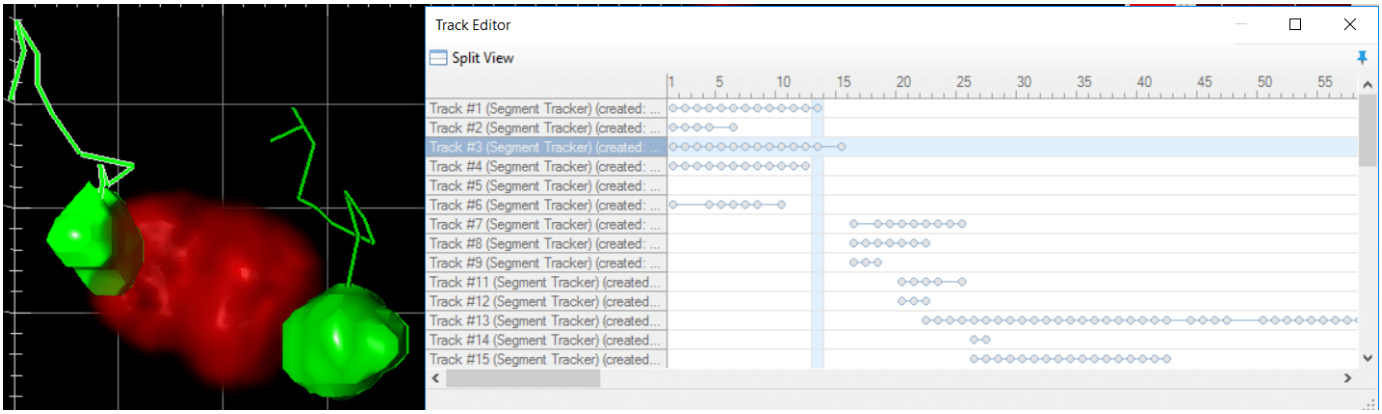

Figure 3: *4D segmentation and 4D tracking of spindle dynamics; (left) 3D representation of male meiosis I in living* C. elegans*; spindle poles are marked by GFP fused to γ-tubulin, chromosomes are marked by mCherry fused to histone H2B. Segmented spindle poles are shown in green, chromosome are shown in red using the 4D Viewer of ZEISS arivis Pro. Tracks of segmented spindle poles over time are depicted as lines. The left track is selected and can be easily identified using the track editor (right), highlighted in blue. Within the track editor, individual tracks are shown on the left, time frames on the top. Every segment is visualized as a small circle. A series of connected segments that form a track is visualized by a horizontal line connecting the segment circles. Merging or splitting of tracks can be easily done by clicking on segments and dragging them to their desired position.*

Case Study 4:
Migration of Interneurons in the Mouse Brain

The laboratory of Univ. Professor Dr Tibor Harkany at the Center of Brain Research in Vienna is interested in the diversification of neurons and their integration into neuronal networks during development. To add knowledge to the diversity of interneurons, Daniela Calvigioni and colleagues analyzed a subtype of GABAergic interneurons producing the neuropeptide cholecysteokinin (CCK). Due to difficulties in histochemical approaches, the migration of CCK interneurons and their population of the cerebral cortex at prenatal stages is poorly understood. Therefore, the researchers developed a novel transgenic mouse line marking CCK interneurons *in vivo*. This opened the possibility to localize this interneuron subtype for the first time within the intact brain structure and to analyze its migratory behavior. To do so, dissected brains of different embryonic time-points were fixed, cleared (CUBIC clearing) and imaged on a ZEISS Lightsheet Z.1 microscope using tile scanning to cover the size of the entire brain.

After image acquisition, the individual z-stacks had to be stitched to generate a 3D image of the mouse brain. However, the researchers were confronted with the enormous data set of ~100 GB for one channel only. This difficulty was only solved by ZEISS arivis Pro.

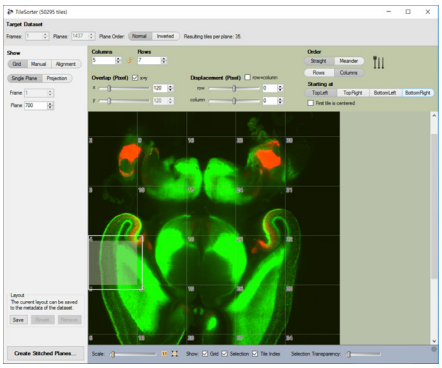

Figure 4a: *Stitching of individual tiles using the Tile Sorter; shown are 35 individual z-stacks covering a dual labelled mouse brain (CCKBAC/DsRed::GAD67gfp/+) at embryonic day 16.5; GABAergic neurons are marked by GFP (green) due to expression of GAD67; CCK expression is marked by DsRed (red). Tile 4 is selected and highlighted by a white box. For aligning, the Tile sorter provides different methods including Grid, Manual and Alignments using advanced algorithms. In this case, the z-stack tiles were sorted using the Grid mode; Pixel overlap was set to 10% as during imaging. Several planes were checked to assure correct alignment. Due to stability during image acquisition no further adjustments as algorith-ms were used. Scale options and transparency settings (see selected Tile 4) help to assure correct alignment.*

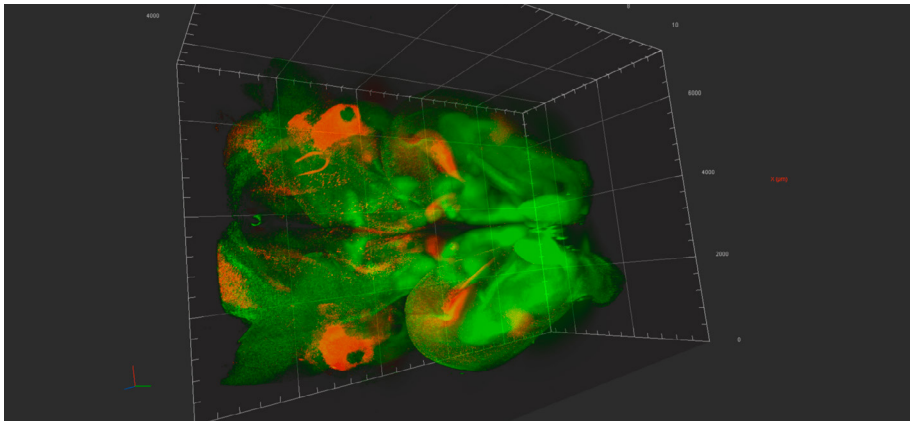

Figure 4b: *Final visual representation of the dataset. Specifications: Microscope: Lightsheet Z.1 microscope (ZEISS), ×5 (EC Plan Neofluar 5×/0.16) detection objective, ×5/0.1 illumination optics, PCO edge sCMOS camera; Imaging settings: ×0.7 zoom, laser power of 20v%, exposure times of 200 ms, tile scan overlap 10%; Data size: 100-300 GB; Picture size: up to 48 tiles, each 1200 px × 1200 px; Voxel size: 1.3 µm × 1.3 µm × 3.5 µm; z-depth: up to 1500 planes.*

About the authors

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"We greatly benefitted from ZEISS arivis Pro, which allows the fluent processing of imaging data with unlimited size on standard hardware. The software can handle big data, it can easily open hundreds of GB files, stitch them and create your 3D image without slowing your system down."

This study benefitted from arivis Pro as the only software with the ability to easily stitch, visualize and share large data sets. Therefore, only the combination of Light-Sheet Microscopy and arivis Pro allowed the researchers to obtain 3D information of the entire brain and share this via movies in a very demonstrative way. Based on this, a classical tangential migratory route of CCK interneurons while populating the cerebral cortex was observed. Overall, this study identified several characteristics of prenatal CCK interneurons and integrates important information for unravelling the diversity of interneuron functionality.

Conclusion

The ZEISS arivis Pro analysis strategy and iterative approach allow processing and segmentation of a small field of view, a 3D/4D subset, or the entire dataset. Analysis results can be viewed in synchronized split windows in 2D and 3D views simultaneously, which is particularly useful for densely packed structures and tracking experiments. The software's integrated Machine Learning/Deep Learning functionality (available as local training option or in the ZEISS arivis Cloud) allows for setting up an image segmentation and analysis pipeline with ease and without extensive knowledge of AI analysis methods.

Analyze complex and challenging samples with a few clicks. No matter the source and format of the image, the arivis scientific image analysis platform is designed to easily process and analyze it. Our software tools are highly integrated and can benefit customers in academia, across industries and various applications, including biotech, pharma, life science and material science.

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