Three-Dimensional Cephalometric Landmark Annotation Demonstration on Human Cone Beam Computed Tomography Scans

Konstantinia Almpani[1,2], **Anna Adjei**[2], **Denise K. Liberton**[1], **Payal Verma**[1], **Man Hung**[*,2,3,4], **Janice S. Lee**[*,1]

[1] National Institute of Dental and Craniofacial Research [2] College of Dental Medicine, Roseman University of Health Sciences [3] University of Utah School of Medicine [4] George E. Wahlen VA Medical Center

[*] These authors contributed equally

Corresponding Author

Konstantinia Almpani
nadine.almpani@nih.gov

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Abstract

Craniofacial cephalometric analysis is a diagnostic tool used for the assessment of the relationship of various bones and soft tissues in the head and face. Cephalometric analysis has been traditionally conducted with the use of 2D radiographs and landmark sets and restricted to size, linear and angular measurements, and 2D relationships. The increasing use of 3D cone beam computed tomography (CBCT) scans in the dental field dictates the need for the evolution to 3D cephalometric analysis, which incorporates shape and a more realistic analysis of longitudinal development in all three planes. This study is a demonstration of 3D cephalometric analysis with the use of a validated set of skeletal tissue landmarks on human CBCT scans. Detailed instructions for the annotation of each landmark on a 3D volume are provided as part of a step-by-step protocol. The generated measurements and 3D coordinates of the landmarks can be exported and used both for clinical and research purposes. The introduction of 3D cephalometric analysis in basic and clinical craniofacial studies will lead to future advancements in the field of craniofacial growth and development.

Introduction

Cephalometric analysis, which examines the dental and skeletal relationships of the human skull, is the clinical application of cephalometry. In addition to anthropologists, developmental biologists, forensic experts, and craniofacial researchers who study human evolution and craniofacial development, it is utilized by oral health professionals, including dentists, orthodontists, and oral and maxillofacial surgeons, as a treatment planning tool. The earliest institutions that used cephalometric analysis in orthodontics were Hofrath in Germany and Broadbent in the USA in 1931[1,2,3]. The primary objective of the analysis was to provide a theoretical and practical resource to evaluate the craniofacial proportions of an individual and to define the anatomical source of malocclusion[1]. This allowed for the growth pattern of the maxilla and mandible to be tracked, their relational positions in space to be monitored, and changes in soft tissue and teeth displacement to be observed. As a result, changes brought about by orthodontic treatment could be monitored, and skeletal and dental relationships could be characterized for a diagnosis to be made for treatment planning. Evaluation of the dentofacial complex was done by comparing a patient's cephalometric tracing with reference values that were representative of a normal population of similar age, race, and ethnicity[1].

The traditional method of analysis consisted of a two-dimensional (2D) depiction of three-dimensional (3D) structures[4,5]. A major setback of this technique is the distortion and magnification of anatomical structures *via* conventional x-ray imaging on plain film or digital formats, which can lead to inaccurate cephalometric tracings and interpretations[6,7]. The initial introduction of 3D imaging in the form of axial computed tomography (CT) and spiral CT did not include dental or non-medical applications due to high cost and high radiation doses. However, the emergence of cone beam computed tomography (CBCT) scans mitigated these concerns, as expenses and radiation doses were significantly lower than CT[1]. The shift in this imaging narrative galvanized the widespread use of CBCT in orthodontics for improvement in diagnosis and treatment planning. The main advantage of 3D imaging over the conventional 2D image technique is that 3D allows the examiner to view anatomical structures without superimpositions and spatial distortions (i.e., head position of the individual). Therefore, a much more accurate positioning of the anatomical landmarks used for the conduction of cephalometric analysis is possible, especially in cases of facial asymmetry. Moreover, a much larger anatomical area can be analyzed.

One of the most recent advances in the field of cephalometry is the implementation of deep learning (DL) for automated landmark detection[8,9,10,11]. Although the results of these studies are promising, the levels of accuracy in the placement of the landmarks are not yet satisfactory. Moreover, most of these studies use relatively small landmark sets that are derived from previous 2D cephalometric analyses, providing insufficient coverage of the cranial base, which is an important structure for the study of craniofacial growth and development. This demonstration video showcases in detail a methodology for the conduction of manual, high-accuracy 3D cephalometric analysis with the use of a validated set of 3D skeletal tissue landmarks covering the areas of the face, cranial base, mandible, and teeth for use in clinical and research studies involving CBCT imaging[4]. An example of a completed 3D analysis can be seen in **Figure 1**.

Protocol

This protocol follows the guidelines of the human research ethics committees of the Institutional Review Boards of the National Institutes of Health (NIDCR IRB #16-D-0040) and Roseman University of Health Sciences. See the **Table of Materials** for details related to the software used in this protocol. The same protocol can be followed with the use of different software, after adjustments based on their specific settings and technical details. The CBCT scans used for creation of the figure included in this paper, as well as the video demonstration, have been anonymized prior to their use, and informed consent has been acquired from the subjects, allowing the use of their scans in research related publications. Both subjects were seen at the NIH Dental Clinic, where the scans were acquired (Planmeca ProMax 3D system; low dose mode, 400 µm resolution) and had been consented onto an NIH IRB-approved protocol (NCT02639312).

1. Uploading the CBCT scan and view in the 3DAnalysis module

1. Open the referenced software and click on **browse file**. Select the scan to be analyzed and click **open**.
2. Go to the **3DAnalysis module**.

2. Uploading a landmark configuration file

1. In the **3DAnalysis module**, click on the **Save information** floppy disk icon. Then, select **Load a configuration** and browse for the configuration file.
   NOTE: The configuration file including the landmarks used by the authors is included as **Supplementary File 1**.

3. Coordinate system setup

1. Click on the **Reorientation** icon.
2. In the window that opens, select the **By picking landmarks** option. This allows the user to orientate all the scans in the same way, which is important if their 3D coordinate values are to be compared. The options selected for this protocol are: **N** as the O**rigin Landmark**, **Three-point definition** with landmarks **Or R**, **Or L**, **Po L**, and **Define A-P Axis (Mid-Sagittal Plane)** with landmarks **N** and **Ba**.

4. CBCT scan image adjustments

1. Adjust the **Brightness** and **Contrast** to reduce image noise from the menu on the left side of the screen.
2. Zoom in and out by holding down the **Ctrl** key and left-clicking simultaneously and sliding across the screen. Move the image in a bodily manner by holding down the **Shift** key and left-clicking simultaneously and sliding across the screen. Enable **Clipping** from the **settings** menu to create sectional views in all planes of space.

5. Addition of new landmarks

1. From the **Settings** menu, which has a tool icon, click on **landmarks** to reveal a list of available landmark options, and then select the landmark of choice.
2. To set a default view for landmarks, select **tracing task**, click on **set up**, choose **landmark**, set the view as desired, and click on **use current view settings**. Repeat the above steps to change the default view for any additional landmark.

6. Annotation of 3D anatomical landmarks

1. From the top of the menu on the left of the screen, select **Create Tracing**. In the window that opens, click on **Start** in the left lower corner of the window. Start annotating the 3D landmarks by left-clicking directly on the 3D volume at the location where the landmark should be placed based on its definition.
2. Confirm and adjust the location of the landmark using the section views on the right of the screen. In case they cannot be seen, from the layout selection menu on the left, select **Slice locator**. To confirm the placement of a landmark, click **Stop** and select the desired view to visualize the landmark. Once confirmed, proceed to placing the remaining landmarks.
3. To change the position of the landmark with the use of the 3D volume, stop the analysis by clicking on **Stop** at the bottom of the **Tracing tasks** menu, click on the landmark point to be moved, and drag it to the new desired position.

4. To re-annotate a landmark, double-click on the ticked square next to the landmark and then answer **Yes** to the follow-up question.

7. Definition and specific annotation instructions for each 3D landmark

1. Basion (Ba)-the midpoint on the anterior border of the anterior curvature of the foramen magnum
    1. For the **axial section**, look for the deepest end of the curvature of the section of the foramen magnum. For the **sagittal section**, look for the most posterior point of the mid-section of the foramen magnum. For the **coronal section**, look for the inferior midpoint of the curvature of the foramen magnum.
2. Porion (Po_R, Po_L)-the most superior, posterior, and external point located at the upper margin of each ear canal (external auditory meatus)
    1. For the **axial section**, look for the edge of the margin of the external auditory meatus. For the **sagittal** section, look for the point of intersection of the eustachian tube with the bony canal. For the **coronal section**, look for the midpoint on the inferior border of the superior curvature. The vertical line through the point roughly bisects the ear canal.
3. Nasion (N)-the intersection of the suture between the frontal bone and the nasal bones (fronto-nasal suture)
    1. For the **axial section**, look for the midpoint/height of curvature of the suture. For the **sagittal section**, look for the anterior point of the suture where the frontal and nasal bones meet. For the **coronal section**, look for the center of the fronto-nasal suture. The vertical line through it roughly bisects the nose.
4. Orbitale (Or_R, Or_L)-the most antero-inferior point on the inferior orbital rim
    1. Set the frontal view (bony window) as default and clip axially from inferior to superior until the curvature of the lower margin of the orbit is reached to locate the inferior-most point of the lower curvature of the orbit.
    2. Use 2D views to confirm that the landmark is on the bone. Adjust the sagittal and coronal sections to reflect the anterior positioning of orbitale. Ensure that the landmark is anterior in position just to the point where the orbital rim starts to curve out.
5. Supraorbitale (SOr_R, SOr_L)-the most superior and anterior point of the superior orbital rim
    1. Set the frontal view as default in the 3D volume and gradually clip axially from superior to inferior until the curvature of the upper margin of the orbit is reached to locate the most superior point of the upper border of the orbit.

       NOTE: Avoid marking the supraorbital notch due to its variable anatomy.
    2. Adjust the sagittal and coronal sections to reflect the anterior positioning of the landmark. Ensure that the landmark is anterior in position just to the point where the orbital rim starts to curve out.
6. Sella midpoint (sella)
    1. Look for the center of the sella turcica or hypophyseal fossa, which is a saddle-shaped depression in the body of the sphenoid bone, where the pituitary gland or hypophysis is positioned. Adjust the landmark to the center of the sella turcica in all planes.

2. For the **sagittal section**, place the landmark in the center of the sella turcica. For the **axial** and **coronal sections**, adjust the views accordingly.

7. Sella inferior (Si)-the most inferior and central point on the contour of the sella turcica in the same plane as the sella
    1. For the **sagittal section**, start by positioning the landmark at the most inferior point of the sagittal section of the sella turcica. For the **axial** and **coronal sections**, adjust the position to be in the middle of the section.
8. Sella posterior (Sp)-the most posterior and central point on the contour of the sella turcica in the same plane as the sella
    1. For the **sagittal section**, start by positioning the landmark at the most posterior point of the sagittal section of the sella turcica. For the **axial** and **coronal sections**, adjust the position to be in the middle of the section.
9. Clinoid process (Cl)-the antero-superior point on the contour of the anterior clinoid process in the same plane as the sella
    1. For the **sagittal section**, start by positioning the landmark at the most antero-superior point of the contour of the clinoid process. For the **axial** and **coronal sections**, adjust the position to be in the middle of the section.
10. Zygomatic arch (ZygArch_R, ZygArch_L)
    1. Look for the most latero-inferior point on the outline of the zygomatic arch. Ensure that the skull is properly oriented and the zygomatic arches are seen clearly and "perpendicularly" from the submental view.
    NOTE: If the skull is tilted, accurate landmark annotation will be compromised.
    2. For the **axial section**, position the landmark at the most lateral and inferior point of the curvature of the zygomatic arch. For the **sagittal section**, position the landmark at the most inferior point of the section. For the **coronal section**, position the landmark at the most lateral point of the section.
11. Frontozygomatic suture (FronZyg_R, FronZyg_L)-the antero-lateral point on the frontozygomatic suture.
    1. For the **sagittal section**, ensure that the suture is clearly visible in the section. Position the landmark on the most anterior point of the section of the frontal bone next to the suture. For the **axial** and **coronal sections**, look for the most superior point of the section.
12. Nasal cavity (NasCav_R, NasCav_L)-the junction of the lateral wall of the nose, pyriform rim/nasal floor, and the upper border of the maxilla
    1. The triple junction is best viewed in the **coronal section**. Start by positioning the landmark at the mesial side of the junction. For the **axial section**, position the landmark at the endpoint of the section. For the **sagittal section**, position the landmark at the most lateral point of the suture on the maxillary border.
13. Jugal point (J_R, J_L)
    1. Look for the deepest midpoint of the jugal process of the maxilla. Annotate the landmark so that it is mostly in line with the maxillary first molar.

2. For the **coronal section**, start by adjusting the landmark position to the deepest end of the curvature of the section of the jugal process. For the **axial section**, look for the most lateral point of the section, at the point where the bone density changes. For the **sagittal section**, look for the most inferior point of the section, at the point where the bone density changes.

14. Articulare (Ar_R, Ar_L)-the most posterior point on the condylar head, closer to the glenoid fossa

 1. For the **axial section**, look for the most posterior point around the center of the posterior curvature of the condyle. For the **sagittal section**, look for the most posterior point on the posterior curvature of the condyle. For the **coronal section**, look for the small radiopaque area indicating that the landmark is on the bone.

15. Coronoid process (Cor_R, Cor_L)-the most superior point of the coronoid process

 1. For the **sagittal** and **coronal sections**, position the landmark on the top of the coronoid process. For the **axial section**, look for the small radiopaque area indicating that the landmark is on the bone.

16. Glenoid fossa (G_Fos_R, G_Fos_L)

 1. Look for a point on the glenoid fossa where the condylar head is in maximum articulation with the fossa. This is the approximate center of the dome shaped fossa.

 2. In **3D**, choose the **coronal section** to obtain a view that shows both the condyles reasonably well. Choose a point on the inferior border of the fossa in this view such that a vertical line through this point roughly bisects the condyle; note that this might not appear to be the exact center of the fossa. Target the point of maximum articulation (i.e., roughly close to the center or the exact center in some cases).

 3. For the **coronal section**, look for the most approximate point to the glenoid fossa dome section. For the **sagittal section**, look for the most superior point of the glenoid fossa dome section. For the **axial section**, do not apply any specific fine-tuning if the two other views have been adjusted.

17. Mandible profile (right and left): condylion (Co_R, Co_L), gonion (Go_R, Go_L), antegonion (Ag_R, Ag_L)
NOTE: The landmarks are annotated automatically after tracing the mandibular profile.

 1. Identify the **condylion** as the most posterior and superior point on the condyle. Identify the **gonion** as the most outward point on the angle formed by the junction of the ramus and the body of the mandible. Identify the **antegonion** as the highest point of the concavity of the lower border of the ramus where it joins the body of the mandible.

 2. Trace the mandibular profile with a series of points (double-click or right-click to finish tracing). Start from the mandibular notch and include the condylar and ramus profiles. Follow the curve at the angle to include the gonion by using multiple points every few millimeters. Be sure to choose points on the inferior or lateral margin of the mandibular border.

18. Prosthion (Pr)

 1. Look for the most anterior point of the maxillary alveolar process in the midline. Choose a sagittal section bisecting the maxillary central incisors. In

lateral view, select **Prosthion** and confirm the 2D views.

2. For the **axial section**, identify the middle of the root sections of the maxillary central incisors on the labial alveolar bone. For the **sagittal section**, look for the most anterior point of the maxillary alveolar process. For the **coronal section**, look for the midline between the maxillary central incisors.

19. A-point - the deepest midline point on the premaxilla of the curvature between the anterior nasal spine and the prosthion point

    1. In **3D**, choose a sagittal section bisecting the maxillary central incisors. This plane will have the prosthion (already marked). In **lateral view**, select **A-Point** and confirm the 2D views.

    2. For the **axial section**, identify the tip of the section. For the **sagittal section**, identify the deepest point of the curvature of the premaxilla between the anterior nasal spine and the alveolar process. For the **coronal section**, ensure that the point is in the midline.

20. Anterior nasalsSpine (ANS)

    1. Look for the most anterior point of the nasal spine. Choose a sagittal section bisecting the maxillary central incisors. This plane will have the prosthion and the A-point (already marked). In **lateral view**, select **ANS** and confirm the 2D views.

    2. For the **axial section**, look for the tip of the section. For the **sagittal section**, look for the most anterior point of the nasal spine. For the **coronal section**, look for the middle of the small bone section.

21. Posterior nasal spine (PNS)-the midpoint of the base of the palatine bones at the posterior margin of the hard palate

    1. In **3D**, choose a sagittal section bisecting the maxillary central incisors. This plane will have the prosthion, A-point, and ANS already marked. In **lateral view**, select **PNS** and confirm the 2D views.

    2. For the **axial section**, look for the most inferior point of the midline of the palatine base. For the **sagittal section**, look for the most posterior point of the mid-section of the palatine bone. For the **coronal section**, look for the midpoint of the section of the mid-section of the palatine bone.

22. Infradentale (Id)

    1. Identify the point of transition from the crown/tooth of the most prominent mandibular medial incisor to the alveolar projection.

    2. In **3D**, choose a sagittal section bisecting the mandibular central incisor. In **lateral view**, select **Id** and confirm the 2D views. If three incisors are present, ensure that the plane bisects the middle tooth.

    3. For the **axial section**, confirm that that the landmark annotation point is the most anterior point on the alveolar bone of the selected incisor. For the **sagittal section**, identify the most anterior point of the mandibular alveolar process. For the **coronal section**, ensure that the midline bisects the selected incisor.

23. B-point (B) - the deepest midline point on the mandible between infradentale and pogonion

1. In **3D**, choose a sagittal section bisecting the mandibular central incisors. In **lateral view**, select **B-Point** and confirm the 2D views.
2. For the **axial section**, look between the mandibular central incisors, or in the middle of the section of the middle incisor if an incisor is missing. For the **sagittal section**, identify the point of the deepest concavity anteriorly on the mandibular symphysis. For the **coronal section**, look between the mandibular central incisors or the vertical grid line intersecting the middle incisor.

24. Pogonion (Pog)
    1. Identify the most anterior point on the symphysis of the mandible.
    2. In **3D**, choose a sagittal section bisecting the mandibular central incisors. In **lateral view**, select **Pogonion** and confirm the 2D views.
    3. For the **axial** section, identify the vertical grid line intersecting the mandibular section. For the **sagittal section**, look for the most anterior point of the symphysis. For the **coronal section**, look for the small osseous area indicating that the landmark is placed on the bone surface.
25. Anatomic gnathion (Gn) - the lowest point on the anterior margin of the lower jaw in the mid-sagittal plane
    1. In **3D**, choose a sagittal section bisecting the mandibular central incisors. In **lateral view**, select **Gn** and confirm the 2D views.
    2. For the **axial** and **coronal sections**, look for the vertical grid line intersecting the mandibular section. For the **sagittal section**, identify the lowest point on the anterior margin of the lower jaw.
26. Menton (Me) - the lowest point of the mandibular symphysis.
    1. In **3D**, choose a sagittal section bisecting the mandibular central incisors. In **lateral view**, select **Me** and confirm the 2D views.
    2. For the **axial section**, identify the small osseous area indicating that the landmark is placed on the bone surface. For the **sagittal section**, identify the lowest point of the symphysis section. For the **coronal section**, look for the lowest midpoint of the symphysis section.
27. Upper/lower right/left incisor profile

    NOTE: Three points are required: the root of the upper/lower incisor; **Axial**: the midpoint of the section of the apex; **Sagittal, Coronal**: the tip of the apex.
    1. In the crown of the upper/lower incisor: in the **axial** and **coronal sections**, look for the midpoint of the incisal edge; and in the **sagittal section**, identify the tip of the incisal edge.
    2. In the labial point of the upper/lower incisor: in the **axial section**, identify the midpoint of the tooth section; in the **sagittal section**, look for the most prominent point of the labial surface; and in the **coronal section**, ensure that the point is in the vertical line bisecting the tooth.
28. Upper/lower right/left molar profile

    NOTE: The following three points are required.
    1. Root of the upper/lower molar: in the **axial section**, look for the midpoint of the section of the mesial root apex; and in the **sagittal** and **coronal sections**, identify the tip of the mesial root apex.

2. Anterior cusp of the upper/lower molar: in the **axial section**, identify the mesial-buccal cusp of the maxillary/mandibular first molar; in the **sagittal** and **coronal sections**, look for the mesial-buccal cusp of the maxillary/mandibular first molar.

3. Posterior cusp of the upper molar: in the **axial section**, look for the distal-buccal cusp of the maxillary/mandibular first molar; in the **sagittal** and **coronal sections**, identify the distal-buccal cusp of the maxillary/mandibular first molar.

29. Cribriform plate (Cr) - the middle superior point on the crista galli

    1. In the **sagittal section**, choose the most inferior point of the crista galli. In the **axial section**, look for the most inferior point of the small section of the crista galli. In the **coronal section**, choose the most superior point of the crista galli.

30. Foramen ovale (ForOval_R, ForOval_L) - the most antero-medial and superior point of the foramen ovale

    1. In the **axial section**, make sure that this point lies approximately on a plane bisecting the foramen in the antero-medial direction. In the **sagittal** and **coronal sections**, look at the bottom of the canal entrance.

31. Opisthion (Opi) - the midpoint on the posterior margin of the posterior curvature of the foramen magnum in the axial section

    1. In the **sagittal section**, look for the most inferior point of the sagittal section of the foramen magnum. In the **axial** and **coronal sections**, position the landmark in the midline.

32. Anterior cranial fossa (AntCF_R, AntCF_L) - the most anterior superior point on the border separating the anterior and middle cranial fossa

    1. In **3D**, use the axial section, going from anterior to posterior until the curvature of the fossa is reached. If more than one border is present, choose the most anterior border.

    2. In the **axial section**, identify the most anterior point of the section of the anterior cranial fossa. In the **sagittal section**, look for the most superior point of the border of the anterior cranial fossa. In the **coronal section**, identify the most inferior point of the section of the anterior cranial fossa.

33. Internal acoustic meatus (AcM_R, AcM_L) - the most posterior lateral point on the internal acoustic meatus on the petrous part of the temporal bone

    1. In the **axial section**, look for the point reflecting the beginning of the canal where the curvature ends. In the **sagittal section**, look for the posterior point on the curvature. In the **coronal section**, identify the deepest point on the curvature.

34. Hypoglossal canal (Hypog_R, Hypog_L)
    NOTE: This is the most antero-medial point of the canal. In case there are two canals, choose the posterior of the two canals and mark the point on the anterior border of the posterior canal.

    1. In the **axial section**, identify the point reflecting the beginning of the canal where the curvature ends. In the **sagittal section**, look for the deepest end of the curvature. In the **coronal section**, make sure that the point lies roughly on the axis bisecting the canal in the antero-medial direction.

8. Saving of the CBCT scan with annotated landmarks

1. From the **File** menu, select **Save** or **Save as** to save as a separate file and then opt for the preferred type of file.

9. Export measurements and/or landmark 3D coordinates

1. Click on the **Save Information/ floppy disk icon** from the toolbar and then select either **Export Measurements** or **Export Landmarks**. Export the results in .csv file format.

Representative Results

The annotation of a validated 3D landmark configuration is described in detail with the use of a step-by-step protocol and video demonstration. Specific instructions are provided for the annotation of each landmark on the 3D volume, as well as the refinement of their initial positions with the help of the 2D section views that correspond to each plane of space. By following the detailed methodology provided in the protocol in combination with the video instructions, the user can learn how to conduct cephalometric analysis with the use of human CBCT scans.

Figure 1 represents frontal and three-quarter views of a full head CBCT scan of a human skull with the annotated 3D landmarks included in the current configuration. All the described landmarks are Type 1 and Type 2. Type 1 landmarks represent clearly recognizable points usually observed at the intersection of distinct anatomical structures. Type 2 landmarks represent points of maximal curvature on the contour of recognizable anatomical structures[12]. No Type 3 or semi-landmarks were included in this analysis.

After the completion of the annotation of the landmarks, there are two types of data that can be exported and further analyzed by the user: cephalometric measurement and 3D coordinate values. The values of key cephalometric measurements required for the diagnosis and assessment of dentoskeletal malocclusion are provided. These measurements provide a detailed assessment of the skeletal and dental relationships in all three planes of space: sagittal, vertical, and transverse. The 3D coordinate values (x, y, z) of each landmark can be exported and used for the calculation of angles and linear distances. The values of the same coordinates can be used for the conduction of multivariate geometric morphometric analysis (GMA). GMA is a method for studying shape that can capture morphologically different shape variables utilizing Cartesian landmark and/or semi-landmark coordinates. Several statistical techniques can be used to examine shape, without taking into account the size, location, or orientation of the examined structures. Geometric morphometrics is currently the most established body of morphometric theory for handling landmark-based data.

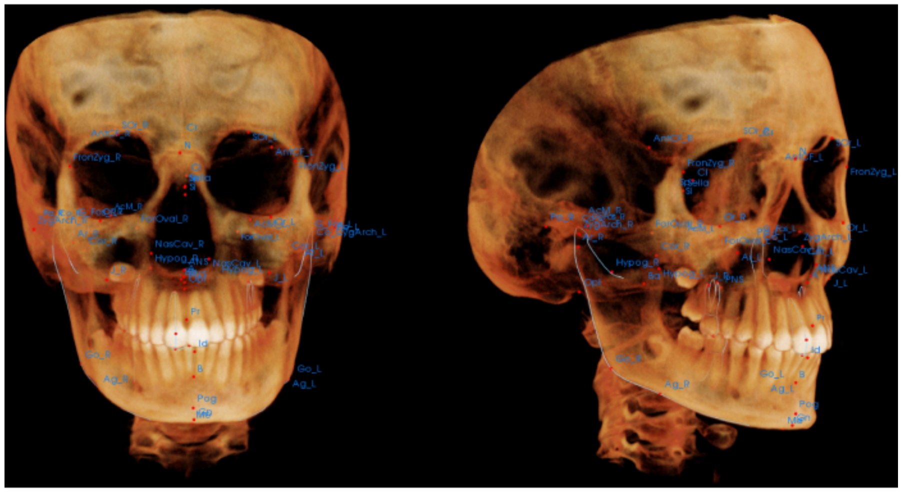

Figure 1: Frontal and three-quarter views of a full head CBCT scan of a human skull with the annotated 3D landmarks included in the current configuration. Please click here to view a larger version of this figure.

Supplementary File 1: Configuration file including the landmarks used in this protocolwhich can be directly uploaded to the software for analysis. Please click here to download this File.

Discussion

Medicine and dentistry have already entered the 3D imaging era. In the disciplines of craniofacial and dental imaging, CBCT scans are increasingly being used, due to the low radiation and decreased cost of the updated systems in comparison to traditional CT machines, easy personnel usage calibration, relatively quick and easy acquisition with minimal patient cooperation, as well as the ability to generate multiple other diagnostic images and analyses from one single scan. Therefore, it is essential for clinicians and researchers to know how to read, diagnose, and analyze these 3D images, as well as learn how to study craniofacial growth and development in 3D.

To assist clinicians and researchers in this field, we present a step-by-step protocol and video demonstration for the conduction of 3D cephalometric analysis with the use of human CBCT scans. These landmarks have been previously defined and validated in a previous publication, where their accuracy and repeatability were confirmed[4]. The detailed refinement instructions for each landmark also assist users in the correct annotation of each landmark. The landmark annotation process is further simplified with the use of preset views of the scan that correspond to the area that each landmark should be positioned. This function saves significant time and effort for the user. Nevertheless, there is a learning curve involved, and practice is required by users to achieve accurate landmark annotation.

The validated 3D landmark configuration used in this protocol provides sufficient coverage of the skeletal tissue of the face, maxilla, mandible, and cranial base. In this way, the true morphology of the craniofacial structures is more accurately represented for evaluation of the dimensions, configuration, and orientation of the craniofacial complex and its component structures. Soft tissue landmarks are not included in this protocol, but users can add landmarks of choice to the provided configuration, as described in the protocol. In addition, for practical reasons, this protocol could not include specific instructions for other 3D analysis software, but can be adapted accordingly by each user.

Apart from the diagnostic value of the generated standard cephalometric measurements, mainly for clinicians, the freedom offered with the use of this analysis to compute angles and linear distances between any 3D landmarks will allow the establishment of new cephalometric analyses that will provide more detailed and complete assessments. Nevertheless, our future direction includes establishing new respective normative values, in the same way that 2D normative values were created in the past.

Moreover, the applications of landmarked based GMA in the craniofacial clinical and research field are developing at a fast pace. Researchers in evolutionary and developmental biology and anthropology for have been using this analysis for more than a decade, but new clinical applications have also been recently presented in the fields of orthodontics, dentofacial orthopedics, and craniofacial surgery. GMA can be also used as part of a quantitative phenotyping in the case of congenital diseases with craniofacial manifestations, as well as for the detection of subtle morphological differences attributed to gene mutations[13,14,15,16]. In addition, the integration of different quantitative approaches by linking morphometric data with functional analysis as well as genetic data can provide new knowledge regarding craniofacial development in healthy, as well as disease, groups.

Because of recent advances in computation and visualization, the conduction of this type of analysis is now feasible on personal computers, with several software packages already available, including Checkpoint, Geomorph (a package of R statistical software), Amira-Avizo, and SlicerMorph. These programs can assist researchers in the medical fields that may be unfamiliar with multivariate statistical analyses to conduct GMA with the availability of built-in automated functions.

Disclosures

The authors declare no conflicts of interest.

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