# Anatomical Landmarks and Measurements of Craniofacial Structures: A Micro-CT Analysis

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## ABSTRACT

**Background:** In this study, an adult rat's calvaria was used to examine craniofacial anatomical landmarks of maxilla and mandible using Micro-Computed Tomography (micro-CT) as the marker of craniofacial growth and development in rat. It is used to assist research, translating landmark points for diagnosis. Landmarks on the rat's calvaria were determined to set the measurements of two specified points. One of the ways to validate craniofacial growth and development is to look at the differences between craniofacial before and after a specified period or treatment. With micro-CT analysis, the researchers are more precise in determining the difficult points in the cranium. **Method:** The sample analysis was carried out and measured by DataViewer version 1.6.0.0 64-bit Application. CTVox version-3.3.1 64-bit is used to display 3D visuals. Reconstruction results after adjusting the data range were 30-255 (the color range 0-255). The soft tissue with a lower density was reduced in order to entirely expose the hard tissue. The machine used was Bruker SkyScan-1173 High Energy Micro-CT.

**Result:** Findings anatomical landmarks for the examples, the maxilla determined I for the point on premaxilla between jawbone and lingual surface of upper incisors. In the mandible, I' for the most anterior edge of the alveolar bone on the convexity of the lower incisor. The unevenness of the cranium made manual measurements difficult and not possible. However, this micro-CT method makes the landmark point determination more detailed and dependable. This makes geometric measurements of craniofacial more reliable.

**Conclusion:** Micro-CT analysis is a more dependable method to determine anatomical landmarks and measure craniofacial structures as the marker of craniofacial growth and development in rat.

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#### INTRODUCTION

Micro-computed tomography (micro-CT) is a non-destructive imaging technique that produces three-dimensional (3D) images of the internal structure of objects with very high resolution, even down to the micron level. The main benefit of analysis using micro-CT is a visualization of detailed internal Structure where micro-CT allows observing the internal structure of objects without need to cut or damage the specimen. This is especially important for morphological analysis in materials science, biology, or medicine(1). Micro CT is also a multidisciplinary analysis, it is applied in various fields including geology to study rock porosity, materials science to observe weaknesses or defects in materials, biology to view biological tissue, and medicine to help diagnose clinical conditions(2).

Micro-CT has high resolution, so it can detect fine details that can not be seen with other imaging techniques, such as MRI or clinical-scale CT, making it very suitable for microstructural studies. Micro-CT is also a non-destructive analysis because this method is non-destructive, the samples analyzed remain intact, thus allowing further observation with other methods or preservation of high-value specimens. Micro-CT images are also 3D reconstructions that can be rotated and analyzed from various angles(3). This was useful for getting a full perspective of the object being analyzed, including more accurate morphometric analysis. And finally, quantitative observation of the volume and distribution of materials, which can be used to calculate the volume, density and distribution of certain materials within an object. For example, in biology, it can measure mineral density in bones(4).

In this research, micro-CT's principles as a technological advancement, and utility in various preclinical applications, particularly in high-resolution anatomical imaging. It highlights the strengths of micro-CT, such as high resolution and scanning efficiency, and its limitations, proper sample placement at the time of scan procession, and poor soft tissue contrast(5). This phase contrast determines for improved imaging of bone biological tissues in mice calvaria. The aim of the research micro-CT also helps determine landmark points as can be done in CBCT and cephalometry previously(3). This is an option for researchers who can carry out further studies and determine in detail the landmark points with all their difficulties.

#### **RESEARCH METHOD**

This study is a descriptive analysis, a research method by collecting data according to truth, then the data is compiled, processed, and analyzed to provide an overview of the existing problem. It is approved with a research ethic number: 0512/HRECC.FODM/V/2024. There were two calvarias of female adult rats, samples were taken from the experimental animal laboratory of the Wijaya Kusuma Faculty of Medicine, Surabaya. The equipment for making a preparate are dappen glass, lighting, tweezers, scalpel, and scissors. For Micro-CT examination, use a Micro-CT Device machine (Bruker SkyScan 1173 High Energy Micro-CT, Kontich, Belgium) with CTVox software version 3.3.1 (64-bit) and CTAn version 1.20.8.0 (64-bit). The scan stage produces a projected image in grey-scale values in 16-bit Tagged Image File Format (TIFF). Preparations were prepared by placing the preserved cranium in a 10% formalin solution for maximum 24 hours, fixed with styrofoam and sticky wax then arranged on an iron plate.



Figure 1. Making calvaria's preparations

The preparation is confirmed in a fixed position meanwhile the plate moves or rotates. This is for the sake of recording validity during the data recording process by the micro-CT machine. The scanning process is carried out for 2-2.5 hours, then the data validity is checked. The data is taken according to the researcher's needs assisted by the equipment operator. The Micro-CT eximination was conducted in the Micro-CT Scan Integrated Physics Laboratory, Faculty of Mathematics and Natural Sciences, Institute of Technology Bandung (ITB).



Figure 2. Micro CT scanning process

# RESULTS

## **Visual Data Reconstruction Record**

After scanning, a DICOM file is obtained, before being converted to JPG format. Then JPG starts to make reconstruction records.



Figure 3. Three-dimention (3D) images of the maxilla extracted as stl file from the DICOM-files



Figure 4. Figure reconstruction record for skull (a) and mandibula (b)

According to the image description, the left image is the reconstruction result without adjusting the data range. In the right image is the reconstruction result after adjusting the data range where the color range 0-255 becomes 30-255. The purpose of soft tissue with a lower density can be reduced so that only hard tissue is exposed.

# Visualization After Data Range Adjustment:

- <image>
- A. Cephalometric Landmarks Points

Figure 5. Cephalometric landmarks points from ventral view of skull



Figure 6. Point of Cephalometric Mandible Landmarks from lateral view



Figure 7. Dorsal view of mandible the angle of mandibular arch

Cephalometric	Description
Landmarks Point	
Ν	A point on the nasofrontal suture
Α	The most anterior point on the nasal bone
E	The intersection between the frontal bone and the most superior-anterior point
	of the posterior limit of the ethmoid bone
So	The intersection between the posterior border of the basisphenoid and the
	tympanic bulla
Pr	The most inferior and anterior point on the alveolar process of the premaxilla
Bu	A point on the premaxilla between the jaw bone and the lingual surface on the
	upper lingual incisors
lu	The most prominent point between the incisal edges of the lower incisors
Mu	A point on the intersection between the maxillary bone and the mesial surface
	of the upper first premolar
li	The most prominent point between the incisal edges of the power incisors
ld	The most inferior and anterior point on the alveolar process of the mandible
Mn	A point in the deepest part of the ante gonial notch curvature
M1	A point on the intersection between the mandibular alveolar bone and the
	mesial surface on the first premolar
B1	A point on the intersection between the lingual surface of the lower incisors
	and the most anterior part of the lingual alveolar bone
Со	The most posterior and superior point on the mandibular condyle
Go	The most posterior point on the mandibular ramus
P(R/L)	The most right/left anterior and lateral point of maxilla
Me(R/L)	The most inferior and anterior point on the right/left lower border of the
	mandible
PM1(R/L)	Most left anterior and lateral point of premaxilla
PM2(R/L)	Most right/left posterior and lateral point of premaxilla
M1(R/L)	First molar on the right/left maxilla
M2(R/L)	Second molar on the right/Left maxilla
M3(R/L)	Third molar on the right/left maxilla
M1'(R/L)	First molar on the right/left mandible
M2'(R/L)	Second molar on the right/Left mandible
M3'(R/L)	Third molar on the right/left mandible
PA(R/L)	Most posterior of the third molar alveolar maxilla
PA'(R/L)	Most posterior of the third molar alveolar mandible
I	Point on premaxilla between jawbone and lingual surface of upper incisors
<b>]</b> ,	The most anterior edge of the alveolar bone on thr convexity of the lower
	incisors

Ро	Most posterior point on cranial vault
θ	Angle of the mandibular arch

 Table 1. Craniofacial cephalometric landmarks

# B. Cephalometric landmarks distances

# The Skull



Figure 8. Lateral view of the Skull





Figure 9. Dorsal view of the skull





Figure 10. Ventral view of skull: skull base length (a), maxillary length (b)



# The Mandible

Figure 11. Lateral view of mandible





Figure 12. Dorsal view mandible: Distance I' to middle line PA'R and PA'L to measure length of the mandibular arch



Figure 13. Dorsal view mandible : to meassured the mandibular width



Figure 14. Dorsal view of mandible : to measured the length of dental arch from M3'R to M3'L

Cephalometric	Description	
Distance		
A. Lateral view of the skull and mandible		
1-2	Maximum length of the cranium	
5-6	Maxillary length	
7-8	Mandibular ramus lenght	
7-9	Mandibular lenght	
B. Lateral view of the skull and mandible		
8-9	Mandibular ramus length	
C. Dorsal view of the mandible		
1-12	Nasal lenght	
13-14	Nasal width	
15-16	Skull width	
D. Dorsal view of mandible		
10-11	Mandibular width	
E. Ventral view of the skull		
17-18	Skull base length	
17-19	Maxillary length	
Table 2. Measurement of craniofacial structures from cephalometric landmarks distance		

The landmarks point of the skull observed with micro-CT can be seen in Figures 8, 9, and 10. The images showing with blue dots were obtained from the lateral, dorsal, and ventral views. The landmarks point of the mandible with micro-CT can be seen in Figures 11 and 12. This also shows trough blue dots was obtained from the lateral and dorsal view of mandible. From the dots of landmarks points were connected into line distances from the outermost end of the skull structure.

#### DISCUSSION

Cranial growth centers are certain areas of the skull where skull bone growth occurs. The bones that make up the cranium develop through a regular growth process in certain growth centers. Following are some of the important growth centers of the skull bones such as the anterior cranial growth center. This center is located at the front of the skull and plays a role in the growth of facial bones and the forehead, middle cranial growth center plays a role in the growth of the middle bones of the skull, including the temporal bone and the sphenoid bone and posterior cranial growth center this center is located at the back of the skull and plays a role in the growth of the cranium, such as the occipital bone(4). According to figure 8 where the lateral view of the cranium shows the outermost point from the back is called the Po point. Po is this point most posterior point on cranial vault. Measurements were also made for the distance between point 1 and 2, which serves as a guide for researchers to determine skull growth from the anteroposterior dimension. For maxillary growth, using points 5 and 6. Where point 5 is the outermost point of the anterior alveolar bone, while point 6 is the most posterior point of the maxillary bone. The difference between pre and post-measurements in a study can be used as valid data in this micro CT analysis examination.

The craniofacial center of anterior growth, in the human cranium refers to certain areas at the front of the skull that involve the growth and development of the facial bones and forehead. This anterior growth center plays an important role in forming proportional and symmetrical facial and forehead structures(6). There are some parts of the cranium involved in the anterior growth center including the Frontal Bone, the forehead bone is an important part of the skull that forms the front of the cranium. In Figure 9, the correct measurements are obtained sagittal and transverse growth. Viewed from points 1-12 Nasal length, 13-14 Nasal width and 15-16 Skull width. The growth of the forehead bone is regulated by the anterior growth center. Maxilla is the upper jaw bone which forms the main part of the facial arch. Maxilla growth is also controlled by the anterior growth center. Nasal Bone is located in the middle of the face and plays a role in forming the structure of the nose. The growth of the nasal bones is also affected by the anterior growth center(7). In the mandible there are also specific points for measuring growth. In Figure 7, the mandibular angle is obtained which can be measured from growth in the transverse direction. Where the larger the angle indicates progressive. Otherwise, in Figure 11 where the triangular shape is taken from the outermost point of processus coronoideus, the outermost point of the gonion, and the most inferior and most anterior points of the mandibular alveolar bone. The distance from this triangle can be used as a reference for measuring mandibular growth over time.

In this research used high resolution imaging techniques such as microcomputed tomography (micro-CT) can provide complementary quantitative information about the progressive changes in threedimensional (3-D) skeletal morphology that occur during skeletal development and postnatal growth. Cephalometric landmarks are specific points on the skull that are used as reference points for analyzing and measuring craniofacial structures. Micro-CT imaging can be a valuable tool for studying and identifying cephalometric landmarks with high precision due to its ability to provide detailed threedimensional images of bone structures. This research aims to determine landmarks cephalometric points that can be seen from micro-CT scans in the cranium area and growth points(4).

Cephalometric landmarks are specific points on the skull that are used as reference points for analyzing and measuring a rat's craniofacial structures. Micro-CT imaging can be a valuable tool for studying and identifying cephalometric landmarks with high precision due to its ability to provide detailed three-dimensional images of bone landmarks. Some common cephalometric landmarks can be identified using Micro-CT(8). Findings anatomical landmarks for the examples, the maxilla determined I for the point on premaxilla between jawbone and lingual surface of upper incisors. In the mandible, I' for the most anterior edge of the alveolar bone on the convexity of the lower incisor(9). The unevenness of the cranium made manual measurements difficult and not possible.

However, this micro-CT method makes the landmark point determination more detailed and dependable. This makes geometric measurements of craniofacial more reliable. From this study micro-CT analysis is a more dependable method to determine anatomical landmarks and measure craniofacial structures as the marker of craniofacial growth and development in rat(10).

## CONCLUSION

Micro-CT analysis is a more dependable method to determine anatomical landmarks and measure craniofacial structures as the marker of craniofacial growth and development in rat.

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