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Confocal Scanning Optical Microscopy of a 3-Million-Year-Old 
Australopithecus afarensis Femur

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Summary: Portable confocal scanning optical microscopy (PCSOM) has been specifically developed for the noncontact and nondestructive imaging of early human fossil hard tissues, which here we describe and apply to a 3-million-year-old femur from the celebrated Ethiopian skeleton, “Lucy,” referred to Australopithecus afarensis. We examine two bone tissue parameters that demonstrate the potential of this technology. First, subsurface reflection images from intact bone reveal bone cell spaces, the osteocyte lacunae, whose density is demonstrated to scale negatively with body size, reflecting aspects of metabolism and organismal life history. Second, images of a naturally fractured cross section near to Lucy’s femoral mid-shaft, which match in sign those of transmitted circularly polarized light, reveal relative collagen fiber orientation patterns that are an important indicator of femoral biomechanical efficacy. Preliminary results indicate that Lucy was characterized by metabolic constraints typical for a primate her body size and that in her femur she was adapted to habitual bipedalism. Limitations imposed by the transport and invasive histology of unique or rare fossils motivated development of the PCSOM so that specimens may be examined wherever and whenever nondestructive imaging is required.

Key words: confocal microscopy, early hominid, Australopithecus afarensis, collagen fiber orientation, osteocyte lacunae

Introduction

Most fossils are either translucent or, if they are surface reflective, are not flat. In both cases, light interacts with the sample over a considerable vertical range and is reflected (or the fluorescent light emanates) from a thick layer. The challenge we face for the nondestructive examination of unique early human fossil ancestors (hominins) is how to obtain research-grade images of microanatomical features of the skeleton in the field setting from such specimens as has been demonstrated for recent tissues (Boyde et al. 1983). We have found a solution in development of portable confocal microscopy, our objective being to image microanatomical features in fossil bones and teeth for the purpose of describing aspects of the hard tissue biology and the organismal life and evolutionary histories of our extinct ancestors.

The principle of the portable confocal scanning optical microscope (PCSOM), as for any confocal
microscope, is to eliminate the scattered, reflected, or fluorescent light from out of focus planes, allowing only light originating from the plane of focus of the objective lens to contribute to image formation. It does this at the several conjugate focal planes (each plane representing the image of the other i.e., intermediate, eye point, and image recording device), and thus eliminates light coming from all out of focus planes. In practice, an illuminated spot in the plane of focus is scanned across the field of view and an image is compiled. Confocal scanning optical microscopy thus differs from conventional light microscopy, where light from the focal plane of the objective lens, as well as from all out of focus planes across the entire field of view, is observed. The history and various technical achievements in confocal microscopy are summarized by Boyde (1995).

Studies of early hominin life history and skeletal functional adaptation typically focus on the study of bone at the organ level. There are, however, bone microanatomical features that reflect physiological processes in relation to developmental and mechanical loading histories. For instance, osteoblasts are cells that produce the bone organic matrix that is subsequently mineralized, which regularly become incorporated into the bone matrix during the growth of bone. Osteoblasts, once embedded in the bone matrix, are called osteocytes, which occupy microanatomical spaces called lacunae. Osteocyte lacunar density in mammalian bone is inversely proportional to body mass (Mullender et al. 1996), which is an indicator of organismal life history; the smaller the mammal the faster its metabolism and life history, which is reflected in faster rates of osteoblast proliferation and osteocyte lacunar density.

The preferred orientation of collagen fibers in bone is another microanatomical feature, which has been demonstrated to shed light on the biomechanical history and competence of bone (Ascenzi et al. 1967; Simkin and Robin 1974). Indeed, examinations of several material properties of bone have determined that collagen fiber orientation makes the most significant contribution to the overall strength of bone tissue (Martin and Boardman 1993; Martin and Ishida 1989; Riggs et al. 1993).

The orientation of collagen (and its associated mineral) is most appropriately investigated by circularly polarized light (CPL) microscopy of bone histological thin cross sections (e.g., 100 µm thick). Collagen fibers oriented transversely, or those perpendicular to the long axis of the bone, resist axial compressive forces and appear bright (i.e., white), those oriented in the longitudinal axis of the bone resist tensile forces and appear dark (i.e., black), and fibers having intermediate orientations appear in different levels of gray in a monochrome image. The brightness in histological thin sections observed by CPL is identical in sign to PCSOM images of the same fields of view, and though we as yet have no explanation for this similarity, it is a characteristic of PCSOM that may be exploited for interpreting collagen fiber orientations in bone.

The PCSOM provides Z-axis through focus imaging of topographically complex surfaces at relatively high magnifications revealing a plane view of fossil hominid hard tissue microstructure. Osteocyte lacunae are visible because of specularly reflected light from the inner walls of the space and crystals precipitated from ground waters within lacunae during the fossilization process. With the employ of CPL-like images, the PCSOM also provides information on subsurface collagen orientation contrast as well. Here, we examine bone microanatomical details from the femur of “Lucy,” a celebrated 3-million-year (my) fossil hominin skeleton from Ethiopia, representative of Australopithecus afarensis.

Materials and Methods

The Microscope

We employ a PCSOM based on the Nipkow disk technique (Nipkow 1884) described in detail by Petran and Hadravsky (1966) and first commercialized in the early 1980s. The Petran and Hadravsky design uses a so-called “two-sided” disk; the specimen is illuminated through an array of pinholes on one side of the disk while detected through a conjugate array of pinholes on the other (via a number of delicately aligned mirrors). Applications of this technology to bone and tooth microanatomy have been described (Boyle et al. 1983). Another Nipkow disk design (the one used here) employs a “single-sided” disk in which the illumination and detection pinhole is one in the same (Kino 1995): that is, illuminating light and its reflections from the object pass through the same pinhole, which is imaged by the eyepiece objective or camera. This latter design is robust and able to tolerate our relatively extreme portable applications.

The PCSOM is illustrated in Figure 1, which employs a one-sided Nipkow disk Technical Instrument Co. K2S-BIO confocal module (Zygo Corp., Sunnyvale, CA). Like other confocal scanning optical microscopes, the final image derives from the plane of focus, thus it eliminates the fog owing to the halo of reflected, scattered, or fluorescent light above and below the plane of focus, which otherwise confounds image content in conventional light microscopy.

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An interesting feature of the single-sided disk design used here (Kino 1995) is the approach taken to suppress internal, nonimage-related reflections that are a significant problem in this type of system; light reflecting from internal components of the microscope, having nothing to do with forming an image, degrades the image. This method is the classical method of illuminating with polarized light to stop light reflections from within the optical system (e.g., from optical hardware within the body of the microscope), but not the useful light reflecting from the specimen and returning through the objective lens. Linear polarizing light filters and a single quarter-wave plate filter, described further below, provides the means for eliminating the unwanted reflected light. A consequence of the single-sided disk design is that it significantly reduces the number of mirrors in the light path making the alignment of the optics less critical. The result is a very robustly constructed instrument able to tolerate transport and relatively rough handling (e.g., as checked-in baggage for air travel).

The microscope configurations include several other features critical to our research. Consideration was given to obtaining objective lenses with relatively long working distances (i.e., ca. 20 mm) because often we have little control over the geometry of broken fossil bone surfaces examined under remote field or museum conditions, and so we must be prepared to image through long Z-height positions to avoid interference between the fossil surface and the objective nosepiece. Objectives chosen include 5× and 10× lenses (34 and 19 mm working distances respectively; Thales-Optem Inc., Fairport, NY) and Mitutoyo 20× and 50× lenses (20 and 13 mm working distances respectively; Mitutoyo Asia Pacific Pte Ltd, Singapore). Flexibility in magnification is achieved by both the introduction of a Thales-Optem 0.5× or 1.9× CCD adapter or by converting the fixed magnification optical assembly described above into a zoom system, which involves the introduction of a Thales-Optem 70×L zoom module (1–7×) between the K2S-BIO module coupler and the manual coarse/fine focus module. For fully automated image acquisition, we motorized the Z focus (below).

Automation in X, Y, and Z axes has been variously implemented onto the PCSOM, which includes a KP53 motorized precision micro-stepping XY stage from the Semprex Corporation (Campbell, CA), and a Vexta 2-phase Z-axis stepping motor (Oriental Motor USA Corp., Torrance, CA). Integrated XYZ movement is performed by an Oasis 4i PCI stepper motor controller board for XY stage and Z focus. A three-axis trackball/mouse control of XYZ axes allows manual stage and focus movement to aid real-time viewing.

Portable image acquisition are transmitted through the FireWireIEEE 1394 digital interface now common on notebook and desktop computers, thus eliminating the need for a framegrabber. The PCSOM uses a JVC KY-F1030U 6-pin IEEE 1394 digital camera containing a \( \frac{1}{2} \) in color progressive scan interline CCD and 1360×1024 output pixels, operating at 7.5 frames per second live.

The 300W Lambda LS Xenon Arc Lamp (Sutter Instrument Company, Novato, CA) transmits a flat and intense beam of light via a liquid light guide. It operates at wavelengths suitable for both fluorescence and white light illumination (320–700 nm output in an ozone-free bulb), is robustly constructed and pre-aligned, and is economically packaged and lightweight, housing its own power supply.

A Shuttle XPC SB52G2 computer with a Pentium4 Intel processor and Windows XP Professional (Shuttle Computer Group Inc., Los Angeles, CA) supports fully automated XYZ stage movement and image acquisition. A reasonably lightweight and thin standard 1024×768 15 in monitor (Dell Inc., Round Rock, TX) was chosen for our real-time viewing.

The microscope returns image detail from a very thin optical plane at and immediately below the object surface (1–50 μm, depending upon specimen characteristics). To obtain two- or three-dimensional projections from a surface which is anything but perfectly flat, potential fields of view must be compiled from a through-series of captured images at all optical planes represented in the Z-axis. Computerized control over image acquisition using

Fig 1. Diagram of the portable confocal scanning optical microscope. See text for details.

T. G. Bromage et al.: Portable CSOM of a 3 my femur
Syncroscopy Auto Montage software (Syncroscopy Inc., Frederick, MD) permits an even and fully representative image of either a pseudo-planar field of view or a three-dimensional reconstruction of surface or subsurface details. For extensive automated XY image montaging, Syncroscopy Montage.Explorer software is employed, which can operate in “3D mode” to acquire useful Z focal planes over fields as large as 40,000 × 40,000 pixels.

A simple and lightweight stand is manufactured from aluminum and includes an upright cylinder, containing within a lead screw operable from above, which drives the Nipkow disk module platform up or down; the drive is sensitive enough to be used as a coarse focus adjustment. The cylinder inserts into a sleeve at the base from which two hollow rectangular feet slide forward and rotate out at any angle appropriate for the balance of weight and required workspace. The platform for holding the K2S-BIO attaches to a sleeve around the cylinder, which rides on a bearing that conveys the module in any rotational position within the workspace.

Microscope electronics automatically switch between 110 and 220 V electrical supplies (only the Nipow disk motor requires an optional 110/220 V adaptor), fits into two suitcases (Pelican Products, Inc., Torrance, CA), and may be set up and tested within 1 h of arrival at museum locations.

**Circularly Polarized Light**

In a conventional CPL microscope, unpolarized light passing through the linear polarizer filter becomes in-phase linearly polarized light (LPL). LPL becomes polarized into helical vibration planes when passing through a quarter wave retardation plate. A quarter wave plate has two transmission axes at 90° to one another: fast and slow. Although these axes do not diminish the intensity of incoming polarized light, they do resolve the beam into two emerging orthogonal components, one along the fast axis and one along the slow axis when the incident polarized light lies between them at 45°. LPL resolved by the quarter wave plate along the slow axis lags that of the fast axis and emerges from the filter one quarter wave length behind and out of phase; the light is no longer linearly polarized. The net vector of each component now vibrates around an elliptical axis, originally turned toward the slow axis to produce a right circular trajectory (clockwise toward the observer). This is CPL.

Polarizing filters in the K2S-BIO are arranged so that stray light within the optical system is rejected by an analyzer crossed to a polarizer. A quarter wave plate diagonally oriented between the objective front lens and the specimen surface imprints a wave shift, which results in LPL in a direction of vibration of which is rotated 90° from the original direction. This puts it parallel to the analyzer and what is transmitted forms the classic incident light bright field image at reduced glare. This filter arrangement invites an explanation for how the K2S-BIO generates reflected CPL.

CPL will refract in a birefringent material such that its elements oriented parallel with the plane of section will transmit peak light intensities irrespective of their 360° rotational position. Incident right CPL (i-RCPL) reflecting from the specimen does not maintain a right circular trajectory (as would be transmitted through a birefringent thin section observed by a typical transmitted light microscope), but reverses to take a reflected left CPL helical path (r-LCPL; clockwise away from the observer). This light is converted back into LPL as it passes once again through the quarter wave plate whose fast and slow axes are effectively reversed (extinction position) from the first, when one considers the reversal from right to left CPL. The LPL polarization sense...
is shifted 90° owing to birefringence of the collagen fibers, aligning now with the transmission axis of the analyzer.

Despite this logic, we remain unsure of its veracity. Irrespective, repeated comparisons of same field of view images of bone by conventional transmitted CPL with those obtained by the K2S-BIO reveal the same signal. Thus, for all tense and purposes, gray-level images of bone obtained by the K2S-BIO may be interpreted in respect of variation in collagen fiber orientation.

The Specimens

We examine here the right femur from the 3 my early human fossil ancestor attributed to *A. afarensis*, catalogue number AL 288-1a-p and commonly referred to as “Lucy.” The long bone shaft fragments had been previously assembled, but were de-glued by National Museum of Ethiopia staff, thus presenting a distal shaft whose intact surfaces and mid-shaft cross section were available for imaging.

Microanatomical details acquired from Lucy were compared with 67 modern human (Goldman et al. 1999) and 13 chimpanzee (*Pan troglodytes*) (McFarlin et al. 2008) mid-shaft femurs derived from individuals of partially known life history. Those employed in the determination of osteocyte lacunar density, together with a variety of other primates and nonprimate mammals, are listed in Table I. Comparisons of the distributions of Lucy’s mid-shaft collagen fiber orientations were made relative to quantitative observations represented as color-coded maps acquired from the human and chimpanzee study sample.

We cleaned all modern bone and tooth specimens with 1% Terg-a-Zyme (Alconox, NY) solution at 50°C, subject to dehydration by graded ethanol substitution and then 50:50 isopropanol:heptane reflux (intermittent exchange of fresh solution in a Soxhlet apparatus) for 7–14 days. All modern and fossil samples excepting the AL 288-1a-p hominin specimen were polymerized in plastic (poly-methylmethacrylate). Each cured block was sectioned to a uniform thickness of 100 µm, ±4 µm in most cases. AL 288-1a-p was examined by PCSOM and thus required no preparation.

Microscopy and Analysis

**Osteocyte density**

Imaging of the early hominin was obtained by PCSOM. A 270 µm field width by 200 µm field height field of view was imaged through 50 µm of focus from the first surface. The focal plane was translated vertically from the outer shaft surface into the bone cortex by ca 5 µm virtual increments; we used the formula 1.52/C² ± 3 µm absolute vertical translation, where 1.52 is taken as the refractive index (RI) of ethylene glycol coverslip medium and

<table>
<thead>
<tr>
<th>Specimen (common name)</th>
<th>Genus</th>
<th>Age and sex¹</th>
<th>Osteocyte density</th>
<th>Body mass (kg)²</th>
<th>Specimen source³</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesser Galago</td>
<td><em>Galago moholi</em></td>
<td>Adult</td>
<td>51,724</td>
<td>0.244</td>
<td>DUPC</td>
</tr>
<tr>
<td>Greater Galago</td>
<td><em>Cheirogaleus major</em></td>
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<td>31,526</td>
<td>0.4</td>
<td>DUPC</td>
</tr>
<tr>
<td>Dwarf Lemur</td>
<td><em>Otolemur crassicaudatus</em></td>
<td>Adult</td>
<td>44,353</td>
<td>1.15</td>
<td>DUPC</td>
</tr>
<tr>
<td>Vervet</td>
<td><em>Chlorocebus aethiops</em></td>
<td>Adult female</td>
<td>32,012</td>
<td>3.515</td>
<td>UCSC</td>
</tr>
<tr>
<td>Macaque</td>
<td><em>Macaca mulatta</em></td>
<td>4.16 yr female</td>
<td>22,222</td>
<td>3.0</td>
<td>MIBP</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td><em>Pan troglodytes</em></td>
<td>M2 erupted (ca. 6 yr)</td>
<td>18,706</td>
<td>33.7</td>
<td>MNH</td>
</tr>
<tr>
<td>“Lucy”</td>
<td><em>Australopithecus afarensis</em></td>
<td>Female adult</td>
<td>23,333</td>
<td>27.5¹</td>
<td>NME</td>
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<tr>
<td>Human</td>
<td><em>Homo sapiens</em></td>
<td>49 yr female</td>
<td>19,166</td>
<td>62</td>
<td>VIFM</td>
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<td><strong>Nonprimate mammal</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Rat (Wistar)</td>
<td><em>Rattus norvegicus</em></td>
<td>3 mo female</td>
<td>58,148</td>
<td>0.3</td>
<td>HTRU</td>
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<tr>
<td>Pygmy Hippo</td>
<td><em>Phanourious minutus</em></td>
<td>Adult</td>
<td>23,641</td>
<td>200¹</td>
<td>TRNC</td>
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<tr>
<td>Hippo</td>
<td><em>Hippopotamus amphibius</em></td>
<td>Adult</td>
<td>16,667</td>
<td>2,000¹</td>
<td>HLD</td>
</tr>
</tbody>
</table>

¹Age and sex are given when known. No adult specimen was geriatric.
²Except where indicated, body mass was measured directly from study individuals.
³DUPC, Duke University Primate Center; HLD, Hessisches Landesmuseum Darmstadt; HTRU, Hard Tissue Research Unit, NYUCD; MIBP, Morgan Island Breeding Program, Yemassee, South Carolina; MNH, Museum für Naturkunde der Humboldt Universität; NME, National Museum of Ethiopia; TRNC, Turkish Republic of Northern Cyprus, Department of Antiquities; UCSC, Sherwood L. Washburn collection, University of California at Santa Cruz; VIFM, Victorian Institute of Forensic Medicine, Melbourne, Australia.
⁴Smith et al. (2002).
⁵Jungers (1982).
⁶Simmons (1999).
⁷Eltringham (1999).
a 170 μm thick coverslip. The through-series of images from each ca. 5 μm virtual focal plane was acquired using Syncroscopy Automontage until 50 μm depth had been achieved. A red-green anaglyph image with 20° angular separation was calculated from this series with Automontage.

Imaging of the comparative samples was performed with the Edge R400 real time 3D microscope, configured to provide transmitted oblique CPL contrast when useful. Histological sections of 100 ±4 μm were coverslipped with ethylene glycol, and XYZ fields of view conforming to 270 μm field width by 200 μm field height, respectively, and containing no dehydration cracks or vascular canals were located. Subsequent imaging procedures followed the same PCSOM imaging routine.

Each through-focus series of images was presented for analysis on a PC configured with dual monitors. The Automontage through series was placed on one monitor, whereas the anaglyph image was opened separately in Adobe Photoshop CS2 (San Jose, CA) on the other. This side-by-side arrangement allowed the viewing of lacunae with red-green anaglyph glasses through the full 3D thickness of the histological section while also scrolling through and cross checking source image details in Automontage.

All lacunae were counted for each XYZ volume according to standard stereological procedure; those intersecting each of one X, Y, and Z surface were labeled as excluded, while those intersecting their contralateral X, Y, and Z surfaces were labeled as included. After all lacunae had been labeled, they were manually counted.

For each histological thin section a single XYZ volume was selected wherever a complete field could be located without vascular canals or cracks. The periosteal cortex was imaged in all individuals, though for purposes of another study on femoral biomechanics, five periosteal and five endosteal volumes were measured around the entire cross section of the human sample, which were averaged into the value given in Table I. All measurements were standardized by simple extrapolation of the number of lacunae per measured bone volume, typically from a 5,400,000 μm cubic measurement volume, to a 1 mm³ unit value.

Collagen fiber orientation

Imaging of the early hominin was obtained by PCSOM. The distal AL 288-1a-p shaft was situated upright so that the naturally fractured surface was more or less plane to the incident beam. There was, however, an approximately 1 cm relief that had to be negotiated by extended focus to be presented as a single two-dimensional image. Extensive automated XYZ image montaging was performed by Syncroscopy Montage Explorer software control of the XYZ stage, which was operated in “3D mode” to acquire all pertinent Z focal planes using the Z-axis stepping motor.

Images of whole histological cross sections obtained on the comparative human and chimpanzee specimens were acquired by transmitted CPL microscopy using a Leica DMRX/E Universal microscope (Leica Inc., Wetzlar, Germany) configured with an automated Marzhauser stage controlled by Syncroscopy Montage Explorer.

Results

A count of 63 osteocytes was made in the sample volume of AL 288-1a-p, which extrapolates to 23,333 osteocytes per mm³ (Fig. 3), which is consistent with her body size of 27.5 kg. Osteocyte lacuna densities calculated from several comparative primate and nonprimate mammals are presented in Table I and graphically represented in Figure 4. It is noted that the primate regression of osteocyte lacuna density vs. body mass falls below that of a generalized mammalian relationship, which indicates that, for their body size, primates incorporate fewer osteoblasts into the bone matrix than other mammals.

Quantitative analyses of CPL employ a simple method for mapping preferential collagen fiber orientation patterns in whole cross sections (Boyde and Riggs 1990). Eight-bit gray-scale images are visualized and pixels distributed by color into eight gray-level bins. Although the comparative humans and chimpanzees were so evaluated, interpretations of whether the AL 288-1a-p bone cortex contains primarily transversely or longitudinally oriented collagen is qualitative, because it is not now possible to arrive at a quantitative solution to the results owing to the vagaries of fossil preservation (Bromage et al. 2003). Hence, the comparison between fossil and modern specimens is relative.

Readily apparent from color-coded gray level images of collagen fiber orientation, where cool colors denote collagen lying longitudinal with the axis of the femur and hot colors represent collagen lying transverse to the femoral axis, the human medial femoral cortex (that cortex along the inner leg) contains relatively more transverse collagen than other cortices (Fig. 5). This is interpreted to mean that the medial cortex experiences significant alignment of collagen to resist compression during locomotory function. The chimpanzee anterior (front), lateral (outer leg), and posterior (rear) cortices are impressively dark, though the intermediate posterior-medial cortex does contain some transverse collagen (Fig. 6). The entire AL 288-1a-p cross section was imaged, providing basic details of the microanatomy (Fig. 7). Preliminary examination of the fossil hominid femur at higher magnification illustrates
that it is more similar to that of a modern human femur, containing more transverse collagen, particularly in the medial cortex than elsewhere (Fig. 8).  

Discussion

There is much interest in obtaining details of early hominid skeletal microanatomy from natural
and fractured surfaces, but such surfaces are rarely giving of all the desired detail. The resolving power, such as that of stereo-zoom microscopy, and the recovery of microanatomical detail from significantly below the surface by scanning electron microscopy, has been wanting. With the development and employ of portable circularly polarized confocal scanning optical microscopy, we are now able to resolve microanatomical details relevant to reconstructions of early hominid development and functional life history.

It is now possible to use bone microanatomy to place early hominids in the organismal life history gamut (Harvey and Clutton-Brock 1985). For instance the lower height of the primate regression of osteocyte lacuna density vs. body mass (Fig. 4) agrees with growth law predictions indicating that primate production energy (i.e., that energy required to develop body mass) is significantly less compared with other mammals of the same body size (Charnov and Berrigan 1991). These predictions are part of a larger model, which explains that growth energy determines also how much energy is available for reproduction later. This suggests that energy-limited rates of production, bone and body size, and reproduction are centrally linked to the regulation of organismal life history according to metabolic constraints (Brown and Sibly 2006).

Collagen fiber orientations in humans are complex because of the variety of axial and compound forces during the various stages of bipedal locomotion, but in general the anterior and the intermediate anterior–lateral aspects of the cortex are subject to tension and the posterior–medial and medial aspects are subject to compression, which more or less agrees with the average human fiber distribution (Goldman et al. 2003). The posterior cortex is consistently dark, however, because of the net tensile influence of muscles attaching by collagen fibers at a high near-longitudinal angle to the femur shaft. Of note, the load axis of a chimpanzee femur is entirely medial to the shaft, thus some resistance to compressive strain may be expected on the medial cortex as would be resistance to tension on the lateral cortex, as reflected in their preferred fiber distributions (McFarlin et al. 2008). That the AL 288-1a-p bone cortex contains a modicum of transversely oriented collagen in all anatomical sectors indicates that Lucy was adapted to habitual bipedalism and unlike the locomotory strategies of living apes.

Conclusion

The portable confocal scanning optical microscope was specifically developed to offer superb analytical light microscopy of early hominid skeletal material. The microscope is, in the main, a heuristic device, enabling us to access and learn about the microstructures of objects normally out of reach from such instrumentation. Because it is both a superb circularly polarized light and reflection microscope, we have added new information on developmental and functional processes in the bone tissues of an early human ancestor.
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