TECHNICAL REPORT

Three-dimensional reconstruction of a fibro-osseous lesion using binary images transformed from histopathological images

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The aim of this report was to introduce a new method of three-dimensional (3D) reconstruction for fibro-osseous lesions (FOLs) using binary images transformed from histopathological images and to describe its usefulness.

A sample of multiconfluent FOL was used (one of the five types of FOL according to a radiographic classification) which was diagnosed histopathologically as ossifying fibroma. Approximately 30 pathological images were assembled into a composite image of the slide using Tiling Boutique software version 3 for Windows (Sanyo Electric, Osaka, Japan). The tiling images were transformed into 8-bit scale images and then into binary images using ImageJ software ver.1.37 (National Institutes of Health, Bethesda, MD). These images were used for 3D reconstruction using ImageJ software. Images were loaded at the same matrix size and were reconstructed into layers of two-dimensional image stacks, adjusted so that contiguous images were aligned based on their centre points, and arranged with long axes horizontal.

3D findings aided the visual understanding of morphological features in the lesion. The 3D reconstruction can be displayed with arbitrary rotation. In this case, the 3D reconstruction, using Real Image software version 4.01 for Windows (KGT, Tokyo, Japan), was created from an arbitrary section. This allowed us to determine the pattern of calcification between groups of connected osteoids and to compare the internal structure of such lesions that are not visible on histopathological findings. Differentiation of features was even more pronounced with a two colour display indicating fibrous connective tissue and osteoid tissue.

A 3D reconstruction of a multiconfluent ossifying fibroma was created using binary images transformed from histopathological images. The quality of the images depends above all on the functionality of the image-processing software. Comparison of each pattern of FOL might allow more simple assessment of the morphological features of FOLs.

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Introduction

For the past 10 years, the authors have investigated the most useful method of differential diagnosis for fibro-osseous lesions (FOLs) of the jaws. In 2003, FOLs were classified into five radiographic patterns.¹ Research has

since focused on using binary images reconstructed from histopathological images to determine the quantity of osteoid tissue within FOLs² and the difference in morphological features such as bone complexity and distribution;³ however, the results have so far been inconclusive.

Consequently, a different approach to morphological features in FOLs has been used where the newly developed technique of computer generated

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three-dimensional (3D) reconstruction to evaluate the shape and quantity of osteoid tissue within the lesions was applied. 3D images are remarkably informative in many areas of clinical practice, although their benefits in FOLs are debatable. Radiographic images of FOLs are complex because of the mixture of fibrous connective tissue and osteoid tissue. Therefore, it is hypothesized that 3D findings could help identify morphological features in these lesions.

Viewing images on a screen in a stereoscopic form has several advantages. Such 3D reconstructions exhibit nicely the connections between every section of two-dimensional (2D) images. In other words, this 3D volume rendering can demonstrate configuration and volume in an arbitrarily specified section, and it is possible to differentiate bone from fibrous connective tissue stereoscopically.

In this study, the aim is to confirm bone configuration that is unobtainable from 2D displays and to generate precise images from various angles. It was attempted, initially, to create a 3D reconstruction using histopathological images from serial sections taken from an ossifying fibroma. The present study describes the methods of 3D reconstruction using binary images transformed from histopathological images and assesses the usefulness of this method.

Materials and methods

The images used were of an ossifying fibroma classified radiographically as multiconfluent (Figure 1) according to a method the authors reported in 2003¹ as an improvement on Eversole's 1984 classification.⁴ The new classification consists of focal, target, radiolucent, calcified and multiconfluent patterns. For this study, a multiconfluent pattern ossifying fibroma from a 63-year-old Japanese woman was selected because it appeared to contain moderate amounts of both osteoid

Figure 1 Intraoral radiography showed a mottled radiopacity, consistent with a multiconfluent ossifying fibroma

tissue and fibrous connective tissue. Figure 2 shows a schematic diagram of the 3D volume rendering process.

Preparation of histopathological images

The excised specimen was fixed with 10% buffered formalin and decalcified with K-CX solution (Falma, Tokyo, Japan). The decalcified specimen was cut in half perpendicular to the long axis, embedded in paraffin and serial sectioned at 10 μ m thickness. Sections were mounted on slides and stained using haematoxylin and

Figure 2 The process of producing three-dimensional (3D) images

eosin. Photomicrographs were taken under $\times 20$ magnification at 5-slide intervals. In this case, one half was used for another investigation and, therefore, only half of the surgically resected tissue was available. The microscope system comprised the following: an AX-80 microscope (Olympus, Tokyo, Japan); an HC-2500 CCD camera (Fuji Film, Tokyo, Japan); and Photograb-2500 version 1.1 software (Fuji Film). The resolution of the CCD camera was 1.4 million pixels. Approximately 20–45 photomicrographs were assembled into a composite image of the slide using Tiling Boutique software version 3 for Windows (Sanyo Electric, Osaka, Japan). Assembled images were saved in tagged image file format (tiff).

Transformation to binary images

The tiled images of the multiconfluent ossifying fibroma (Figure 3) were transformed into 8-bit scale images and then into binary images (Figure 4) using ImageJ software version 1.37 (National Institutes of Health, Bethesda, MD). When histopathological images are modified to create binary images, the threshold between osteoid and bone is determined carefully and often differs among sections because of the degree of bone tissue staining and the duration of decalcification. In this study, therefore, the difference between bone tissue and fibrous connective tissue was determined and a threshold was selected for bone tissue equivalent, between 160 and 256 grey scale for each case. Moreover, as it was difficult to manage small scattered areas of osteoid tissue of less than 100 pixels, such areas were excluded from analysis.

Figure 3 A tiled image in the central area of the multiconfluent lesion shows histopathological features (haematoxylin and eosin, magnification $\times 20$)

Figure 4 A binary image reconstructed on the basis of a visually determined threshold between bone tissue and fibrous connective tissue

Method of 3D reconstruction

After transformation of the composite pathological image into 8-bit scale images and binary images, the resulting image of the ossifying fibroma consisted of 30 serial section images. These images were used in 3D reconstruction with ImageJ software. Data files were loaded in the same order as for the binary images and 8-bit scale images. Images were loaded at the same matrix size and were reconstructed into layers of 2D image stacks, adjusted so that contiguous images were aligned based on their centre points and arranged with long axes horizontal to prevent misalignment of the composite image. Next, stacks were reconstructed by rotation and the resulting 3D reconstruction was displayed on the screen according to the projection (Figure 5).

Colour display and measurement in the 3D reconstruction

Display of the image in two colours to represent fibrous connective tissue and osteoid tissue further delineated morphological features. Figure 6 shows a 3D reconstruction using dedicated Real Image software version 4.01 for Windows (KGT, Tokyo, Japan). Figure 7 shows an arbitrarily specified section and its 3D reconstruction. These were reconstructed from digital imaging and communications in medicine (DICOM) data, which were based on bit-map data.

Results

Figure 5 shows the 3D image reconstructed from the multiconfluent pattern ossifying fibroma. This image gives only a rough outline of morphological features, possibly due to insufficient functionality of the software. However, built-in functions allowed easy measurement of variables such as area, volume, super-

Figure 5 A three-dimensional reconstruction as it appears on the screen. (a) Lateral view. (b) Aerial view

ficial area and perimeter. The 3D reconstruction was of a cross section of the resected lesion (Figure 5). Figure 6 shows a two colour display of the 3D reconstruction, with fibrous connective tissue and osteoid tissue. Figure 7 shows the array structure of fibrous connective tissue and osteoid tissue as detected from the arbitrary section. The observation of resected tissue sample from the reconstruction is useful to demonstrate internal feature complexity linked to bone formation in each section.

Figure 6 Two colour display of the 3D reconstruction: fibrous connective tissue and osteoid tissue

Discussion

Recently, 3D visualizations of anatomical structures have been used in the medical field for improved diagnostic accuracy and specialized medical treatment, and to improve the safety of various procedures.^{5–9} This has rapidly led to improved outcomes for patients.

This study has investigated the differential diagnosis of FOL in the jaws, which is often difficult because the internal structure of these lesions changes over time.^{4,10–12} In the dentomaxillofacial region, the diagnosis of bone sclerosis of the jaws can require rotational panoramic or periapical radiography. When it is unclear if the lesion is a tumour CT can be a straightforward method of observing the internal morphology, and cone beam CT is becoming increasingly popular in this regard. Hence, radiographic methods are selected according to the purpose. FOLs are often thought to have the same appearance as benign periapical tumours,^{10,13–16} but these lesions can differ to some extent. The quantity of osteoid tissue formation in a FOL is always linked to its radiodensity. However, the configuration, distribution and relationship between bone formations are not clear from radiographs; hence, another visualization method is required.

In the present study, ImageJ software allowed data to be easily represented in 3D form; however, the reliability of this method is strongly dependent on the accuracy of the 3D structure and of the transformation to binary images. Image stacks are used to create the 3D structure, and it is often difficult to match the margins of stacked images with those of images from the next section. For this reason, it is useful to mark the paraffin-embedded material with ink in advance. The marking position is selected from the centre of the slide

Figure 7 An arbitrarily specified section (a) and its three-dimensional reconstruction (b). The images show the differences in bone formation in the arbitrary section

and two or three other positions are marked before the paraffin-embedded sections are made. With regard to transformation to binary images, the authors believe that it is important to strictly differentiate bone tissue from fibrous connective tissue. However, this can be difficult, as bone tissues can resemble mixed mature connective tissue and osteoid tissue. In particular, the combination of osteoid tissue and immature osteoid tissue (a transition phase in the formation of mature bone) can be difficult to differentiate from mature bone. Therefore, selection of a threshold is important because of the degree of staining in osteoid tissue. So it is recommended that manual adjustment be used to decide the threshold, while automatic adjustment is useful and accurate in terms of reconstructing 2D image stacks into layers and in adjusting so that contiguous images are aligned based on their centre points.

The 3D display of morphological features in FOLs can be observed through an image generated in two colours to represent fibrous connective tissue and osteoid tissue (Figure 6). This method of generating binary images from histopathological images provides additional morphological information about these complex conditions. The 3D reconstruction allows the visualization of morphological elements that cannot be seen in other ways. 3D reconstruction may demonstrate that two radiopaque masses that appear similar on imaging have a different pattern in terms of distribution or amount of osteoid and bone. Therefore, the authors believe that this technique is useful in the analysis of FOLs.

The use of 3D reconstructions has increased markedly in many medical fields.^{17–30} The method of displaying imaging data is useful in observing areas of the body previously not easily visualized. This has increased competition among manufacturers of CT

volume rendering software, leading to recent technological advances.^{5,6,8,9} In the dental region, the development of cone beam CT has already demonstrated several advantages over conventional CT, with the exception of reconstruction of 3D images.^{20,27,31–34}

The literature contains few descriptions of transforming binary images from histopathological images and the present method is, therefore, informative. However, several issues need to be addressed in the future. Reconstruction of an arbitrary image layer as a 3D image was attempted in this study and these images spearhead the rise of the simplified method for manifestation of bone formations in lesions. However, it was found that the quality of an image made from these data only was poor compared with that shown at dissection. Reconstructing images from sections taken at 10 μm intervals might demonstrate better serial changes in osteoid tissue. Furthermore, more advanced functional programs or 3D-dedicated software can display very detailed 3D reconstructions from arbitrarily selected sections with any degree of rotation, deformation or specification. Nonetheless, this study suggested that 3D reconstruction could be useful in stereological analysis, observation of calcified structures and explaining pathology to patients.

In conclusion, it is believed that the present method of 3D reconstruction using binary images transformed from histopathological images was useful. If serial sections are made of the whole lesion, 3D reconstruction would appear to have a role in observation of internal structure and focal features.

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