# Imaging skeletal growth and evolution

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**Abstract**

This chapter reviews the application of medical imaging and associated techniques to the study of primate skeletal ontogeny and phylogeny. Following a short discussion of plain film radiography and pluridirectional tomography, the principles and practical use of...
computed tomography (CT), micro-CT, magnetic resonance imaging (MRI) and high-resolution MRI are considered in more detail. Aspects discussed include, among others, the specific problems encountered when CT scanning highly mineralised fossils, and ways to avoid common image artefacts in MRI. The second half of the chapter gives an overview of techniques of three-dimensional reconstruction based on CT and MRI, quantitative analysis of images and some practical considerations of handling digital images in a personal computer environment. A glossary explains a range of common terms in medical imaging.

INTRODUCTION

The potential of radiography in the study of vertebrate fossils began to be explored within a year after the discovery of X-rays in November 1895 (Brühl, 1896). The reason for its immediate appeal to palaeontologists is obvious; for the first time it was possible to assess the internal morphology of rare and valuable specimens in a non-destructive way. In the same period the first radiographs of human fetuses and infants, visualising normal and abnormal skeletal growth, were published (see e.g. Mould, 1993, for a review of the early history of radiography). Up to then growth and development could only be studied through dissections or serial sections of specimens with different ages of death. Radiography, being non-destructive and far less laborious than these methods, allowed for large samples to be examined, thus creating the possibility of a more quantitative approach. Also, postnatal growth could now be studied in living subjects, either in cross-sectional studies, simultaneously examining different age groups, or in longitudinal studies, following skeletal growth of individuals over time (see Hunter et al., 1993, for a review of longitudinal human growth series). More recently the development of computed tomography (CT) and magnetic resonance imaging (MRI), in combination with increasingly sophisticated computer graphics applications, has provided a range of new opportunities to qualitatively and quantitatively study soft tissue and bony structures in a much more comprehensive way than was previously possible on the basis of radiographs.

By giving an overview of various imaging techniques in relation to the study of skeletal growth and evolution, this chapter primarily aims to be an introductory text for those researchers who wish to apply digital imaging techniques, but do not have a background in either radiology or medical physics. It thus follows in the footsteps of earlier reviews with a similar scope, such as Jungers & Minns (1979), Tate & Cann (1982), Ruff & Leo (1986) and Vannier & Conroy (1989). The focus is on the use of CT and MRI, with an emphasis on basic concepts and practical considerations, rather than on the underlying technology or on providing a comprehensive review of past imaging-based research. Specific terminology, indicated in bold in the text, is explained in a glossary (Appendix).

PLAIN FILM RADIOGRAPHY

In conventional or plain-film radiography, an object is placed between an X-ray source and X-ray-sensitive film. The image of the object thus formed represents the distribution and degree of X-ray attenuation in the path through the object. A consequence is that all structures in the path of the X-ray beam appear superimposed in the image and cannot be distinguished from each other (Fig. 1a). Radiographs, therefore, provide only limited
Figure 1  CT examination of the *Homo ergaster* cranium KNM-WT 15.000. (a) Lateral topogram; (b) parasagittal CT scan at the level of the right dental row and inner ear. Unlike the radiograph-like topogram, the CT scan has the ability to distinguish between fossil bone and the sedimentary matrix in the maxillary sinus (⋆), and to resolve details such as the root canals of the molars (arrow head), and structures of the bony labyrinth (arrow). Scale bar = 10 mm.
information of more complex three-dimensional objects. Moreover, in the case of fossils any morphological information is blocked out by the presence of sedimentary matrix of a higher density (X-ray opacity or attenuation) than the fossil itself (see examples in Wind & Zonneveld, 1985). X-rays emerge as a diverging conical beam from the source, the focal spot on the anode of the X-ray tube, and the radiographic projection will therefore tend to show a variable degree of distortion. This effect can be minimised by maximising the source–object distance relative to the object–film distance, and by using collimators which let through parallel X-rays only. The choice of film and of added fluorescent screens, which indirectly expose the film, can be important as they influence the contrast resolution and spatial resolution, but a discussion of these aspects is beyond the scope of this chapter. Compared with medical CT and MRI, radiography has the advantage of a higher spatial resolution, and it is inexpensive and easy to use, but it has the disadvantage of a lower contrast resolution.

Given its limitations, conventional radiography is most informative and comprehensive, both qualitatively and quantitatively, when applied to morphology with relatively simple shapes, such as the dentition (e.g. Skinner & Sperber, 1982; Dean et al., 1986; Wood et al., 1988) and postcraniual bones (e.g. Ruff, 1989; Runestad et al., 1993; Macchiarelli et al., 1999). Moreover, lateral radiographs have traditionally taken a central role in studies comparing cranial shape and growth of humans and other primates (e.g. Angst, 1967; Levihn, 1967; Swindler et al., 1973; Dmoch, 1975, 1976; Cramer, 1977; George, 1978; Siriani & Van Ness, 1978; Siriani & Newell-Morris, 1980; Lestrel & Roche, 1986; Ravosa, 1988; Lestrel et al., 1993; Ross & Ravosa, 1993; Lieberman, 1998; Lieberman & McCarthy, 1999; Spoor et al., 1999). They show ontogenetically and phylogenetically important aspects of cranial form, such as basicranial flexion and the relationship between the neurocranium and the facial complex, including the palate and the orbits. A drawback, inherent to the superimposition of structures in radiographs, is that the focus on cranial morphology projected onto a single sagittal plane tends to portray growth and evolutionary change as two-dimensional rather than three-dimensional processes. Some studies, however, have used radiographs with an axial projection to consider morphological change in the transverse plane (Bossy et al., 1965; Putz, 1974; Dean & Wood, 1981; Bach-Petersen et al., 1994).

Pluridirectional tomography is a special form of radiography that was invented in the 1930s in an attempt to solve the problems associated with the superimposition of morphology. In this technique both the X-ray source and the film are moved in opposite directions during exposure, which results in blurring of all details except in one focal plane. Outside clinical practice a few studies have used this technique to investigate fossils (e.g. Fenart & Empereur-Buisson, 1970; Price & Molleson, 1974; Hotton et al., 1976; Wind & Zonneveld, 1985). Furthermore, tomography is the key technique of the so-called vestibular method, in which cranial morphology in lateral projection is compared using a reference plane defined by the lateral semicircular canals of the inner ear (see Fenart & Pellerin, 1988, for a review of this method and its applications). The vestibular method has been used for both interspecific and growth studies (e.g. Fenart & Debloc, 1973; Cousin et al., 1981).

COMPUTED TOMOGRAPHY

Since its development in the 1970s (Hounsfield, 1973), computed tomography (CT) has taken over from conventional radiography and pluridirectional tomography as the imaging method of choice when investigating complex skeletal morphology whether in the context
of palaeoanthropology or human craniofacial growth studies dealing with, for example, congenital craniofacial deformities. In medical CT scanners an X-ray source and an array of detectors rotate about the specimen and measure its attenuation within the confines of a slice-shaped volume in a great number of directions using a fan beam. By repositioning the table with the specimen, the plane in which measurements are taken can be changed. Digital cross-sectional images, which map the different degrees of attenuation (expressed as attenuation coefficients) in the slice, are calculated from the measurements and are shown on a computer monitor using a grey scale with black representing the lowest and white the highest density (see Newton and Potts, 1981; Swindell & Webb, 1992, for reviews of the principles of CT).

Prior to making the cross-sectional scans, the CT scanner is normally used to obtain one or more radiograph-like reference images, as a way of indentifying and documenting where the scans are to be made. These so-called scout scans, topograms or scanograms are prepared by keeping the X-ray source and the detectors stationary, and dragging the specimen through the fan-beam by moving the table (Fig. 1a).

In spiral or helical CT, a variant introduced in 1989, the X-ray source and detectors continuously circle the specimen while the table is translated simultaneously. Consequently, the attenuation measurements are taken in a spiral trajectory, rather than as individual slices at fixed table positions. Cross-sectional images can be reconstructed at any given position by means of interpolating these spiral measurements. An important advantage in clinical practice is the short scan time, which avoids motion artefacts and is needed in vascular studies using intravenous contrast media. However, spiral CT is not advantageous when scanning scientific specimens, and the interpolation process necessary to reconstruct planar images from spiral data results in a reduction in image quality (Wilting & Zonneveld, 1997; Wilting & Timmer, 1999).

By producing cross-sectional images CT overcomes the problems caused by the superimposition of structures in conventional radiographs, and thus provides detailed anatomical information without interference from structures lying on either side of the plane of interest (compare Fig. 1a and b). Moreover, there is no parallax distortion because the density of the object is measured in multiple directions. Whereas the spatial resolution of CT is not as good as that of conventional radiography, it has a better contrast resolution, so that it can resolve small density differences such as between fossilised bone and attached rock matrix or white and grey brain matter.

Applications

Given its properties, CT is ideal to examine fossils, and has been applied in numerous palaeoanthropological studies to assess, among others, midline cranial architecture, the paranasal sinuses, the middle and inner ear, the brain cavity, the structure of the cranial vault, the dentition, cortical bone geometry of long bones and the mandible, as well as any structure or surface hidden by attached matrix (e.g. Jungers and Minns, 1979; Tate & Cann, 1982; Ward et al., 1982; Maier & Nkini, 1984; Wind, 1984; Senut, 1985; Zonneveld & Wind, 1985; Ruff & Leo, 1986; Conroy & Vannier, 1987; Conroy, 1988; Hublin, 1989; Zonneveld et al., 1989b; Conroy et al., 1990, 1995; Demes et al., 1990; Conroy & Vannier, 1991a,b; Daegling & Grine, 1991; Macho & Thackeray, 1992; Montgomery et al., 1994; Spoor et al., 1994; Bromage et al., 1995; Ross & Henneberg, 1995; Schwartz & Conroy, 1996; Hublin et al., 1996; Spoor, 1997; Spoor et al., 1998a). Some of these structures are shown in the parasagittal scan in Fig. 1b.
CT scans have also been used in a limited number of studies dealing with fetal growth of the human cranium and brain (Virapongse et al., 1985; Sick & Veillon, 1988; Imanishi et al., 1988; Dimitriadis et al., 1995). In all of these studies individual two-dimensional CT images were analysed, but increasingly a stack of contiguous CT scans covering all or part of a specimen is used as the basis for three-dimensional reconstructions, an application that will be discussed separately.

Some technical background

When dealing with CT in morphological studies, an understanding of a number of basic concepts is important to appreciate the possibilities and limitations of the technique. Here ‘CT scan’ is strictly used to refer to one slice or image, but sometimes this term is also used to indicate a CT examination or a series of images (as in ‘to do a CT scan of a specimen’). Like any digital image, a CT scan is composed of an array of a limited number of image (‘picture’) elements or pixels (Fig. 2). As each slice has a given thickness, each pixel actually represents a volume element or voxel (Fig. 2). Each pixel is associated with one CT number which is a measure of the average density, the attenuation coefficient, in the voxel. Medical scanners produce images with a fixed matrix of pixels, and the pixel size therefore depends on the field of view (FOV), the area covered by the image. For example, a typical CT scan with a matrix size of \(512 \times 512\) pixels and a FOV of \(240 \times 240\) mm has a pixel size of \(0.47\) mm. The thickness of the slices can be selected, but generally has a minimum of between \(1.0\) and \(2.0\) mm.

Figure 2  Diagram of a CT or MR scan with a given slice thickness, demonstrating the concept of the two-dimensional picture elements, or ‘pixels’, and their associated volume elements, or ‘voxels’ (after Zonneveld, 1987).
The best possible spatial resolution in the plane of the scan that can be obtained with current medical CT scanners is about 0.3–0.5 mm. This resolution, mainly determined by the X-ray beam geometry, can only be achieved when it is not limited by the pixel size of the image. This is the case when the pixel size is half the spatial resolution or smaller (Blumenfeld & Glover, 1981). Perpendicular to the scan plane, the spatial resolution is significantly poorer as it is limited by the minimum slice thickness of 1.0–1.5 mm. The ability to visualise small structures can, however, be improved by making overlapping rather than contiguous scans.

The attenuation coefficients, calculated for each voxel from the detector measurements, are expressed on a CT number scale of typically 4096 Hounsfield units (H). This scale is defined by a value of –1000 H for air, and 0 H for water, with very dense tissue, such as dental enamel, closer to the maximum value of 3095 H. However, when viewing the image this number of possible density levels is too high to be discriminated by the human eye if each level is shown as a slightly different shade of grey. Shown on a computer monitor the 4096 unit (12 bit) scale is therefore converted into a grey scale with a maximum of 256 (8 bit) levels using a window technique. When tissues with widely different densities are to be shown, for example bone or teeth as well as soft-tissue or air, a large window width is chosen so that the full extent of the CT scale is represented in shades of grey. On the other hand, when tissues with little density difference are to be distinguished, for example grey and white matter of the brain, muscle and fat or fossil bone and attached rock matrix, a small window width has to be selected together with an appropriate window level (centre) so that all grey levels are employed to bring out maximally the contrast. Tissues with CT numbers outside the window range are simply represented as white and black. When examining a CT scan the viewer can interactively adapt the window setting by changing the width and level. Changing the window setting of a CT image influences the apparent size of structures in the image, and familiarity with this effect is therefore crucial for anybody using windowed images in morphological research (see the section on quantitative use below, and Spoor et al., 1993 for more details).

CT scans can be archived in three different ways. Most commonly, the CT image is stored as a computer file describing the CT numbers of each pixel (see the section Working with Images below). Occasionally, the raw data, the processed detector measurements before image reconstruction, are kept in addition to these image data. This allows for future reconstruction of additional images from the same scan data, for example using a different field of view, different convolution filters, or an alternative CT number scale. A main reason not to keep the raw data other than in special circumstances is that they take about six times as much digital archive space than image data. The third way of archiving scans is by saving or making photographic prints (hardcopies) of images as shown on the screen. However, as a consequence of the window technique, such images represent only part of the full digital description and much information is lost. Hence, on its own this latter form of archiving is not advisable for scientific purposes.

Practical aspects

In practice, a few basic points can help to obtain the best possible results when using CT to investigate the morphology of soft tissue, skeletal or fossil specimens.

Scan plane

Selection of the best plane in which the scans are to be made depends on the purpose of the CT examination, and deserves serious consideration. In the case of imaging a specimen for
general study, for example as the basis for three-dimensional reconstructions, it is usually best to orient the specimen in such a way that the smallest possible field of view can be selected, because a reduced pixel size will, up to a certain point, provide a better resolution (see below). On the other hand, when assessing specific and detailed structures, scans should take advantage of the significantly better spatial resolution in the scan plane than between the slices. For example, to investigate the developing dentition sagittal or coronal scans, parallel with the direction of eruption and perpendicular to the enamel caps, are more appropriate than transverse ones. However, when the root morphology is of special interest transverse scans may be more informative. For many studies scans will have to be made in more than one plane.

**Gantry orientation**
When the scan plane has to be adjusted it is advisable to rotate the specimen accordingly rather than to tilt the gantry. In a series of scans made with a tilted gantry the morphology systematically shifts in location from scan to scan. This is confusing when examining the stack of slices and results in skewed three-dimensional reconstructions in cases where the software cannot cope with tilted scans.

**Slice thickness**
Using the smallest available slice thickness minimises partial volume averaging and the best possible spatial resolution perpendicular to the scan plane is obtained.

**Slice index or interval**
Scans are usually made contiguously (the slice index equals the slice thickness), or overlapping when focusing on detailed morphology (a slice index is half or less of the slice thickness). A situation in which the use of spiral CT could be advantageous, is when overlapping scans are required but a slice increment smaller than the slice thickness is not available in the non-spiral mode of the scanner.

**Field of view**
When studying detailed morphology it is advisable to make zoom reconstructions to reduce the pixel size to half the spatial resolution or less. This way the pixel size does not limit the resolution in the image. For example, overview scans of a human cranium with a matrix size of $512 \times 512$ mm and a field of view of over $200 \times 200$ mm will not show the best-possible spatial resolution of the scanner of, say, 0.4 mm. To achieve this, zoom reconstructions with a field of view of $102 \times 102$ mm or less have to be made of the specific areas of interest. Reducing the pixel size via zoom reconstructions should not be confused with magnification of an existing image.

**Kernel**
It is best to use a neutral convolution filter or kernel for the reconstruction of the images, as these give the most truthful representation of the boundaries of structures. They are known, for example as Ramp or Shepp-Logan filters, and are those used for scanning the abdomen. Edge-enhancement filters, which may be recommended for skeletal applications in clinical practice, provide images which appear crisper, but the artificial enhancement of interface contrast results in inaccuracies when analysed quantitatively (Spoor, 1993; Spoor et al., 1993). Edge-enhancement filters are also referred to as bone or head-and-neck kernels. Smoothing filters have the opposite effect of edge enhancement, and should not be used either.
Archiving
Images are best saved in digital form, for example on optical disk, and not just as hard copies (photographic prints). Saving images with data compression should be avoided as this may give problems when they are to be read with software other than that of the scanner company. Moreover, data compression via a reduction in matrix size, for example from $512 \times 512$ pixels to $256 \times 256$ pixels, or a reduction of the pixel depth, for example from $4096$ (12 bit) to $256$ (8 bit) possible CT numbers, will result in a loss of spatial and contrast resolution, respectively. Ideally the images saved in the scanner format are converted into a universal format such as DICOM. Saving the raw data is necessary when additional image reconstructions are required in the future.

Scanning fossils
CT scans of highly mineralised and/or matrix-filled fossils made with medical scanners may show a reduced image quality. The main reasons are that the density or overall mass of the fossil may be outside the normal range found in patients for which the scanner is designed. The following three phenomena are most commonly encountered (see Zonneveld & Wind, 1985; Zonneveld et al., 1989b; Spoor & Zonneveld, 1994 for previous discussions of practical problems with scanning fossils).

Overflow of the CT number scale
Both fossilised bone and the surrounding sedimentary matrix may contain minerals with a density greater than the range covered by the standard CT number range of a scanner. Pixels with attenuation coefficients that exceed the scale’s maximum are assigned the highest possible CT number. In images this shows up as overflow artefacts, i.e. homogeneous white areas in which no detail is visible. It should be noted that in older scanners such areas sometimes showed up as black because after reaching its maximum the CT scale would drop to the lowest value ($-1000$ H = black) and then rise again. Apart from masking any detail in the affected area, overflow artefacts tend to deform the outline of the structure involved. For example, a dental enamel cap with overflow will look swollen, and any thickness measurements greatly exaggerate the true value (Spoor et al., 1993). Overflow artefacts may remain undetected since they are barely distinguishable from very dense areas within the CT number range (see e.g. the enamel in Fig. 1b). By plotting the CT numbers in a suspicious area it can be seen that in a normal dense area without overflow every pixel has a high, but slightly different CT number (owing to the noise). In overflow areas, on the other hand, all pixels have a CT number of exactly the maximum value (see example in Spoor et al., 1993).

Some modern CT scanners are relatively tolerant, correctly representing areas with densities well above the traditional 3095 H. A few others have the option of selecting an extended CT number scale which can accommodate attenuation coefficients up to ten-times as high as the standard scale (this option is used in clinical practice to deal with metal of dental fillings and artificial joints). Recalibration of the scanner to cope with particularly high densities is sometimes possible (Spoor & Zonneveld, 1994), but this is only possible with full technical support from the scanner company and rarely feasible in a hospital setting. If overflow artefacts obscure the morphology under investigation and the fossil cannot be examined on a more appropriate scanner, it may be worthwhile, as a last resort, to save the raw data for image reconstruction with an extended CT number scale at a later date, perhaps with help from the scanner company. Overflow artefacts may occasionally follow from beam hardening (see below).
Lack of detector signal
When fossils with a high density and a large mass are scanned using thin slices, an insufficient amount of X-rays may reach the detectors. This lack of detector signal results in streaks of high noise in the direction of the highest attenuation (‘frozen noise’ artefacts) and increased noise levels in the images, which obscure details, in particular those with low contrast (Fig. 3). This problem is mostly encountered with highly mineralised crania, in particular when their endocranial cavities are filled with matrix. Moreover, it particularly affects those scans which show the largest cross-sectional surface of the specimen because the severity is directly dependent on the distance traversed by the X-rays. If lack of signal artefacts occur, while using the maximum X-ray tube load (kV and mAs), the only option is to increase the signal by increasing the slice thickness, which unavoidably reduces the spatial resolution (Fig. 3).

Thus, there is a balance between noise levels and spatial resolution, and the best compromise can only be found experimentally. When perforce increasing the slice thickness it is obviously best to keep the originally intended slice increment. Sometimes it is worth scanning a morphological area that is severely affected in one scan plane in a different, less affected plane, and use this stack of images to reconstruct the originally intended ones (see multiplanar reformatting, and the section on three-dimensional imaging below). For example, transverse scans at the level of the petrous bones of a fossil cranium may be too noisy to reveal any detail of the bony labyrinths. However, sagittal scans of the petrous bones, in which X-rays traverse significantly smaller distances through the fossil, will likely give acceptable results (see Figure 3 in Spoor & Zonneveld, 1994). Transverse images can be reconstructed from a

Figure 3  Midsagittal CT scans of the *Australopithecus boisei* cranium KNM-ER406 with a slice thickness of (a) 1 mm and (b) 3 mm. The high density and mass of the matrix-filled fossil result in a lack of detector signal causing high noise levels and ‘frozen noise’ streak artefacts when using a 1 mm slice thickness. Increasing the slice thickness to 3 mm improves the image quality so that details such as the clivus (arrow heads) become visible, but decreases the spatial resolution.
stack of sagittal ones, which show the labyrinthine structures clearly although with a reduced resolution due to the discrepancy between the in-plane spatial resolution and slice thickness.

**Beam hardening**

This is the progressive removal of the softer (low energy) X-rays from the spectrum as the X-ray beam passes through the object, because they are more readily attenuated. The remaining harder, more penetrating, radiation results in lower CT numbers. All CT scanners are calibrated to compensate for the expected beam hardening in patients, and artefacts may occur when the actual amount of beam hardening is significantly smaller or larger (Joseph, 1981). More beam hardening may occur in fossils, owing to their higher density and mass, which may give dark streak artefacts. Scanners can be recalibrated to compensate for higher degrees of beam hardening (Zonneveld & Wind, 1983), but again this is rarely feasible in a hospital setting. In fossils less beam hardening occurs when a specimen is very small, as is the case with isolated teeth or bone fragments. This results in higher CT numbers, giving overflow artefacts when the actual density is close to the upper limit of the CT number scale. Such artefacts can be suppressed by surrounding the specimen with sufficient mass, such as a cylinder of plexiglass or water bags. Similarly, it is advisable to scan small extant specimens, such as fetuses or small primates, with some added mass, to avoid beam hardening-induced overflow artefacts in the teeth.

**Micro-CT/X-ray microtomography**

Dedicated industrial and research micro-CT scanners have been developed, which can provide images with a much higher spatial resolution and a thinner slice thickness than medical CT scanners (see e.g. Flannery et al., 1987; Holdsworth et al., 1993; Anderson et al., 1994; Bonse, 1997; Denison et al., 1997; Illerhaus et al., 1997). These scanners generally differ from medical ones in that it is the specimen, mounted on a turntable, rather than the source/detector system that rotates. Some are otherwise not unlike medical scanners in that attenuation is measured using a line of detectors from which cross-sectional images are calculated. Others calculate a three-dimensional volume of CT numbers from radiographs recorded in a great number of directions using an image-intensifier and a framegrabber. Cross-sectional images can be calculated from this data volume in any direction. The drawback of the latter method is that the limited dynamic range (latitude) of many image intensifiers means that relatively small contrasts cannot be reproduced accurately in the image reconstruction. Consequently, this system tends to be less useful for scanning matrix-filled fossils with little contrast between the matrix and the mineralised bone.

The in-plane spatial resolution and the slice thickness that can be obtained with micro-CT varies between 1 and about 200 µm and depends on the size of the specimen that is scanned. Unlike medical scanners, many of these microtomographs produce isometric voxels, i.e. the pixel size is identical to the slice thickness. Whereas the scan time per slice is in the order of a few seconds with a medical scanner, it is typically minutes with micro-CT.

Micro-CT has predominantly been developed for material research or to assess small tissue samples, for example to study mineral content or trabecular structure of bone (Flannery et al., 1987; Kuhn et al., 1990; Anderson et al., 1994, 1996; Müller et al., 1994; Davis & Wong, 1996; Rüegsegger et al., 1996). However, it has also been used successfully to visualise the morphology of extant and fossil crania in great detail (Rowe et al., 1993, 1997; Shibata & Nagano, 1996; Thompson & Illerhaus, 1998; Spoor et al., 1998b;
Figure 4  Micro-CT of a skull of *Microcebus rufus*. (a) Coronal scan at the level of the external acoustic meatus, showing e.g. the spiral of the cochlea on either side. Scale bar = 10 mm. (b) Lateral view of a 3-D reconstruction, with the position of the coronal scan indicated (scale as in a).
Spoor & Zonneveld, 1998). An example of a coronal micro-CT slice of a *Microcebus* cranium (pixel size 60 µm) is shown in Fig. 4a.

**MAGNETIC RESONANCE IMAGING**

MRI was developed in the 1970s (Lauterbur, 1973; Mansfield *et al.*, 1976; Mansfield & Pykett, 1978), on the basis of techniques and principles developed for chemical nuclear magnetic resonance (NMR) spectroscopy, and was later applied in diagnostic imaging as a non-invasive imaging modality (Edelstein *et al.*, 1980).

MRI can produce cross-sectional images or volumetric datasets, using pulses of radio-frequency (RF) energy to map the relative abundance and other physical characteristics of hydrogen nuclei, i.e. protons sometimes also referred to as spins, in the presence of a strong, static magnetic field. Before image data can be collected, the protons are aligned into a state of equilibrium by the static field, where their spin axes begin to precess about the axis of the field with a specific frequency, the Larmor frequency. This state is maintained by the field until the spin axes are ‘flipped’ out of alignment into a higher energy, more excited, state using a sequence of RF pulses. The frequency of these pulses must be similar to the Larmor frequency for the protons to absorb the energy and become excited, i.e. the Larmor frequency is the resonant frequency of the protons in the magnetic field. After the pulses cease, the protons begin to relax back to their original, unexcited, state and in doing so emit energy equivalent to the difference between the two energy states. This energy is picked up by a coil, analogous to a TV aerial, as a free induction decay (FID) signal called the echo. The amplitude of echo is proportional to proton concentration and decays exponentially with a time constant that is dependent on the chemical environment of the protons. Spatial information is encoded in the spins prior to excitation by introducing three magnetic gradients within the main field, one for each dimension (i.e. orthogonally directed). This information appears in the echo as differences in phase and frequency, and is used to form a two-dimensional plane, or occasionally a three-dimensional block, of elements into which the signal intensities are mapped.

As in CT, the MR signal calculated for each voxel and its associated pixel are shown on a computer monitor using a grey scale with black representing the lowest and white the highest intensity. Images can be reconstructed using information from two different relaxation processes, known as T1 and T2, and also via T2*, which incorporates the effects of local variations in the magnetic field. T1 and T2 are the most common measures used, and they can be assessed by varying the periods between subsequent pulses, and between excitation and sampling the echo (see section on manipulating image contrast). In T1-weighted images, fat gives a more intense signal than water and thus appears brighter, whereas T2-weighted images show the reverse pattern.

Since the concentration of fat and water varies between different tissues, it is relatively straightforward to differentiate, by their echoes, tissues with a sufficient number of protons. Soft tissues contain the largest concentrations of protons in the form of ‘free’, intermediate and bound hydrogen (or as water). Proton-deficient tissues, like mineralised bone, produce very little echo and appear as signal voids in the image. Nevertheless, it is possible to see most, if not all, of the ossified architecture of the skeleton silhouetted against the signal from proton-rich tissues. More detailed accounts of MRI can be found in Foster & Hutchinson (1987), Bushong (1988), Young (1988), Newhouse & Weiner (1991), and Westbrook & Kaut (1993).
In the study of skeletal growth and evolution MRI and CT are complementary tech-
niques. Whereas CT provides excellent visualisation of hard-tissues in extant and fossil
specimens, MRI is the technique of choice to investigate the interaction between the devel-
oping skeletal and soft-tissue systems. For example, brain development has been proposed
as one of the major factors underlying cranial morphology, both from a phylogenetic and
an ontogenetic perspective (e.g. Ross & Ravosa, 1993; Spoor, 1997). MRI provides an
excellent visualisation of brain morphology, whereas CT has difficulty distinguishing
between brain tissue and the narrowly surrounding cerebrospinal fluid (Zonneveld &
Fukuta, 1994). Moreover, because MRI is thought to be harmless to living tissue, as
opposed to the ionising effect of X-rays used in CT, it is ideal for longitudinal growth stud-
ies. The nature of MRI thus even allows for in utero imaging of fetal development (Weinreb
et al., 1985; Powell et al., 1988; Girard et al., 1993; Colletti, 1996).

Most clinical MRI units are designed to image adult human morphology, providing in-
plane spatial resolutions in the region of 0.7–1 mm and a slice thickness of about 1–3 mm,
and are not, therefore, suitable for imaging smaller specimens or detailed morphology. For
example, in a study of fetal development or of smaller primate species, smaller voxels are
needed to locate detailed morphology. Resolution can, however, be improved with stronger
static magnetic fields, which ensure that the spins are more coherently aligned and thereby
increasing signal strength, and with stronger gradients for more sensitive spatial encoding.

High-resolution magnetic resonance imaging

High-resolution magnetic resonance imaging (hrMRI) uses magnetic fields of around
4–8 tesla (T) and gradient strengths of about 0.1 Tm⁻¹, as opposed to 2 T and 0.01 Tm⁻¹
in medical MRI, to obtain spatial resolutions in the region of 156–300 µm and slice thick-
nesses of 300–600 µm (see Effmann & Johnson, 1988; Johnson et al., 1993; Smith et al.,
1994, for descriptions of a similar technique called MRI microscopy). A limitation, as with
micro-CT, is that hrMRI requires long imaging times (typically 24 h per specimen).
Consequently, there is a greater chance that the specimen will move or currents in the
system will fluctuate, causing small motion artefacts and temporal field distortions (see later
section on limiting image artefacts). Some examples of midsagittal T2-weighted hrMR
images of formalin-preserved human fetuses, obtained with a 4.7 T field, are shown
in Fig. 5.

Practical aspects

Many aspects inherent to working with digital images, such as the image matrix and the
graphical representation of the data, are similar to what has been discussed in relation to
CT. Others specific for medical MRI and hrMRI are as follows.

Image resolution and slice thickness

These are determined by the strength of three magnetic gradients within the main magnetic
field. These are known as the slice selection gradient, phase encoding gradient, which
determines the number of pixel columns in the matrix, and the frequency encoding grad-
ingent, which determines the number of pixel rows in the matrix. In MRI, the term ‘matrix’
does not necessarily refer to the matrix of pixels in the image, or ‘image matrix’, rather it
refers to the number of data points taken from an area of the object, which is defined by the
field of view (FOV). These points are then represented by the pixels in the image matrix.
Figure 5  Mid sagittal T2-weighted hrMR images of human fetuses. (a) 14-week-old (slice thickness 390 µm; in plane spatial resolution 195 µm); (b) 18-week-old (slice thickness 500 µm; in plane spatial resolution 250 µm); (c) 25-week-old (slice thickness 625 µm; in plane spatial resolution 313 µm). Ages determined from crown–rump lengths following Streeter (1920). Scale bars = 10 mm.
Matrices and FOVs need not always be the same size or shape, but they must always contain a corresponding number of columns and rows, and therefore elements. The need for this distinction will become clear later on.

To minimise slice thickness, the slice selection gradient must be as large as possible within its operational limits. This is usually less than its absolute limits, so that the frequencies describing a slice width cover a steeper gradient and are therefore easily differentiated from frequencies in adjacent slices. To produce thinner slices, the frequency range describing the slice width can be reduced. It is not, however, advisable to reduce the range too far, since the inherent instability in the system, including small frequency fluctuations, becomes gradually more pronounced as the range becomes smaller. Refer to the manufacturer for further details on the operational limitations of the gradients.

Both the phase and frequency encoding gradients should also be as large as possible to maximise the number of data points in the matrix and therefore pixels in the image matrix. Matrices can be made square by making the phase and frequency gradients the same, or rectangular by making one larger than the other. In both cases, the shape of the field of view must reflect the shape of the matrix to keep the pixels in the image matrix isometric. For example, when collecting data from a rectangular matrix of 256 × 512 (ratio 1:2), use a field of view (FOV) of 6 cm by 12 cm to keep the pixels in the image matrix isometric (234 × 234 µm). A square matrix with a rectangular FOV will produce rectangular pixels in a square image matrix and can complicate postacquisition data processing such as interpolation. The size and shape of the matrix and FOV should be selected according to the size and shape of the object under investigation, remembering that the larger the matrix and the smaller the FOV, the greater the resolution in the image matrix.

**Signal to noise ratio (SNR)**

As with most medical imaging systems, a number of compromises must be made in MRI to obtain optimal results. The most important of these is the balance between higher spatial resolution and poorer signal to noise ratio and vice versa. Achieving the right balance is something of a challenge, requiring an intuitive understanding of the physics involved or a process of trial and error. This is compounded by the variability in the numerous factors that influence the signal and can change between one specimen and another (Hoult & Richards, 1976; Foster & Hutchinson, 1987). Nevertheless, the signal to noise ratio can generally be increased when imaging large specimens by reducing resolution and increasing slice thickness. This is not, however, suitable for smaller specimens, such as fetuses and small primates, for obvious reasons. Instead, each point in the matrix can be sampled more than once, so that the random effects of noise gradually average out with each additional repetition. The drawback of this approach is that the improvement in SNR is proportional to the square root of the number of averages, thus more and more averages are needed for each significant increase in SNR and imaging times become longer. Other ways in which signal to noise can be increased whilst maintaining resolution include: increasing the period (TR) between subsequent excitation pulses, reducing the period between the excitation pulse and sampling the signal (TE), and by using spin echo pulse sequences where possible.

**Interleaving**

This is a technique used in MRI to reduce the effects of signal transfer between adjacent slices. It is particularly important for thin slices, less than 1 mm thick, where the excitation pulse for one slice can easily overlap into neighbouring slices, known as cross excitation, or
the remnants of signal from previous excitations in other slices can leak into the target slice, known as cross talk. The combined echo, if sampled, contains erroneous information about the distribution of spins in more than one slice and therefore affects image contrast. Interleaving solves this problem by exciting slices alternatively rather than sequentially, i.e. 1,3,5,7 and then 2,4,6 rather than 1,2,3,4, etc. This process thereby limits the amount of signal that can leak into the target slice by increasing the distance.

**Manipulating image contrast**

In current practice, image contrast in MRI is almost exclusively based on the T1 recovery and T2 decay times of the excited spins in the sample. Most soft tissues have characteristic T1 and T2 values (Bottomley *et al.*, 1984) and can, therefore, be readily differentiated by weighting the acquisition to either process. In general, image contrast depends on the differences in these values between tissues containing varying amounts of fat and water. Fat has short T1 and T2 times and appears bright with T1 weighting and dark with T2 weighting. Water, on the other hand, has long T1 and T2 times, and appears dark with T1 weighting and bright with T2 weighting. Tissues containing intermediate concentrations of fat and water occupy the remaining grey values according to the weighting. Tissues containing very little fat or water, such as cortical bone, produce insufficient signal and appear dark with both T1 and T2 weighting. Blood vessels in soft tissue specimens containing deoxyhaemoglobin, a paramagnetic substance which disturbs the local field, also appear dark with both weightings.

With these few tissue characteristics, either T1 or T2 weighting can be selected to emphasise the image contrast between the morphological features under investigation. For example, morphology of the inner ear is more readily seen with T2 weighting than T1 weighting because the endolymph, perilymph or preservative fluid (provided it contains sufficient water) within the bony labyrinth appears brighter than the surrounding bone. With T1 weighting, both the fluid and surrounding bone appear dark, giving very little contrast with which to distinguish the two.

Although some preservative solutions contribute to image contrast, as in the above example, they can cause a number of problems. Besides tissue shrinkage, the most important of these in MRI are the changes to T1 and T2 values, caused by the sometimes volatile chemistry of the solutions (Thickman *et al.*, 1983; Isobe *et al.*, 1994). The severity of this effect depends on how long the material has been preserved and the concentration of the solute (see section on interference from preservative fluids).

**Image artefacts**

MRI artefacts have been reviewed in great detail in, for example, Henkelman & Bronskill (1987) and Foster & Hutchinson (1987). Some of their more common causes and possible remedies are as follows.

**Coil filling**

The ratio of the sample to the RF-coil volume is known as the volume filling factor. This is an important factor which determines how efficient the coil is at picking up signal from the sample. If the volume of the coil is much larger than that of the sample SNR is reduced compared with a larger sample. If, on the other hand, the volume of the sample is nearly the same as that of the coil the magnetic and electrical properties of the sample reduces coil efficiency and lowers SNR. Furthermore, ferromagnetic contaminants in the coil material and large gradients close to the coil produces variations in flip angle; both of these effects can
degrade the image at the sample periphery. The remedy is to choose or construct a coil that has a cross-sectional diameter about 1–3 cm larger than that of the sample and is less sensitive to the field gradients.

**Wrap around**

This occurs when the size of the specimen in the imaging plane is larger than the FOV. Signal from outside the FOV is folded, and mapped onto the opposite side of the image. To avoid wrap around, increase the FOV or image in another plane, which reduces the sample’s cross section.

**Cross talk and cross excitation**

See section on interleaving.

**Movement artefact**

If the sample moves during imaging, even very slightly, the spatial information encoded in the spins is invalidated and the misregistration appears in the image as ‘ghosting’. To avoid movement simply ensure that the specimen is securely fastened in the coil before imaging. Strips of Velcro™ and foam rubber, moulded to the shape of the coil, are particularly useful in this situation.

**Interference from preservative fluids**

If a specimen has been preserved for some time in a solution containing comparatively little water, the relaxation characteristics (T1 and T2) of its constituent tissues will all tend to shift towards the solute’s values and image contrast is lost. Experience shows that strong ethanol/methanol solutions are in general more detrimental to image contrast than formalin solutions, especially with T2 weightings, and should therefore be avoided if possible. It is, however, in many cases sufficient to keep the specimen in a formalin solution for a few weeks to obtain a significantly improved result. Alternatively, try using a different weighting and experiment with different values of TE and TR. Frozen specimens do not provide any MR signal at all.

**Geometric distortion**

Inhomogeneity of the static field and the non-linearity of the gradients may result in geometric distortion in MR images (Derosier et al., 1991; Bakker et al., 1992; Michiels et al., 1994; Sumanaweera et al., 1994). When using a scanner for which this effect has not been evaluated and accurate representation of morphology is required, it is worth measuring the degree of distortion by scanning appropriate test phantoms. By using correction techniques, significant reduction of distortion-based errors has been reported (Sumanaweera et al., 1995; Maciunas et al., 1996).

**THREE-DIMENSIONAL IMAGING**

By using computer graphics techniques, a series of contiguous or overlapping CT or MR images can be stacked to provide a three-dimensional (3-D) data set of the scanned object, which can be analysed and visualised in a variety of ways (see e.g. Robb, 1995, for a general overview). This technique is now commonly applied in medical practice (Höhne et al., 1990; Hemmy et al., 1994; Zonneveld, 1994; Zonneveld & Fukuta, 1994; Linney &
Alusi, 1998; Udupa & Herman, 1998), as well as in palaeoanthropology (Zollikofer et al., 1998; Spoor & Zonneveld, 1999).

**Multiplanar reformatting**

A basic use of a 3-D data set is multiplanar reformatting, the technique to extract images in planes other than the original stack. For example, the midsagittal images of the human fetuses shown in Fig. 5 are resampled from an original stack of transverse hrMR scans. The spatial resolution of reformatted images is not as good as in the original ones, unless the voxels of the image stack are isometric (i.e. the pixel size equals the slice thickness) and the new image is exactly perpendicular to the original image plane. Thus, if the best possible spatial resolution is to be achieved it is important not to rely on reformatted images, but to choose the most appropriate plane when making the scans.

**3-D visualisation by surface rendering**

The second application of 3-D data sets is to obtain reconstructions of all or selected parts of a specimen. Even for those who are experienced with interpreting the cross-sectional shapes shown in individual CT or MR scans, 3-D reconstructions provide a much better and more realistic topographic impression of the overall morphology. Usually the reconstructions are based on either CT or MR data sets, but in so-called multimodality matching different sets are combined, for example visualising the cranium based on CT and the brain on MRI (Gamboa-Aldeco et al., 1986; Zuiderveld et al., 1996).

In studies of skeletal morphology the most common technique of visualising the 3-D dataset is surface rendering, in which surfaces of selected tissues are extracted from the data volume and imaged. It generally involves three steps. The first one, known as segmentation, is to isolate the tissue or material to be imaged in the 3-D reconstruction. This process is performed separately in each CT or MR slice, most commonly by thresholding for the range of CT or MR numbers characterising the relevant tissue. Segmentation can be improved by manually drawing regions of interest to exclude specific parts from the 3-D reconstruction and by using specialised region growing and edge detection software tools. In the second step the border lines of the selected tissues in each slice are combined to form a three-dimensional surface description of the structure to be imaged. This often involves interpolation between the slices to create a smooth surface. The last step is the illumination of this surface by means of one or more virtual light sources, thus improving a sense of three-dimensionality and bringing out surface details. Examples of surface-rendered reconstructions on the basis of regular CT scans and micro-CT scans are shown in Figs 6 and 4b, respectively.

Internal structures can be demonstrated in 3-D reconstructions by making cut-away views in which part of the selected tissue is left out, for example to demonstrate the paranasal sinuses, or the endocranial cavity (Fig. 6a). Visualisation of such hollow structures can be improved by their representation as solid objects (flood-filling; Fig. 6b). When dealing with reconstructions of soft-tissue specimens cut-away views can be shown with multiplanar reformatted images mapped onto the cut surfaces. The 3-D effect of reconstructions can be enhanced by generating stereo pairs of images and animation sequences simulating movement. A particularly appealing method of presenting surface rendered 3-D reconstructions is through stereolithography which provides life-sized or enlarged plastic models that can be handled manually (Zonneveld, 1994; Zollikofer & Ponce de Leon, 1995; Zollikofer et al., 1995, 1998; Seidler et al., 1997).
Figure 6. Surface rendered CT-based 3-D reconstructions of middle Pleistocene hominids, using surface rendering. (a) Anterosuperior view of the Petralona specimen, based on 2.0 mm thick slices. The cut-away view demonstrates the large frontal sinus (f) and the endocranial cavity (b). Anterolateral aspect of the Broken Hill specimen, based on 1.5 mm thick slices. Cut-away view of the left anterior quadrant of bone shows the flood-filled frontal (f), ethmoidal (e) and maxillary (m) sinuses and endocranial cavity. Scale bars = 10 mm.
Three-dimensional imaging has been applied in palaeoanthropological studies and a few comparative primatological analyses to visualise internal morphology, such as unerupted dentition, root morphology, the paranasal sinuses, the inner ear or the endocranial surface (Zonneveld et al., 1989b; Koppe et al., 1996; Seidler et al., 1997; Conroy et al., 1998; Thompson & Illerhaus, 1998; Zollikofer et al., 1998; Spoor & Zonneveld, 1999; Ponce de Leon & Zollikofer, 1999). A second category of applications is the reconstruction of fossils by complementing missing parts through mirror imaging (Zollikofer et al., 1995), with the possibility of combining fossils of more than one individual, which requires the additional step of scaling to obtain matching sizes (Kalvin et al., 1995). The bones of a crushed fossil can be ‘electronically dissected’ and reassembled, and plastic deformation can be corrected (Braun, 1996). In a third type of application, 3-D reconstructions are used as the basis for morphometric studies (see next section).

Skeletal growth studies using CT-based 3-D reconstructions predominantly deal with the clinical assessment of various cranial deformities and their surgical treatment in children (Marsh & Vannier, 1985; Zonneveld et al., 1989a; David et al., 1990; Leboucq et al., 1991). Moreover, it has also been applied to study the ossification process of the developing fetal cranium (Neuman et al., 1997).

Rapid developments on the computer graphics side of 3-D reconstruction have led to increasingly realistic images. However, improved visual representation of, for example, the surface of a cranium, does not imply that the image is more accurate as well. The extent to which the reconstruction reflects reality primarily depends on limitations inherent to CT or MRI. The best possible accuracy of three-dimensional reconstructions is limited by the spatial resolution within the scan plane and by the slice thickness and slice increment in the direction perpendicular to the scan plane (Vannier et al., 1985). This is clearly demonstrated by comparison of Figs 6a and 4b, based on 2 mm and 0.06 mm thick contiguous CT slices, respectively. However, the original voxel size may not always be obvious from the final 3-D reconstruction because increasingly sophisticated interpolation algorithms lead to excellent smoothing of the steps between the stacked slices (compare Fig. 6a and b).

An important factor influencing the accuracy of the reconstruction is the segmentation process. Software packages for 3-D reconstruction generally select each structure to be shown by thresholding for a single range of numbers characterising the relevant material. A major problem with thresholding in MR images is that many different tissues tend to give MR signals in overlapping ranges. Hence, selecting a single structure, such as the brain, by thresholding only is not possible, and requires either extensive manual interaction, or more-specialised tissue characterisation techniques (Vannier et al., 1991b; Clarke et al., 1995; Kapur et al., 1995). Consequently, surface rendering is less frequently used in MRI-based 3-D reconstruction other than for images showing the skin surface in combination with cut-away views showing the internal morphology on the cut surfaces.

In CT-based reconstructions a major source of segmentation artefacts is partial volume averaging, the effect that if contrasting materials occupy a voxel, the CT number is a mixture of the CT numbers of those materials (this phenomenon equally occurs in MR images, but is less obvious because segmentation is problematic anyway). Hence, if bone and air both occupy a voxel its CT number may be below the range that is representative for bone. In scans of crania this effect results in artificial low CT numbers of thin bony structures, such as parts of the temporal squama and part of the ethmoid region. In fossils the segmentation of bone by thresholding is further complicated by local differences in mineralisation and matrix penetration of the bone, and by rock matrix which locally may have a similar density as bone (see Spoor & Zonneveld, 1999, for a review of segmentation
artefacts in fossils). Hence, for optimum segmentation of a surface more than one thresh-
old may be required, but in practice a compromise range is selected. Although manual
interaction can improve the reconstruction this perforce leads to inaccuracies, such as cor-
tical bone that is locally imaged too thick, too thin or even shows artificial holes. To avoid
the problems associated with thresholding for a preselected CT number range, so-called
’snake’ edge detection techniques have been developed which locally compare the CT
numbers on either side of a gradient and select the most likely position of the tissue inter-
face (Gourdon, 1995; Lobregt & Viergever, 1995; McInerney & Terzolpoulos, 1995).

3-D visualisation by volume rendering

An alternative technique of representing 3-D data sets, other than surface rendering, is
volume rendering, in which all of the data volume contributes to the images (Drebin et al.,
1988; Levoy, 1988; Toga, 1990; Robb, 1995). Tissue segmentation, the crucial step in sur-
face rendering, is therefore skipped, unless it is used to isolate the structure that is to be
volume rendered. Different CT or MR numbers in the stack of slices are assigned different
colours and different degrees of opacity. Subsequently, this volume description is projected
onto a plane for viewing. If the assigned opacity is directly correlated with X-ray attenua-
tion the resulting image is similar to a radiograph, predominantly showing bony structures.
However, by making alternative assignments other structures can be brought out. For
example, in Fig. 7 voxels representing either skin or brain tissue have been given an opac-
ity higher than those representing other tissues.

Volume rendering and surface rendering techniques both have their strong and weak
points, and which of the two is most appropriate depends on the specific application
(Rusinek et al., 1991; Udupa et al., 1991). Volume rendering has the advantage that many
aspects of the internal and external morphology of a specimen can be shown in relation to
each other without the need for a laborious segmentation process and complicated cut-away
views. It is especially useful where tissue segmentation is problematic or impractical, as in
MRI. However, the computational cost of volume rendering is high, requiring more time
than surface rendering. Moreover, the fuzzy representation of surfaces and some degree of
superimposition of structures that characterise volume rendering make surface rendering
the more appropriate technique to reconstruct skeletal morphology.

QUANTITATIVE ANALYSIS

Morphometry

Both CT and MR images and 3-D reconstructions can form an excellent basis for mor-
phometric analyses. For example, CT has been used to obtain basicranial angles of adult and
fetal specimens (Dimitriadis et al., 1995; Spoor, 1997), as well as linear or area measure-
ments of long bone shaft geometry (Jungers & Minns, 1979; Ruff, 1989; Ohman et al.,
1997), cranial vault thickness (Hublin, 1989; Garcia, 1995; Zollikofer et al., 1995), inner
ear morphology (Spoor et al., 1994; Spoor & Zonneveld, 1995; Hublin et al., 1996;
Thompson & Illerhaus, 1998) and enamel thickness (Macho & Thackeray, 1992; Conroy
et al., 1995; Schwartz et al., 1998). CT-based 3-D reconstructions typically provide
craniofacial landmark data (Hildeboldt et al., 1990; Richtsmeier, 1993; Richtsmeier et al.,
1995) and volume measurements of the paranasal sinuses and the endocranial cavity
Figure 7  Volume rendered 3-D reconstructions based on the hrMRI data set of the 25-week-old fetus shown in Fig. 5c. (a) Three-quarter frontal view, with the highest opacity assigned to the skin and the top of the head removed to demonstrate the brain morphology. (b) Anterior view, with the skin made transparent to demonstrate the outline of the brain, spinal cord, eyes and inner ears.
MRI has been used, among others, to obtain linear and volumetric measurements of the brain (Falk et al., 1991; Vannier et al., 1991a; MacFall et al., 1994; Semendeferi et al., 1997; Rilling & Insel, 1999) and of aspects of joint morphology (Smith et al., 1989; Eckstein et al., 1994; Pilch et al., 1994).

The accuracy of morphometric measurements depends on the spatial resolution, the pixel or voxel size and on the window setting used when the measurements are taken. Ways of obtaining the best possible spatial and contrast resolution for a given specimen have been described in the Practical aspects sections. When taking measurements in scans of large structures the accuracy will be determined by the pixel size and not by the spatial resolution, and positioning the landmarks is usually no problem. However, taking measurements of detailed morphology from images with a pixel size sufficiently small not to limit the spatial resolution may be more difficult. Structures are unavoidably shown with blurred boundaries, owing to the limited resolution, and the apparent size of structures changes when the window setting is altered. However, the window setting showing the exact position of a boundary can be calculated from the local CT or MR numbers on either side of the interface, a procedure described in detail elsewhere (Spoor et al., 1993; Spoor & Zonneveld, 1995; Feng et al., 1996; Ohman et al., 1997; Schwartz et al., 1998). A consequence is that measurements between two interfaces with very different contrasts, for example dentine–enamel and enamel–air when measuring enamel thickness, require two very different window settings to obtain accurate results. Taking such measurements from hard copies or 3-D reconstructions is therefore prone to inaccurate results, because both have fixed window settings. An important limitation of the ability to take accurate measurements from digital images is that when the distance between two tissue interfaces is less than twice the spatial resolution the position of neither interface can be determined accurately, owing to effects of interference (Spoor et al., 1993). The tables of the cranial vault and dental enamel in many primate species are therefore too thin to allow accurate measurements when examined with medical scanners.

Density measurements

CT and micro-CT are used to obtain density measurements of bone and teeth, examining absolute and relative values and their distribution (Genant & Boyd, 1977; Cann & Genant, 1980; Adams et al., 1982; Glüer et al., 1988; Glüer & Genant, 1989; Steenbeek et al., 1992; Anderson et al., 1996). Being more a physiological than a morphological topic, this application falls somewhat outside the scope of this chapter. However, some discussion is warranted given the fact that density measurements can be important in the context of investigating the biomechanical properties and functional morphology of a skeletal structure (Ruff & Leo, 1986).

Absolute density measurements vary when taken with different CT scanners or at different times (Cann et al., 1979), for example after scanner maintenance or after changing an X-ray tube or a detector segment. This variation can be corrected for by scanning a standard calibration object along with the examined specimen. When studying either absolute or relative density measurements, artificial density distributions should be taken into account. For example, beam hardening in scans of a homogeneous object tends to result in somewhat lower CT numbers in the centre than in the periphery of the cross-section (Newton & Potts, 1981; Ruff & Leo, 1986). In CT scans of small or thin structures, such as the subchondral bone of joint surfaces and trabecular bone, the CT numbers do not reach the values representative of the actual bone substance because of the limited spatial resolution. Finding relatively low CT values can thus ambiguously imply that the bone is
locally less dense or relatively thin. This phenomenon occurs when a structure is less than two to three times the spatial resolution in cross-sectional size (Spoor et al., 1993), and using micro-CT instead of medical CT may thus be critical to obtain meaningful results. Any density measurements of fossils are problematic because of unknown taphonomic factors. Fossils found in caves are often partially penetrated by calcite with a very high density, whereas air-exposed parts may have been decalcified.

WORKING WITH IMAGES

For a long time manipulating CT and MR images was confined to the specialist’s realm of UNIX-based computer environments. This, together with the multitude of brand-specific image formats used to handle these images, made post-acquisition image manipulation almost impossible without the help of a medical physicist or software engineer. With the increase in power of personal computers, and the emergence of appropriate software and universal formats, such as DICOM (Digital Imaging and Communications in Medicine), it is now possible to do basic manipulations on a PC or Mac. This section describes some basic techniques and suggestions on handling images on computers.

CT and MR images

First of all, the image data should be retrieved from the imaging modality, and must be written in a file format suitable for storage or transfer between computers. How this is done is usually defined by local protocols and will therefore require the help of an on-site radiologist or medical physicist. Once the image files are brought into the personal computer environment, for example by FTP transfer to the hard disk or on CD-ROM or optical disk, attempts can be made to view their contents. Although there are numerous image formats available, the majority contain two basic elements: the body and the header.

Body

The body part of the file contains the data which describe one or more images by giving a binary value for each pixel. The range of such values supported by the format, known as the pixel depth, is quoted in terms of bits and is usually 12 or 16 bits in the case of CT and MR images. A 12-bit file can store data with up to 4096 different values per pixel, whereas this is 65 536 values for a 16-bit file. In most image formats 2 bytes of computer memory are used to store the binary value of each pixel. The number of pixels that make up an image can be calculated from the matrix size. For example, a $512 \times 512$ matrix consists of 262 144 pixels, which at 2 bytes per pixel occupies 524 288 bytes of memory. If the body contains not one but, for example, three images the total body size is $3 \times 524\,288$ or 1 572 864 bytes (1.6 Mb).

Header

The header is usually located at the beginning of the file and contains descriptive information, like patient details, dimensions of the image matrix, number of slices, slice thickness and technical parameters used in the examination. If an image file contains multiple images, there is usually only one header, but sometimes each image has a header (some DICOM formats) or the header file is altogether separate from the image file (ANALYZE™ format has separate files with the extensions ‘.img’ and ‘.hdr’). Different configurations are summarised in Fig. 8.
If a format is unfamiliar it is often possible to calculate the header length, provided the body size is known (or can be estimated). This enables some software packages, many of which are available on the World Wide Web as freeware, to read the image data without knowing the file format by simply skipping the calculated length of the header. The header length, and thus the offset needed, is given by the total file size minus the estimated body size. If in the above example of a three-image file the total file size is 1,577,514 bytes, then the header is 4,650 bytes. If each image has a header, then the offset required for each image is the estimated header size divided by the number of images, i.e. 1,550 bytes. Some software packages prompt for the relevant information and automatically calculate the offset values, others require the user to do the arithmetic. Of course, removing or skipping the header means that identification, demographic and technique information is lost or inaccessible. It is therefore advisable to record the essential image parameters such as matrix size, pixel size, slice thickness and slice index at the time when the images are made.

Many packages will prompt for information, other than the matrix size, number of images, and pixel depth, before loading an unfamiliar format. Most common aspects that are queried include the byte order and whether the pixel values are signed or not. Image files have a byte order that is either normal or swapped. The latter, generated by specialised computers and some Apple Macintosh’s, puts the most significant bit of information first by reversing the byte order in the file. The information in a normal, unswapped 12-bit file would be ordered from 0 to 11, whereas in a swapped file the order is reversed to 11 to 0. Indicating ‘unsigned’ or ‘signed’ determines whether the software recognises negative values when interpreting the numeric values in the file. For example, the 4,096 values in a 12-bit file can be seen as either 0 to 4,095 (unsigned) or, for example, as the typical Hounsfield CT number scale from –1000 to +3095 (signed). In some software packages the minimum and maximum values that occur in an image can be indicated.

If any of the parameters is incorrectly set whilst loading an unfamiliar format, the images appear with characteristic artefacts, some of which are summarised in Fig. 9. More than one incorrect parameter obviously results in combinations of such artefacts. Unknown settings are best found through a trial and error approach of testing different combinations, while being guided by the type of resulting artefact(s).
Figure 9  The CT image of Fig. 1b imported using incorrect parameters. (a) Unswapped image opened as byte-swapped; (b) 16-bit image opened as 8 bit; (c) image opened with a header length that is too short; (d) image opened with a header length that is too long; (e) $512 \times 512$ image matrix opened as $256 \times 256$; (f) $256 \times 256$ image matrix opened as $512 \times 512$. 
produced by medical scanners, the pixel depth is usually 12 or 16 bit and the matrix size is 256 × 256, 512 × 512 or occasionally 320 × 320. If the option of a 12-bit pixel depth is not available a 16-bit depth can be used instead. The mere 4096 values of the 12-bit depth would disappear among the 65 536 possible values, resulting in an almost black image. However, usually the software automatically detects the limited range that is read in, or the minimum and maximum values known or estimated to be present in the image can be indicated manually. Tips on dealing with unknown image formats can be found in the help files of particular software packages, and by consulting the newsgroups that are frequently formed by users on the World Wide Web. Essential information may also be available from the proprietors of the format in question, many of whom have technical staff that can be contacted via the Web.

**Graphic image files**

Occasionally, images may be needed in formats more generally compatible with PC or Mac software for further analysis, such as morphometrics, or for publication. In this situation it is important to remember that the type of graphic file used can affect the spatial resolution and contrast resolution of the image. The two main types are bitmapped and vector or object-orientated images. Vector files are used for storing discrete geometric forms and are not suitable for medical image data. The bitmapped family of image formats includes, amongst others, PCX, BMP (windows bitmap), GIF (Graphics Interchange Format), TIFF (Tag Image File Format), and JPEG (Joint Photographic Experts Group). The first problem with these formats, is that they support 24-bit colour ranges (8 bits for red, green and blue each), except for TIFF which supports 8 bit, and thus, the original pixel values may be extrapolated to fill the extra bits. Secondly, some of these formats employ ‘lossy’ as opposed to ‘lossless’ compression techniques. With lossless compression, like for example Run-Length Encoding (RLE), none of the image data is lost, instead it is replaced by a string expression. For example, five consecutive black pixels would be stored as 5,0 (string) rather than 00000. Lossy formats are more efficient at compression, but at the expense of pixel depth, matrix size or both. Thus, lossy formats, like JPEG, should be avoided if the image is to be used for anything other than pure illustrative purposes. Saving data as greyscale images in a bitmapped lossless format should minimise any changes made to the pixel values and resolution.

**FUTURE PROSPECTS**

In the short term, the application of CT and MRI in morphological research will see its main advances in relation to aspects of data processing rather than data acquisition. Driving factors include the opportunities created by the ever-increasing computer power and the demand for fast and life-like 3-D rendering techniques coming from the film industry and from advanced medical applications such as surgical simulation and virtual endoscopy. Three-dimensional rendering software will become quicker, easier to use and less expensive as the gap between Unix workstations and personal computers narrows further. Multimodality matching, combining CT and MRI based 3-D datasets, will become a more mainstream technique, and will improve the integrated morphological information that can be extracted from soft-tissue specimens. The use of 3-D datasets as the basis for complex multivariate morphometric studies will increase, including the possibility of morphing
surface-rendered reconstructions between different taxa and developmental stages. Emerging technologies that offer potential in this regard are discussed by O’Higgins in Chapter 7.

More widespread application of CT and MRI in palaeontology and developmental biology is starting to result in extensive scientific image archives which document, for example, the hominid fossil record and human fetal growth series. The Internet forms the ideal structure to provide worldwide access to such reference collections. The availability of the common DICOM image format, supported by the major medical imaging companies, will further contribute to routine exchange of datasets.

The image quality that can be obtained with medical CT has largely remained stable over the past decade. What has changed dramatically, however, is the speed of scanning. Faster scanners make the acquisition of 3-D sets of a large number of specimens for a given research project increasingly feasible. Whereas micro-CT used to be confined to experimental, purpose built set-ups, there will likely be a trend towards affordable and commercially available scanners. Portability will have the consequence that such scanners will increasingly be taken to the specimens in a museum collection.

MRI, being a more complex imaging technique than CT, is still well in development. Stronger magnets, better coil designs and improved encoding gradients will increase the image quality, and faster pulse sequences will shorten imaging times. The potential of hrMRI in studying skeletal morphology in relation to the associated soft-tissue structures has only recently been realised, and a wide range of applications remains to be explored.

In short, the development of CT and MRI in combination with powerful computer graphics software has provided a range of new opportunities to qualitatively and quantitatively study skeletal ontogeny and phylogeny. Interestingly, the application of these techniques is going through its own process of growth and evolution. Following an initial ‘pretty pictures’ phase of researchers and a more general audience marvelling at all the possibilities of producing beautiful 2-D and 3-D images, the true scientific merit of purposefully uncovering evidence not accessible by other means has now become abundantly clear.

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APPENDIX: GLOSSARY OF IMAGING TERMS

Attenuation (of X-rays). The decrease in radiation intensity as a result of its interaction with the matter encountered (absorption and scattering).

Beam hardening. The progressive removal of the softer (low energy) X-rays from the
spectrum as the X-ray beam passes through the object, as they are more readily attenuated. The remaining harder, more penetrating, radiation results in lower CT numbers.

**Bit.** A single digit of the binary numbers (0 or 1) used by computers. A combination of eight bits can express $2^8$ or 256 different values, 12 bits 4096 values and 16 bits 65 536 values.

**Bit range.** See Pixel depth.

**Byte.** Computer memory used to store a group of eight bits.

**Contrast resolution.** The ability to resolve small contrast between different tissues.

**Convolution filter.** Mathematical filter function used during the reconstruction of a CT image. Different types of convolution filters, known as edge-enhancement, smoothing or Ramp filters, can be selected according to the characteristics of the tissue interfaces that are to be imaged. Also named ‘kernel’.

**CT number.** Value on the Hounsfield scale assigned to a pixel corresponding to the X-ray attenuation within the voxel represented by the pixel.

**Field of view (FOV).** The diameter of the area of the scanned object that is represented in the reconstructed image.

**Free induction decay signal (FID).** The electrical current induced in the MRI receiver coil by the relaxing spins. It gradually decreases as more and more spins return to their unexcited state.

**Frequency encoding.** The introduction of spatial information using a magnetic gradient to produce characteristic frequencies along one dimension of the magnet.

**Hounsfield scale.** Linear scale of CT numbers given in Hounsfield units (H), and defined by values representing the attenuation of air (–1000 H) and that of water (0 H).

**Kernel.** See Convolution filter.

**Matrix.** The square two-dimensional array of pixels that makes up an image.

**Matrix size.** The number of rows and columns of a matrix.

**Magnification.** A post-reconstruction enlargement of an area of an image by interpolation of its CT or MR numbers. In contrast to a zoom reconstruction the enlarged image is therefore not based on the raw data of the scan but on the image itself.

**MR number.** Value assigned to a pixel corresponding to the magnetic resonance signal within the voxel represented by the pixel.

**Multiplanar reformatting.** Technique to extract new images from a stack of images in planes other than that of the original stack.

**Noise.** Random fluctuation of CT or MR numbers representing a homogeneous medium.

**Overflow.** The phenomenon in CT scans that the attenuation exceeds the maximum value of possible CT numbers of the Hounsfield scale.

**Partial volume averaging.** The averaging of different densities within a voxel, in particular along the thickness of the slice. The different densities are therefore represented by a single CT or MR number, which decreases the sharpness of the image.

**Phase.** The position of the spin in its rotational path.

**Phase encoding.** The introduction of spatial information using a magnetic gradient to produce characteristic phases along one dimension of the magnet.

**Pixel.** Abbreviation of ‘picture element’ representing the (two-dimensional) building blocks of the matrix of an image.

**Pixel depth.** The range of possible values (CT or MR numbers) assigned to a pixel, given in bits.

**Pixel size.** The size of the imaged area represented by one pixel, calculated by dividing the field of view by the matrix size.

**Raw data.** The processed detector output before image reconstruction. In CT this is the

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natural logarithm of the quotient of the unattenuated and attenuated detector signals after calibration for fluctuations in tube output and for beam hardening (so called ‘calibrated line integrals’). In MRI this is a measure of the free induction decay signal frequencies against time. These are mapped in a theoretical space in which phase and frequency data is stored during the acquisition.

**Signal to noise ratio (SNR).** The difference between wanted signal and unwanted background signal.

**Slice thickness.** Thickness of the slice of an object represented in a CT or MR image.

**Spatial resolution.** A quantitative measure of the ability to resolve small details, i.e. to visualise to small details separately. It is not the smallest isolated detail that can be visualised.

**Spin echo pulse sequences.** RF pulse sequences that flip the spins 180° before the echo is sampled.

**Surface rendering.** Technique of representing 3-D data sets of images in which surfaces of selected tissues are extracted from the data volume and imaged.

**T1.** The time taken for 63% of spins to return a state of alignment with the magnetic field.

**T1 recovery.** The restoration of spins to a state of alignment with the magnetic field, also called spin–lattice relaxation.

**T1 weighting.** A method for showing differences in T1 times between tissues as image contrast.

**T2.** The time taken for 63% of the excited spins to decay.

**T2 decay.** The loss of spins from the excited state, also called spin–spin relaxation.

**T2 weighting.** A method for showing differences in T2 times between tissues as image contrast.

**TE.** Time to echo; the time between the excitation pulse and the echo.

**TR.** Repetition time; the time between excitation pulses.

**Volume rendering.** Technique of representing 3-D data sets of images in which all of the data volume contributes to the images and different CT or MR numbers are assigned different colours and different degrees of opacity.

**Voxel.** Abbreviation of ‘volume element’. The (three-dimensional) volume represented by a (two-dimensional) pixel. In volume it therefore equals the squared pixel size times the slice thickness.

**Window technique.** The method of displaying only a range (window) of the possible CT or MR numbers by a maximum of 256 grey levels between black and white during the display of an image.

**Window level.** The average of the maximum and minimum CT or MR numbers of the range displayed as grey levels. Also called the window centre.

**Window width.** The difference between the maximum and minimum CT or MR numbers of the range displayed as grey levels.

**Zoom reconstruction.** An enlarged reconstruction of a part of an overview scan calculated using the raw data of the scan.

**REFERENCES**


