A Procedure for Removing the Blurring in Microdensitometric Analysis*

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Summary: The bone width of the femur and fibula was measured by three techniques, osteometry, radiogrametry and microdensitometry, in six adult crab-eating monkeys (Macaca fascicularis). The measurement was taken five times at midshaft and at proximal and distal epiphyseal sites in each bone. Based on the paired t test, the bone width obtained microdensitometrically was significantly greater than that measured by other techniques (p < 0.05). It seems to be one of the causative factors for these differences that X-rays produce a blurring zone around the shadow of an original object on the film. The blurring is not visually identified, but it can be distinguished from the real image in microdensitometric analysis. A procedure was devised for removing errors by the blurring in microdensitometric measurement of the bone width. According to its algorithm, a program was assembled using a personal computer.

In microdensitometry (MD), some parameters are specified on the MD pattern scanned with a microdensitometer on a X-ray film. These parameters are classified into two categories, ones related to bone density such as the maximum and minimum heights and total area of the MD pattern, and the others related to the bone size such as bone width and cortical thickness. Some of the latter are measured with proper instruments on the bone or on its film directly. For example, the bone width can be obtained by three techniques, osteometry, radiogrametry and microdensitometry. However, even on the same sample, its value is not necessarily identical by these techniques of measurement. The MD analyzing system is usually constituted the core microdensitometer with communicated a personal computer. In an established application for the MD works, the data scanned with the microdensitometer are transferred into a computer and analyzed automatically in mm standardized Al equipment values (SAE). Investigators obtains only the analyzed results from the computer, but they are often unknown of a calculating procedure in the computer.

Material and Methods

Six femora and fibulae of two males and four female monkeys (Macaca fascicularis) were used for this study. The third molar was completely erupted in all animals. Sex differences were not mentioned here.

Three measuring sites were marked with a pencil on each bone at the midshaft and at the proximal and distal epiphyseal regions (Figure 1-a). The proximal and distal epiphyseal points are located on the lines run transversely at the distal end of the lesser trochanter and the superior border of the patellar surface in the femur, and at the proximal and distal thinnest necks medially laterally in the fibula, respectively.

Radiographs of the bone were taken with the ultra soft X-ray apparatus (Softex, type C-SM). The bone was laid on the film cassette inserted the sheet film, contacting with its posterior surface at the major trochanter and the medial and lateral condyles in the femur, and all over in the fibula. Each measuring sites were indicated with steel pins affixed on the bone (Figure 1-b). Two type sheet film, FR and FG (Fuji), were used. While the sensitizer is spread only on one side of the base in the film FG which is not on the market, it is spread on both sides of the base in its improved one, the film FR.

The midpoint of bones was carefully positioned in the center of divergent area of X-ray beams. The film face was kept at right angles to the long axis of the
X-ray beam. In each bone, the standard X-ray exposure condition is as follows:

<table>
<thead>
<tr>
<th>exposure factors</th>
<th>femur</th>
<th>fibula</th>
</tr>
</thead>
<tbody>
<tr>
<td>voltage</td>
<td>45kVp</td>
<td>40kVp</td>
</tr>
<tr>
<td>ampire</td>
<td>3.0mAs</td>
<td>2.5mAs</td>
</tr>
<tr>
<td>exposing time</td>
<td>120sec</td>
<td>80 sec</td>
</tr>
<tr>
<td>focal film distance</td>
<td>72cm</td>
<td>72cm</td>
</tr>
</tbody>
</table>

The exposure factors were appropriately varied to the bone size in each case. The exposed sheet films were developed manually in normal process; 3 minutes in development, 1 minute in washing and 5 minutes in fixation. The solution for development and fixation were kept a constant temperature of 20°C.

We used a microdensitometer system, UltroScan XL Laser Densitometer Type 2222-020 (LKB Produkt, Sweden) contacted with the IBM personal computer PC/AT. The microdensitometer scans on the film with a 100 μm diameter spot beam and outputs one datum at 20 μm intervals. These data were recorded by two ways; one is a polygraph drawn onto a line printer, called the MD pattern, and other is digital data transferred into the computer, called optical density value (OD value) by Inoue et al. (1984).

The bone width was measured by three techniques at three sites in each bone. The osteometric measurement (OS) was taken on the bone with a sliding caliper directly, determined to the nearest 0.05mm, and the radiogrametric (RA) was using the digitizer (Professional Digitizer, Mutoh Comp.) on the film, graded to the 0.001mm, and the microdensitometric (MD) was calculated from the OD value. The OD value always indicates 0 (zero) on the base line. However, it changes to a plus value at the points A and B on the MD pattern. These points can be clearly identified by the computer analysis (Figure 2). When the points A and B are the mth and nth data respectively, the bone width is calculated according to the following formula:

\[
\text{Bone Width (in } \mu\text{m}) = \text{absolute}(n-m) \times 20 \mu\text{m} \ (n > m)
\]

Measurement was taken at three sites for each bone of 6 samples five times over. Thus, the means and standard deviations were calculated from 30 determinations in three techniques.

**Results and Discussions**

Table 1 shows the means and standard deviations...
of the bone width at the proximal site, midshaft and distal site in the OS, RA and MD, respectively. The paired t test was used to assess the difference between the means of two samples in the present paper.

1. Comparison of the films FR and FG in radiogrametry and microdensitometry.

In radiogrametry, although the FG value was arithmetically somewhat greater than the FR in the femur at least, no significant differences were found between the FR and FG values in both the femur and fibula. The level of probability ranged 0.486 to 0.813 in the femur and 0.652 to 0.966 in the fibula based on the paired t test. Thus, in the following discussion, the results of the film FR were referred only.

In microdensitometry, the FG value was somewhat greater than the FR in both femur and fibula. For the difference between the FR and FG values, the levels of probability by the paired t test between the FR and FG values were under 0.001 at the proximal site, 0.01 at the mid-shaft and 0.01 at the distal site of the femur, and 0.038, under 0.001 and 0.039 at each sites of the fibula respectively. Apparently, significant differences are found between the mean values obtained from two types of film at the 0.05 significance level.

Some differences such as the thickness of the sensitive film base, the particle size and thickness of sensitizer may be causally related to these differences.

2. Osteometry and Radiogrametry.

Table 2 compares the RA and MD values with the OS value. No significant differences are found between the RA and OS values at each site of both bones at the 0.05 significance level, though the RA value is somewhat greater than the OS at all sites of the femur and at mid-shaft of the fibula. The RA/OS ratio ranges from 97.54 to 101.31. Usually, on the film, a shadow becomes larger in size than its original object, as it is projected by radiated X-ray beam. As the measuring point of the bone is apart from the film or from the center of the X-ray beams, the difference between a real position and its image becomes greater. According to Engström (1962), an extending ratio of the projective shadow (M) calculated the following function;

\[ M = \frac{(c+d)}{c} \]

The d means a distance from the superior surface of an object to the sensitizer on the film base and the c a distance from the focus to the superior surface of an object.

At the proximal and distal sites of the fibula, the RA value is slightly smaller than the OS. In the fibula, because the cortex becomes thin towards the epiphyses, its image is projected more darkly on the film at the epiphyseal end than at the mid-shaft. “When two faces are bordered with the marked differences in the

\[
\begin{array}{|c|c|c|c|c|c|c|}
\hline
\text{bone} & \text{film} & \text{proximal} & & \text{midshaft} & & \text{distal} & \\
\hline
\text{Osteometry} & & & & & & \\
\text{femur} & & 9.27 & 0.75 & 8.75 & 0.66 & 13.43 & 0.88 \\
\text{fibula} & & 4.86 & 0.52 & 3.88 & 0.19 & 4.60 & 0.34 \\
\text{Radiogrammetry} & & & & & & \\
\text{femur} & FR & 9.28 & 0.70 & 8.79 & 0.67 & 13.59 & 0.66 \\
\text{FG} & 9.34 & 0.67 & 8.84 & 0.70 & 13.56 & 0.79 \\
\text{fibula} & FR & 4.77 & 0.53 & 3.89 & 0.17 & 4.49 & 0.40 \\
\text{FG} & 4.77 & 0.55 & 3.92 & 0.20 & 4.50 & 0.43 \\
\text{Microdensitometry} & & & & & & \\
\text{femur} & FR & 11.82 & 0.78 & 10.53 & 0.87 & 14.80 & 0.99 \\
\text{FG} & 12.73 & 0.65 & 11.67 & 0.93 & 15.93 & 1.02 \\
\text{fibula} & FR & 6.82 & 0.93 & 6.87 & 0.54 & 8.35 & 1.20 \\
\text{FG} & 7.51 & 1.07 & 7.54 & 0.32 & 9.02 & 1.03 \\
\hline
\end{array}
\]

\( n = 30 \)
Table 2. The comparison with the osteometry (OS) and radiogrametry (RA), the microdensitometry (MD).

<table>
<thead>
<tr>
<th>bone</th>
<th>site</th>
<th>difference</th>
<th>ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA/OS</td>
<td>femur</td>
<td>OS minus RA</td>
<td>RA to OS</td>
<td></td>
</tr>
<tr>
<td>proximal</td>
<td>-0.01 ± 0.20mm</td>
<td>100.19 ± 2.47</td>
<td>0.951 -</td>
<td></td>
</tr>
<tr>
<td>midshaft</td>
<td>-0.04 ± 0.09</td>
<td>100.47 ± 1.13</td>
<td>0.813 -</td>
<td></td>
</tr>
<tr>
<td>distal</td>
<td>-0.16 ± 0.43</td>
<td>101.31 ± 3.29</td>
<td>0.438 -</td>
<td></td>
</tr>
<tr>
<td>fibula</td>
<td>proximal</td>
<td>0.09 ± 0.11</td>
<td>98.0 ± 4.44</td>
<td>0.500 -</td>
</tr>
<tr>
<td>midshaft</td>
<td>-0.01 ± 0.05</td>
<td>100.39 ± 1.35</td>
<td>0.772 -</td>
<td></td>
</tr>
<tr>
<td>distal</td>
<td>0.11 ± 0.14</td>
<td>97.54 ± 3.31</td>
<td>0.264 -</td>
<td></td>
</tr>
<tr>
<td>MD/OS</td>
<td>femur</td>
<td>OS minus MD</td>
<td>MD to OS</td>
<td></td>
</tr>
<tr>
<td>proximal</td>
<td>-2.55 ± 0.18</td>
<td>127.69 ± 2.88</td>
<td>0.001 ***</td>
<td></td>
</tr>
<tr>
<td>midshaft</td>
<td>-1.78 ± 0.39</td>
<td>120.35 ± 3.95</td>
<td>0.001 ***</td>
<td></td>
</tr>
<tr>
<td>distal</td>
<td>-1.37 ± 0.57</td>
<td>110.25 ± 4.21</td>
<td>0.001 ***</td>
<td></td>
</tr>
<tr>
<td>fibula</td>
<td>proximal</td>
<td>-1.95 ± 0.68</td>
<td>140.25 ± 14.3</td>
<td>0.001 ***</td>
</tr>
<tr>
<td>midshaft</td>
<td>-2.99 ± 0.63</td>
<td>177.61 ± 18.6</td>
<td>0.001 ***</td>
<td></td>
</tr>
<tr>
<td>distal</td>
<td>-3.75 ± 1.04</td>
<td>180.74 ± 19.4</td>
<td>0.001 ***</td>
<td></td>
</tr>
</tbody>
</table>

n = 30

darkness, the dark face is recognizes more dark and the light face is lighter (Hoshino, 1974).” He called it a visual edge effect.

3. Osteometry and Microdensitometry.

The MD value is compared with the OS in Table 2. The MD values was significantly greater than the OS at each measuring site of both bones (p<0.001), especially in the fibula. The MD/OS ratio ranged from 110.25 to 180.74. Its caution may be principally on the “blurring” around the real image, though the blurring is hardly recognized on the soft X-ray film compared with on the hard X-ray film. According to Engström (1962), an extent of the blurring calculated the following function;

\[ \Delta u = W(d + s) / c \]

The \( \Delta u \) means an extent of blurring, the \( W \) a diameter of the focus, the \( d \) a distance from the superior surface of object to the sensitizer on the film base, the \( s \) a thickness of sensitizer, the \( c \) a distance from the focus to the superior surface of object. As the blurring region is extended irregularly in each film, it could not be distinguished in naked eyes from the density of non-shadow of an object on the film. With a high precisional microdensitometer, the blurring region is recorded in the data. Thus, the MD values may be estimated to be greater than the value of true image.

4. Treatment for the blurring.

As shown in Figure 2, after gentle slopes at both ends (A-C and B-D), the MD pattern arises suddenly from the points C and D. The data output from the microdensitometer were analyzed using a personal computer for finding the start (A and B) and end (C and D) points of the blurring region. A set of N measurements from the microdensitometer were transferred into the personal computer. An individual datum of them was denoted by \( X_1, X_2, X_3, \ldots, X_n \). Figure 3 presents a variation curve of the difference between adjoining OD values (\( d_i = X_k - X_{k-1} \), \( k:1-n \)). The \( d_i \) value becomes greater abruptly at the border from the blurring region into the real image, showing two peaks C’ and D’. These points probably correspond to the points C and D in Figure 2, respectively. It is estimated that the distance between the points C’ and D’ gives a real bone width. The results were shown in Tables 3 and 4. There are no significant differences between the OS and revised MD values at the midshaft in the femur and fibula and at the proximal in the femur (p<0.05). Although significant differences are found between the OS and revised MD values at other sites, the revised MD value becomes to be close to the OS. Its ratio to the OS value is still about 113% at the proximal site of the fibula. The shadow extends on the X-ray film (Engström, 1962).

It seems that this treatment provides a simple, ob-
Table 3. The means and standard deviations of the revised bone width, the distance between C' and D' in Fig. 3 (in mm).

<table>
<thead>
<tr>
<th>bone</th>
<th>site</th>
<th>proximal mean</th>
<th>proximal SD</th>
<th>midshaft mean</th>
<th>midshaft SD</th>
<th>distal mean</th>
<th>distal SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>femur</td>
<td>FR</td>
<td>9.52</td>
<td>0.67</td>
<td>8.80</td>
<td>0.68</td>
<td>14.20</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>9.60</td>
<td>0.71</td>
<td>8.83</td>
<td>0.69</td>
<td>14.32</td>
<td>0.89</td>
</tr>
<tr>
<td>fibula</td>
<td>FR</td>
<td>5.53</td>
<td>0.84</td>
<td>3.93</td>
<td>0.20</td>
<td>5.11</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>5.69</td>
<td>0.83</td>
<td>3.95</td>
<td>0.22</td>
<td>5.12</td>
<td>0.41</td>
</tr>
</tbody>
</table>

n = 30

Table 4. The comparison of revised microdensitometry (RE) with the bone width by osteometry (OS).

<table>
<thead>
<tr>
<th>bone</th>
<th>site</th>
<th>OS minus RE</th>
<th>RE to OS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>femur</td>
<td>proximal</td>
<td>-0.26 ± 0.26mm</td>
<td>102.95 ± 2.34</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>midshaft</td>
<td>-0.06 ± 0.08</td>
<td>100.57 ± 0.97</td>
<td>0.772</td>
</tr>
<tr>
<td></td>
<td>distal</td>
<td>-0.77 ± 0.21</td>
<td>105.77 ± 1.64</td>
<td>0.002 ***</td>
</tr>
<tr>
<td>fibula</td>
<td>proximal</td>
<td>-0.67 ± 0.44</td>
<td>113.47 ± 8.22</td>
<td>0.005 ***</td>
</tr>
<tr>
<td></td>
<td>midshaft</td>
<td>-0.05 ± 0.06</td>
<td>101.37 ± 1.63</td>
<td>0.292 -</td>
</tr>
<tr>
<td></td>
<td>distal</td>
<td>-0.50 ± 0.26</td>
<td>111.09 ± 6.31</td>
<td>0.001 ***</td>
</tr>
</tbody>
</table>

n = 30

An objective and representative way to divide at the border from the blurring to the real image of bones. Finally, we present an example program by Turbo Pascal for a personal computer for this procedure in Figure 4.

References

Program DIFFERENCES_of_OD_VALUE;

type DIM = Array [1..1000] of Integer;

var
  FILE_NAME : String[20];
  DRIVE_NO : Char;
  FYLE : String[50];
  FILE_VAR : File of Integer;
  I, J, N, COUNTER, MidPoint : Integer;
  STARTING, ENDING : Integer;
  X, MAX : Integer;
  BW : Real; ( Bone Width )
  Z : DIM;

Begin
  ClrScr; LowVideo;
  Write('< < < Read in the Optical Density Value');
  Writeln(' from the Densitometer > > > ');

  NormVideo; Writeln;
  Write('File_Name:  '); Readln(FILE_NAME);
  Write('Drive  No:  '); Readln(DRIVE_NO);
  LowVideo;

  FYLE  := Concat(DRIVE_NO,':V',FILE_NAME);
  Assign(FILE_VAR, FYLE);
  (SI-)

  Reset(FILE_VAR);
  (SI+)
  While IOresult <> 0 do begin
    Writeln(t$7); ( Bell )
    Writeln('File < ', FYLE, ' > not Exist. ');
    NormVideo; Writeln;
    Write('File_Name:  '); Readln(FILE_NAME);
    Write('Drive  No:  '); Readln(DRIVE_NO);
    LowVideo;
    FYLE  := Concat(DRIVE_NO,':V',FILE_NAME);
    Assign(FILE_VAR, FYLE);
    (SI-)

    Reset(FILE_VAR);
    (SI+)
  end; [ of While IOresult ]

  (File Operation )

  N := FILESIZE(FILE_VAR);

  ClrScr; LowVideo; Clear Screen
  Write('< < < Read in the Optical Density Value');
  for J:=1 to N-1 do begin
    Seek(FILE_VAR,J); Read(FILE_VAR,I);
    GotoXY(10,10); Write('Operating Data No:',J:3,', Now ... ');
    GotoXY(10,16);
    Writeln('Optical Density =',I:5);
    if I>0 then begin
      COUNTER:=COUNTER+1;
      Z[COUNTER] := I
    end ( of if I )
  end ( of for J )

  Close(FILE_VAR); File Close

  BW:= N*0.02; Data_No * 20 Micron
  Writeln;

  Write ('Bone Width except Base_Data ...... ');

  BW:=Abs(ENDING-STARTING)*0.02; Data_No * 20 Micron
  Writeln;

  Write ('Bone Width after Procedure ......... ');