To Build a Fish

Structuring Space and Material in Skeletons

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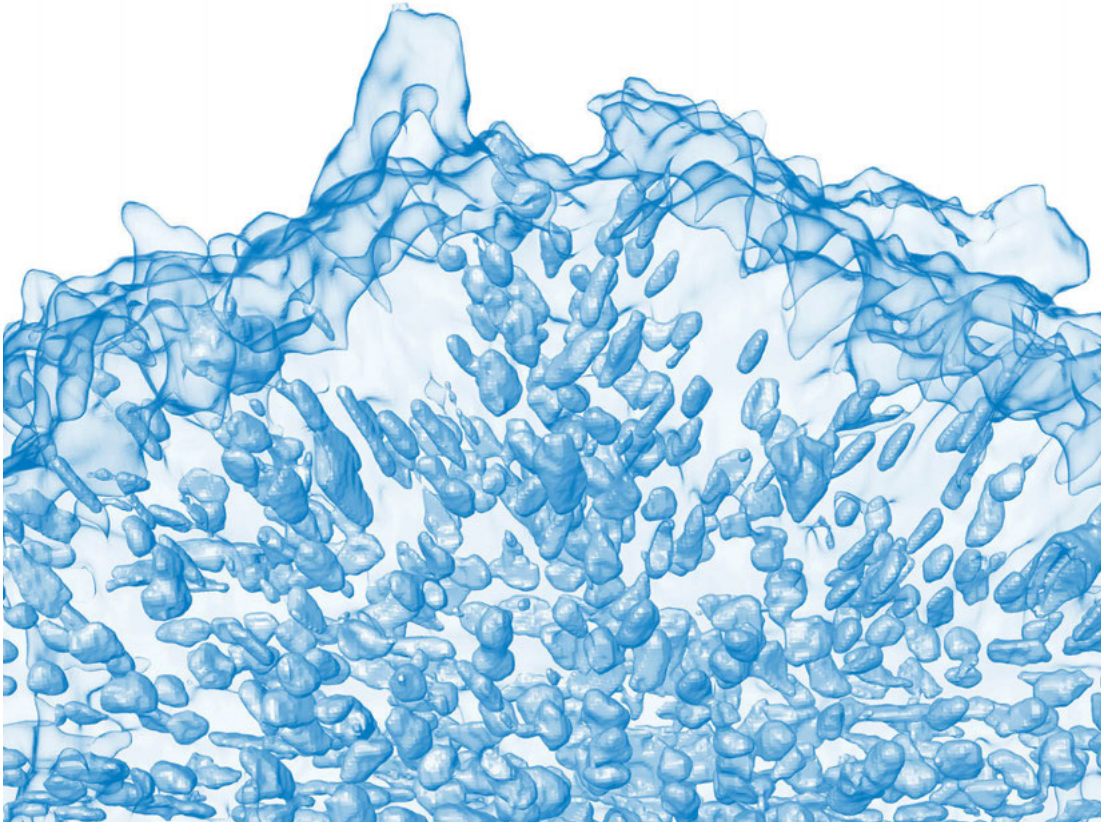


The old adage "There are many fish in the sea" is, with respect to animal diversity, quite true. Of the ~40,000 species of vertebrates (animals with backbones), more than 50 percent are fish. Fish occupy almost every body of water on the planet (from tropical to polar seas and all freshwater habitats) and are exceptionally diverse in diet, anatomy, and ecology. In our research work group, this huge variation of form and function offers a backdrop for studying how the tissues that build skeletons grow, vary, and evolve, adapting to conditions and challenges. Observing tissues outside of their biological context, only in the vials and slides of the lab, is like trying to suss out what pistons or valves do separately from the engine they belong in. We strive to fold the natural context of tissues into our work, how they are used in the animal's habitat and life history. Can they tolerate extreme temperatures or damage? Do they grow indefinitely? Do they assimilate particular elements from food? By cataloguing the natural diversity of tissue form and composition within the frames of ecology (where and how the animal lives) and evolution (species' relationships), we can start to make sense of the mosaic of animal forms and functions, and to decode the factors driving how tissues adapt—in short and long-time scales—to solve new problems.

Biomedical science is somewhat myopic with regard to the study of skeletal tissues: our understanding of the function and anatomy of bone and cartilage comes from just a few, closely related species of mammals. This restricts our perspective on the scope of what skeletal tissues actually are and what they can do. The skeletons of sharks and rays, for example, represent an opposite materials design strategy to ours: whereas our skeletons are made of bone, filled with cells charged with fixing damage, the skeletons of sharks are cartilage, which can be added to and patched up, but not repaired. Despite this, we know that shark and ray skeletons perform just as well as ours—and perhaps better, considering the extreme loads some species deal with in their lifetimes. These fascinating, alternative design solutions make fantastic fodder for engineering applications: How can a low-density material (cartilage) perform as well as a high-density one (bone)? How can a skeletal tissue be made resistant to damage so it doesn't need a cellular repair service?

Our work group combines engineering and biology approaches to study the development and mechanics of skeletons, in particular how tissue materials and architecture interact. We first characterize the geometries and tissue properties of natural systems using high-resolution engineering and materials science tools. Then to get a feel for how tissues manage and distribute forces, we build physical and digital mimics from biological data, scaling them up to sizes that make them easier to handle and test. With a multimaterial 3D printer, for instance, we manufacture biorealistic models with both rigid and flexible parts, which can be pushed, pulled, and fractured in ways that teach us about biological conditions. As in any design process, when the model raises more questions or fails to work, we return to the source—the biology—for a deeper understanding of the template.

The tools we use to look at our samples actually dictate what we see: no imaging tool is all-seeing, and so there will always be trade-offs and decisions made, building a curious subjectivity—but also creativity—into science. For example, some techniques create 3D images but are limited by how large the sample can be. Others can map chemical composition in a tissue, but only in 2D on the sample surface. In our work, the micro-CT scanning we often use won't show soft tissues unless we stain them with chemicals that add contrast, but even those agents have affinities, binding to some tissues and ignoring others. To some degree, it is a matter of choosing approaches that fit our imaging goals and limitations, the right tools for the particular job. In the following images of boxfish armor and stingray tesserae, we only wanted to visualize hard tissues and in 3D, which made micro-CT the perfect choice. Even so, targeting specific tissues doesn't preclude surprises: the many stingray spines we show pincushioning a wedgefish jaw might not have been discovered if another imaging technique had been used. To step around imaging and sampling biases, we often combine multiple tools to study the same tissue: by overlapping our hard tissue data from stingray tesserae (from micro-CT or electron microscopy) with polarized light microscopy images, we bring soft tissue architectures also into focus. We can even leverage techniques' biases to our advantage, for example, digitally filling

voids in our tesserae micro-CT data (what was NOT imaged) to reveal the complex, internal cell networks. In these ways, the data we generate are reflections of our interests, but also the imaging and analysis tools we know of from experience and collaboration, those we have available, and those we choose.

There is a rich history of animal biomechanics study at organismal scales—including Stephen Wainwright's classic "To Bend a Fish," a treatise on the importance of fish skin[1] —but we push to understand form-function relationships at smaller sizes. The following pictures highlight how imaging tools can provide windows into the microscopic, hidden architectures of anatomy. By combining biology and engineering insights, we illuminate the functional roles of tissues while also pointing to generalizable features useful for building manmade composites. Given the impressive diversity, long lives, and ancient lineages of many fishes, their skeletons have much to teach us, if we are creative in how we look.

Further readings

• *Daniel Baum et al., "High-throughput Segmentation of Tiled Biological Structures Using Random Walk Distance Transforms,"* Integrative & Comparative Biology *59, no. 6 (2019): 1700–12.*
• *Júlia Chaumel et al., "Co-aligned Chondrocytes: Zonal Morphological Variation and Structured Arrangement of Cell Lacunae in Tessellated Cartilage,"* Bone *7, no. 134 (2020).*
• *Mason N. Dean et al., "Large Batoid Fishes Frequently Consume Stingrays Despite Skeletal Damage,"* Royal Society Open Science *4, no. 9 (2017): 170674.*
• *Lennart Eigen et al., "Ontogeny of a tessellated surface: carapace growth of the longhorn cowfish Lactoria cornuta,"* Journal of Anatomy *(2022). In press.*
• *Peter Fratzl et al., "The Mechanics of Tessellations: Bioinspired Strategies for Fracture Resistance,"* Chemical Society Reviews *45, no. 2 (2016): 252–67.*
• *Ronald Seidel et al., "Cartilage or Bone? Collagens in the Skeleton of 'Cartilaginous' Fishes Answer an Old Question,"* Journal of Structural Biology *200, no. 1 (2017): 54–71.*

1 Stephen A. Wainwright, "To Bend a Fish," in *Fish Biomechanics*, ed. Paul W Webb and Daniel Weihs (New York: Praeger, 1983), 68–91.

Dusty Jars and Hidden Scars

Natural history museums are libraries for Nature's works. Behind the public exhibits are countless shelves of specimens waiting to be studied and, in some cases, harboring secrets. When this jaw of a guitarfish (a large relative of stingrays) was micro-CT-scanned (fig. 1), a battery of broken stingray spines were discovered, lodged into the soft tissue of the mouth (here, colored red against the gray, translucent renderings).

Although the guitarfish was thought, due to its pebble-like teeth, to eat only small animals from the sand, this finding shows they are also voracious predators of their own relatives. This chance observation also gave unexpected insight into how shark and ray cartilage deals with tissue damage, while showing that shelved museum specimens hold clues to the habits of living animals, reminding us that we should not judge books by their covers (or jaws by their teeth).

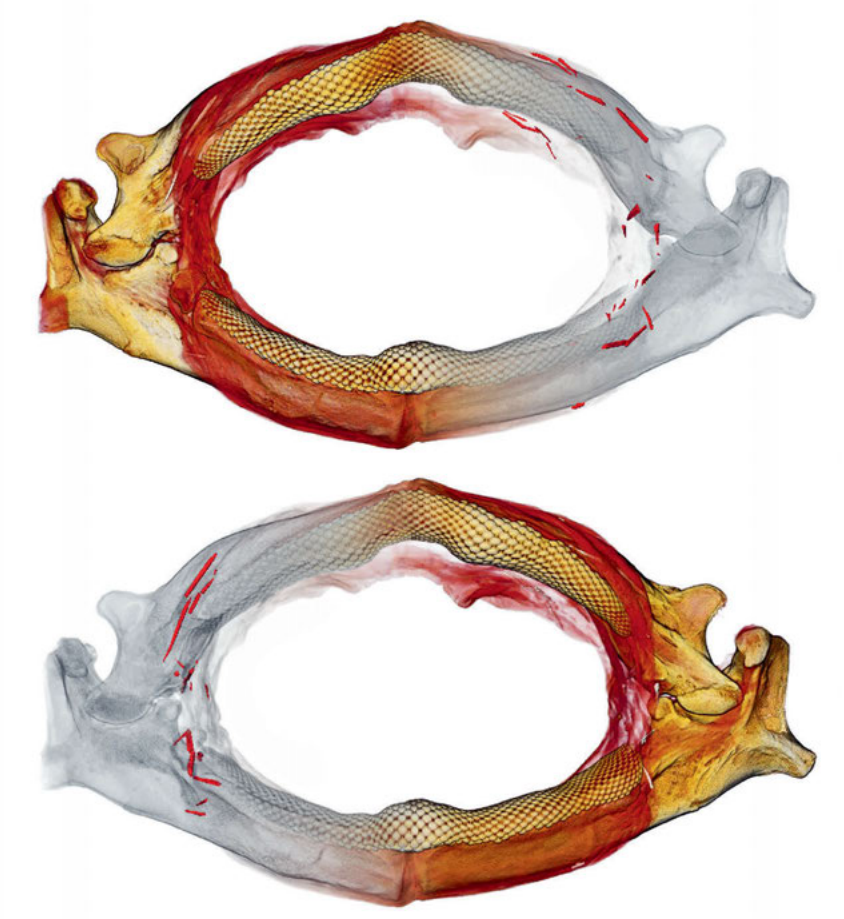


Fig. 1: Micro-CT-scanned jaw of a guitarfish, rendered half-transparent to show embedded stingray spines (red).

To Build a Stingray

To understand how Nature builds complex architectures, we can deconstruct them into their parts, but this is often easier said than done. Sharks and rays have skeletons made entirely of a curious armored cartilage, covered in an outer hull of many thousands of mineralized tiles called tesserae. This tessellated cartilage has been unique to sharks and rays for hundreds of millions of years, but has proved difficult to study and visualize due to tesserae being both numerous and small: the piece of a stingray skeleton shown here is just ~2 cm long, but it is covered by more than 3,000 tesserae of different shapes and sizes.

The image (fig. 2) is a visual record of a workflow, developed by combining materials and computer science approaches, starting from digital micro-CT data of a real specimen and, through image processing, digitally dissecting the tesserae from one another to color them according to their size. This is the first window into the architectural rules that define this skeletal design, a roadmap for the assembly of a complex biological pattern.

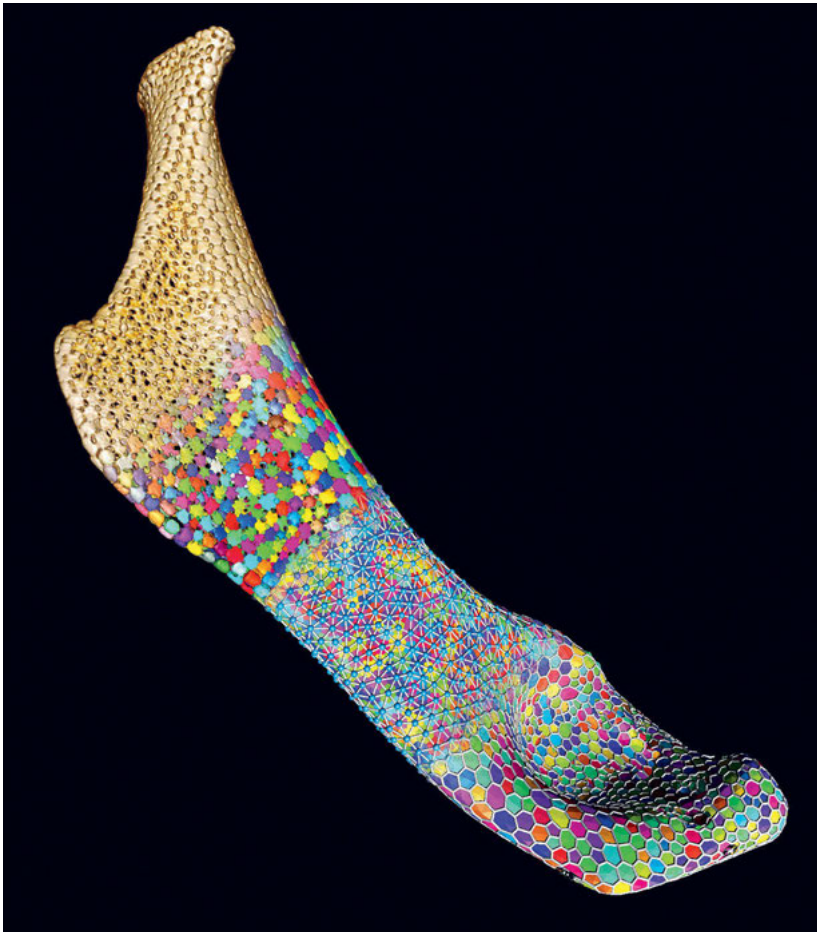


Fig. 2: Piece of a stingray skeleton, rendered to depict our analysis workflow, from micro-CT data, to isolated tesserae, to quantifiable networks (from left to right).

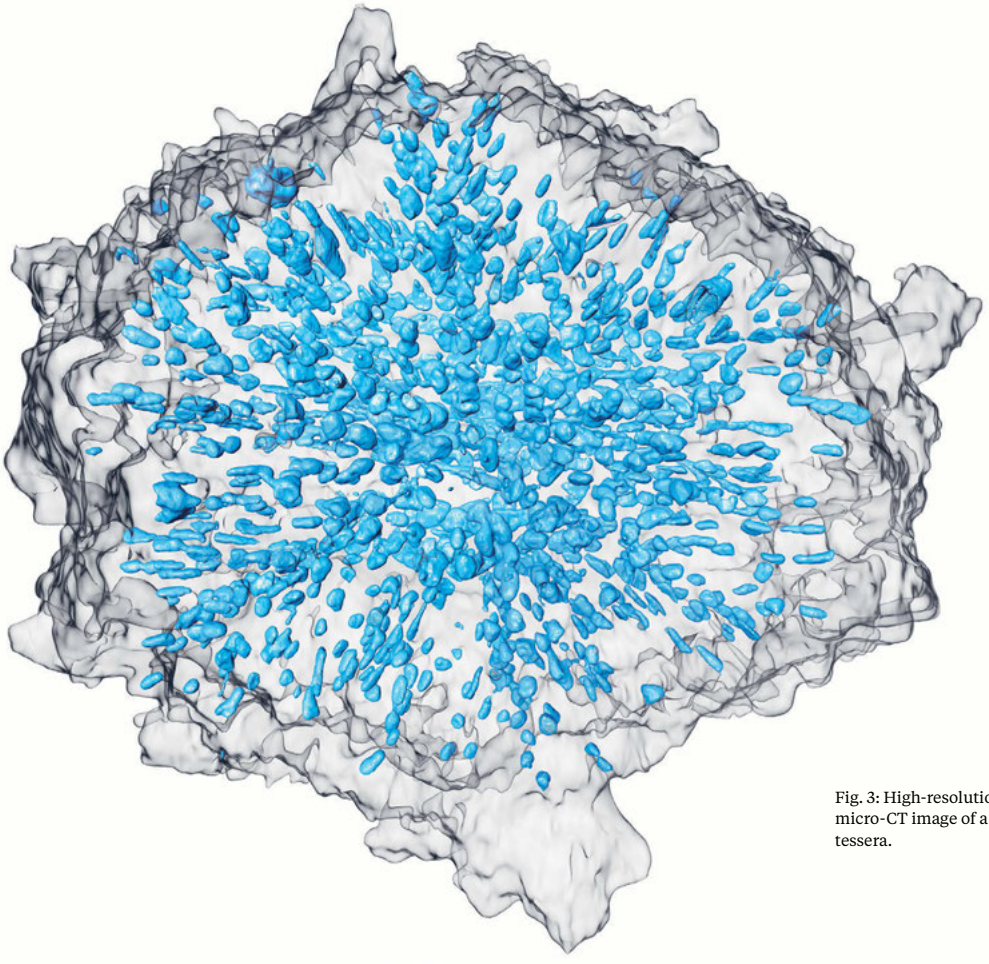
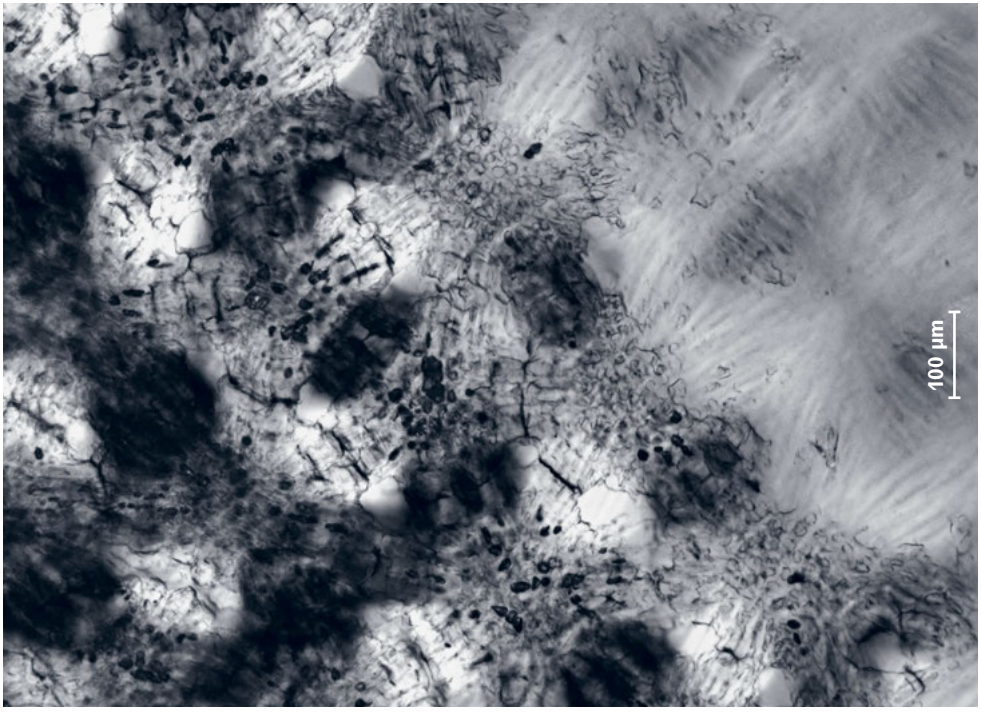


Fig. 3: High-resolution micro-CT image of a single tessera.

The Microchip of Shark and Ray Skeletons

The tesserae covering the skeletons of sharks and rays cobble together to form a hard crust (fig. 2), but are not simple blocks. The edges of each tessera are convoluted, as can be seen in this high-resolution micro-CT image of a single tessera (fig. 3), just a fraction of a millimeter wide. Moreover, each of the many thousand tesserae covering each piece of the skeleton harbor a rich population of cells that live ensconced in cavities within the hard material. By digitally filling those cavities (shown here in blue), we discovered that the cells are organized radially, connected to one another by small passages in a rich, communicating network.

The communication network is even broader than appreciated from this image: cells can interact within a tessera, but also across the gap between tesserae, like tenants in a building talking to their neighbors across the alley, perhaps allowing tesserae to connect into a broader interactive community.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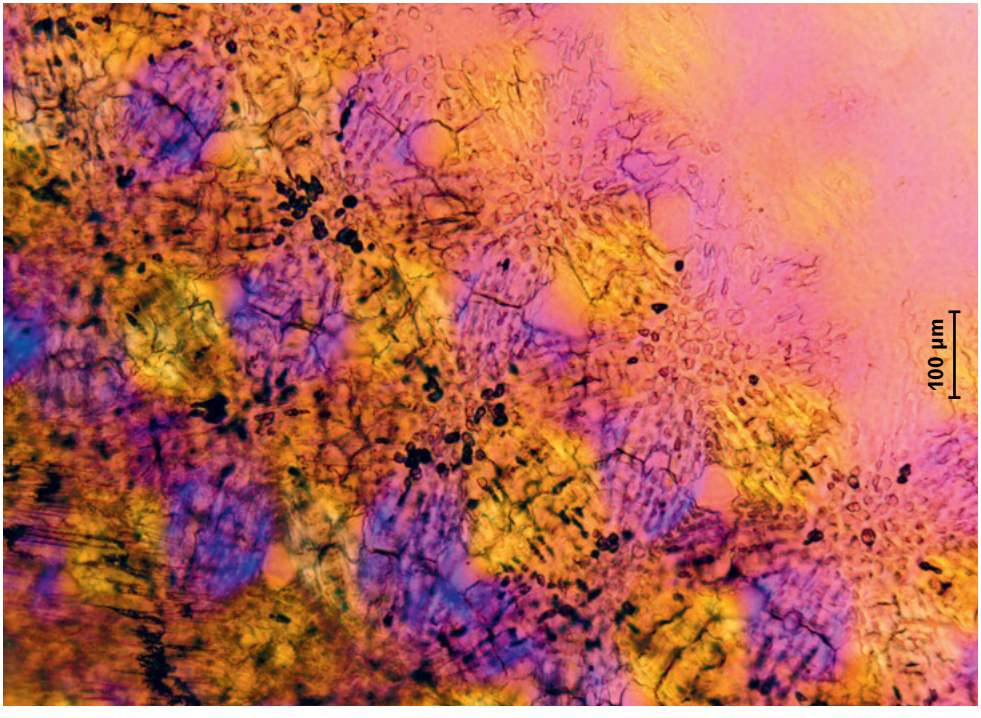
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Holding It All Together

What controls biological patterns? In growing mineralized tissues—like bone or shark and ray tesserae—collagen fibers often form scaffolds to guide where mineral crystals are tucked and packed, but the tight association of mineral and fiber can make this collagen scaffold hard to see.

The technique that produces these images, however, allows us to exploit the structure of collagen itself and to track its path through tissues. We take advantage of a tool originally developed to look at geologic crystals, but now often co-opted for visualizing fiber directions in biology: using polarizing filters (waveplates or retardation plates) in the light path of the microscope, we reveal the gross orientation of organic fibers within the tessellated layer of a stingray skeleton (fig. 4a). Both images show the same magnified region of the tessellated layer; however, in the colored image (fig. 4b), the different hues signify distinct fiber orientations, resulting from the light's being split into two perpendicular polarization directions, which pass the retardation plate at different speeds. The result is a window into the long-ranging fiber highways that act as blueprints for skeletal mineralization.

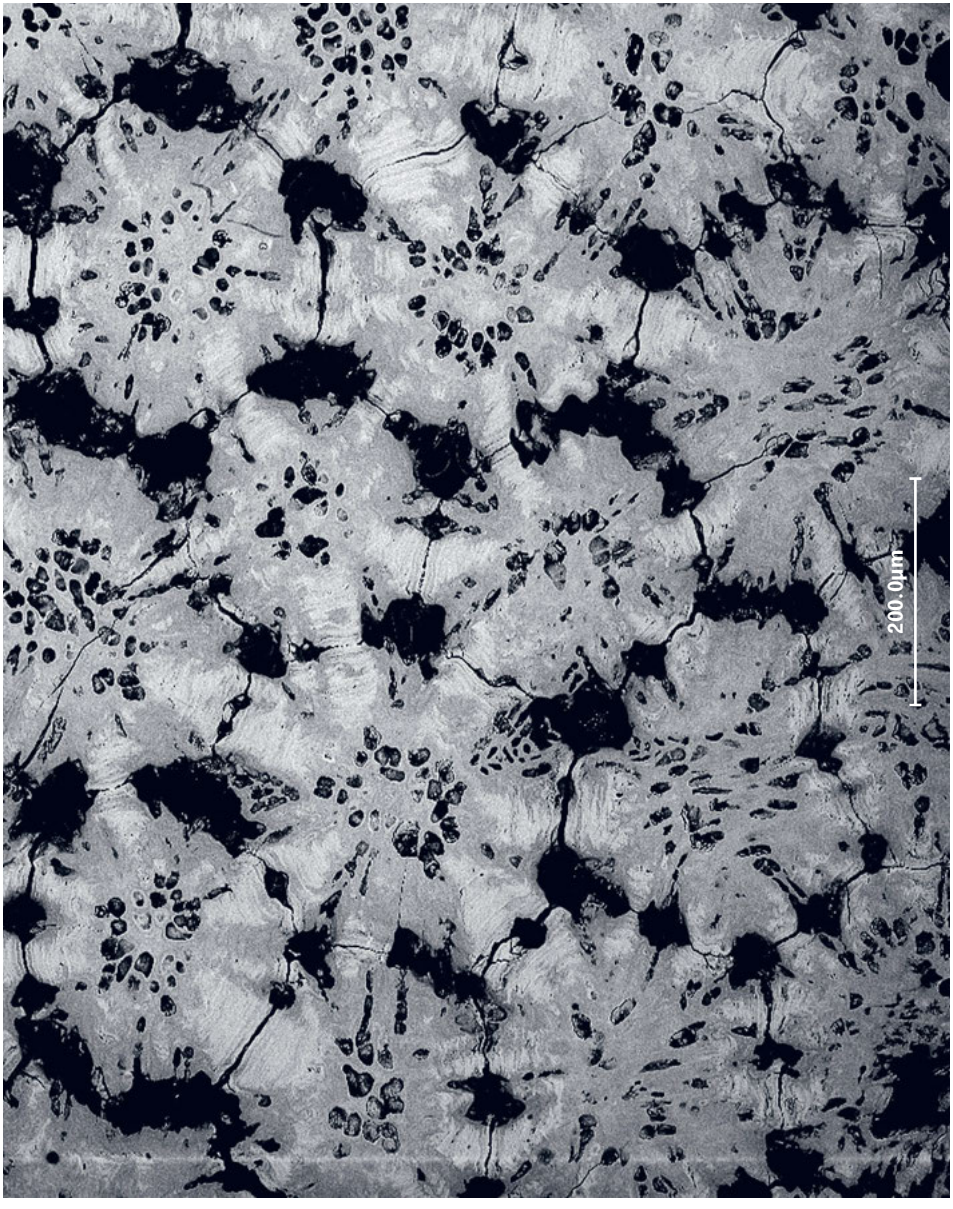
Inside Natural Building Blocks

One of the most striking aspects of biological tissues is their complexity at different length scales, which also makes them difficult to examine in their entirety, across scales. However, some microscopes can facilitate this, with a simple switch of the filter or tool used to look at the tissue. These environmental scanning electron microscope images obviously aren't identical, but actually show the same array of tesserae, the microscopic building blocks that cover all shark and ray skeletons.

The right image (fig. 5b), from a secondary electron detector, provides the topography of the sample, showing tesserae that are linked by string-like organic fibers to form flexible joints. In contrast, the left image (fig. 5a) was taken with a backscattered electron detector, revealing the distribution of heavier elements in the tissue (such as those forming mineral). The grayscale variation shows that tesserae are not uniform bricks of mineral, but rather play with how and where mineral is packed. The black regions harbor soft tissue, while the whitest regions are hypermineralized, reinforcing points where tesserae collide as the skeleton twists and turns.

Fig. 4a (opposite, top); 4b (opposite, bottom): Polarized light microscopy images of tesserae, illustrating fiber organization in the tissue, linking tesserae. The colors in the bottom image provide a visual map of fiber orientation, with similar colors indicating common fiber direction.

Figs. 5a & 5b (overleaf): Electron micrographs of a field of tesserae, a backscattered electron detector showing mineral density variation (left) and a secondary electron detector showing tissue topography (right).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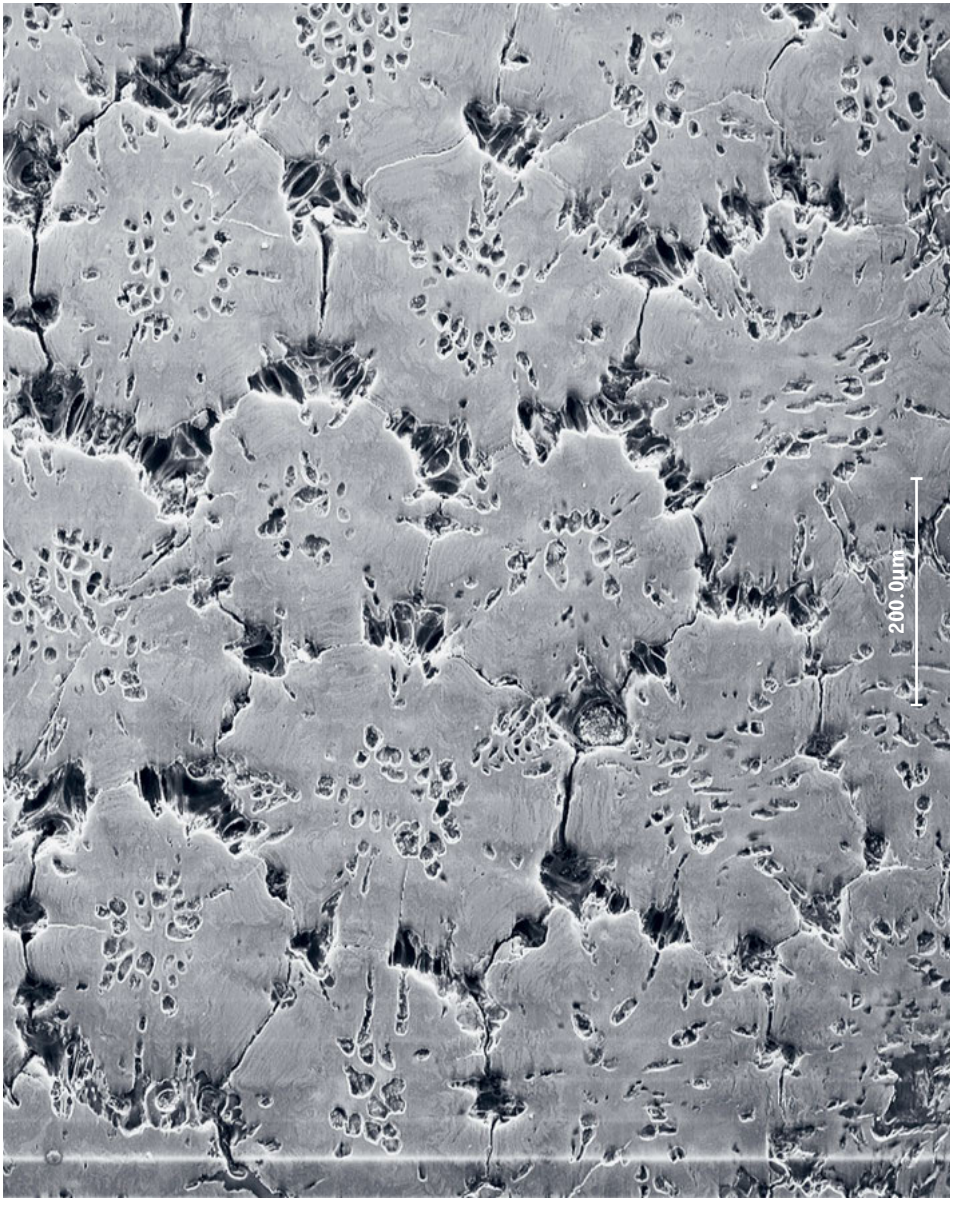
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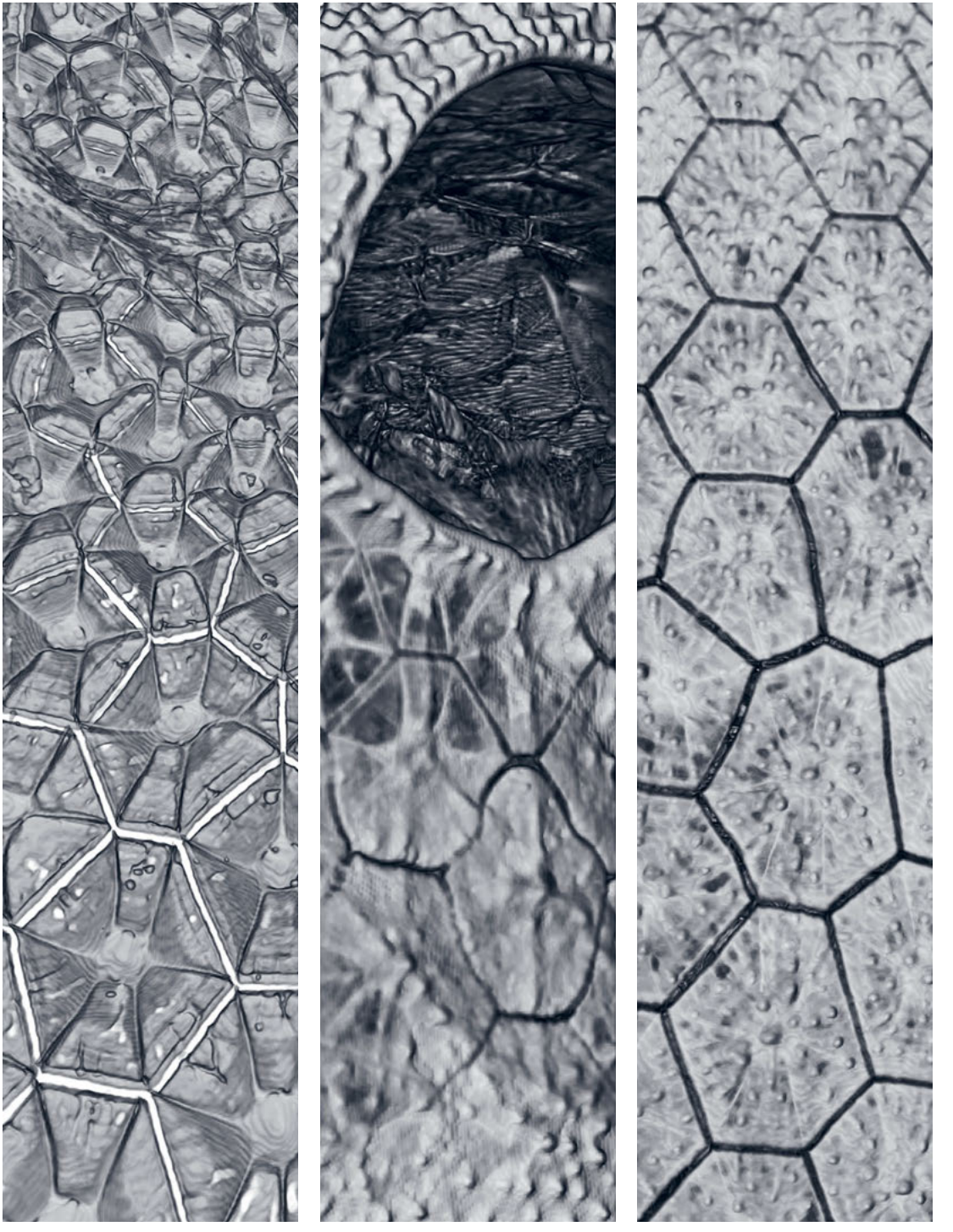

The Geometry of Armor

These micro-CT scan images (fig. 6) show the impressive plated body armor of a fish, which takes advantage of geometric principles to fortify and cover its body completely. Shark and ray cartilage is not the only biological material bearing a geometric tessellation: geometric patterning is a pervasive motif in Nature's toolkit for building tissues. This is sometimes an artifact of how the tissues grow; however, geometries like hexagons and pentagons are simply efficient shapes for covering nonplanar surfaces (as any soccer ball will attest).

These images show interior and exterior views of the body scutes of a boxfish, a small species that putters around tropical reefs. The fish's name comes from its awkward, boxy appearance, but this is a small sacrifice for protection from predators.

Fig. 6 (opposite): Micro-CT scan of boxfish armor, the digital nature of the data allowing exploration of scute structure from the interior (left) and exterior (right two images).