

Unlock the Hidden World Inside Fish Specimens

Exploring Natural History Collections with Micro-CT Imaging



Seeing beyond

The Scientific Value of Museum Collections

Natural history museums are like time capsules of biodiversity. Serving as both repositories of current and past global biodiversity, and as research centers for the study of this diversity, they provide a window to the many plants and animals that live on our quickly-changing earth. These museums are the only places where entire organisms from all over the world are stored and catalogued. Together, they hold millions of preserved specimens — many of them one-of-a-kind. Some are especially rare, collected centuries ago or representing species that no longer exist. These unique samples are crucial for identifying species and understanding how animals have evolved over time.



Some specimens are of particular scientific value given their rarity or their status as so-called **type specimens**. These form the basis of scientific descriptions for a plant or animal species—that is, for the first time these organisms are scientifically named. Type specimens are the ultimate benchmark for species identification and comparison when new species are scientifically described.



The species description was based on a single specimen. This specimen is known as the **HOLOTYPE** and is the sole name-bearing representative of the species.



The species description was based on several specimens (without a **HOLOTYPE** being designated). These specimens are known as **SYNTYPES** and are equal name-bearing representatives of the species.



A later scientist selects a single specimen from among the **SYNTYPES** to be the sole name-bearing representative of the species. This specimen is now known as the **LECTOTYPE** and the remaining specimens are known as **PARALECTOTYPES** with no name-bearing status.



The species description was based on several specimens. One of these specimens was designated the **HOLOTYPE** and is the sole name-bearing representative of the species. The remaining specimens are known as **PARATYPES** with no name-bearing status.

Type specimens may have been collected relatively recently although many were collected years ago; some even hundreds of years ago, and these are therefore often fragile. Among other rare and highly valuable specimens are collections from remote or hard to reach habitats. They may even represent individuals from species that have gone extinct in the time between their collection and today. If lost or damaged, these specimens cannot be replaced and certain scientific questions may remain forever unanswered.

Peering Inside Without Cutting It Open

Studying such rare and important specimens has historically been limited to external features to avoid damage by dissection. This leaves a wealth of information unexplored—details such as the skeleton and internal soft tissues that can be crucial to uncovering characteristics that reveal evolutionary relationships. While this is a problem for scientists in every zoological field of study, it is perhaps most frequently experienced by ichthyologists in their study of fishes, that most speciose and diverse group of vertebrates. Fish morphologists are especially interested in the internal anatomy of this highly diverse assemblage and often need to look at rare or otherwise highly valuable specimens.



Thus X-ray tomography has brought about a quantum leap in the study of such specimens. Micro-CT (μ -CT) scanning in particular has allowed biologists to overcome this research challenge, blowing open the doors to the study of even globally unique specimens.



Take a Look Inside One of the World's Oldest Collections

Welcome to the fish section of the ZEISS Xradia Context μ -CT where we carry out 3D visualizations of fish specimens housed at the Senckenberg Natural History Collections (SNSD) in Dresden. This particular collection has a long history, dating back as early as 1587 when the first specimens were collected as part of the Naturalienkabinett of the Saxon-Polish Elector-King Augustus the Strong. That makes our collections from the Museum of Mineralogy and Geology and the Museum of Zoology among the oldest natural history collections in the world. The zoological collection alone currently houses over six million specimens from all over the earth, including extensive type specimens.

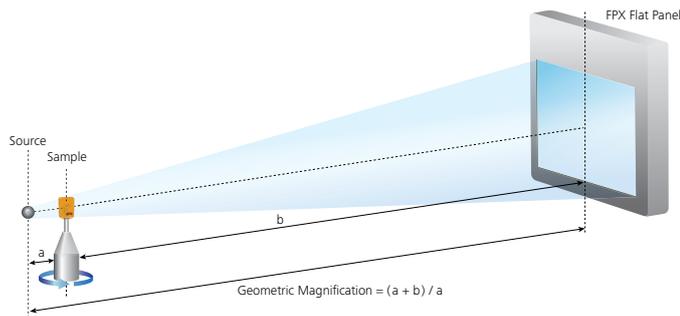
The ichthyology section focuses on research questions regarding the evolution, phylogenetic relationships, taxonomy and comparative anatomy of fishes. With over 36,000 described species, fishes are not only the most species-rich group of vertebrates, but they also include some of both the smallest and largest of all vertebrates in their numbers. Fishes have conquered almost every aquatic habitat on earth, from the deepest oceans to streams on the highest mountain tops, and even underground.

Using ZEISS Xradia Context μ -CT, our research group has now completed a project funded by the state of Saxony to examine a large number of endemic freshwater fishes from the Western Ghats Biodiversity Hotspot in southern India.

Endemic species are those restricted in their distribution to a specific area of our planet. In the case of fishes, this is often a single river or river basin. Among the endemic freshwater fishes of the Western Ghats, several are also highly unusual species that can only be found in underground (or subterranean) habitats. These fishes have evolutionary adaptations that make it possible to live in subterranean ecosystems. For example, their color pigmentation is reduced and their eyes are tiny or even altogether absent. The evolutionary relationships of such strange and very different-looking fishes are often hard to decipher, making a detailed study of their internal anatomy crucial. Yet, because of their rarity, achieving high resolution images of their anatomy without damaging the specimen is key.

How Micro-CT Scanning Works

Here, μ -CT plays a pivotal role. By generating skeleton and soft tissue scans, the inner structure of the whole fish can now be viewed in detail by anyone who has a copy of the scan. To do this, first you must determine the scanning parameters to produce scan data of the highest possible quality. Then mount the sample on the sample holder, making sure the fish, container and sample holder all remain motionless (aside from during rotation). Once in place, the holder offers a wide range of travel between the X-ray source and the flat panel detector so it is easy to navigate to your region of interest.



Microfocus source and geometric magnification

While the sample rotates 360 degrees, multiple 2D projections are acquired. The final dataset will benefit from the number of acquired projections from different angles since each projection carries specific X-ray transmission information. The generated dataset is mathematically reconstructed to a 3D volume dataset. The X-ray source generates 30-160 kV / 4-10 W and the spatial resolution can be as great as 0.95 μ m.

Now, to get the best possible resolution, pack the samples into a low-density container and place it as near to the X-ray source as possible. Several variables will need to be adjusted to provide adequate contrast and image quality for the scan. Much of the post-processing can be automated—reconstructing, as well as putting together multiple scans into one much larger scan—but all steps can be carried out manually, if need be. Often manual work may enhance the data for specimens with very fine ossifications—for example, larvae.

Once complete, simply remove the specimen from the container, unchanged, and put it back into the collection where it will be safely stored and ready for future research.

The Findings—So Far

The team has generated more than 100 datasets and over 20 TB of data of computationally reconstructed 3D volumes to visualize the internal anatomy of endemic fishes in the Western Ghats. This has provided new and important scientific data that can help inform their taxonomy and systematics. The quality of these images is remarkable and second to none in fish anatomy scans that have been available to us previously from other sources. One immediate and unexpected result from our project was the discovery of a new species of catfish, a stygobiont or ground water dwelling species that was collected with the help of numerous people from India as part of a citizen science project (Raghavan et. al., 2023). While the process of analyzing 20TB of data is still ongoing, the use of μ -CT scanning for other extremely valuable material in the collection has already started: The globally unique developmental series of the rope eel, an ancient fish from Africa, is often referred to as a 'living fossil'. Below (above) is the scan of the skull of an adult and (below) a larva.



The exquisite quality of scans from the μ -CT also makes it possible to study the anatomy of tiny fish species, such as *Danionella translucida* and *D. dracula* with a maximum size of only 10-17 mm—smaller than a thumbnail. Additionally, μ -CT scanning was applied to investigate the development of the skeleton of the so-called **living fossils** of the family Polypteridae, from the earliest formation of bones in the head to the fully formed skull.

Beyond the Scan: The Digital Window into Museum Specimens

Museum collections house millions of specimens which bear unique witness to the earth's past and present. Long-term preservation of these specimens is one of the objectives of natural history museums, conserving them for current and future research.

Here, non-destructive technologies play a key role as they help visualize the inner structure of these precious specimens. By generating 3D datasets, Micro CT opens up new ways of accessing the world's collections for scientific evaluation. This is in sharp contrast to light and laser scanning microscopy, which mainly allow imaging of the sample surface, but cannot image

the inner structure, unless specimens are sectioned or otherwise dissected and prepared. The computed and digital version of a natural history museum specimen allows its virtual dissection and contributes to unique insights into its anatomy. In this way scientific questions of taxonomy, systematics, evolution and biogeography of samples can be addressed.

ZEISS Xradia Context μ -CT combines high resolution images with easy-to-use sample navigation. The results show that unexpected discoveries can be made from the high-contrast images. And as a bonus, the digital datasets can be stored in databases and made available for further international research.

Reference

Raghavan, R.; Sundar, R.L.; Arjun, C.P., Britz, R. and Dahanukar, N.(2023) Evolution in the dark: Unexpected genetic diversity and morphological stasis in the blind, aquifer-dwelling catfish *Horaglanis*. *Vertebrate Zoology*, 73, pp.



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