

RESEARCH

Project-6

Sujan Darai (sujad96@zedat.fu-berlin.de), Matanat Mammadli (matanam94@zedat.fu-berlin.de), Samra Hamidovic (samrah96@zedat.fu-berlin.de)

Full list of author information is available at the end of the article

Abstract

Goal of the project: Utilizing Amira's image segmentation tools, we aim to process and visualize the data, thereby gaining deeper insights into the spatial relationships and structures within complex biological datasets.

Main results of the project: Isosurface module with higher threshold results in better head skeleton view without too much noise. Hierarchical watershed segmentation results in better segmented images than connected component analysis (Label Analysis in this case). Minimal Depth port in hierarchical watershed segmentation with around 9000 value results in a good segmentation that is not too over- or under-segmentated.

Personal key learning:

- 1 Sujan Darai: Better documentation of the report
- 2 Samra Hamidovic: Writing report in a more professional way
- 3 Matanat Mammadli: Working with Amira software, learning about threshold segmentation, hierarchical watershed segmentation, and connected component analysis.

Estimated working hours:

- 1 Sujan Darai: 4 hours
- 2 Samra Hamidovic: 5 hours
- 3 Matanat Mammadli: 10 hours

Project evaluation: 1

Number of words: 1628

Keywords: Amira, Threshold Segmentation, Connected Components, Colorwash, Rendering, Label Analysis, Hierarchical Watershed Segmentation

1 Introduction

In recent years, advancements in 3D imaging techniques have significantly enhanced our ability to visualize and analyze complex biological materials. These techniques, such as micro-computed tomography (microCT), routinely produce high-resolution datasets consisting of stacks of grayscale images. However, the challenge lies in quantifying structural aspects of the biological materials, which necessitates the extraction of individual structures from the dataset through a process called segmentation. Traditional distance-based segmentation methods, such as the Euclidean distance transform, are commonly employed but have notable limitations, including susceptibility to noise and ineffectiveness for anisotropic shapes. [1]

The research introduces a novel approach, the random-walk distance transform, which offers improved robustness and suitability for high-throughput segmentation of biological tilings. By examining case studies on the tessellated cartilage of stingrays, the dermal endoskeleton of starfish, and the prismatic layer of mollusk shells, a comprehensive workflow for the segmentation and analysis of these complex

structures is illustrated.[1]

Amira, a powerful 3D visualization and data analysis software, offers a range of image segmentation tools that facilitate the processing and analysis of complex biological datasets. These tools are integral in transforming raw imaging data into meaningful, segmented structures, enabling detailed analysis and interpretation. Visualization, a key feature of Amira, is crucial for understanding and interpreting the data, providing researchers with intuitive insights into the spatial relationships and morphological characteristics of the structures under study. [1]

Despite the advancements in segmentation methods and visualization tools, it is important to acknowledge that these methods do not always work perfectly. The accuracy and effectiveness of segmentation can be influenced by various factors, such as noise in the data, the complexity of the structures, and the limitations of the algorithms used.

The proposed workflow in this paper addresses some of these challenges by incorporating the random-walk distance transform, which is shown to be more robust against noise and capable of handling anisotropic shapes more effectively than the traditional Euclidean distance transform. [1]

In conclusion, while modern segmentation approaches and visualization tools like those in Amira greatly enhance our ability to analyze complex biological structures, researchers must remain aware of their limitations and be prepared to implement additional processing steps to achieve accurate and meaningful results. The integration of innovative methods, such as the random-walk distance transform, represents a significant advancement in this field, enabling more efficient and accurate segmentation of large, complex datasets.

2 Goal of the project

The goal of this project is to segment 3D images using advanced techniques to enhance visualization and understanding of complex biological datasets. The project will utilize thresholding to separate structures from the background based on intensity values, creating a binary image. Connected component analysis will then be applied to identify and label contiguous regions, distinguishing individual structures. Hierarchical watershed segmentation will refine this process by using image gradients to separate structures with complex boundaries. Amira's image segmentation tools will be employed to process and visualize the segmented structures, enabling detailed analysis and interpretation of the data. Through these visualizations, we aim to gain a deeper understanding of our data and the spatial relationships within it.

3 Data and preprocessings

In our report, we have used a dataset entitled "EmodesmusZMB6190A_resampled.am" which represents a micro-CT scan of the Eirenis modestus dwarf snake species skull for modelling. The format ".am" exhibits the Amira Mesh file, which is commonly used for keeping and envisioning 3D imaging data. The skulls dataset is provided by the Museum für Naturkunde, Berlin, and shared by Kristin Mahlow. The main reason behind using this dataset in our scientific research is to advance the understanding of the dwarf snake species named Eirenis modestus. In this dataset, the

micro-CT scan investigates complex details of the skull's structures (morphology and anatomy) for diverse scientific analyses without damaging the physical structure. The data has already been cleaned and thus no preprocessing was required.

4 Methods

Amira is a high-performance 3D visualization and data analysis software designed for scientists and researchers. It is widely used in various fields, including biology, medical imaging, materials science, and engineering. Amira provides a comprehensive suite of tools for visualizing, analyzing, and understanding complex 3D data, enabling users to process and interpret volumetric datasets, segment structures of interest, and create detailed visual representations. The software supports advanced image processing techniques, segmentation algorithms, and quantitative analysis, making it a valuable resource for extracting meaningful insights from high-resolution imaging data. Additionally, Amira's intuitive user interface and powerful visualization capabilities facilitate the exploration and presentation of complex datasets, enhancing the overall understanding and communication of scientific results. [1]

In addition to threshold segmentation and connected components analysis, we applied hierarchical watershed segmentation.

For the software, we used MacOS Sonoma 14.5.

5 Results and discussion

When we first opened the data in Amira software, the Ortho Slice module was automatically attached to it. The first image of the snake head was this image 1.

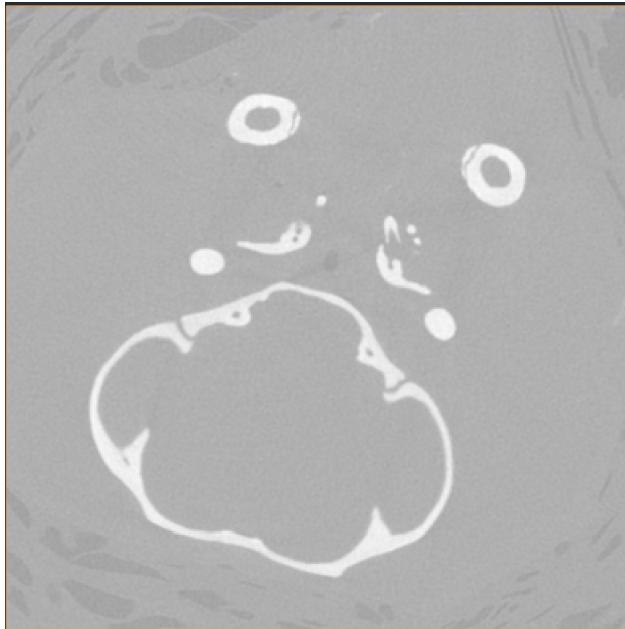


Figure 1: Ortho Slice view of snakehead (grayScale.am colormap)

We could then play with Colormap options in the Ortho Slice module, changing the colors of this first image.

The next task was attaching an Isosurface module to the data and trying several thresholds until we could clearly see the head skeleton without too much noise. We chose threshold 21237.7 and these were the images we obtained (see Figure 2).

Then we attached an Interactive Thresholding module to the data, set the lower value of the Intensity Range to the same value we found most appropriate for the Isosurface visualization, aka to 21237.7, and obtained the binary segmentation data called EmodestusZMB6190A_resampled.thresholded*.

We also attached a Color Wash module to the Ortho Slice and connected its Data port with the binary segmentation data. Then we attached the Voxelized Rendering module to the binary segmentation dataset. This is the image we obtained from this module, with Colormap labels.am, see image 3. These were the tasks from subtask 1: Threshold segmentation.

From subtask 2: Connected Components, we attached a Label Analysis module to the binary segmentation data (EmodestusZMB6190A_resampled.thresholded*) and ran it. One of the outputs was the result of connected component analysis, called EmodestusZMB6190A_resampled.Label-Analysis* (see image 4). We could also export the results (table) of this analysis.

Then we recomputed the threshold segmentation with a much higher threshold (we chose 27607) and reran the connected component analysis. These are the results we obtained (see image 5), as you can see, the numbers of Label Analysis changed and also snake head skeleton does not have as many blue voxels as before, after setting the threshold of the Interactive Thresholding module higher. Again, here we could also export Label analysis results as .am file.

Afterward, we visualized the result with the Voxelized Renderer module. We change the default colormap of the Voxelized Renderer from labels.am to labels256.am. Here are the results (see image 7), as we can see, voxel colors changed and there are fewer voxels on the snakehead than before (after setting the threshold higher and running label analysis).

From subtask 3: Hierarchical Watershed we first attached the Hierarchical Watershed Segmentation module to the EmodestusZMB6190A_resampled.am dataset, set the Global Minimum of the Global Range port to the value we used for isosurface visualization (21237.7) and ran the module. Then we visualized the result with the Voxelized Rendering module using the labels256.am colormap (see image 7). As we can see snake head skeleton seems well-covered and segmented (segmentations are well-separated with different colors).

Then we gradually increased the value of the Minimal Depth port (from 0 to 9102 and to 18204) and updated the segmentation. These are the results (see image 8), as we can see, with Minimal Depth 0 voxels were not well-separated and did not cover snakehead skeleton segments accordingly, Minimal Depth 9102 showed better results in segmentation and beginning from Minimal depth 16000 and forwards

(18204 included and till the max value 65535) we got same results over and over again, and these numbers resulted in over-segmentation of the snake head skeleton. We think the best value of the Minimal Depth port that is a good compromise between over- and under-segmentation is 9102.

Our segmentation results after applying Hierarchical Watershed Segmentation were much better than our segmentation results after Label Analysis (or connected component analysis). The voxels covered the snake head skeleton better and resulted in better segmentation after Hierarchical Watershed Segmentation rather than after connected component analysis.

We could also do the first task from subtask 4: Merging Regions, we had to identify the regions that have not been perfectly segmented and handle the regions that need merging. We used the PickAndMergeLabels module for this, we applied it on the EmodesZMB6190A_resampled.hws dataset and got EmodesZMB6190A_resampled.mergedFragments* dataset as a result. We visualized this result using a Voxelized Rendering module with labels.am Colormap and got this image 9.

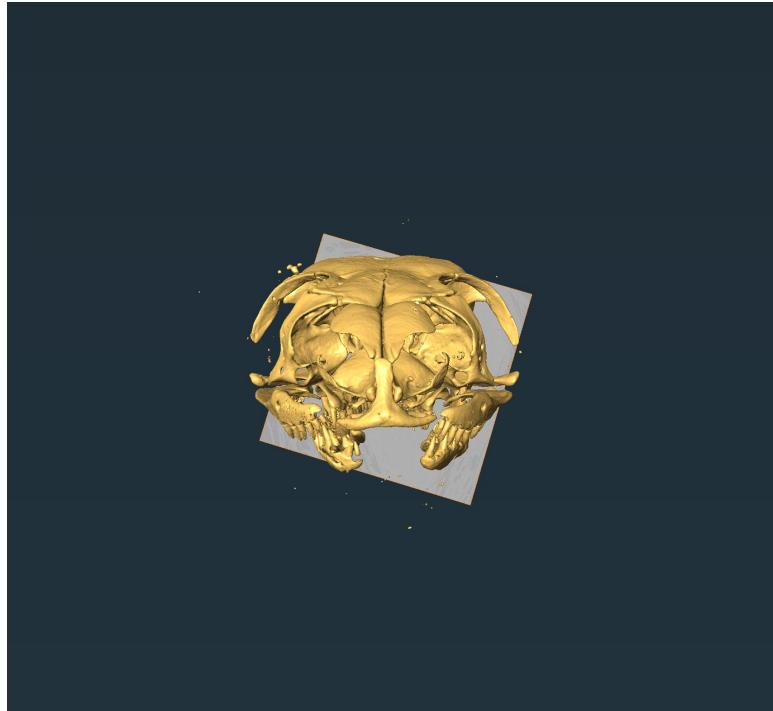
We only did subtask 4 till this part.

6 Contributions

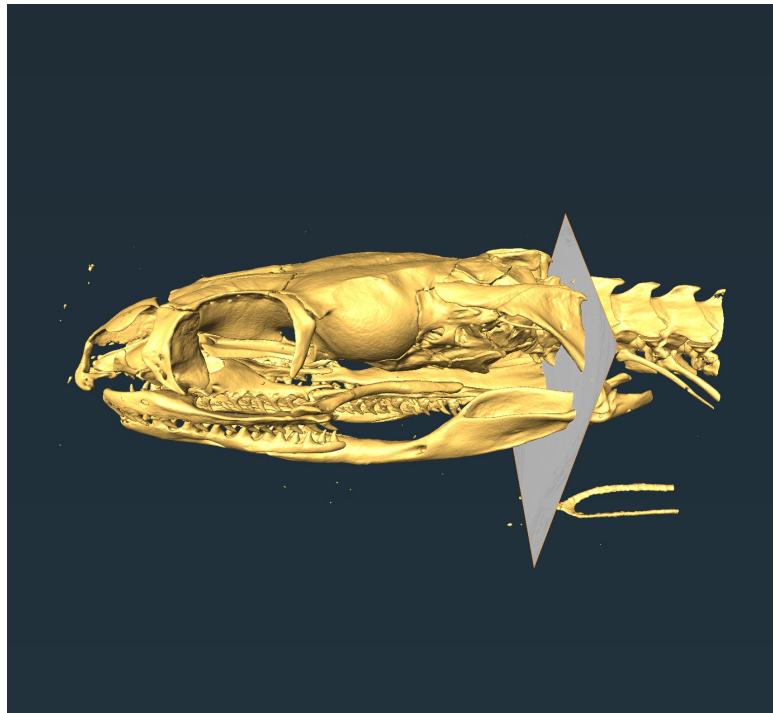
Sujan Darai: Writing Data and preprocessing part and reviewing of whole report

Matanat Mammadli: Doing subtasks 1,2,3 and 4.1 with Amira Software, writing Data & preprocessings and Results & discussion

Samra Hamidovic: Writing Introduction, Goal of the project and Methods



(a) Frontal view



(b) Side view

Figure 2: Snake head skeleton images from Amira (Isosurface module)

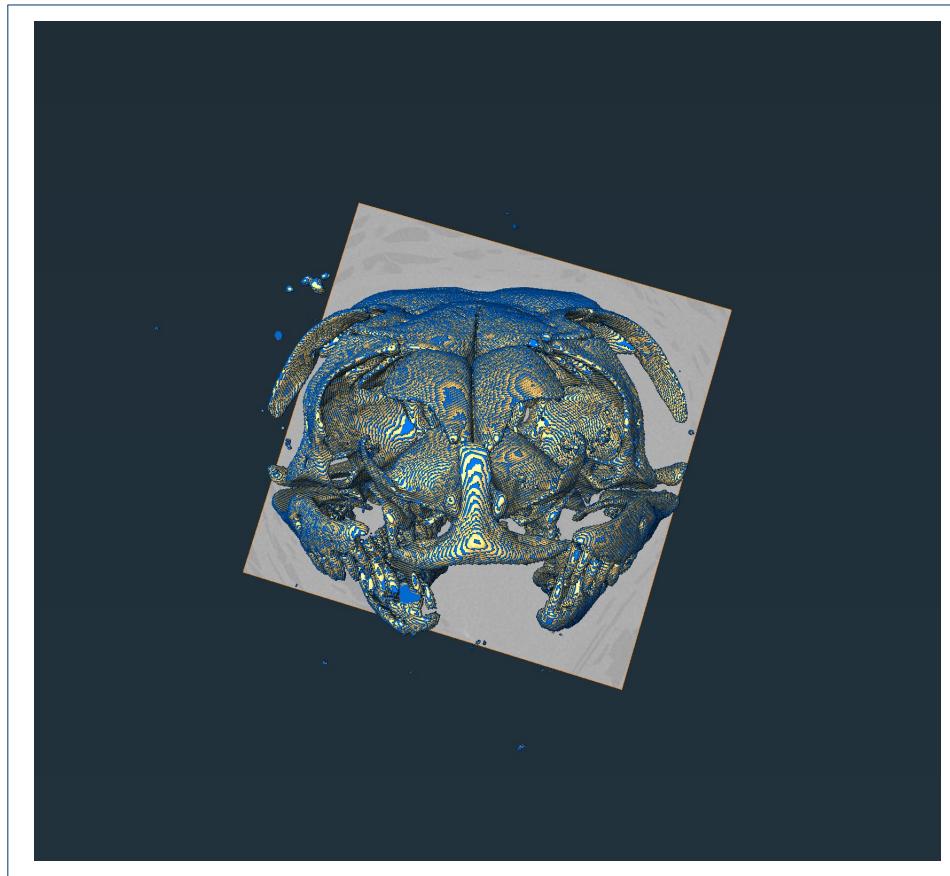


Figure 3: Voxelized Rendering image of snakehead skeleton (labels.am Colormap)

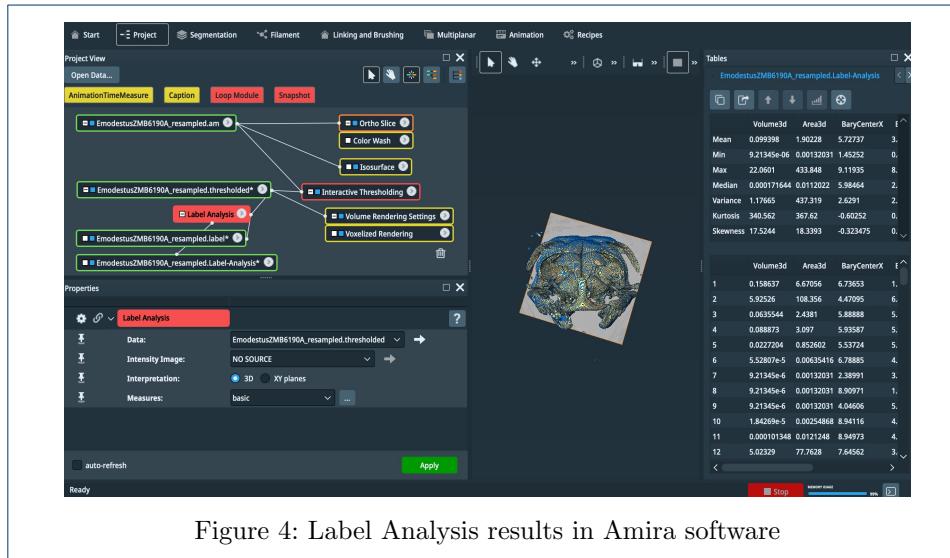


Figure 4: Label Analysis results in Amira software

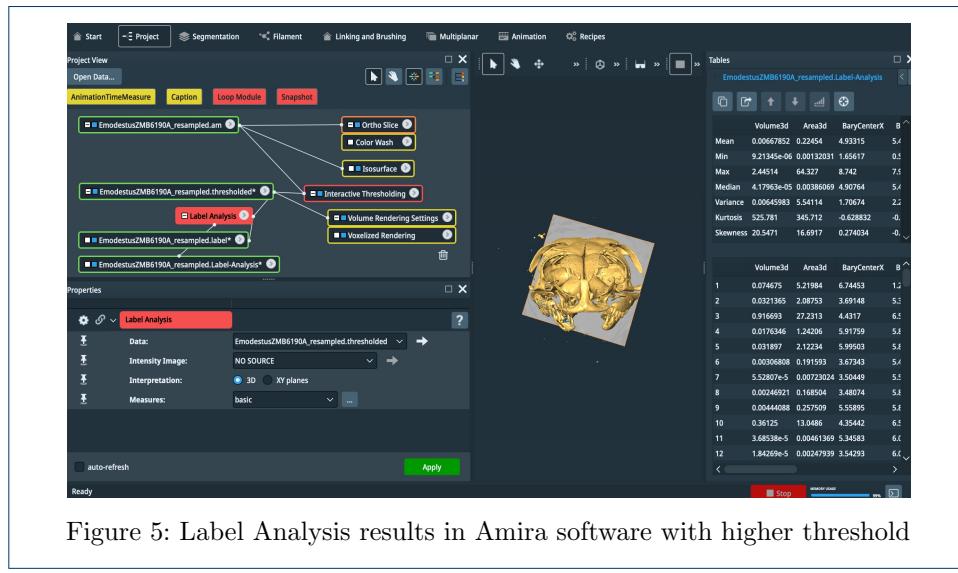


Figure 5: Label Analysis results in Amira software with higher threshold

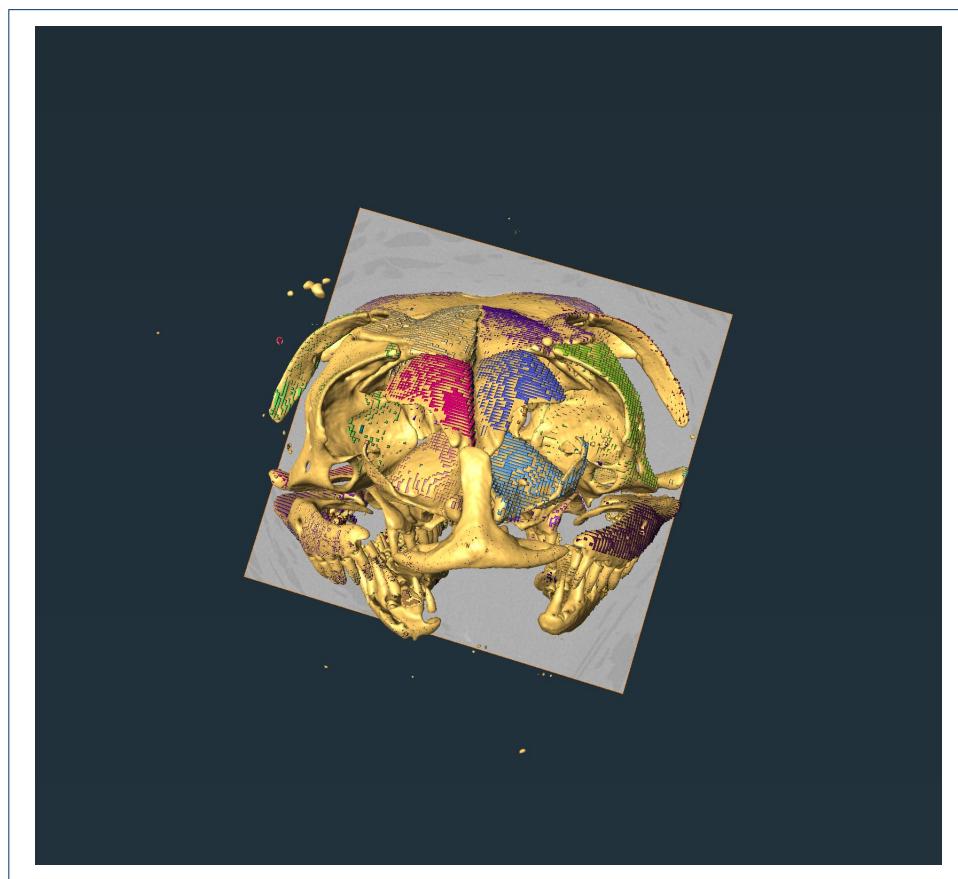


Figure 6: Voxelized Rendering image of snakehead skeleton after Label Analysis with a higher threshold (labels256.am Colormap)

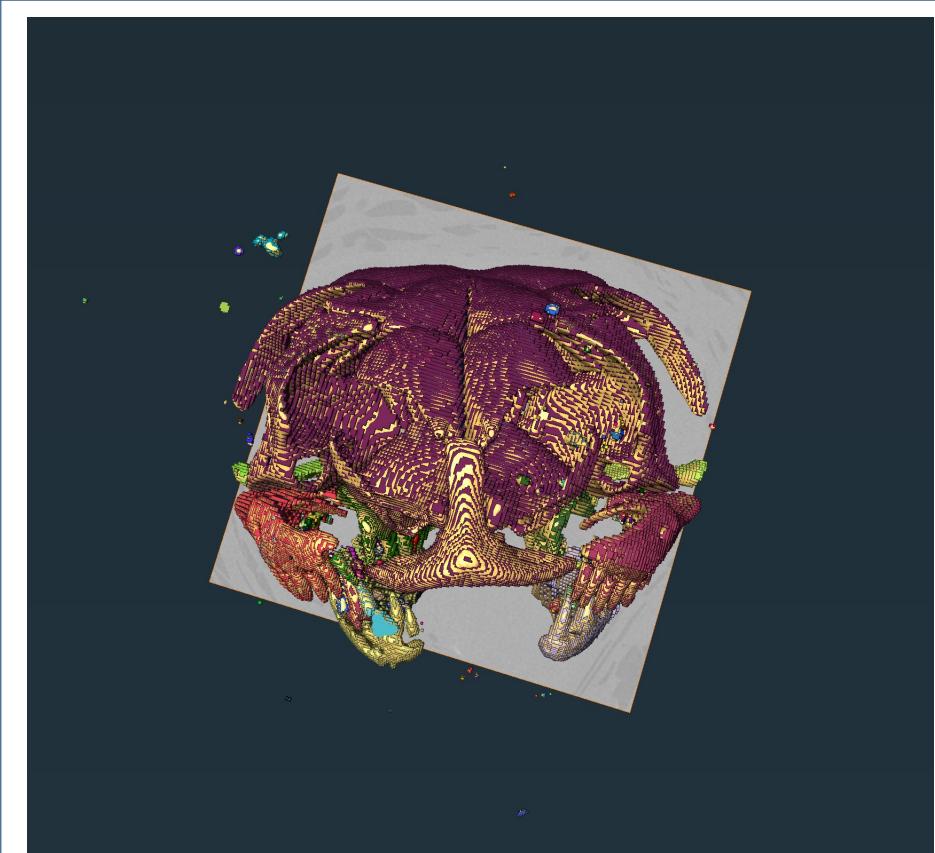
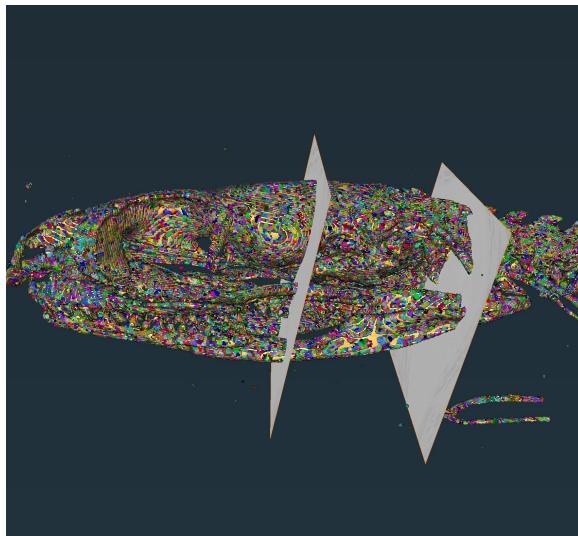
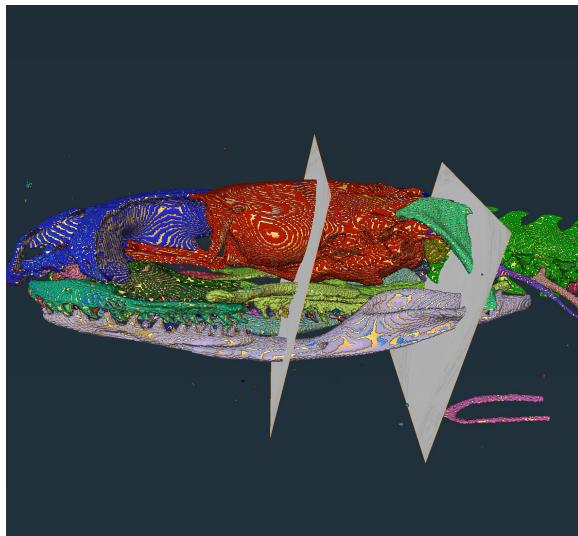


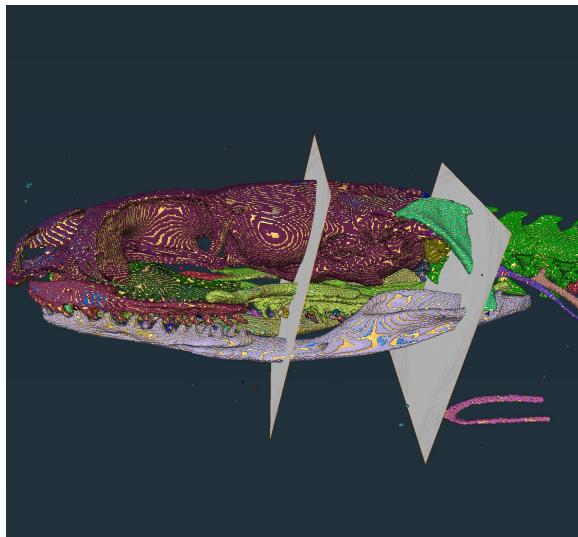
Figure 7: Voxelized Rendering image of snakehead skeleton after Hierarchical Watershed Segmentation (labels256.am Colormap)



(a) Minimal Depth 0



(b) Minimal Depth 9102



(c) Minimal Depth 18204

Figure 8: Hierarchical Watershed Segmentation images of snakehead skeleton visualized with Voxelized Rendering module (labels256.am Colormap)

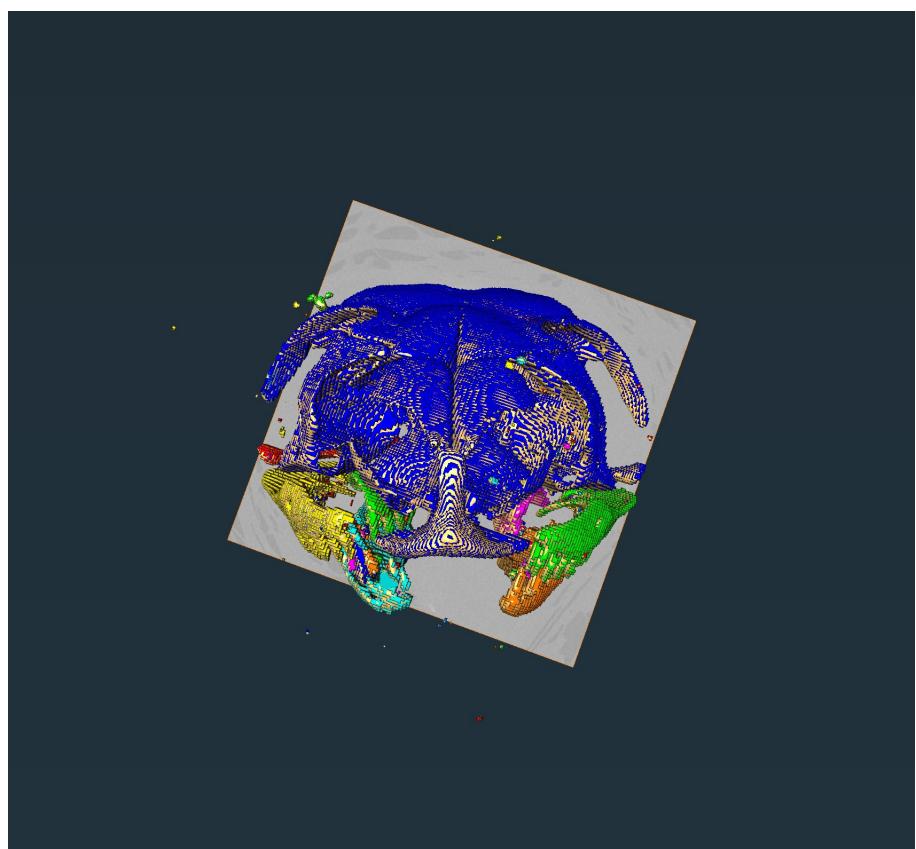


Figure 9: Voxelized Rendering image of snake head skeleton after PickAndMerge-Labels (labels.am Colormap)

7 Appendix

References

1. Daniel Baum, James C Weaver, Igor Zlotnikov, David Knötel, Lara Tomholt, and Mason N Dean. High-Throughput Segmentation of Tiled Biological Structures using Random-Walk Distance Transforms. *Integrative and Comparative Biology*, 59(6):1700–1712, December 2019.