Understanding the Tiling Rules of the Tessellated Mineralized Endoskeleton of Sharks and Rays

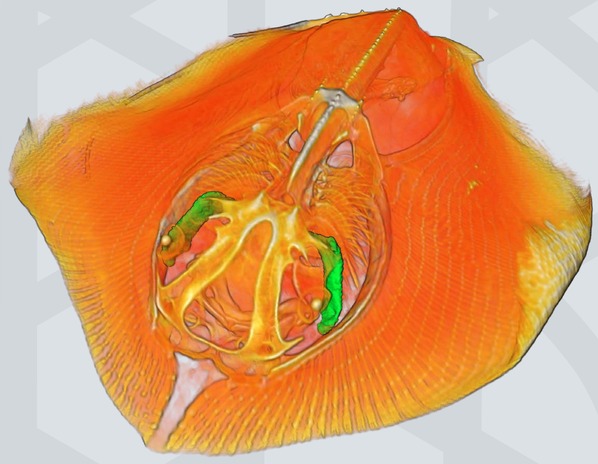
D. Knötel[1], R. Seidel[2], Ahmed Hosny[3], J. C. Weaver[3], D. Baum[1], M. N. Dean[2]

[1]Zuse Institute Berlin, [2]Max Planck Institute (MPI-CI), [3]Wyss Institute

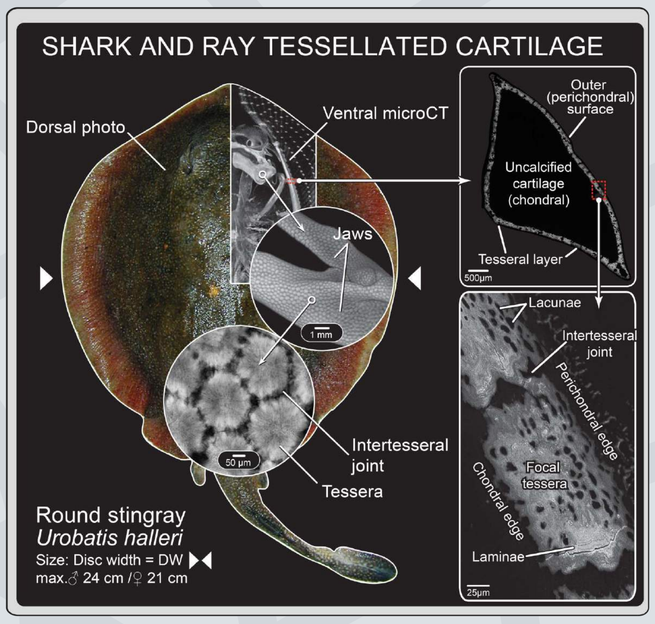
Acknowledgment: Human Frontier Science Program Young Investigator Grant (HFSP RGY0067), Peter Fratzl, Kady Lyons

Introduction

The cartilaginous skeleton of sharks and rays is covered with calcified tiles, called tesserae. It is still unclear what role skeletal tiling plays in the mechanics and growth of the skeleton. Our goal is to understand the tiling rules behind this tesselation of the endoskeleton. This will allow us to artificially mimic this complex biological system, for example through building artificial tesserae-like tilings on given smooth surfaces. A segmentation of tesserae enables three-dimensional quantification and visualization of tesseral networks, and is therefore the first step to learn the tiling rules. However, due to the large number of tesserae, their size (200-400 µm wide, 150-300 µm deep), and the size of µCT datasets, an automatic segmentation algorithm is required.

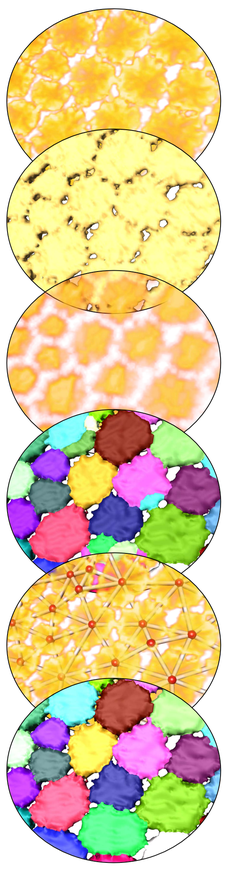


Volume rendering of a stingray with hyomandibulae highlighted in green



Segmentation Algorithm

Manual tesserae separation is not feasible for datasets with thousands of tesserae. The idea of the developed flexible segmentation pipeline is to use a hierarchical watershed algorithm on a two-dimensional distance map followed by manual error corrections. Qualitative evaluation was done by visually comparing the segmentation with an isosurface or volume rendering, quantitative evaluation by computing the RAND index and variation of information on manually segmented parts of the dataset.



Input µCT data
Volume rendering where high intensity values can primarily be seen on the borders between two tesserae.

Smoothing and background segmentation
Use anisotropic diffusion and local thresholding.

2D distance map
Calculate distance of tesserae voxels to the background in tesseral plane to create a function with low values on the borders and higher values around the tesserae centers.

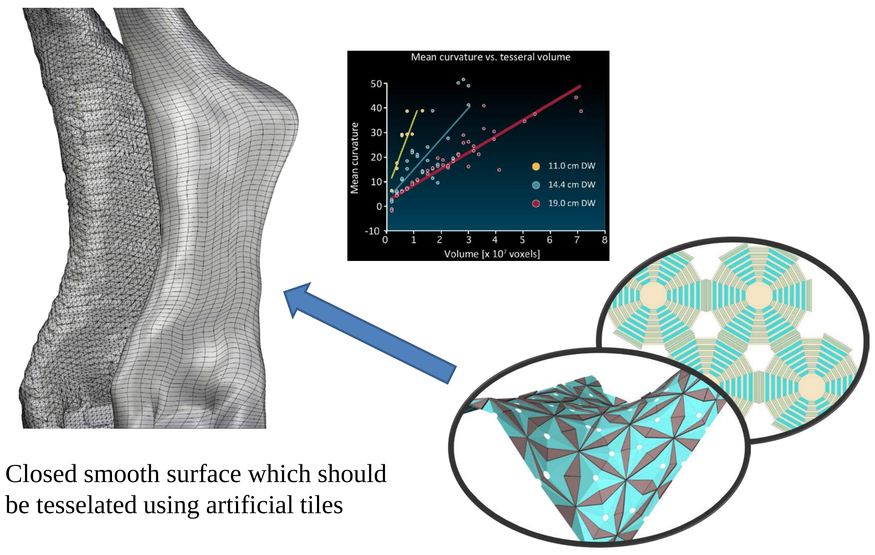
Initial segmentation
Use hierarchical watershed with given minimum segment size to isolate individual tesserae.

Dual graph of segmentation
Create graph with one vertex for each segment for visualization and user interaction.

Manual corrections
Correct segmentation errors using segment merging and splitting.

Future Directions: Artificial Tilings

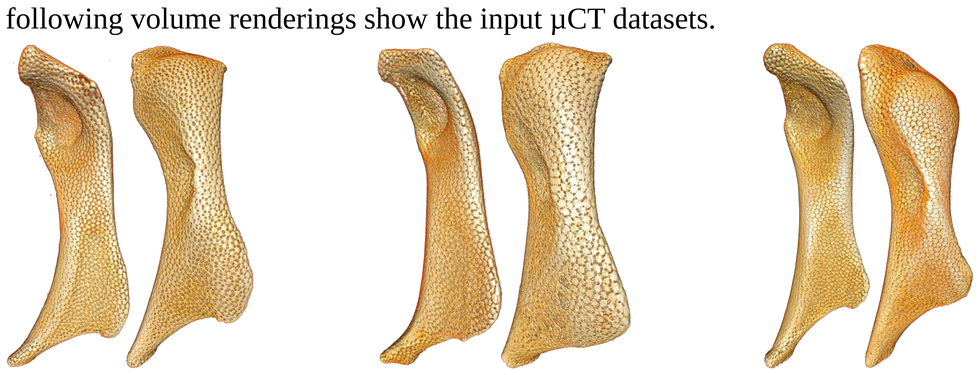
From the insight gained through the analysis of the segmentation, we plan to generate artificial tilings on smoothed closed surfaces. Tilings might then be generated according to different features, for example, mean or Gaussian curvature.



Closed smooth surface which should be tesselated using artificial tiles

Data Analysis

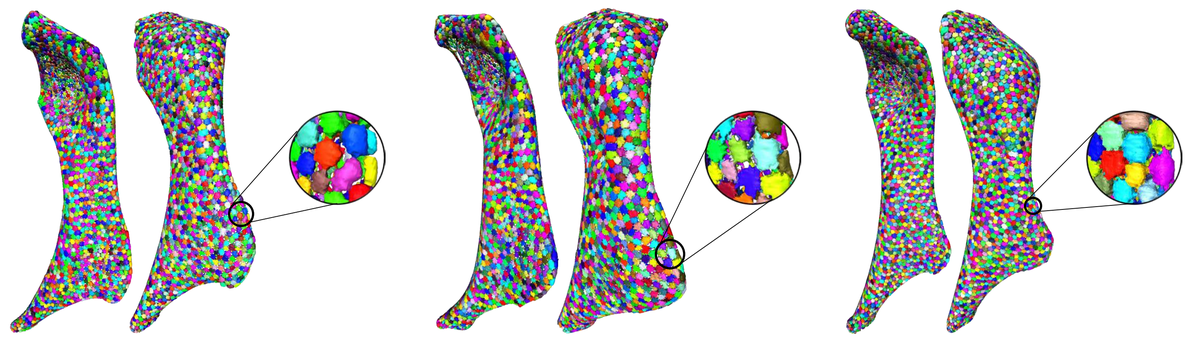
We use our segmentation algorithm to study the tiling structure of the left and right hyomandibulae of three ages of stingray, each consisting of several thousands of tesserae. The following volume renderings show the input µCT datasets.



A B C

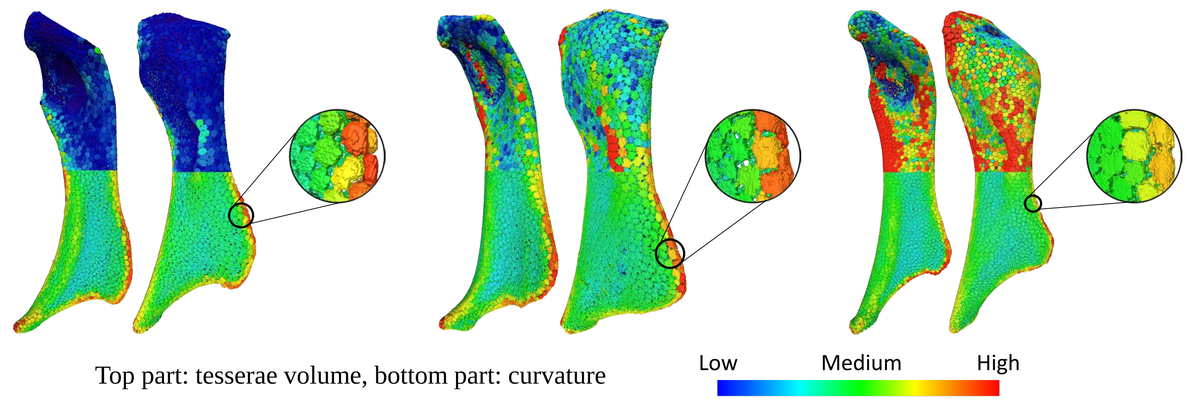
	A	B	C
Disc width (cm)	11	14.4	19
Hyomandibula length (mm)	13	18	24
Sex	Male	Female	Female

For datasets with a resolution of about 800 x 600 x 2000 voxels the creation of the initial segmentation took one to three hours. The following high quality segmentations are manually improved results, each computed in about one day. The needed effort depends on the desired segmentation quality.



	A left	A right	B left	B right	C left	C right
# Tesserae	3025	3081	2729	2759	3481	3488
Average volume (µm³)	$1.23 \cdot 10^6$	$1.08 \cdot 10^6$	$3.53 \cdot 10^6$	$3.30 \cdot 10^6$	$6.43 \cdot 10^6$	$6.73 \cdot 10^6$
Average # neighbors	5.7	5.8	5.8	5.7	5.9	5.9
CC / UC	0.19	0.17	0.15	0.14	0.18	0.19

Successful segmentation of tesserae enables the computation and visualization of several biologically relevant variables, such as curvature, volume, width, thickness or number of neighbors. This data allows further analysis of the tesselated cartilage and therefore deeper insight into design and material properties of cartilaginous skeletons. It is planned to analyze at least three more hyomandibulae which complement the currently available datasets regarding age and sex.



Top part: tesserae volume, bottom part: curvature