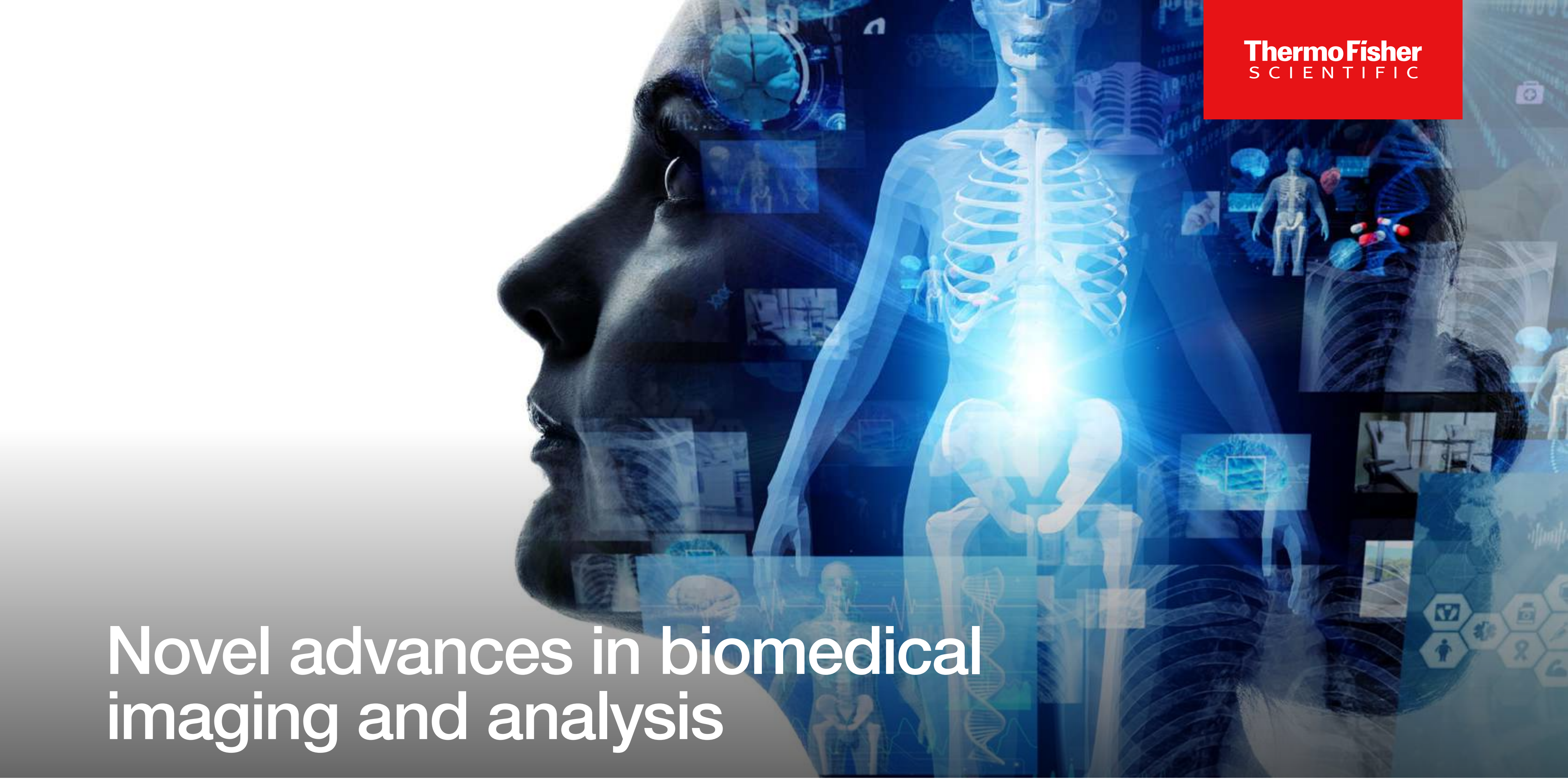

Novel advances in biomedical imaging and analysis

Five innovative applications in clinical and preclinical research enabled by Amira Software

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The biomedical research field is continuously striving to improve diagnostic accuracy, treatment efficacy, and our fundamental understanding of human health and disease. Advanced imaging software plays a pivotal role in these endeavors, enabling researchers to visualize, analyze, and interpret intricate structures and processes with unprecedented clarity and detail.

This eBook features a number of articles that showcase how advanced imaging and analysis with Thermo Scientific™ Amira™ Software is helping to push the boundaries of medical research. Each section provides an in-depth look at how these technologies are applied, the insights that were gained, and the potential implications for future research.

In this eBook, we present five compelling use cases that demonstrate the powerful capabilities of Amira Software in biomedical research

Explore five pioneering studies that demonstrate the transformative power of advanced imaging technologies in biomedical research. Each section delves into a specific application, showcasing how these innovative techniques have provided new insights and improved our understanding of various complex biological systems and diseases.

Clinical challenges of esophageal cancer diagnosis
Learn how 3D reconstruction and segmentation improve the analysis of esophageal tumor growth and T-stage correlations.

Innovative 3D imaging of vascular networks enhances liver disease studies
Explore how high-definition fluorescent micro-optical sectioning tomography provides critical insights into liver disease through 3D reconstruction of vascular structures at single-cell resolution.

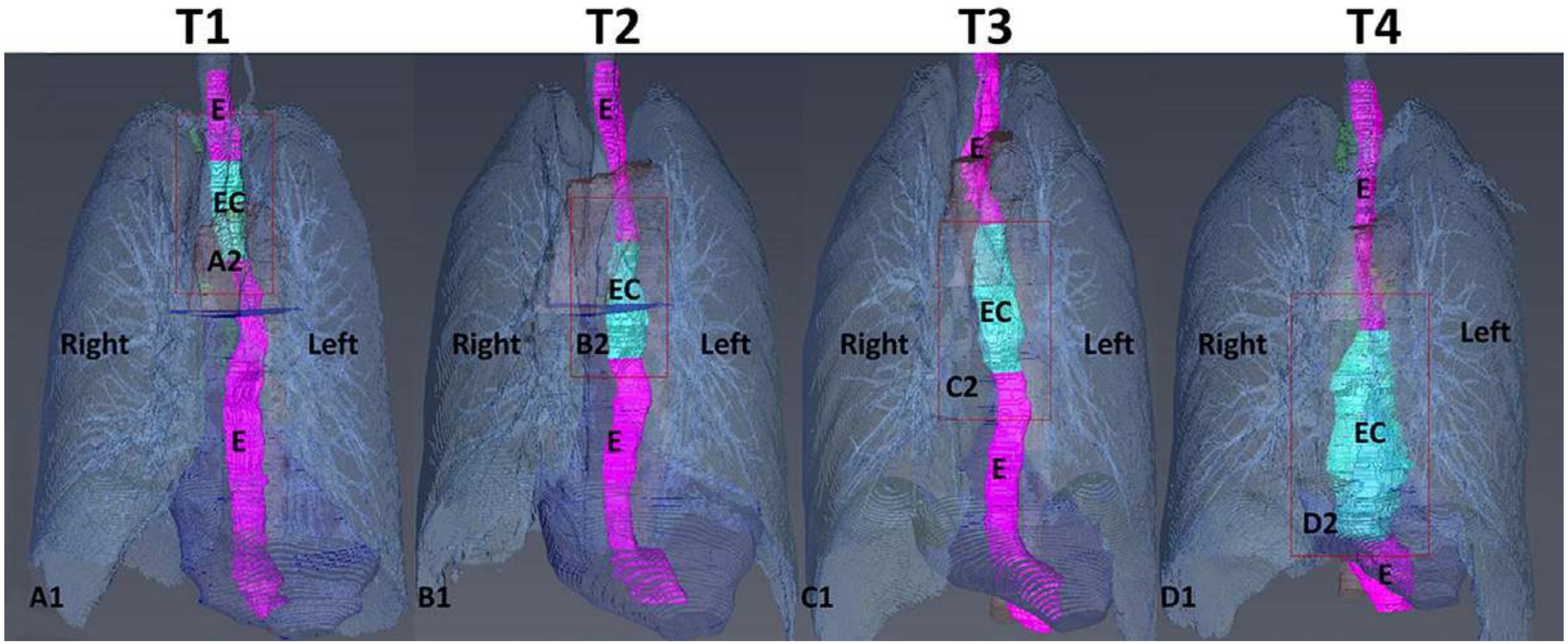
Unraveling brain evolution through the study of teleost fish intelligence
Uncover how high-resolution imaging and 3D visualization reveal the unique brain structures underlying advanced intelligence in wrasses.

A novel strategy for inhaled particle detection at whole-lung scale
Discover how advanced imaging techniques were used to study particle distribution and lung architecture at a whole-lung scale.

4D imaging for the study of middle-ear biomechanics
Understand how advanced 4D imaging techniques were used to study the biomechanics of the middle ear in its natural state

Clinical challenges of esophageal cancer diagnosis

Esophageal cancer is a challenging and dangerous disease, accounting for 5.5% of cancer-related deaths while only making up 3.1% of new cases, according to 2020 GLOBOCAN estimates.[1] Its relative lethality is a result of multiple factors including its difficult diagnosis and rapid progression, resulting in only a 15–25% five-year survival rate.[2] Determining the extent of tumor growth (or T-stage) is a critical part of accurate diagnosis and treatment, but this information can be challenging to obtain for esophageal cancer, particularly at early stages.



Esophageal tumor progression at different T-stages, reconstructed with Amira Software. Figure reproduced in part from Wang et al. under CC BY 4.0.

Computed tomography angiography

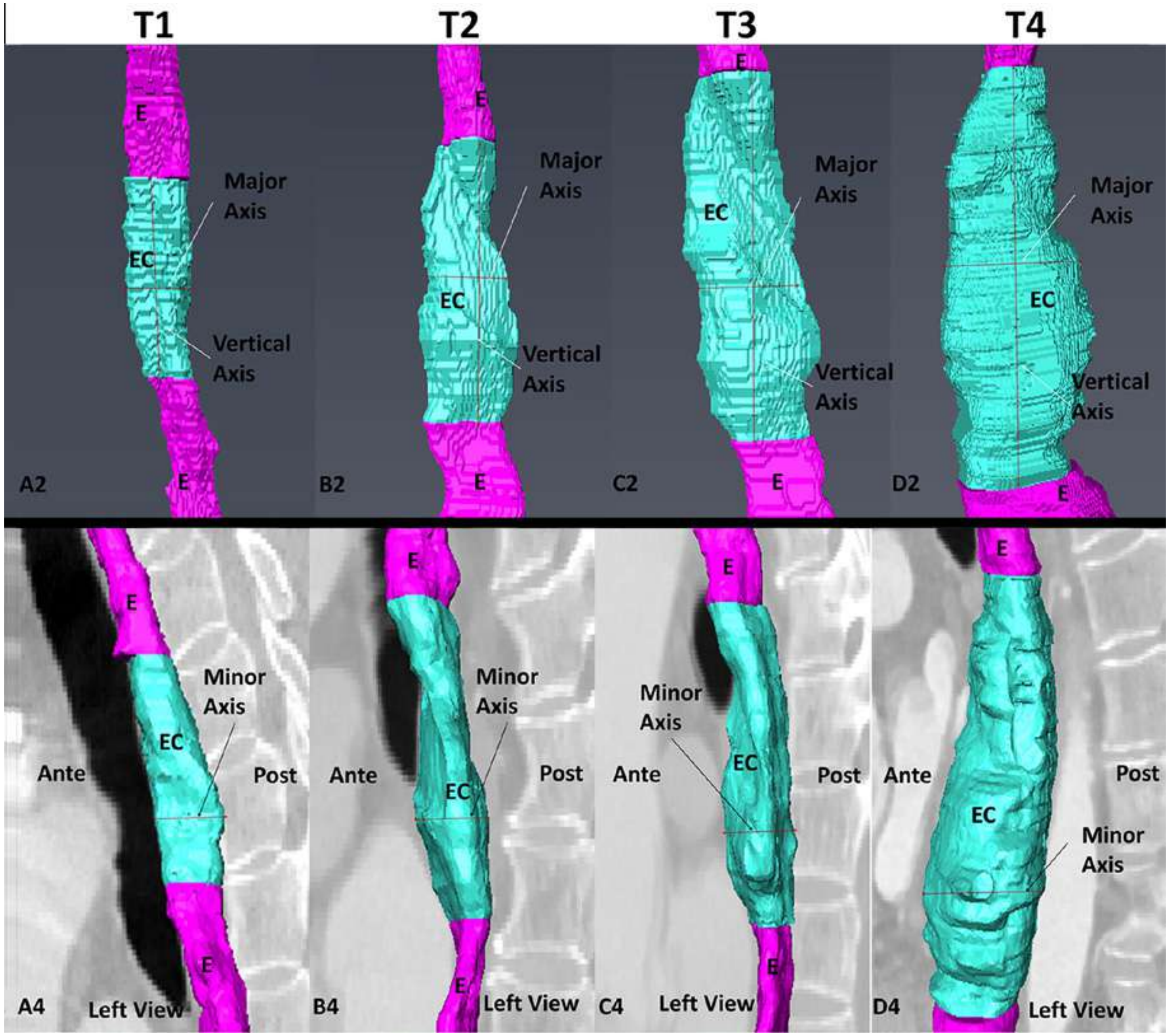
Clinically, doctors utilize a number of complex methods for tumor-stage determination, including computed tomography angiography (CTA). This procedure combines a typical CT scan with the injection of a dyeing agent that provides blood vessel and tissue contrast. While the procedure can be highly accurate for late-stage esophageal tumors, it struggles to accurately identify early tumors while also being invasive and cost-prohibitive.[3] This leaves room for improved methods that could more accurately categorize these tumors earlier, enabling improved treatment.

The increased prevalence of powerful 3D reconstruction software has provided a new avenue for tumor analysis. These tools can perform a range of data post-processing and segmentation to not only identify tissue structures more accurately, but also to analyze the resulting reconstructions for trends and identifying characteristics.

Tumor volume correlates with esophageal cancer progression

Researchers at the Army Medical University (Chongqing, China) and Shanxi Medical University have utilized Amira Software to analyze a wide range of pre-operative CTA data in order to find structural trends that could assist in the diagnosis of esophageal tumor growth.[4] Data was collected from 155 patients with esophageal cancer at various T-stages, from T1 to T4. Utilizing the data segmentation and reconstruction capabilities of Amira Software, they were able to find strong correlation between tumor volume and T-stage. Additionally, the lengths of the major and minor axes of the tumor were also found to correlate with T-stage. Their results provide three critical properties that can be used to assist in the clinical determination of T-stage.

This study highlights the impact that advanced segmentation and 3D reconstruction can have on high-resolution imaging data. As we collect more and more information with advanced tools and techniques, it becomes ever more important that we find ways to analyze and interpret that wealth of data. Versatile tools like Amira Software, with integrated automation and AI capabilities, allow you to extract critical details from your observations, leading to valuable insights that can shape clinical procedures and ultimately improve the lives of patients.



Major and minor axes of esophageal tumors at different T-stages, reconstructed with Amira Software. Figure reproduced in part from Wang et al. under CC BY 4.0.

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1. Sung, H, *et al.* *Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries*. *CA Cancer J Clin* 71:3 (2021). doi: 10.3322/caac.21660
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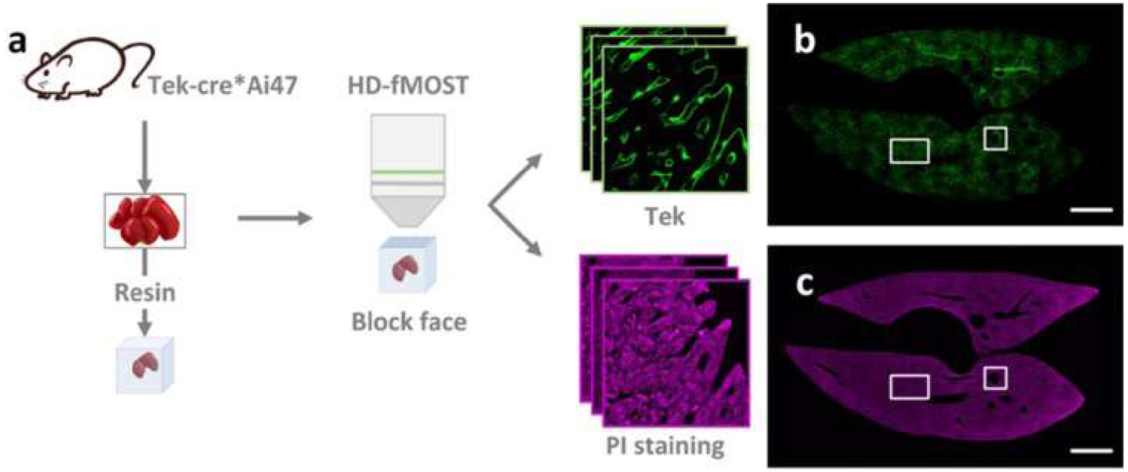
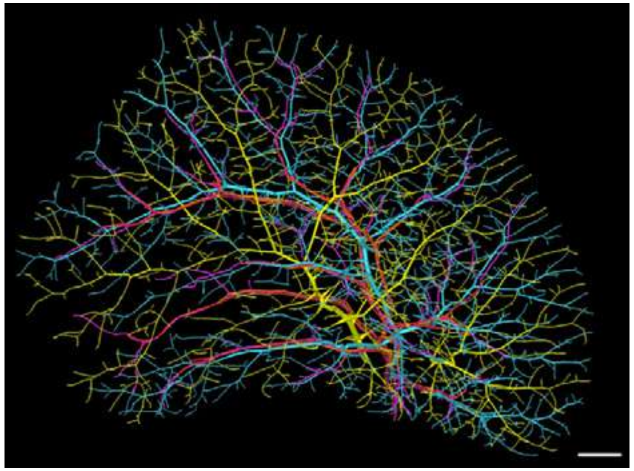
> "…Utilizing the data segmentation and reconstruction capabilities of Amira Software, they were able to find strong correlation between tumor volume and T-stage."

Innovative 3D imaging of vascular networks enhances liver disease studies

Chronic liver diseases cover a broad spectrum of conditions that cause physical damage and scarring to the liver over time, such as fibrosis and cirrhosis. This scarring can exert pressure on the liver's intricate vasculature, potentially obstructing essential veins and arteries. To comprehend the progression and impact of fibrosis, detailed, high-resolution 3D information on vascular structures is needed, ideally at single-cell resolution.



Fluorescent micro-optical sectioning tomography workflow utilized by Zhang *et al*. Figure reproduced in part under CC BY 4.0.



Reconstructed liver blood vessels, bile ducts, and lymphatic vessels. Figure reproduced in part from Zhang *et al*. under CC BY 4.0.

To investigate liver vasculature, researchers of the Huazhong University of Science and Technology used high-definition fluorescent micro-optical sectioning tomography (HD-fMOST) to acquire information on cytoarchitectural and vascular structure for intact mouse liver lobes at single-cell resolution.

Amira Software was used for a range of identification and reconstruction, allowing for the hepatic and portal veins to be distinguished. A dedicated Filament Editor was used to extract the intricate 3D network of filamentous structures from the image data, tracing the hepatic artery, bile ducts, and lymphatic vessels. Additionally, an autoskeleton tool was used to trace sinusoids.

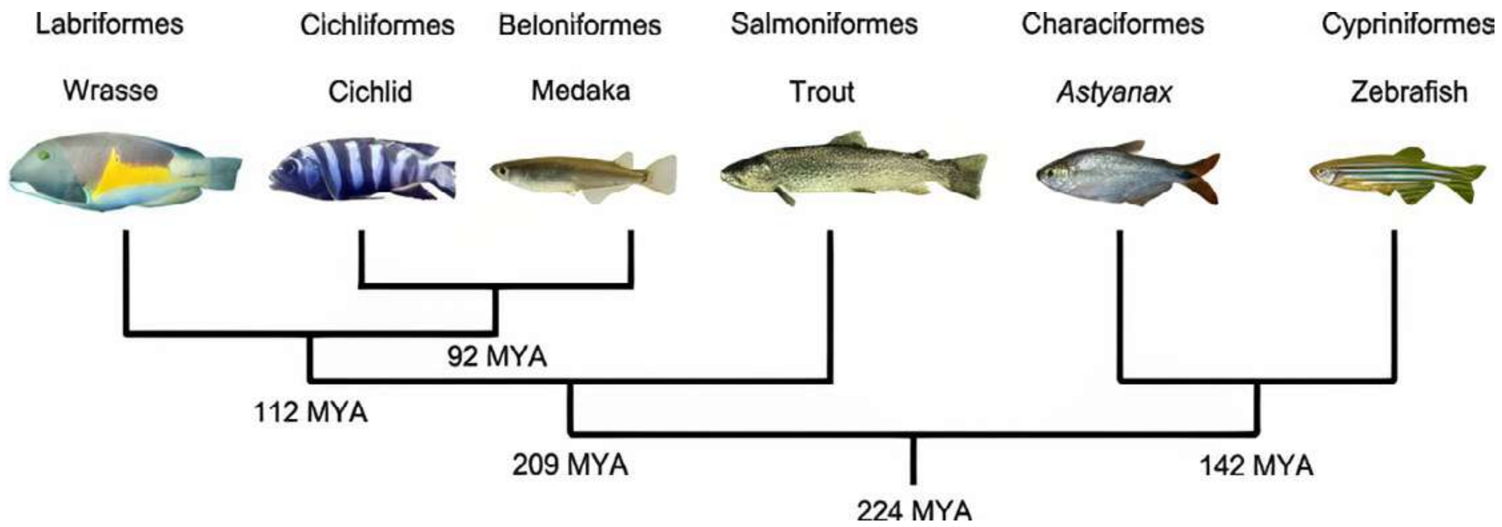
The combination of HD-fMOST and Amira Software enabled systemic observation of the liver vasculature. With additional samples and fluorescent labeling, this approach could even reveal further details on the fine orientation and branching of vessels.

References

1. Zhang, Q, *et al*. **Multiscale reconstruction of various vessels in the intact murine liver lobe**. *Commun Biol* 5:260 (2022). doi: 10.1038/s42003-022-03221-2

“Amira Software was used to for a range of identification and reconstruction, allowing for the hepatic and portal veins to be distinguished.”

Unraveling brain evolution through the study of teleost fish intelligence



Phylogenetic tree of the teleost species sampled by Estienne *et al*. Figure reproduced under CC BY 4.0.

Higher order brain function in different animals

Once, higher order intelligence was thought to be exclusive to primates, as identified by a number of complex cognitive functions such as advanced tool use and self-recognition. However, these hallmarks of intellect have since been recognized in a number of other animals, even outside of mammals. Notably, corvids and parrots have exhibited highly advanced behaviors including creative tool use, which necessitates a combination of complex reasoning, planning, and fine motor skills.

The development of higher brain function is coupled with an increase in relative brain mass, termed encephalization. In primates, this corresponded to an increase in the size of the cerebral cortex compared to other mammals. It would be natural to assume that similar increases in intellect would occur in the same way in birds, but instead, it was found that they had an increase in neuronal density in the pallium (a region of the brain which also encompasses the cortex). This seems to indicate that higher cognitive function can develop in markedly different ways in different animal classes.

Wrasse fish intelligence

Wrasses are a family of marine fish in the teleost fish infraclass, a sub-section of Actinopterygii or "ray-finned fishes." Despite the relatively low encephalization of teleosts, and fish in general, wrasses have been found to exhibit tool-use. Since they lack limbs, they use their mouths to grab shellfish and break them by repeatedly hitting them against a hard flat surface such as a rock or coral.

To determine how this higher order behavior corresponded to encephalization, researchers at the Paris-Saclay Institute of Neuroscience compared the body mass, brain mass, and brain structure of various wrasses and teleosts.

> "Using manual segmentation, the individual regions of the brain could be identified, including lobes and connective fibers."

Brain visualization with Amira Software

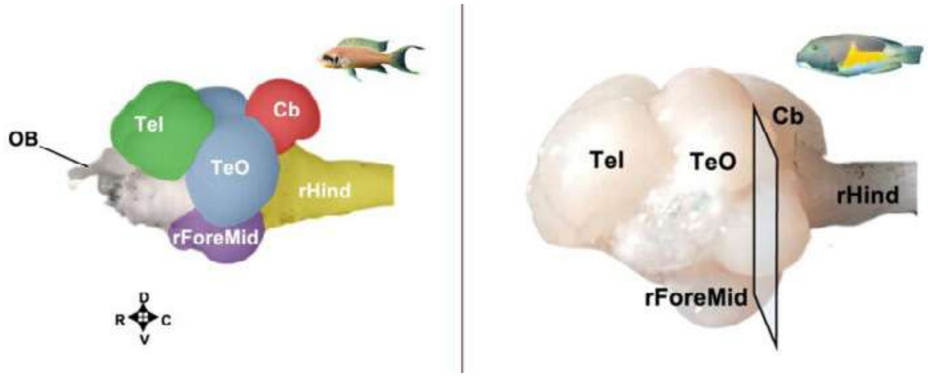
In order to visualize these intricate brain structures, micrometer thick slices of teleost brains were imaged and recombined digitally into 3D representations with Amira Software. Using manual segmentation, the individual regions of the brain could be identified, including lobes and connective fibers. The researchers found that wrasses have a larger telencephalon and general forebrain/midbrain region compared to other teleost fish. Notably, this included a much larger inferior lobe, a structure that has no direct analog in mammals and birds. This lobe also showed unique and substantial connectivity to the pallium, which has already been linked to higher-order thinking in other animals. Overall, this showcases yet another unique way in which higher order thinking can develop in animals, expanding the ways in which we understand brain function and evolution.

Understanding brain evolution through imaging

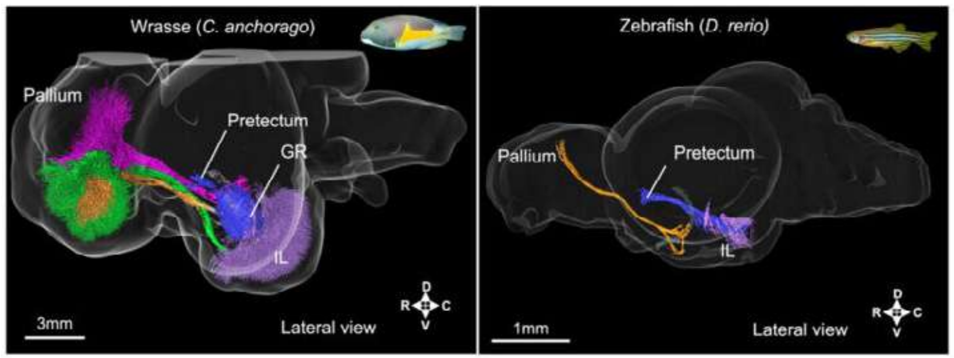
This study has provided unique, novel insights into the development of intelligence in different animal classes, and how intelligent behavior can evolve along different pathways in the brain. These observations were enabled by high-resolution imaging and 3D visualization, which provided the researchers with an intimate view of the entire brain and its intricate structures.

References

1. Estienne, P, *et al.* **Different ways of evolving tool-using brains in teleosts and amniotes**. *Commun Biol* 7:88 (2024). doi: **10.1038/s42003-023-05663-8**



Left) The five major regions of the brain examined in this study, highlighted on the brain of a cichlid, a relative of the wrasse. Right) The same regions highlighted on the wrasse brain. Figure adapted from Estienne *et al*. under CC BY 4.0.



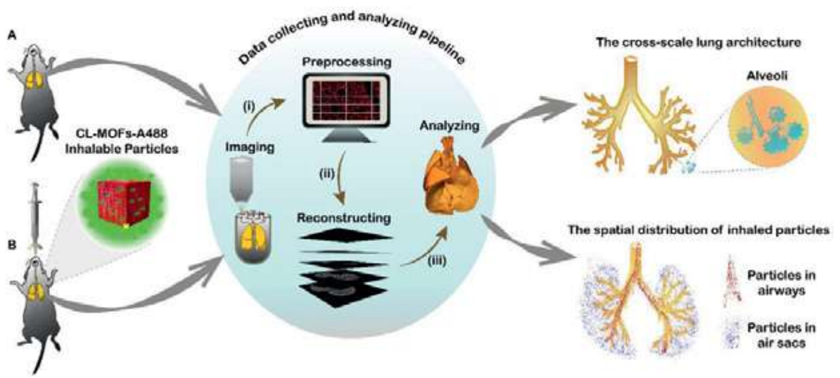
Comparison of the interconnectivity of the pallium and the inferior lobe (IL) in wrasses and zebrafish. The IL of wrasses is distinctly larger compared to zebrafish, indicating a high degree of encephalization. Figure adapted from Estienne et al. under CC BY 4.0.

A novel strategy for inhaled particle detection at whole-lung scale

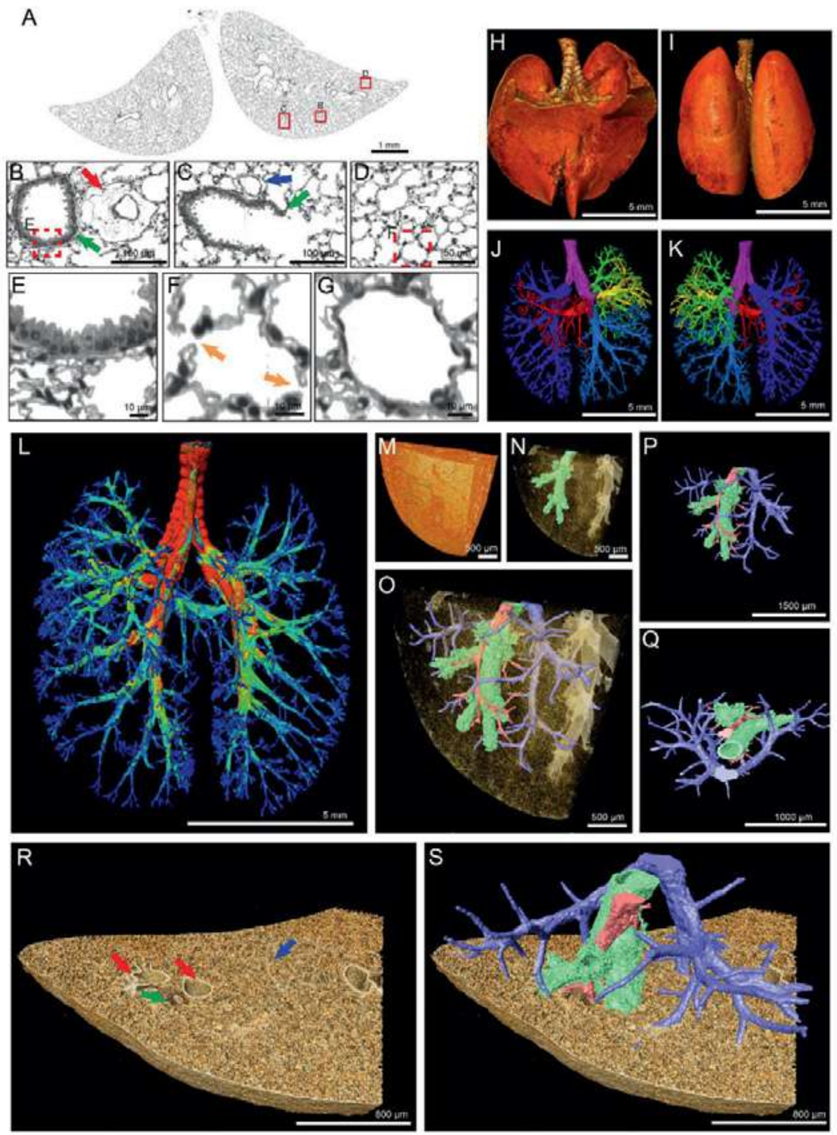
Chronic obstructive pulmonary disease, lower respiratory infections, and lung cancer are among the leading causes of mortality worldwide. A drug delivery methodology called dry powder inhalation (DPI) is being explored as a promising treatment method against pulmonary diseases.

For DPI to be considered effective, it is crucial that the drug is precisely deposited at the desired location within the respiratory tract, which also helps to limit side effects. Although in vitro research has advanced significantly, a gap exists between particle-distribution simulations and the actual distribution of particles in vivo. Beyond this, there is also a need to fully understand the 3D architecture of lungs to ensure accurate particle distribution *in situ*.

Researchers at the Chinese Academy of Sciences used advanced micro-optical sectioning tomography (MOST) coupled with whole-lung Nissl-staining to acquire entire mouse-lung structures.[1] Fluorescence MOST (fMOST) was also used to track fluorescent model particles within the lung datasets. Amira Software provided image segmentation and 3D reconstruction along with qualitative analysis of lung structure and quantitative analysis of particle deposition in different lung areas.



Tomography workflow utilized by Sun *et al*. Mouse lungs with and without inhaled particles (A and B) were processed for imaging analysis. Resin-embedded mouse lung sample were then imaged with MOST and fMOST; whole lung architecture was obtained by overlapping 80,000 coronal images. Figure reproduced from Sun *et al*. under CC BY 4.0



Rendering of trachea and vascular system. A-G) Images of the lung acquired at different magnification. H-S) 3D renderings of the mouse lungs along with reconstructions of airways and the vasculature. Figure reproduced from Sun *et al*. under CC BY 4.0

Volume rendering with Amira Software enabled easy visualization of the whole lung surface. Segmentation tools were used to differentiate various lung structures, including the bronchi, terminal bronchioles, alveoli, arteries, veins, and capillaries. The five lobes of the lung were identified and the number of particles in each lobe was calculated. The Animation Director in Amira Software was also used to edit the recorded frames into movies. The unique flexibility of Amira Software allowed for the visualization of particle distribution within the complex structure of the lungs.

This novel imaging approach could help to optimize DPI drug efficacy through a more complete understanding of particle distribution and how it relates to the architecture of the lung. Ultimately, characterization techniques such as this stand to revolutionize respiratory care and improve the lives of individuals with respiratory conditions.

References

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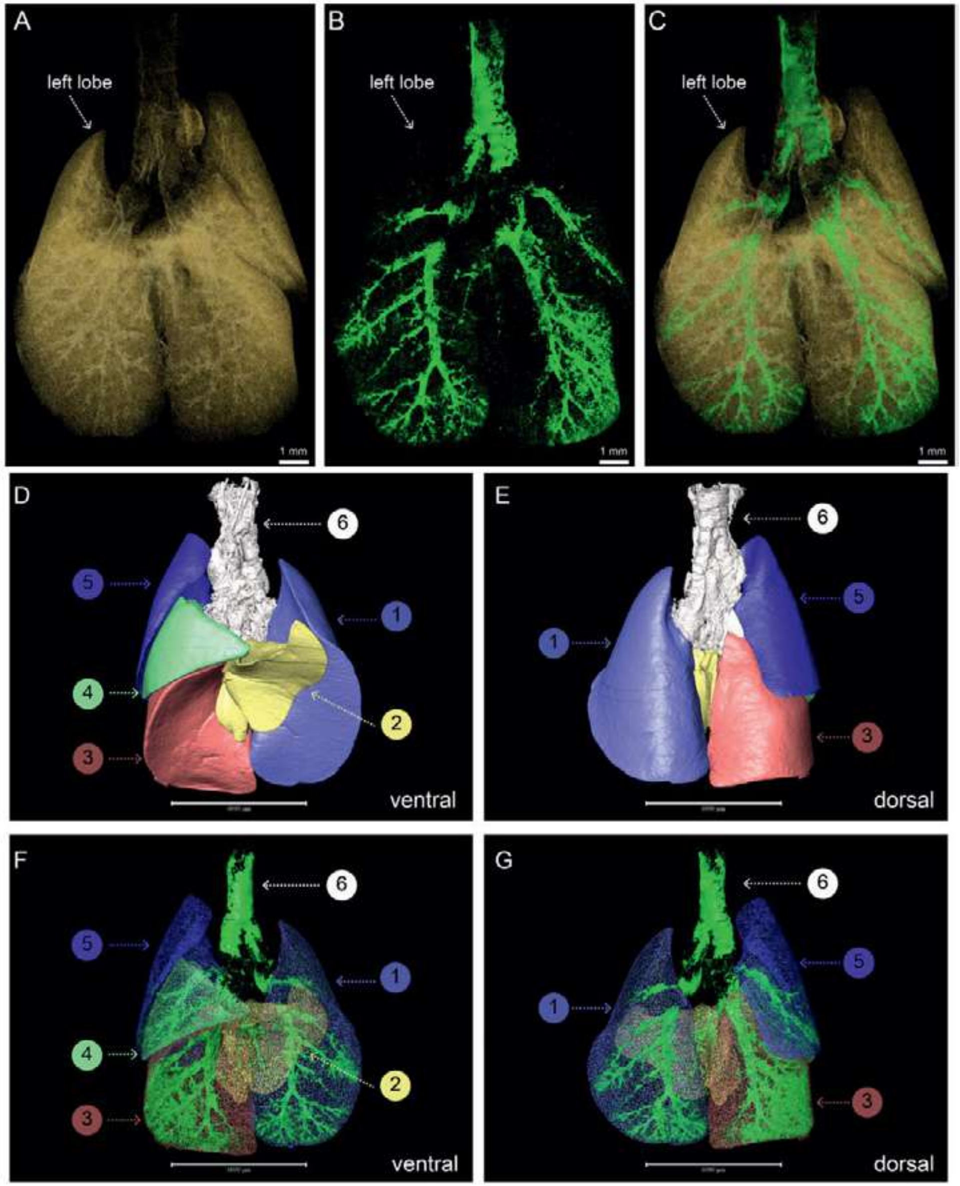
> “Amira Software provided image segmentation and 3D reconstruction along with qualitative analysis of lung structure and quantitative analysis of particle deposition in different lung areas.”



Distribution of fluorescent model particles in a mouse lung. The internal trachea of the lung (A) can be overlaid with fluorescent data (B) for a clear visualization of the particle distribution in the lung (C). D-G) Identification of lung lobes and the extrapulmonary trachea. Almost 50% of the particles were identified in the caudal lobe (3), followed by 27% in the left lobe (1), 15.52% in the extrapulmonary trachea (6), and 5% in the remaining lobes. Figure reproduced from Sun *et al*. under CC BY 4.0

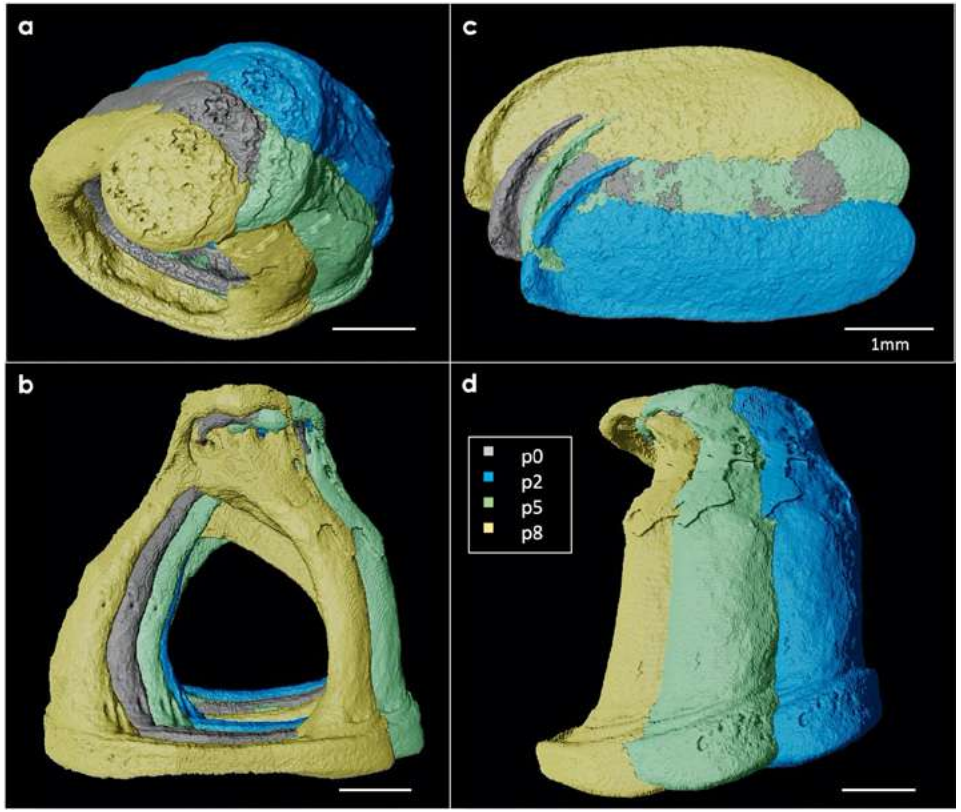
4D imaging for the study of middle-ear biomechanics

Hearing loss is a serious problem that affects all category ages worldwide.[1] It is estimated that by 2050, more than 700 million people will suffer from hearing impairment due to age-related deterioration. Studies of the middle ear, which contains three tiny bones called the malleus, incus, and stapes, are particularly complex due to the small size of this structure. It is also a significant challenge to conduct studies on sound transmission within this part of the ear. Understanding the intricate biomechanics of these ossicles and their role in hearing will be crucial for the development of effective treatments and interventions for hearing loss in the future.

To better understand this structure, researchers used synchrotron-based X-ray imaging to visualize the human ear as it was acoustically stimulated.[2] Combined with Amira Software, this approach was able to produce 3D structures of the human eardrum and ossicular chains in motion, providing a 4D visualization of the ear over time. This approach revealed how these structures move and behave dynamically during sound stimulation.

Amira Software was used to register five sub-scans that captured the ossicular chain and tympanic membrane. The combined image was subsequently processed for segmentation, isolating the stapes, incus, malleus, and tympanic membrane. This segmentation was crucial for accurately identifying and analyzing the movements of each ossicle separately.

In this study, Amira Software delivered essential insights into the intricate biomechanics of the middle ear, thereby facilitating future research endeavors.



3D visualization of stapes movement in a human ear sample. Four views of the stapes position, at four different phases of the movement, are displayed in gray, blue, green, and yellow. Figure reproduced from Schmeltz *et al*. under CC BY 4.0

“Amira Software was used to register five sub-scans that captured the ossicular chain and tympanic membrane. The combined image was subsequently processed for segmentation, isolating the stapes, incus, malleus, and tympanic membrane.”

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Revolutionizing clinical and pre-clinical research with advanced imaging and analysis

Amira Software has significantly advanced biomedical research through its robust imaging and analysis capabilities. In esophageal cancer studies, it enabled precise segmentation and 3D reconstruction of tumor data, revealing crucial correlations between tumor characteristics and T-stages, thereby enhancing diagnostic accuracy. In liver disease research, Amira Software facilitated detailed reconstruction of vascular networks, distinguishing between hepatic and portal veins and tracing intricate 3D filamentous structures. For brain evolution studies, the software's manual segmentation and 3D visualization capabilities allowed researchers to explore unique brain structures in teleost fish, shedding light on higher order cognitive functions. In lung particle detection, Amira Software tools provided comprehensive 3D reconstruction and analysis of lung architecture and particle distribution, helping to guide optimized drug delivery methods. Lastly, in the study of middle-ear biomechanics, 4D visualization and segmentation with Amira Software enabled the detailed study of ossicular chain movements, offering new insights into hearing mechanisms.

Across these diverse applications, the advanced capabilities of Amira Software have been instrumental in extracting critical details, leading to valuable insights and potential improvements in medical treatments and procedures.

"With Amira Software, those at the VetCore facility can analyze the size and shape of organs, tissues, and cells. We can visualize complex details of microscopic tissue anatomy, and we can put together data from different imaging modalities such as clinical CTs and MRIs and microCT, light microscopy, and electron microscopy—which deepens our understanding of biological structures."

Stephan Handschuch, PhD
VetCore, University of Veterinary Medicine, Vienna

Watch testimonial

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