



## Segmentation of the Tessellated Mineralized Endoskeleton of Sharks and Rays



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The cartilaginous endoskeletons of sharks and rays are covered by tiles of mineralized cartilage called tesserae that enclose areas of unmineralized cartilage. These tessellated layers are vital to the growth as well as the material properties of the skeleton, providing both flexibility and strength. An understanding of the principles behind the tiling of the mineralized layer requires a quantitative analysis of shark and ray skeletal tessellation. However, since a single skeletal element comprises several thousand tesserae, manual segmentation is infeasible. We developed an automated segmentation pipeline that, working from micro-CT data, allows quantification of all tesserae in a skeletal element in less than an hour. Our segmentation algorithm relies on aspects we have learned of general tesseral morphology. In micro-CT scans, tesserae usually appear as round or star-shaped plate-like tiles, wider than deep and connected by mineralized intertesseral joints. Based on these observations, we exploit the distance map of the mineralized layer to separate individual tiles using a hierarchical watershed algorithm. Utilizing a two-dimensional distance map that measures the distance in the plane of the mineralized layer only greatly improves the segmentation. We developed post-processing techniques to quickly correct segmentation errors in regions where tesseral shape differs from the assumed shape. Evaluation of our results is done qualitatively by visual comparison with raw datasets, and quantitatively by comparison to manual segmentations. Furthermore, we generate two-dimensional abstractions of the tiling network based on the neighborhood, allowing representation of complex, biological forms as simpler geometries. We apply our newly developed techniques to the analysis of the left and right hyomandibulae of four ages of stingray enabling the first quantitative analyses of the tesseral tiling structure, while clarifying how these patterns develop across ontogeny.

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