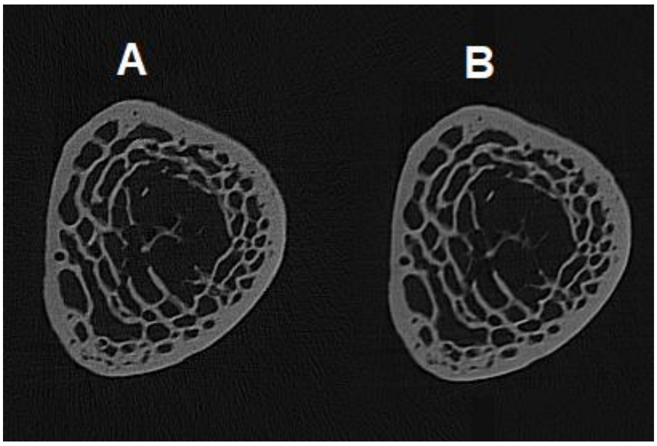
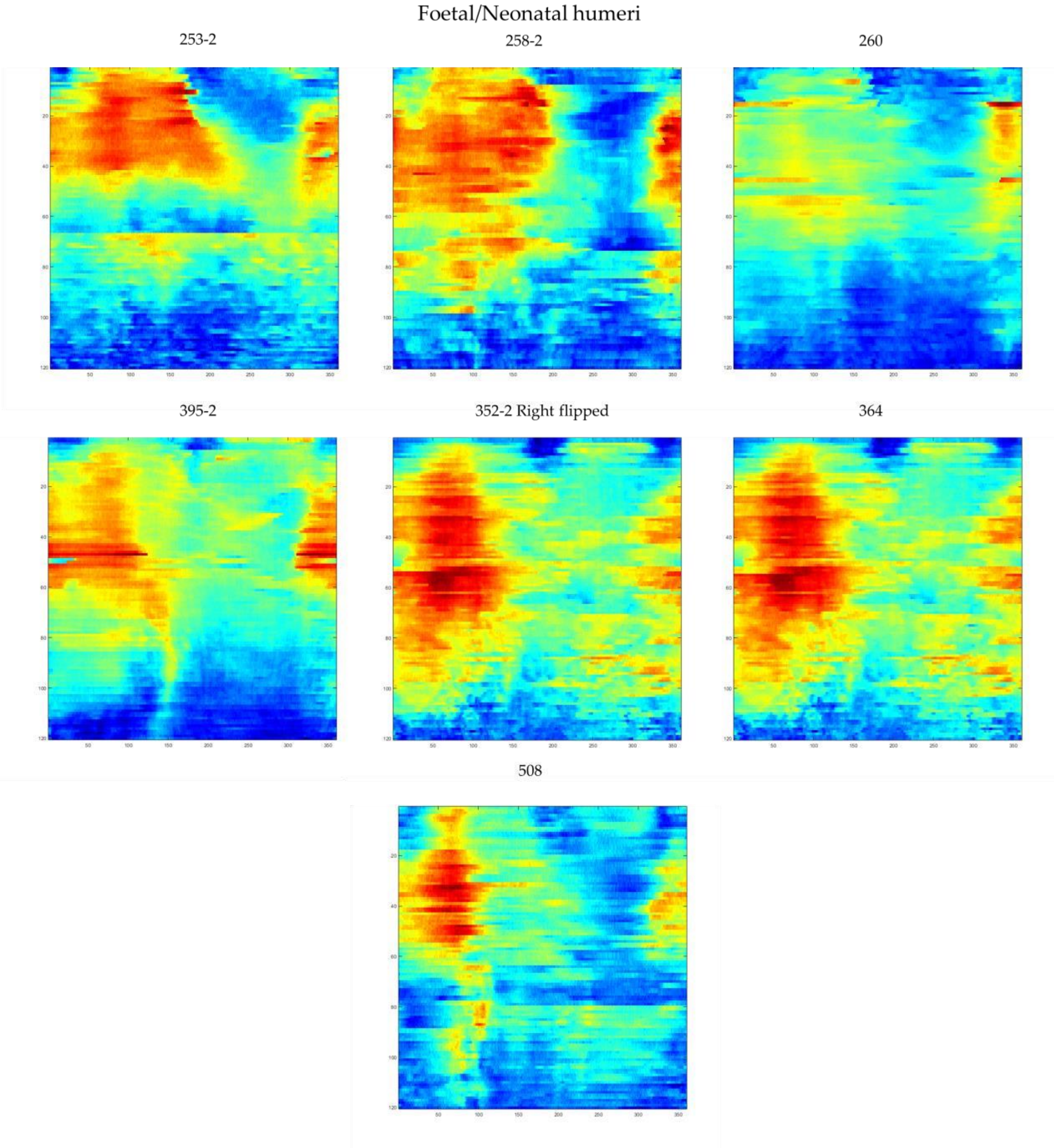
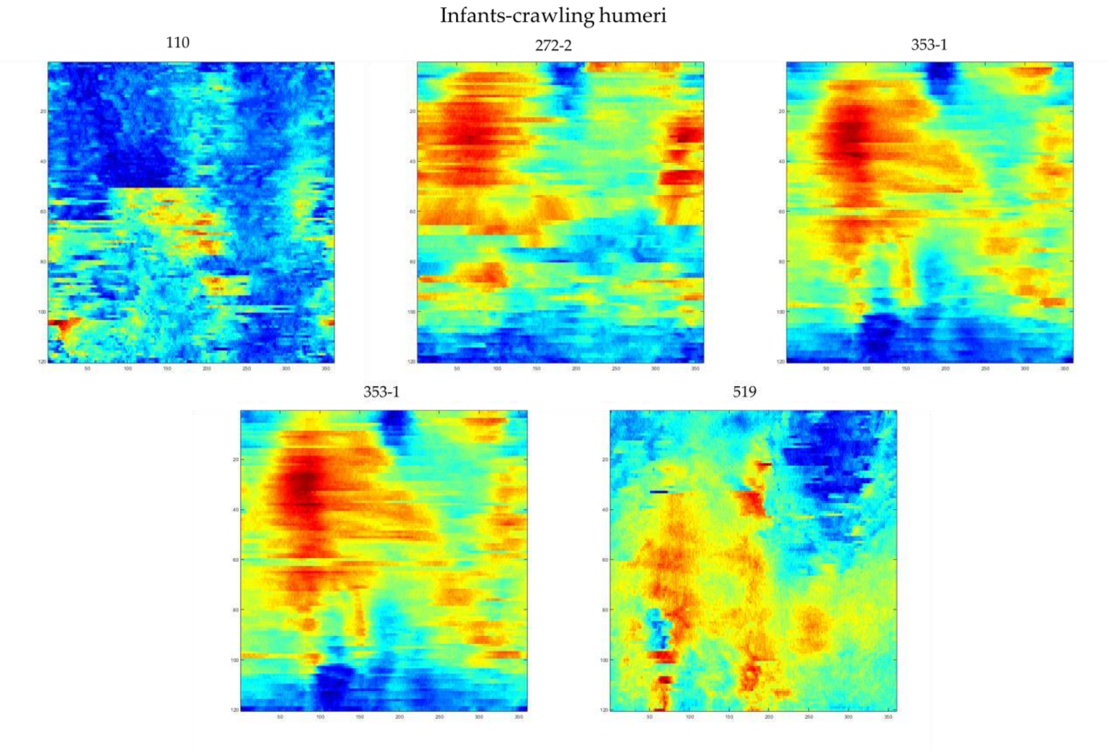
1.1 S1. Example of downsampling

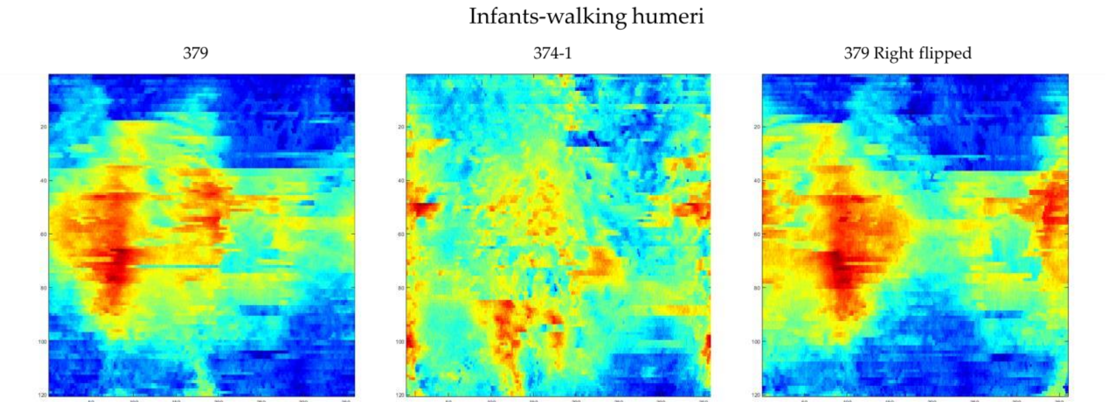


Effects of downsampling. A: Original resolution slice, B: Downsampled slice. As downsampling was in the Z plane, the downsampling had little effect on X-Y resolution.

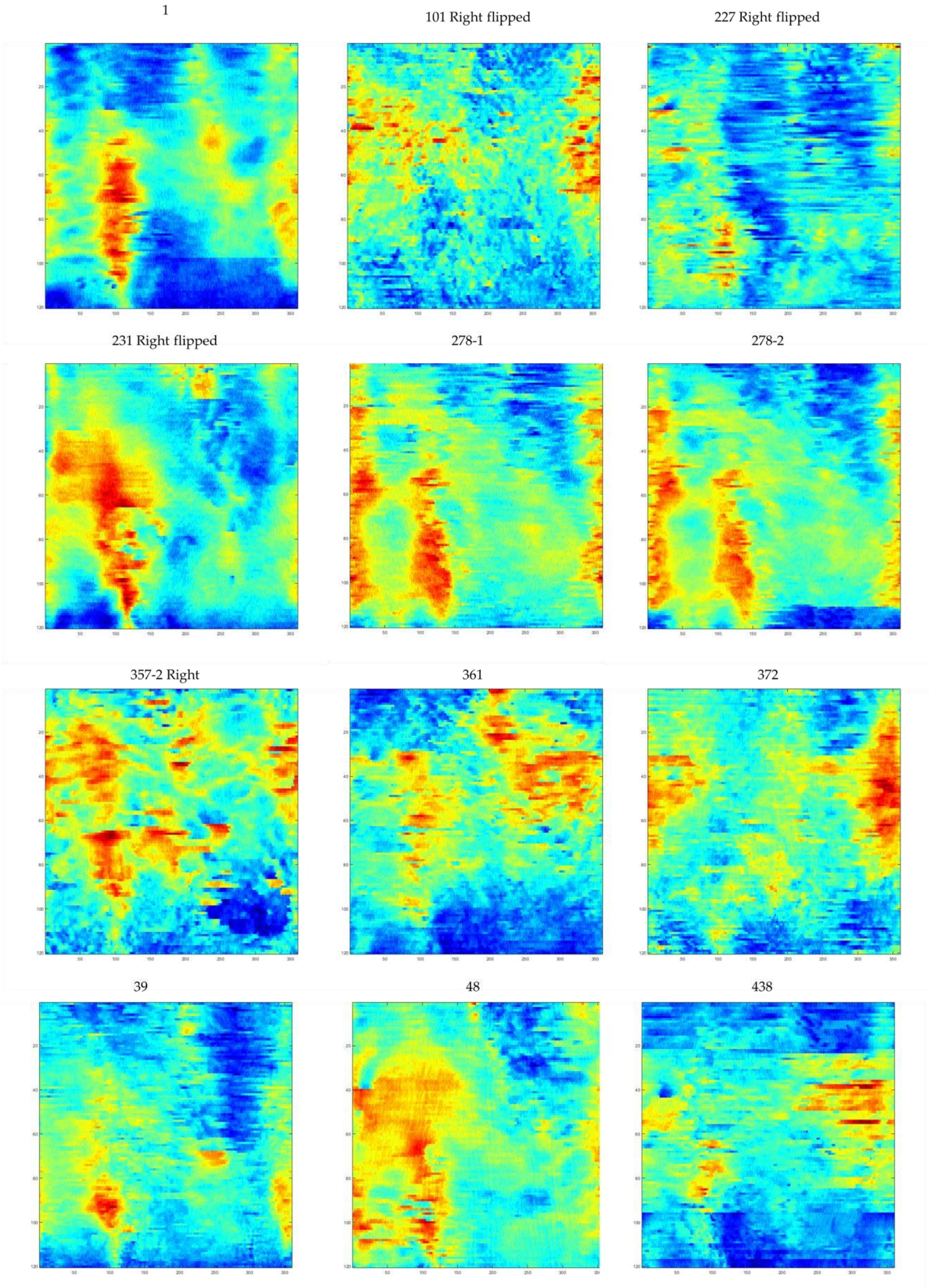
1.2 S2. Cortical thickness maps for all specimens

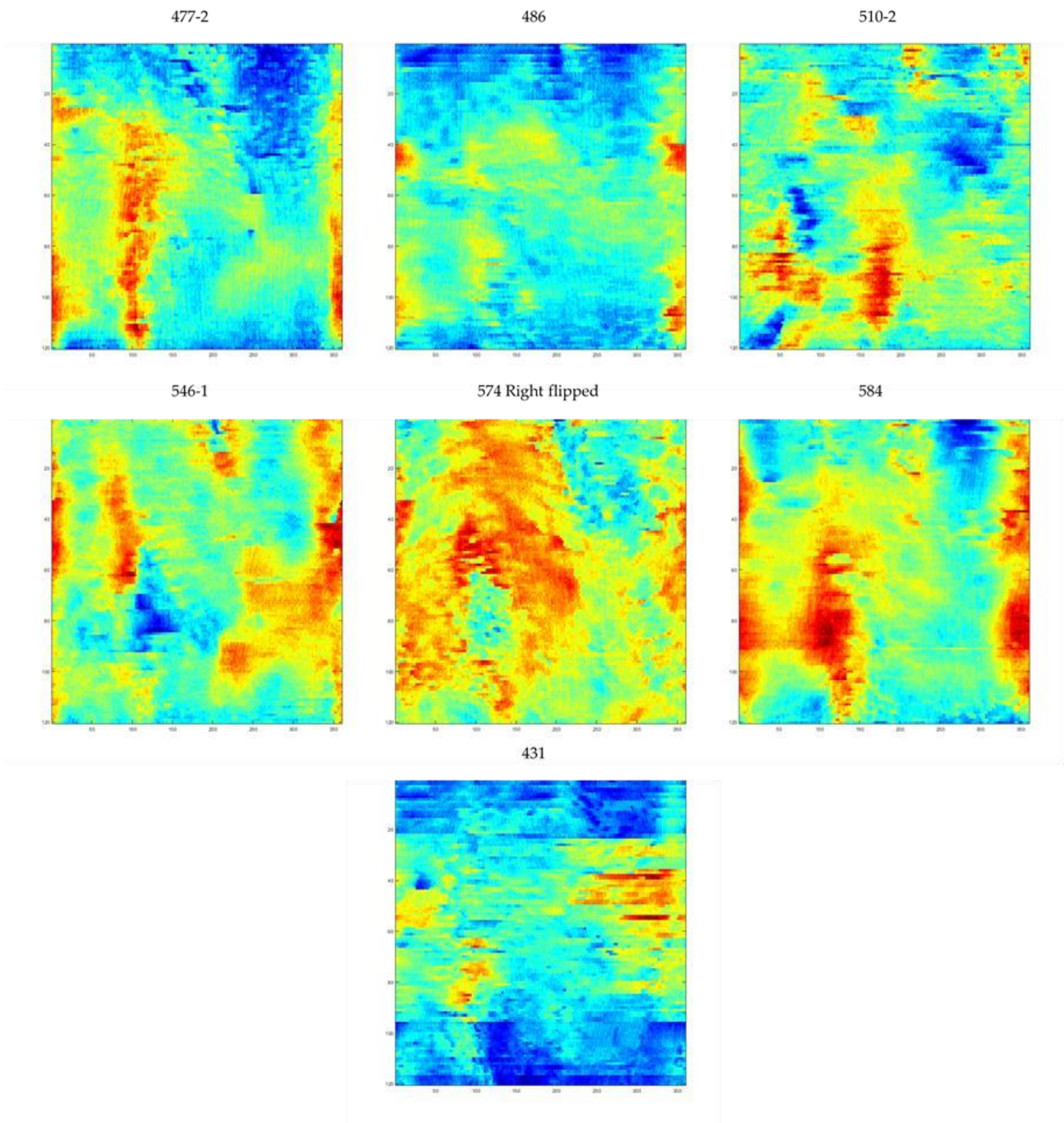



Infants-crawling humeri
110
272-2
353-1
353-1
519

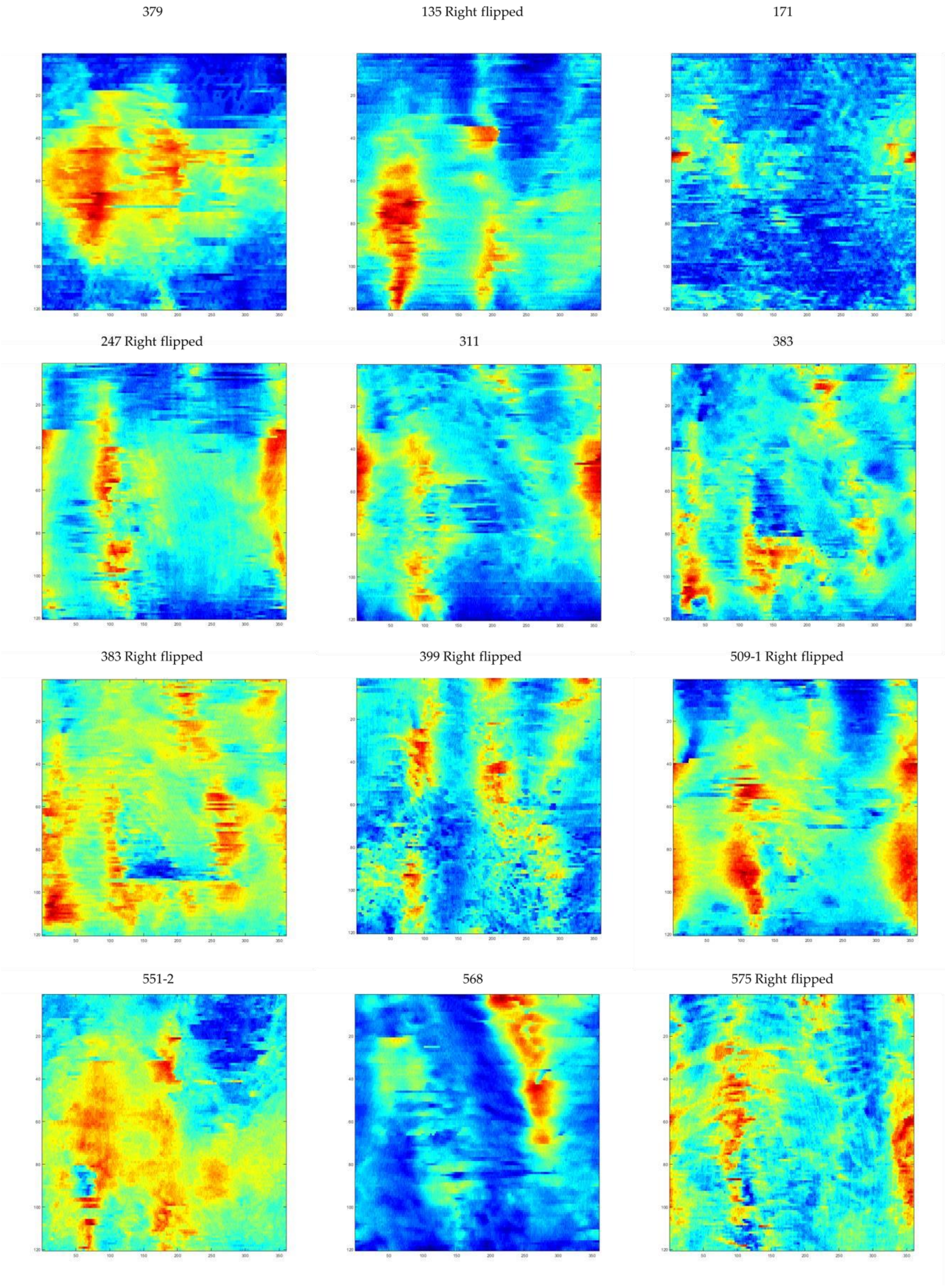

Infants-walking humeri
379
374-1
379 Right flipped

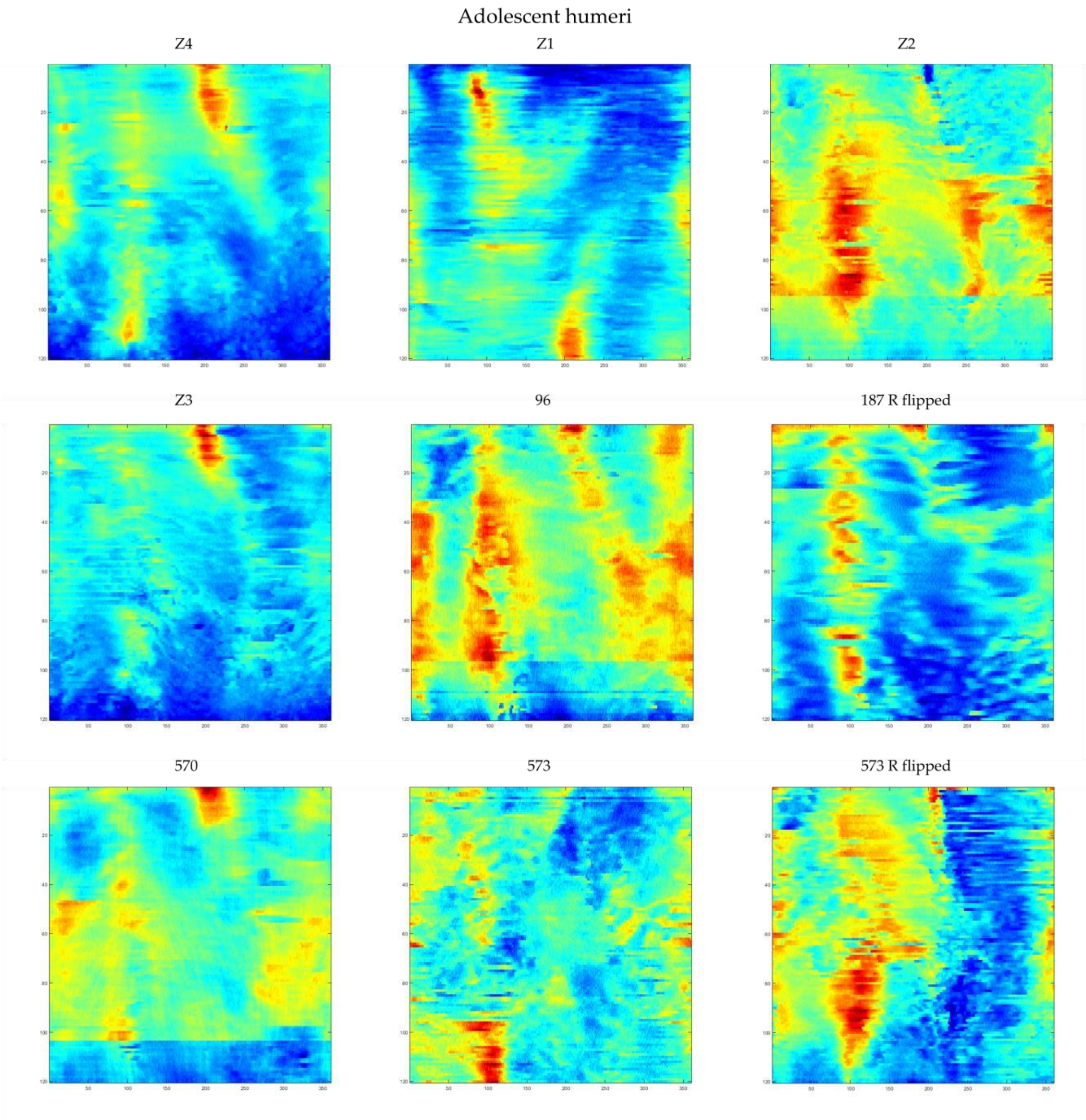
Younger child humeri




477-2
486
510-2
546-1
574 Right flipped
584
431

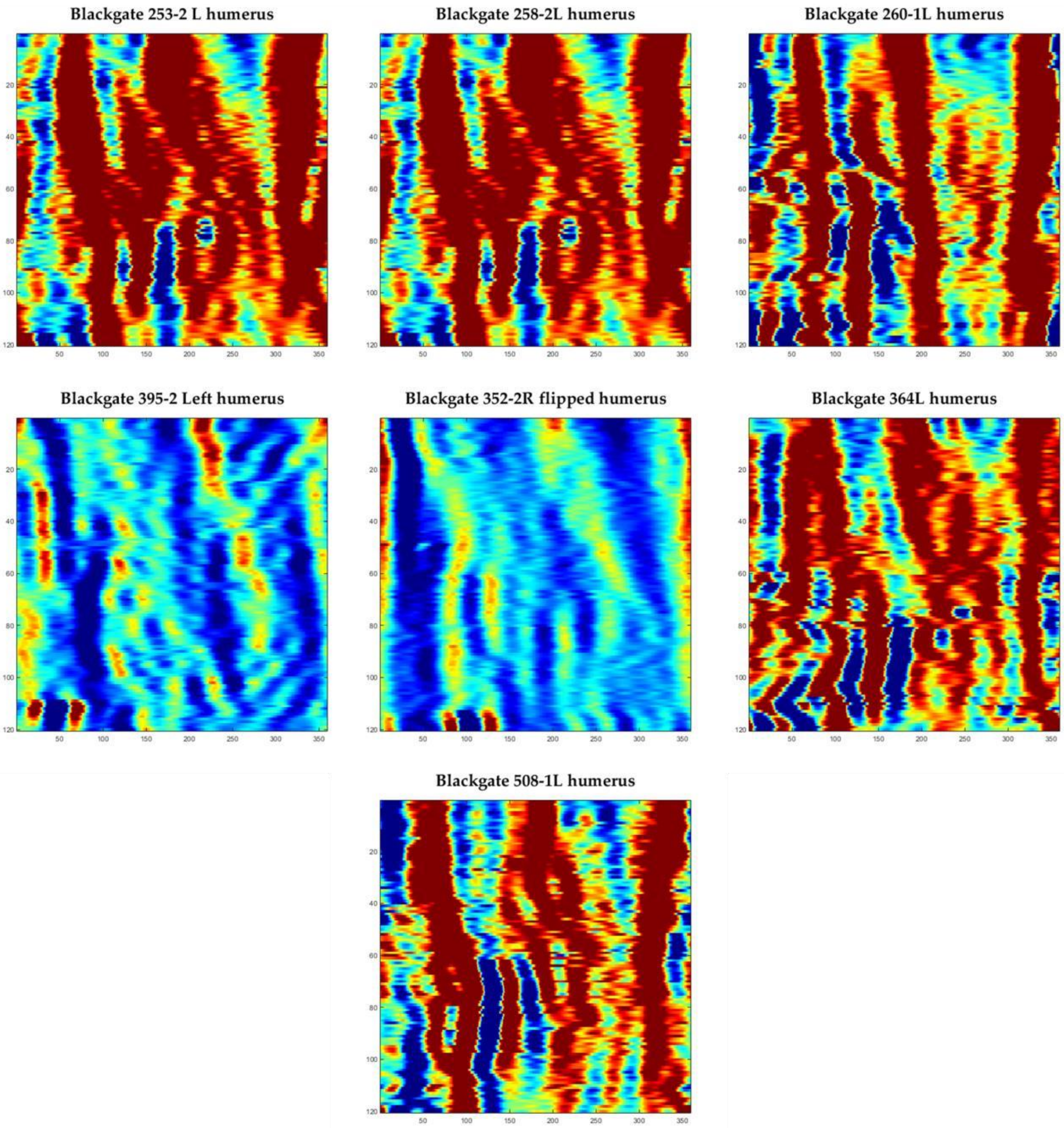
Older child humeri



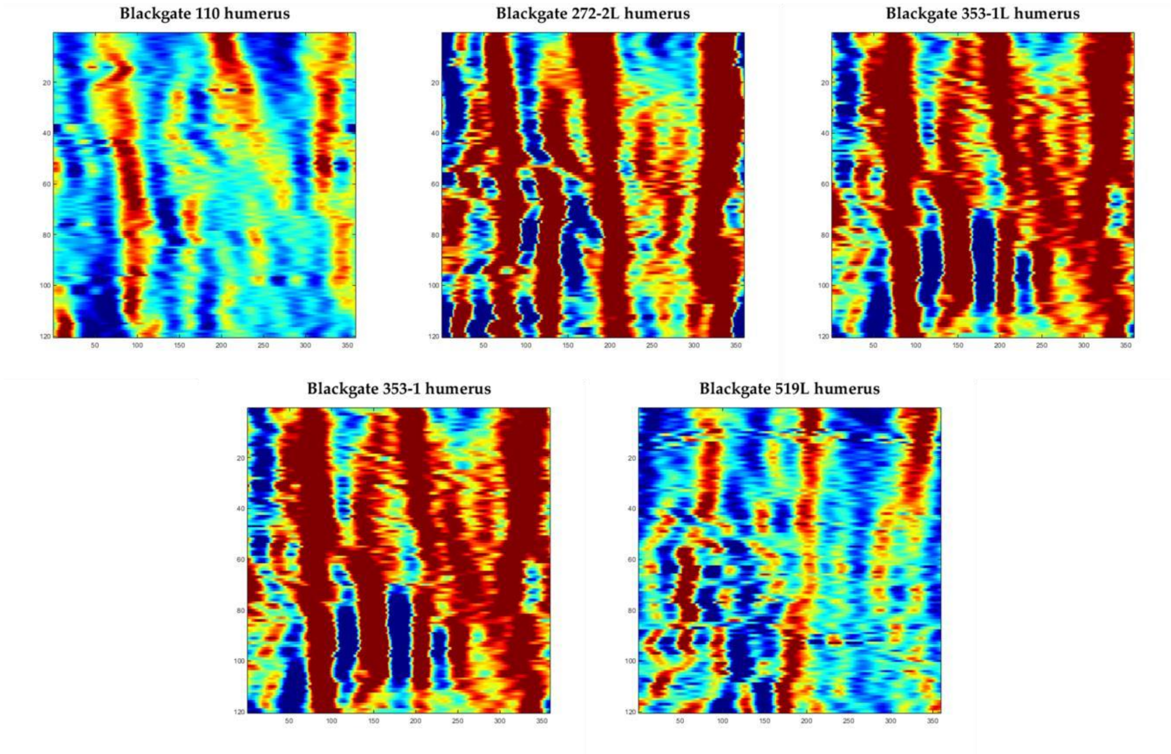

Adolescent humeri
Z4
Z1
Z2
Z3
96
187 R flipped
570
573
573 R flipped

1.3 S3. Periosteal curvature maps for all specimens

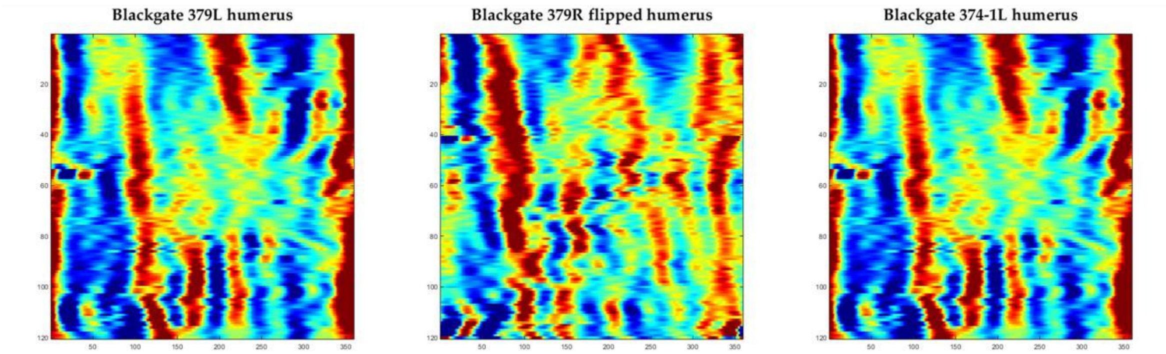
Foetal/neonatal humeri



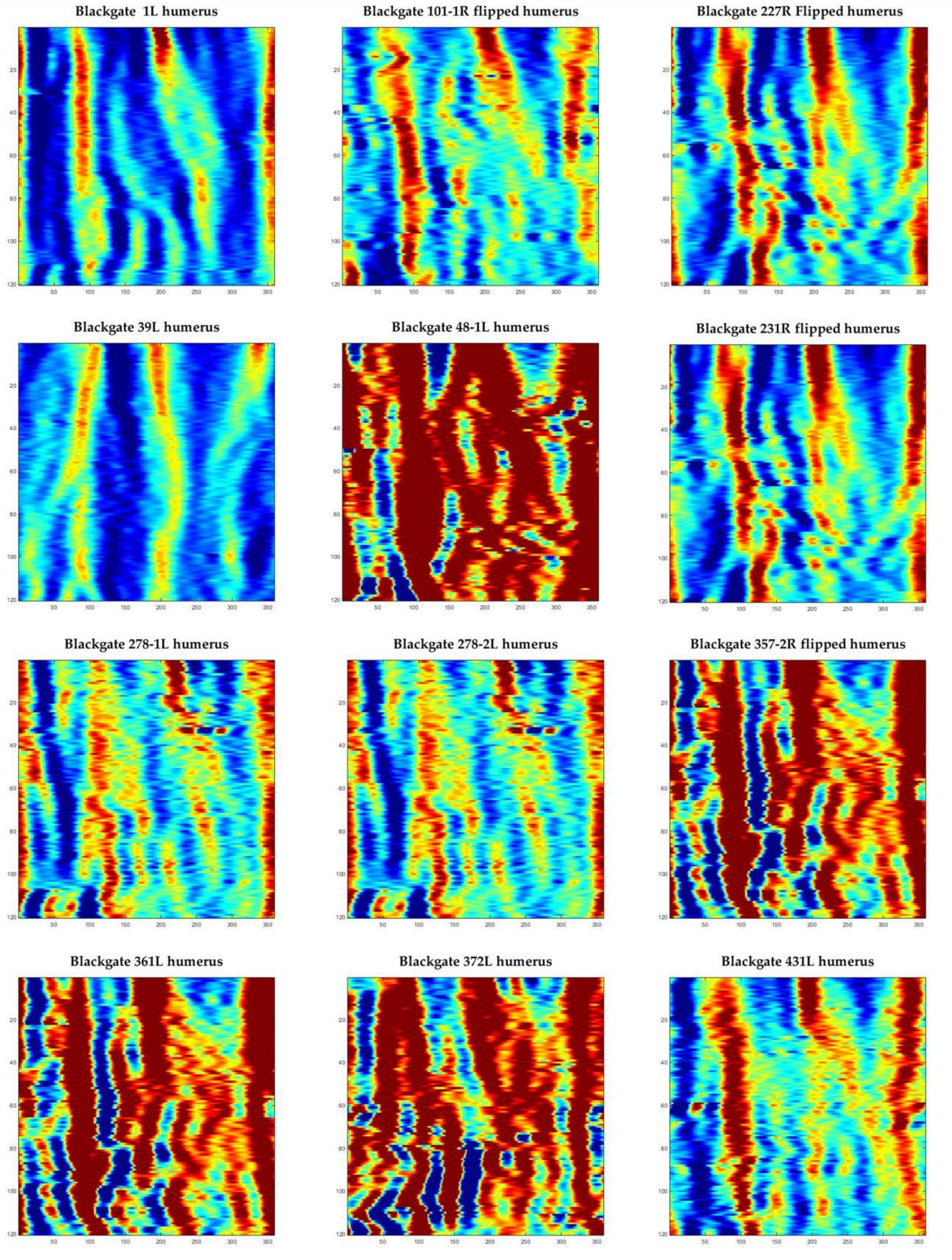
Infant-crawling humeri

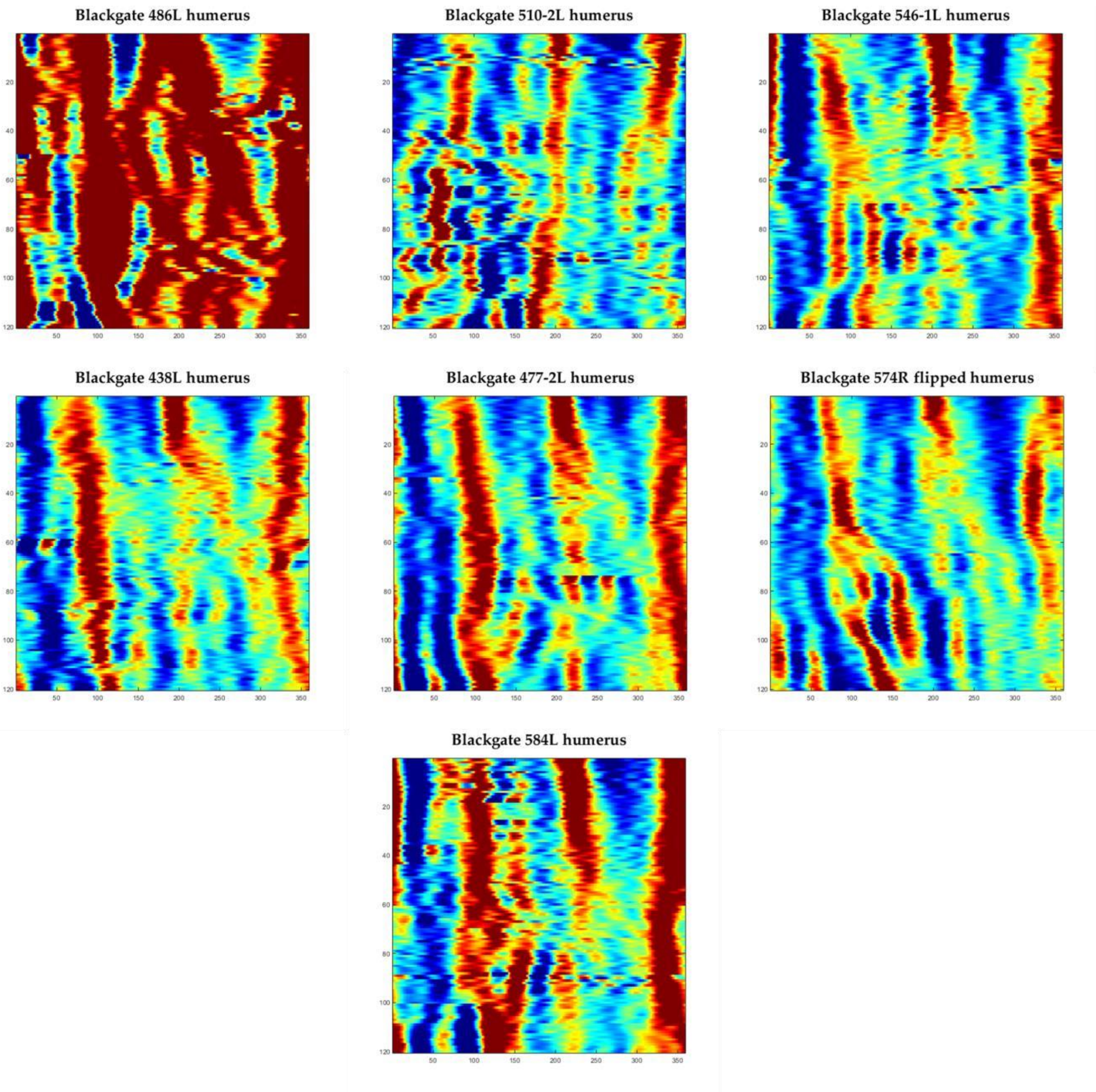


Infant-walking humeri

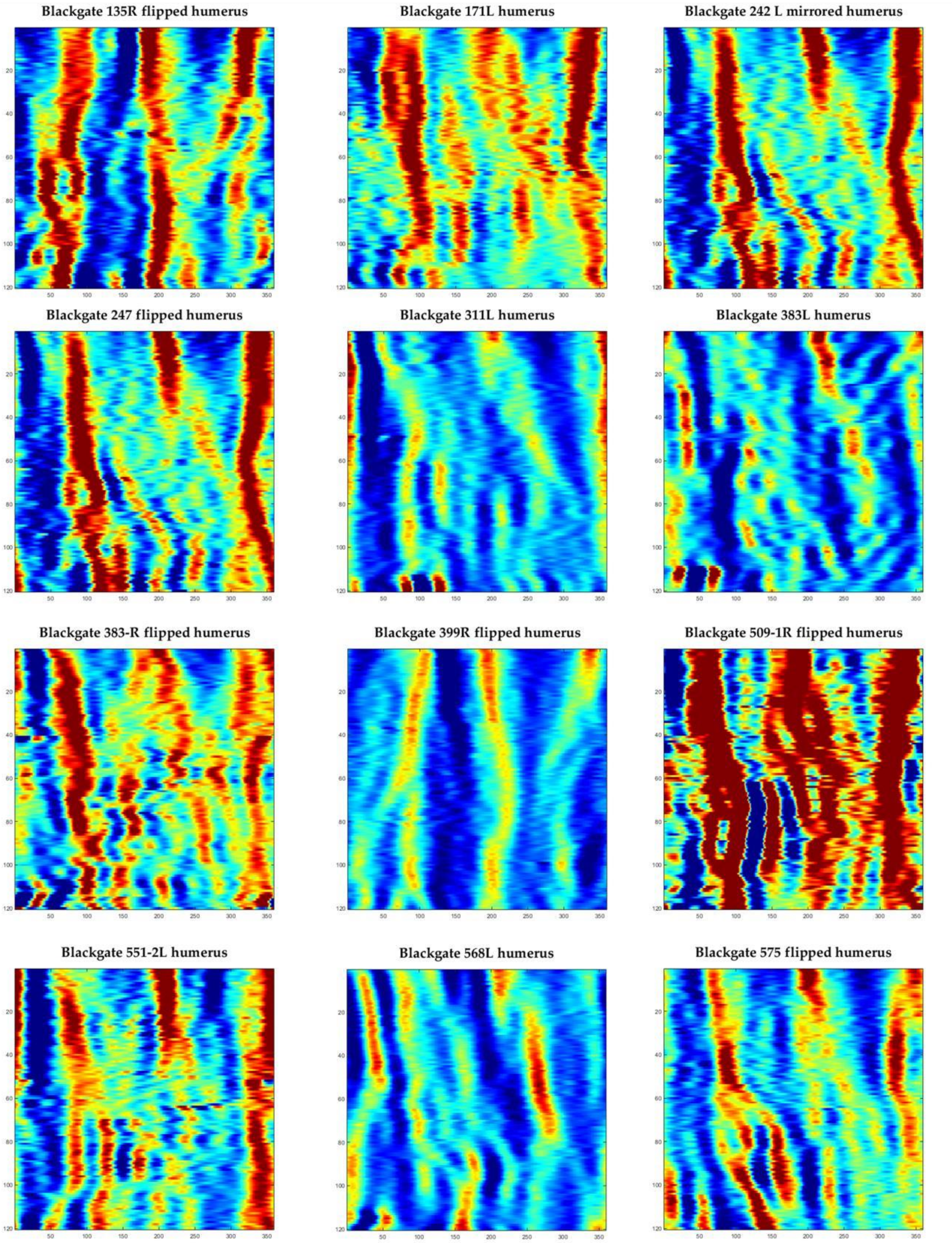


Young child humeri

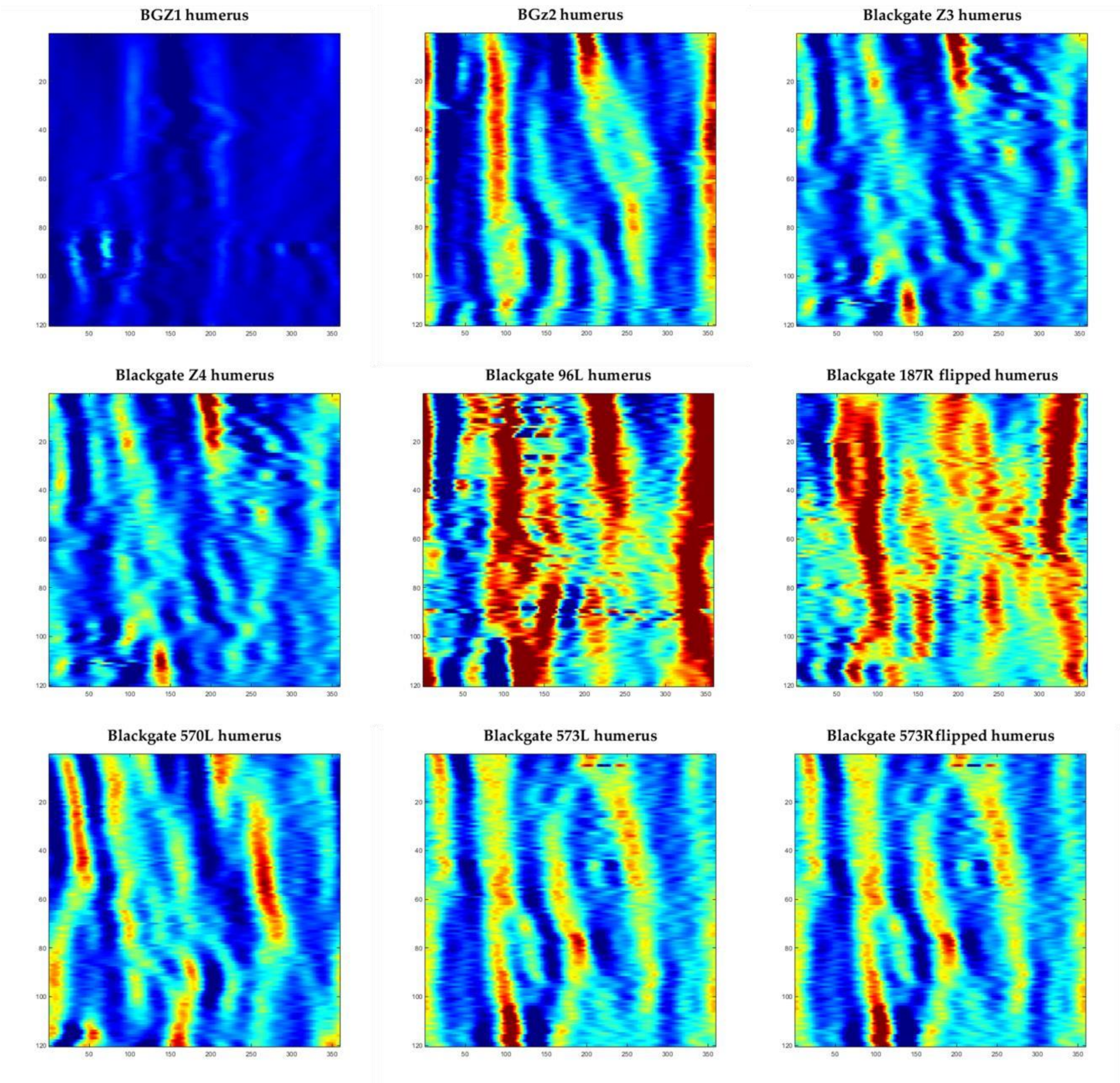



Blackgate 486L humerus
Blackgate 510-2L humerus
Blackgate 546-1L humerus
Blackgate 438L humerus
Blackgate 477-2L humerus
Blackgate 574R flipped humerus
Blackgate 584L humerus

Older child humeri



Adolescent humeri



1.4 S4 specimen age estimates and scan parameters

Specimen no.	Age group	new group	Age Estimate*	Original voxel size	No. of slices	Source to object	Source to detector	Resliced Z res. /mm	Length/ mm
1	Young Child	Young Child	5-6	0.086038	2014	524.4794	1198.746	0.866402	173.3
39	Young Child	Infant	1.5-2.5	0.069528	1723	426.8844	1227.944	0.598986	119.8
48	Young Child	Young Child	5-6	0.069528	1590	426.8844	1227.944	0.55275	110.5
48	Young Child	Young Child	5-6	0.069528	1590	426.8844	1227.944	0.55275	110.5
96	Adolescent	Adolescent	12-15	0.0574	1462	289.1936	1007.005	0.419594	83.9
96	Adolescent	Adolescent	12-15	0.0574	1462	289.1936	1007.005	0.419594	83.9
101	Young Child	Infant	1-1.5	0.069528	1665	426.8844	1227.944	0.578823	115.8
110	Infant	Infant	0.5-1.5	0.0673	1415	356.9686	1061.448	0.476148	95.2
135	Older Child	Older Child	6-8	0.097158	1688	591.4458	1217.49	0.820015	164.0
141	Young Child	Young Child	4.5-5.5	0.086038	1905	507.0948	1178.77	0.819511	163.9
171	Infant	Infant	0.5-1	0.108602	1269	703.7958	1296.104	0.689078	137.8
171	Infant	Infant	0.5-1	0.108602	1262	703.7958	1296.104	0.685277	137.1
187	Adolescent	Adolescent	10-12	0.108602	2005	703.7958	1296.104	1.088733	217.7
227	Young Child	Young Child	2.5-3.5	0.080756	1632	478.9878	1186.264	0.658967	131.8
227	Young Child	Young Child	2.5-3.5	0.080756	1632	478.9878	1186.264	0.658967	131.8
231	Young Child	Young Child	4.5-5.5	0.086038	1827	507.0945	1178.771	0.785956	157.2
247	Older Child	Older Child	8-9	0.097158	1722	591.4458	1217.49	0.836532	167.3
253	Foetal/Neonate	Foetal/Neonate	36-38 weeks I.u	0.097158	1722	591.4458	1217.49	0.836532	167.3
258	Foetal/Neonate	Foetal/Neonate	36-38 weeks I.u	0.0574	1312	289.1936	1007.005	0.376544	75.3
258	Foetal/Neonate	Foetal/Neonate	36-38 weeks I.u	0.0574	1312	289.1936	1007.005	0.376544	75.3
260	Foetal/Neonate	Foetal/Neonate	1-1.25 MO	0.0574	1312	289.1936	1007.005	0.376544	75.3

272	Foetal/Neonate	Infant	3-6 MO	0.051958	1350	261.6095	1007.003	0.350717	70.1
278	Young Child	Young Child	5-6	0.086038	1881	507.0945	1178.771	0.809186	161.8
278	Young Child	Young Child	5-6	0.086038	1881	507.0945	1178.771	0.809186	161.8
311	Older Child	Older Child	9.5-12.5	0.108602	2105	703.7958	1296.104	1.143034	228.6
352	Foetal/Neonate	Foetal/Neonate	32-34 weeks I.u	0.0384	1346	194.01	1007.005	0.258432	51.7
353	Foetal/Neonate	Infant	3-6 MO	0.0574	1439	289.1936	1007.005	0.412993	82.6
353	Foetal/Neonate	Infant	3-6 MO	0.0574	1439	289.1936	1007.005	0.412993	82.6
357	Foetal/Neonate	Foetal/Neonate	0-1 MO	0.044172	1468	228.4256	1034.253	0.324223	64.8
361	Young Child	Young Child	2.5-3.5	0.080756	1560	478.9878	1186.264	0.629894	126.0
364	Foetal/Neonate	Foetal/Neonate	0-2 MO	0.044172	1603	228.4256	1034.253	0.354039	70.8
372	Young Child	Infant	1-1.5	0.064069	1710	359.8926	1123.446	0.547793	109.6
374	Infant	Foetal/Neonate	0-1 MO	0.086038	1701	507.0948	1178.77	0.731753	146.4
374	Infant	Foetal/Neonate	0-1 month	0.086038	1701	507.0948	1178.77	0.731753	146.4
379	Infant	Infant	6-9 MO	0.069528	1351	426.8844	1227.944	0.469664	93.9
383	Older Child	Older Child	8.5-9.5	0.108602	1986	703.7958	1296.104	1.078416	215.7
383	Older Child	Older Child	8.5-9.5	0.108602	1986	703.7958	1296.104	1.078416	215.7
384	Older Child	Older Child	7-8	0.108602	1690	703.7982	1296.102	0.917689	183.5
388	Young Child	Young Child	4-5	0.086038	1779	507.0945	1178.771	0.765307	153.1
395	Foetal/Neonate	Foetal/Neonate	0-1 MO	0.044172	1512	228.4256	1034.253	0.333941	66.8
399	Older Child	Older Child	11-12	0.108602	2131	703.7958	1296.104	1.157152	231.4
406	Young Child	Infant	1.5-2.5	0.080756	1434	478.9878	1186.264	0.579018	115.8
431	Young Child	Young Child	5-6	0.080756	1566	478.9878	1186.264	0.632317	126.5
438	Young Child	Young Child	5-6	0.064069	1669	359.8926	1123.446	0.534659	106.9
477	Young Child	Young Child	3.5-4.5	0.064069	1669	359.8926	1123.446	0.534659	106.9
478	Young Child	Infant	1-2	0.069528	1572	426.8844	1227.944	0.546493	109.3

486	Young Child	Infant	1.5-2.5	0.080756	1550	478.9878	1186.264	0.625857	125.2
508	Foetal/Neonate	Foetal/Neonate	0-2 MO	0.051958	1317	261.6095	1007.003	0.342144	68.4
509	Older Child	Older Child	5.5-6.5	0.108602	1629	703.7982	1296.102	1.841351	176.9
510	Young Child	Young Child	5-6	0.108602	1725	703.7982	1296.102	0.936694	187.3
519	Infant	Infant	1-3 MO	0.051958	1388	261.6095	1007.003	0.360589	72.1
546	Young Child	Young Child	4.5-5.5	0.108602	1611	703.7982	1296.102	0.874791	175.0
546	Young Child	Young Child	4.5-5.5	0.108602	1611	703.7982	1296.102	0.874791	175.0
551	Infant	Young Child	3.5-4.5	0.108602	1611	703.7982	1296.102	0.874791	175.0
568	Adolescent	Older Child	9-11 years	0.108602	2111	703.7958	1296.104	1.146292	229.3
570	Adolescent	Adolescent	15-17	0.108602	2111	703.7958	1296.104	1.146292	229.3
573	Adolescent	Adolescent	15-17	0.108602	2111	703.7958	1296.104	1.146292	229.3
573	Adolescent	Adolescent	15-17	0.108602	2111	703.7958	1296.104	1.146292	229.3
574	Young Child	Young Child	5-6	0.108602	1552	703.7982	1296.102	0.842753	168.6
575	Older Child	Older Child	11-12	0.108602	1756	703.7982	1296.102	0.953528	190.7
584	Young Child	Young Child	2-3 years	0.080756	1530	478.9878	1186.264	0.617781	123.6

*Unless otherwise stated-age is in years

1.5 Supplementary figures S5-S7.

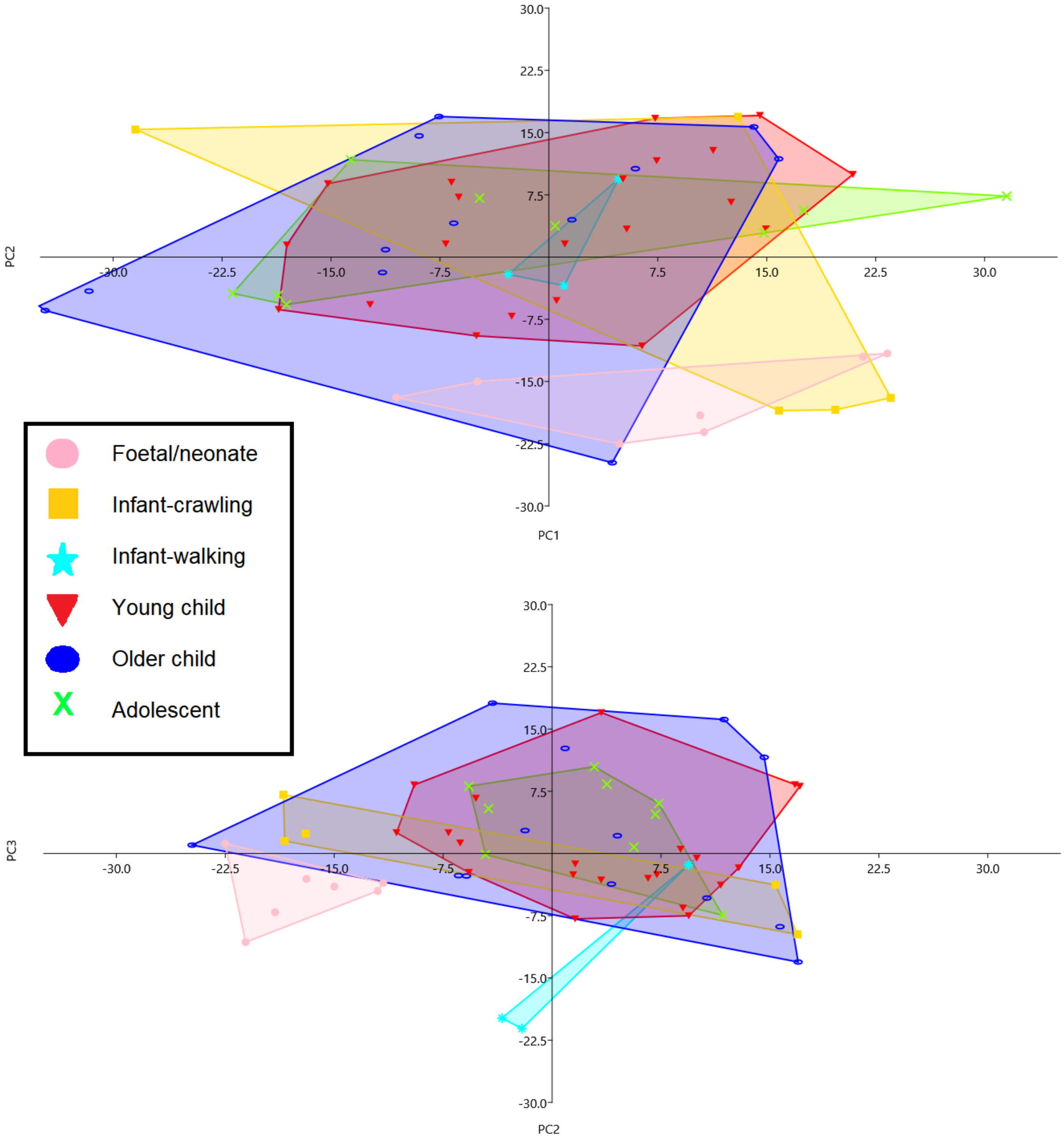


Figure S5. PCA of thickness scores

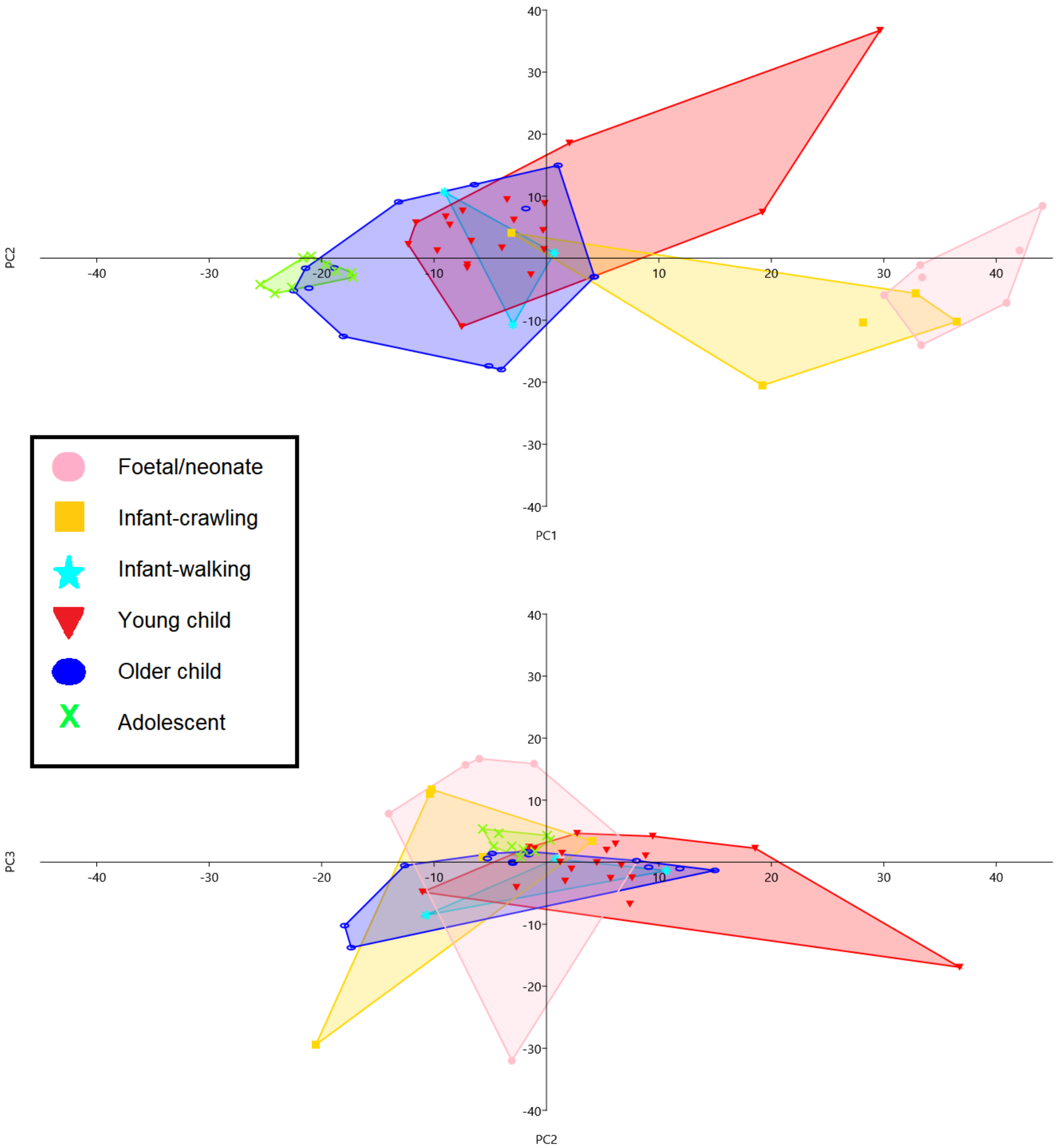


Figure S6. PCA of periosteal curvature scores

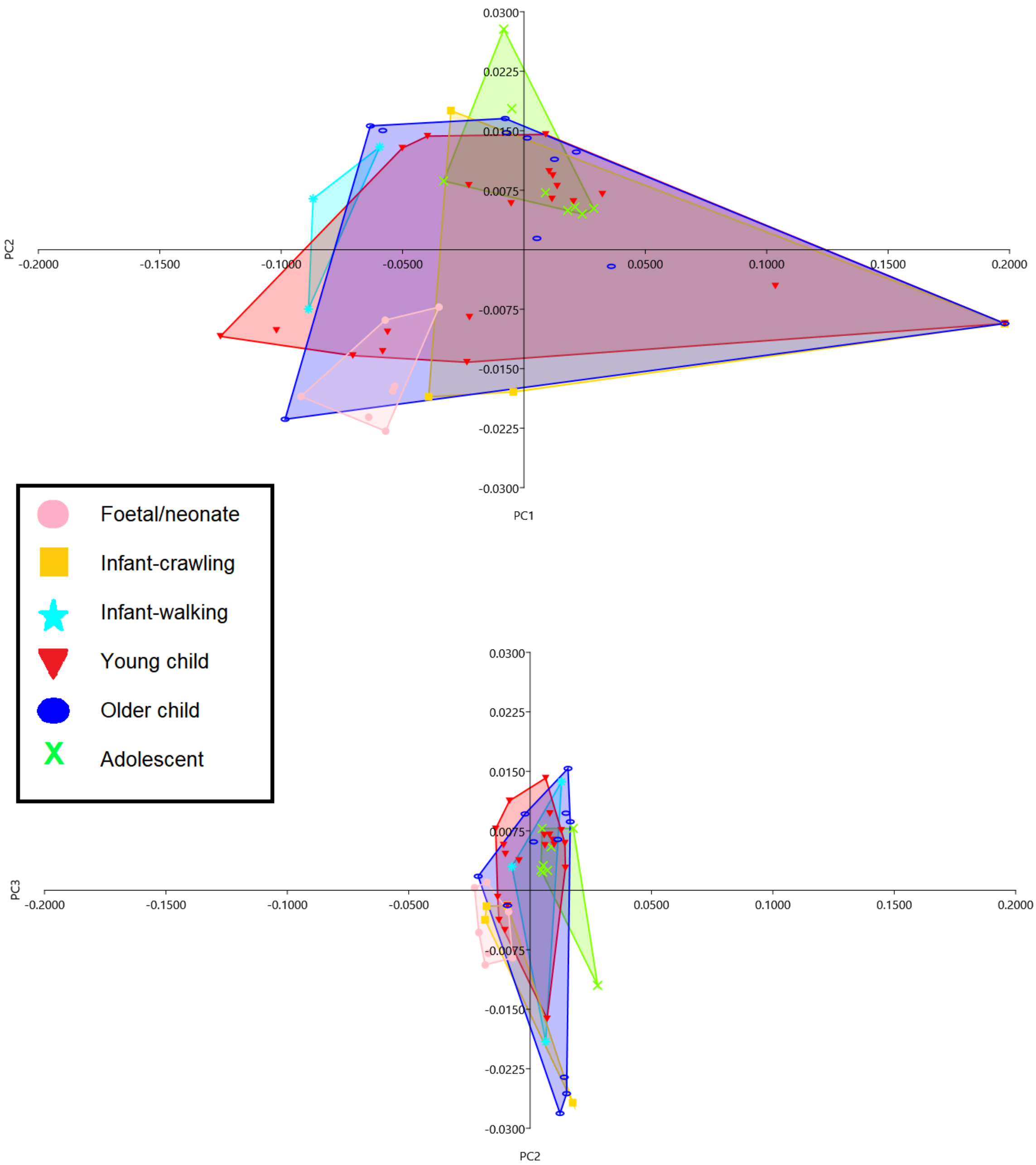


Figure S7. PCA of GMM coordinates

1.6 S8. MATLAB code for thickness calculations

```
function calculate_thickness_map
% This function looks at all the .tif files in a folder and calculates a thickness map
% it assumes that each tiff file is part of an image stack
 num_samples = 360; % this is the number of samples per revolution, so 360 would give a 1 degree
interval
slice_subsample = 6; % this is the subsample factor for the innerpoint and outerpoint files
sample_subsample = 90; % this is the subsample factor for each slice for the innerpoint and
outerpoint files
 % first get a folder from the user
folder_name = uigetdir('', 'Choose the folder that contains the image stack'); if
folder_name == 0     error('No folder selected');
end
%
 %save_name = uigetdir('', 'Choose the folder to save results into');
%if save_name == 0
   %error('No folder selected');
%end
% Change the code below to the directory you want to save the results to if
~exist(['C:\Users\~'],'dir') mkdir(['C:\Users\~']) end
save_name = 'C:\Users\mqbpkto2\Dropbox (The University of Manchester)\Thesis
chapters\thickness mapping\JULY18'

file_list = dir(fullfile(folder_name,'*.tif')); if
isempty(file_list)
   error('No .tif files found in folder')
end
%this code will automatically read a csv with metadata in. Change the columns below to %reflect
where your voxel sizes are
excel_file_list = dir(fullfile(folder_name,'*metadata.csv')); if
isempty(excel_file_list)     error('No .csv files found in
folder')
end
for file_number = 1:length(excel_file_list)      file_name =
[folder_name '/' excel_file_list(file_number).name];     metadata =
xlsread(file_name);
end mm_per_pixel =
metadata(2) slice_thickness =
metadata(3)
%This dialogue lets you tell Matlab the dimensions of your file.
%response = inputdlg({'X-Y resolution in mm','Z resolution in mm'}, 'Image Resolution', [1 50; 1
50]);
%if isempty(response)
 %   error('The size of each image pixel must be specified')
%end

%mm_per_pixel = str2double(response{1});
```

```
%slice_thickness = str2double(response{2});
%this dialogue lets you input the start and end percentage for your sequence. %This
means that you can just leave all your images in the folder, and not %worry about
moving the unwanted slices to another folder.
%responseb = inputdlg({'Start %','end %'}, 'Image Start and End Percent', [1 50; 1 50]);
%if isempty(responseb)
   %error('The start and end Percentage must be specified')
%end
%start_image = str2double(responseb{1});
%end_image = str2double(responseb{2});
% or you can alter this code below, if you are going to be doing the same set of measurements
repeatedly start_image = 40; end_image = 160; start_perc = start_image;
end_perc = end_image;

%start_image = round((length(file_list)/100)*(str2double(responseb{1})));
%end_image = round((length(file_list)/100)*(str2double(responseb{2})));
%start_image = (round((length(file_list)/100)*(start_perc)));
%end_image = round((length(file_list)/100)*(end_perc));
file_numbers = end_image - start_image; file_list_subsample =
file_list(start_image:end_image); if ~exist([save_name
'\outerpoints\'],'dir')  mkdir([save_name '\outerpoints\']) end
if ~exist([save_name '\innerpoints\'],'dir')
mkdir([save_name '\innerpoints\']) end
if ~exist([save_name '\slice_geometry\'],'dir')
mkdir([save_name '\slice_geometry\']) end
if ~exist([save_name '\outerpoints_subsample\'],'dir')
mkdir([save_name '\outerpoints_subsample\']) end
if ~exist([save_name '\thickness\'],'dir')
mkdir([save_name '\thickness\']) end
if ~exist([save_name '\innerpoints_subsample\'],'dir')
mkdir([save_name '\innerpoints_subsample\']) end
if ~exist([save_name '\thickness_plots\'],'dir')
mkdir([save_name '\thickness_plots\']) end
if ~exist([save_name '\thickness_images\'],'dir')
mkdir([save_name '\thickness_images\']) end
if ~exist([save_name '\J\'],'dir')
mkdir([save_name '\J\']) end
if ~exist([save_name '\CA\'],'dir')
mkdir([save_name '\CA\']) end
if ~exist([save_name '\Ix_Iy\'],'dir')
mkdir([save_name '\Ix_Iy\']) end
if ~exist([save_name '\Imax_Imin\'],'dir')
mkdir([save_name '\Imax_Imin\']) end
%thickness_image = zeros(num_samples,(length(end_image-start_image))) ;
thickness_image = zeros(file_numbers, num_samples);

[pathstr, name, ext] = fileparts(folder_name);
```

```
outerpoints_file = fopen([save_name '\outerpoints\' name '_outerpoints.csv'], 'w'); innerpoints_file =
fopen([save_name '\innerpoints\' name '_innerpoints.csv'], 'w'); outerpoints_subsample_file =
fopen([save_name '\outerpoints_subsample\' name
'_outerpoints_subsample.csv'], 'w');
innerpoints_subsample_file = fopen([save_name '\innerpoints_subsample\' name
'_innerpoints_subsample.csv'], 'w');
J_file = fopen([save_name '\J\' name '_J.csv'], 'w');
CA_file = fopen([save_name '\CA\' name '_CA.csv'], 'w');
Ix_Iy_file = fopen([save_name '\Ix_Iy\' name '_Ix_Iy.csv'], 'w');
Imax_Imin_file = fopen([save_name '\Imax_Imin\' name '_Imax_Imin.csv'], 'w'); centroids_file =
fopen([save_name '\slice_geometry\' name '_slice_geometry.csv'], 'w'); fprintf(centroids_file, 'slice,
centroid_x, centroid_y, min_thickness, max_thickness, TA, Cortical area, Trabecular area, J, Ix/Iy,
Imax/Imin\n', 'w');
fprintf(J_file, 'slice, J\n', 'w'); fprintf(CA_file, 'slice,
CA\n', 'w'); fprintf(Ix_Iy_file, 'slice, Ix/Iy\n', 'w');
fprintf(Imax_Imin_file, 'slice, Imax/Imin\n', 'w');
 %thickness_file = fopen([save_name '\thickness\' name '_thickness.csv'], 'w');

for slice_counter = 1: length(file_list_subsample);

   current_file = fullfile(folder_name,file_list_subsample(slice_counter).name);    fprintf('%s\n',
current_file);

   [thickness, thickness_start, thickness_end, centroid_x, centroid_y] =
calculate_thickness(current_file, num_samples);    thickness = thickness .*
mm_per_pixel;    thickness_start = thickness_start .* mm_per_pixel;
thickness_end = thickness_end .* mm_per_pixel;    centroid_x = centroid_x
.* mm_per_pixel;    centroid_y = centroid_y .* mm_per_pixel;
max_thickness = max(thickness);
   min_thickness = min(thickness);

%these following formulae calculate conventional cross sectional properties.     TotalA =
(polyarea(thickness_end(1:1,1:360),thickness_end(2:2,1:360)));
   TrabA = (polyarea(thickness_start(1:1,1:360),thickness_start(2:2,1:360)));
   Cortical_area = TotalA-TrabA;
   %find Imax     distances = sqrt((thickness_end(1:1,1:360)-
centroid_x).^2)+((thickness_end(2:2,1:360)centroid_y).^2);
   [maxRadius, maxRadiusIndex] = max(distances);    maxPointCoord =
thickness_end(:,maxRadiusIndex);    maxPointCoordinner =
thickness_start(:,maxRadiusIndex);    opp_point = maxRadiusIndex+180;
   if opp_point>360       opp_point =
opp_point-360
   else
      opp_point = opp_point
   end
   opp_pointCoord = thickness_end(:,opp_point);
opp_pointCoordinner = thickness_start(:,opp_point);    %extract Imax
```

```
    Imax = sqrt((((maxPointCoord(1))-
(opp_pointCoord(1))).^2)+(((((maxPointCoord(2))(opp_pointCoord(2))).^2))));
    Imax_inner = sqrt((((maxPointCoordinner(1))-
(opp_pointCoordinner(1))).^2)+(((((maxPointCoordinner(2))-(opp_pointCoordinner(2))).^2))));
%find Imin
    [minRadius, minRadiusIndex] = min(distances);    minPointCoord =
thickness_end(:,minRadiusIndex);    minPointCoordinner =
thickness_start(:,minRadiusIndex);    opp_pointmin =
minRadiusIndex+180;
    if opp_pointmin>360
        opp_pointmin = opp_pointmin-360    else
        opp_pointmin = opp_pointmin    end
    opp_pointminCoord = thickness_end(:,opp_pointmin);    opp_pointminCoordinner =
thickness_start(:,opp_pointmin);
    %xtract Imin
    Imin = sqrt(((minPointCoord(1))-(opp_pointminCoord(1))).^2)+(((((minPointCoord(2))-
(opp_pointminCoord(2))).^2)));
    Imin_inner = sqrt(((minPointCoordinner(1))-
(opp_pointminCoordinner(1))).^2)+(((((minPointCoordinner(2))-
(opp_pointminCoordinner(2))).^2)));

    %extract max and min second moments of area
    Second_moment_of_area_min = (pi*((Imin^4)-(Imin_inner^4)))/32
    Second_moment_of_area_max = (pi*((Imax^4)-(Imax_inner^4)))/32

    %extractImax/Imin for circularity measure
    Ix_Iy = Imax/Imin;
    %extract J
    J = Second_moment_of_area_min+Second_moment_of_area_max;
    %Extract moments of area divided
    Imax_Imin = Second_moment_of_area_max/Second_moment_of_area_min;

    fprintf('Max thickness = %f\n', max_thickness);    fprintf('Min
thickness = %f\n', min_thickness);
    %fprintf('TA = %f\n', TotalA);
    %fprintf('Cortical area = %f\n', Cortical_area);
    %fprintf('Trabecular area = %f\n', TrabA);
    %fprintf('J = %f\n', J);
    %fprintf('Ix/Iy = %f\n', Ix_Iy);
    %fprintf('Imax/Imin = %f\n', Imax_Imin);

    fprintf(centroids_file, '%d,%g,%g,%g,%g,%g,%g,%g,%g,%g,%g\n', slice_counter, centroid_x,
centroid_y, min_thickness, max_thickness, TotalA, Cortical_area, TrabA, J, Ix_Iy, Imax_Imin);
fprintf(J_file, '%d, %g\n', slice_counter, J);
    fprintf(CA_file, '%d, %g\n', slice_counter, Cortical_area);    fprintf(Ix_Iy_file,
'%d, %g\n', slice_counter, Ix_Iy);
    fprintf(Imax_Imin_file, '%d, %g\n', slice_counter, Imax_Imin);
```

```
    thickness_image(slice_counter, :) = thickness;



    for sample_counter = 1: length(thickness_start);
        fprintf(innerpoints_file, '%d,%g,%g\n', (slice_counter*slice_thickness), thickness_start(1,
sample_counter), thickness_start(2, sample_counter));
        fprintf(outerpoints_file, '%d,%g,%g\n', (slice_counter*slice_thickness), thickness_end(1,
sample_counter), thickness_end(2, sample_counter));
        %fprintf(thickness_file, thickness);
        if (mod(slice_counter, slice_subsample) == 0 && mod(sample_counter, sample_subsample) ==
0)
            fprintf(innerpoints_subsample_file, '%d,%g,%g\n', slice_counter.*slice_thickness,
thickness_start(1), thickness_start(2));
            fprintf(outerpoints_subsample_file, '%d,%g,%g\n', slice_counter.*slice_thickness,
thickness_end(1), thickness_end(2));        end

    end

end

fclose(outerpoints_file); fclose(innerpoints_file);
fclose(outerpoints_subsample_file);
fclose(innerpoints_subsample_file);
fclose(centroids_file);
fclose(CA_file); fclose(J_file);
fclose(Ix_Iy_file);
fclose(Imax_Imin_file);

 %thickness_image2 = thickness_image(start_image:end);
% write the thickness data to a CSV file
%csvwrite((fullfile([folder_name] 'thickness_output.csv'), thickness_image);
%[pathstr, name, ext] = fileparts(folder_name);
%csvwrite([save_name '\thickness\' name 'thickness2.csv'], thickness_image2);
%csvwrite([save_name '\thickness\' name 'thickness.csv'], thickness_image);

% produce a suitable image of the thickness map

graph_title1 = ('Cortical thickness of '); graph_title2 = ('
humerus. Colour scale is in mm'); graph_bone =
(excel_file_list.name(1:end-12)); %graph_bone2 =
graph_bone(1:end-12);
graph_title = [graph_title1, graph_bone, graph_title2];
[pathstr, name, ext] = fileparts(folder_name); figure(1);
clf('reset'); set(gcf, 'Color', 'w'); set(gcf,'Units','pixels'); set(gcf,
'Position', [50, 50, 1000, 1000]);
```

```
set(gcf,'PaperType','<custom>'); set(gcf,'Units','inches'); set(gcf,'PaperUnits','inches'); position =
get(gcf,'Position'); set(gcf,'PaperPosition',[0,0,position(3:4)]); set(gcf,'PaperSize',position(3:4));
imagesc(thickness_image); colormap(jet(256));
%axes1 = axes('Parent',figure(1),...
    %'XTickLabel',{'Anterior','Medial','Posterior','Lateral','Anterior'},...
   % 'XTick',[0 90 180 270 360],...
    %'Layer','top',...
    %'YDir','reverse',...
    %'YTick',zeros(1,0));
%% Uncomment the following line to preserve the X-limits of the axes
% xlim(axes1,[0.5 360.5]);
%% Uncomment the following line to preserve the Y-limits of the axes
% ylim(axes1,[0.5 4.5]);
%box(axes1,'on');
%hold(axes1,'on');

% Create image
%image(cdata1,'Parent',axes1,'CDataMapping','scaled');

% Create xlabel
xlabel('Bone Position');

% Create ylabel
ylabel({'Distal to proximal humerus.'});

% Create title title(graph_title);
 % Create colorbar colorbar('EastOutside');  % Create arrow
annotation('arrow',[0.0613750000000001 0.0593750000000001],...
    [0.922330097087376 0.107142857142857]); set(gca,
'XTick',[0 90 180 270 360]);
'XTickLabel',{'Anterior','Medial','Posterior','Lateral','Anterior'};,...
 plot_name = fullfile(folder_name, 'thickness_plot');
print(gcf,'-dpdf',[save_name '\thickness_plots\' name 'thickness plot', '.pdf'],'-r 150') print(gcf,'-
depsc2',[save_name '\thickness_plots\' name 'thickness plot', '.eps'],'-r 150') print(gcf,'-
dtiff',[save_name '\thickness_plots\' name 'thickness plot', '.png'],'-r 72') figure(2);
clf('reset'); set(gcf, 'Color', 'w'); set(gcf,'Units','pixels'); set(gcf,
'Position', [50, 50, 1000, 1000]);
set(gcf,'PaperType','<custom>'); set(gcf,'Units','inches'); set(gcf,'PaperUnits','inches'); position =
get(gcf,'Position'); set(gcf,'PaperPosition',[0,0,position(3:4)]); set(gcf,'PaperSize',position(3:4));
imagesc(thickness_image); colormap(jet(256));
plot_name2 = fullfile(folder_name, 'thickness_image');
print(gcf,'-dpdf',[save_name '\thickness_images\' name 'thickness_image', '.pdf'],'-r 150') print(gcf,'-
depsc2',[save_name '\thickness_images\' name 'thickness_image', '.eps'],'-r 150') print(gcf,'-
dtiff',[save_name '\thickness_images\' name 'thickness_image', '.png'],'-r 72')

% ask if the thickness file should be saved somewhere %[pathstr,
name, ext] = fileparts(folder_name); default_name = [name,
'_thickness.csv'];
```

```
%[file_name, path_name] = uiputfile('*.csv', 'Save the thickness image', default_name);
%if file_name ~= 0
 %csvwrite(fullfile(path_name, file_name), thickness_image);  return
 function [thickness, thickness_start, thickness_end, centroid_x, centroid_y] =
calculate_thickness(current_file, num_samples)

% this function calculates the thickness of the bone wall
% it produces num_samples values from angle zero to 360
% zero angle is aligned with the X axis and increases counterclockwise

% read the image data =
imread(current_file); [height,
width, channels] = size(data);

% now do some sanity checking on the image
if channels ~= 1
    error('Only greyscale images supported'); end

if data(1, 1) ~= data(1, width) || data(1, 1) ~= data(height, width) || data(1, 1) ~= data(height, 1)
error('All 4 corners of the image must have the same greyscale value'); end

background = data(1, 1);

% find the centroid xsum
= 0; ysum = 0; count = 0;
for ix = 1: width     for iy
= 1: height
        if data(iy, ix) ~= background
xsum = xsum + ix;           ysum = ysum
+ iy;           count = count + 1;
        end
    end end
 if count == 0 % must be a completely blank image so set all the thicknesses to zero
fprintf('Centroid not found\n');     thickness = zeros(1, file_numbers);     return end
 centroid_x = xsum / count; centroid_y =
ysum / count;
fprintf('Centroid x = %f y = %f\n', centroid_x, centroid_y);
 % now work radially around from centroid
thickness = 9999 * ones(1, num_samples); % create a suitable row vector to hold the thicknesses using
dummy big values
thickness_start = zeros(2, num_samples);
thickness_end = zeros(2, num_samples); for i = 1:
num_samples
    circle_fraction = (i - 1) / num_samples;
angle = 2 * pi() * circle_fraction;    del_x =
cos(angle);     del_y = sin(angle);     % look for
the inner edge
```

```
    for r = 0: 0.5: max([width, height]) % the 0.5 here gets us around any potential rounding error
problems
        x = r * del_x + centroid_x;         y
= r * del_y + centroid_y;         ix =
round(x);         iy = round(y);
        if (ix < 1 || ix > width || iy < 1 || iy > height)
            % if we can't find the inner edge then there is a complete hole here so set the thickness to zero
            thickness(i) = 0;
            break;
        end
        if data(iy, ix) ~= background % found the inner edge
thickness_start(1, i) = ix;             thickness_start(2, i) = iy;
            break;         end
end     if thickness(i) ~=
0
        % look for the outer edge
        for r = max([width, height]): -0.5: 0 % the -0.5 here gets us around any potential rounding error
problems
            x = r * del_x + centroid_x;
y = r * del_y + centroid_y;             ix
= round(x);             iy = round(y);
            if (ix < 1 || ix > width || iy < 1 || iy > height)
                continue; % this isn't an error, we just haven't found the edge of the image yet
end
            if data(iy, ix) ~= background % found the outer edge
thickness_end(1, i) = ix;                 thickness_end(2, i) = iy;
                break;
end         end
        % if the outer edge is the same as the inner edge it means that the radial line has met at the same
pixel
        % so in fact the distance between the outer edge and inner edge is always 1 pixel too small so
%we need to correct for this
        thickness(i) = 1 + sqrt((thickness_end(1, i) - thickness_start(1, i))^2 + (thickness_end(2, i) -
thickness_start(2, i))^2);

    end

end

return
```

1.7 S9. MATLAB code for periosteal curvature calculations

```
    function k_fouriercurvature


 %subsample = 10

% read the csv folder containing the outline coordinates for each stack
folder_name = uigetdir('', 'Choose the folder that contains the image stack'); if
folder_name == 0     error('No folder selected');
end
file_list = dir(fullfile(folder_name,'*.csv')); if
isempty(file_list)
   error('No .csv files found in folder')
end
%make a subdirectory for each output to be saved in.
if ~exist([folder_name '\curvature_rawpoints\'],'dir')
mkdir([folder_name '\curvature_rawpoints\']) end
if ~exist([folder_name '\curvature_heatmap\'],'dir')
mkdir([folder_name '\curvature_heatmap\']) end
if ~exist([folder_name '\curvature_2dgraph\'],'dir')
mkdir([folder_name '\curvature_2dgraph\']) end
if ~exist([folder_name '\curvature_values\'],'dir')
mkdir([folder_name '\curvature_values\']) end
if ~exist([folder_name '\smoothed_values\'],'dir')
mkdir([folder_name '\smoothed_values\']) end

for file_number = 1:length(file_list)

   file_name = [folder_name '/' file_list(file_number).name];
   [pathstr, name, ext] = fileparts(file_name);
   file_name2 = [folder_name '\curvature_values\' file_list(file_number).name(1:end-4)
'curvature.csv'];
   file_name3 = [folder_name '\smoothed_values\' file_list(file_number).name(1:end-4) 'smoothed
outline.csv'];
   heatmap_name = [folder_name '\curvature_heatmap\' file_list(file_number).name(1:end-4)
'curvature_heatmap'];
   smoothoutline_name = [folder_name '\curvature_2dgraph\' file_list(file_number).name(1:end-4)
'curvature_smoothed'];
   outline_name = [folder_name '\curvature_rawpoints\' file_list(file_number).name(1:end-4)
'original_curvature']
graph_bone_name = [name(1:end-12)];
   fourierpoints = csvread(file_name);

slice_number = fourierpoints(:, 1); x_list =
fourierpoints(:, 2);
y_list = fourierpoints(:, 3);
```

```
% count the number of points in a slice
points_per_slice = 1; for i = 1:
length(slice_number) - 1     if (slice_number(i +
1) ~= slice_number(i))        break;     end
    points_per_slice = points_per_slice + 1; end

slice_number = reshape(slice_number, [points_per_slice, length(slice_number) / points_per_slice]);
% this reshape needs checking
x_list = reshape(x_list, [points_per_slice, length(x_list) / points_per_slice]); y_list =
reshape(y_list, [points_per_slice, length(y_list) / points_per_slice]);

[r, c] = size(slice_number); num_slices =
c; num_points_wanted = 360;
k_map = zeros(num_slices, num_points_wanted);
l = zeros(num_slices*num_points_wanted,3);
for i = 1: 3     figure(i)     clf('reset');     set(gcf,
'Color', 'w');     set(gcf,'Units','pixels');
    set(gcf, 'Position', [i + 50, i + 50, 1000, 1000]); end


for slice = 1: num_slices
x = x_list(1:points_per_slice, slice);
y = y_list(1:points_per_slice, slice);

    figure(1);     plot(x,
y);
    hold on;
    plot_name3 = outline_name;
  title(['Original endosteal contour of ' graph_bone_name ' humerus from a μCT scan']);
xlabel('X'); ylabel('Y');
  %print(gcf,'-dtiff',[plot_name3, '.png'],'-r 72');
    %print({(gcf'-dtiff', plot_name3, '.png')} '-r 72');
   %print(gcf,'-depsc2','.eps')
    %hold off;
    %print(gcf,'-dtiff',[plot_name3, '.png'],'-r 72')


    outline = [x, y];
    iNoOfHarmonicsAnalyse = points_per_slice / 2;
    bNormaliseSizeState = 0;     bNormaliseOrientationState
= 0;
    rFSDs = fEfourier(outline, iNoOfHarmonicsAnalyse, bNormaliseSizeState,
bNormaliseOrientationState);

    % the degree of smoothing depends on the number of harmonics we choose
iNoOfHarmonicsReconstruct = 10;     iNoOfPointsReconstruct =
num_points_wanted;
    smoothed_outln = rEfourier(rFSDs, iNoOfHarmonicsReconstruct, iNoOfPointsReconstruct);
```

```
%%%%% Jamie added in bit to output smoothed_oultn variable
smoothed_outln_stored(:,2*slice-1) = smoothed_outln(:,1);     smoothed_outln_stored(:,2*slice) =
smoothed_outln(:,2);

if slice == num_slices
    %B = reshape(smoothed_outln_stored,[],2)
   csvwrite([file_name(1:end-4) '_smoothed_outln.csv'],smoothed_outln_stored)
    %csvwrite([file_name(1:end-4) '_smoothed_outln2.csv'],B)     end
if slice == num_slices
    B = reshape(smoothed_outln_stored,[],2)
    csvwrite([file_name(1:end-4) '_smoothed_outln2.csv'],B)     end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%

%l_map(slice, :) = smoothed_outln;     figure(2);
plot(smoothed_outln(:, 1), smoothed_outln(:, 2));     hold
on;
k_coeff = calc_k_coefficent(smoothed_outln(:, 1), smoothed_outln(:, 2));     k_map(slice,
:) = k_coeff;

title(['Smoothed endosteal contour of ' graph_bone_name ' humerus from a μCT scan']); xlabel('X');
ylabel('Y');
plot_name2 = smoothoutline_name;
%print(gcf,'-dtiff',[plot_name2, '.png'],'-r 72');     end
print(gcf,'-dtiff',[plot_name2, '.png'],'-r 72')
print(gcf,'-dtiff',[plot_name3, '.png'],'-r 72')
figure(3) clf('reset'); set(gcf, 'Color', 'w');
set(gcf,'Units','pixels');
set(gcf, 'Position', [50, 50, 1000, 1000]); imagesc(k_map);

set(gcf,'PaperType','<custom>');
set(gcf,'Units','inches');
set(gcf,'PaperUnits','inches'); position =
get(gcf,'Position');
set(gcf,'PaperPosition',[0,0,position(3:4)]);
set(gcf,'PaperSize',position(3:4)); plot_name =
[heatmap_name];
colormap(jet(256));
colorbar('EastOutside'); xlabel('Bone
Position'); ylabel({'Slice
number.','Lower numbers are more
proximal'}); title(['Surface curvature of '
graph_bone_name ' humerus']);
%print(gcf,'-dtiff',[plot_name3, '.png'],'-r 72');
 %print(gcf,'-dpdf','Curvature Map.pdf','-r 150')
```

```
%print(gcf,'-depsc2','Curvature Map.eps','-r 150')
%print (gcf,'-depsc2','filename2 Curvature Map.eps','-r 150')

print(gcf,'-dpdf',[plot_name, '.pdf'],'-r 300') print(gcf,'-depsc2',[plot_name,
'.eps'],'-r 300') print(gcf,'-dtiff',[plot_name, '.png'],'-r 72')
% output the k values per slice to a file

%if (file_name2 ~= 0)     fout =
fopen((file_name2), 'w');     for slice =
1: num_slices        fprintf(fout, '%d',
slice);        k = k_map(slice, :);        for
t = 1: length(k)            fprintf(fout,
',%f', k(t));        end
        fprintf(fout, '\n');
    end     fclose(fout);
  fout2 = fopen((file_name3), 'w');

end
  function rFSDs = fEfourier(outline, iNoOfHarmonicsAnalyse, bNormaliseSizeState,
bNormaliseOrientationState)
% Forward elliptical Fourier transform - see Kuhl FP and Giardina CR %
"Elliptic Fourier features of a closed contour" Computer Graphics and %
Image Processing 18:236-258 1982 for theory.
% Returns a shape spectrum of input x,y data "outline" with %
iNoOfHarmonicsAnalyse elements.
% The output FSDs will be normalised for location, size and orientation
% if bNormaliseSizeState and bNormaliseOrientationState are TRUE

% Pre-calculate some constant arrays
% n * 2 * pi
% n^2 * 2* pi^2
% where n is the number of harmonics to be used in the analysis rTwoNPi =
(1:1:iNoOfHarmonicsAnalyse)* 2 * pi; rTwoNSqPiSq = (1:1:iNoOfHarmonicsAnalyse) .*
(1:1:iNoOfHarmonicsAnalyse)* 2 * pi * pi;

iNoOfPoints = size(outline,1) - 1; % hence there is 1 more data point in outline than iNoOfPoints
rDeltaX = zeros(iNoOfPoints+1,1); % pre-allocate some arrays rDeltaY = zeros(iNoOfPoints+1,1);
rDeltaT = zeros(iNoOfPoints+1,1);

for iCount = 2 : iNoOfPoints + 1
    rDeltaX(iCount-1) = outline(iCount,1) - outline(iCount-1,1);
rDeltaY(iCount-1) = outline(iCount,2) - outline(iCount-1,2); end

% Calculate 'time' differences from point to point - actually distances, but we are
% carrying on the fiction of a point running around the closed figure at constant speed.
% We are analysing the projections on to the x and y axes of this point's path around the figure for
iCount = 1 : iNoOfPoints
```

```
    rDeltaT(iCount) = sqrt((rDeltaX(iCount)^2) + (rDeltaY(iCount)^2));
end check = (rDeltaT ~= 0);  % remove zeros from rDeltaT, rDeltaX...
rDeltaT = rDeltaT(check); rDeltaX =
rDeltaX(check); rDeltaY =
rDeltaY(check);

iNoOfPoints = size(rDeltaT,1) - 1; % we have removed duplicate points   %
now sum the incremental times to get the time at any point rTime(1) = 0;
for iCount = 2 : iNoOfPoints + 1
   rTime(iCount) = rTime(iCount - 1) + rDeltaT(iCount-1); end

rPeriod = rTime(iNoOfPoints+1); % rPeriod defined for readability
 % calculate the A-sub-0 coefficient rSum1 =
0;
for iP = 2 : iNoOfPoints + 1
rSum2 = 0;     rSum3 = 0;
rInnerDiff = 0;
    % calculate the partial sums - these are 0 for iCount = 1
if iP > 1         for iJ = 2 : iP-1
            rSum2 = rSum2 + rDeltaX(iJ-1);
rSum3 = rSum3 + rDeltaT(iJ-1);        end
            rInnerDiff = rSum2 - ((rDeltaX(iP-1) / rDeltaT(iP-1)) * rSum3);     end
    rIncr1 = ((rDeltaX(iP-1) / (2*rDeltaT(iP-1)))*(rTime(iP)^2-rTime(iP-1)^2) +
rInnerDiff*(rTime(iP)rTime(iP-1)));     rSum1 = rSum1 + rIncr1; end
rFSDs(1,1) = ((1 / rPeriod) * rSum1) + outline(1,1); % store A-sub-0 in output FSDs array - this array
will be 4 x iNoOfHarmonicsAnalyse  % calculate the a-sub-n coefficients
for iHNo = 2 : iNoOfHarmonicsAnalyse
rSum1 = 0;     for iP = 1 : iNoOfPoints
        rIncr1 = (rDeltaX(iP) / rDeltaT(iP))*((cos(rTwoNPi(iHNo-1)*rTime(iP+1)/rPeriod) -
cos(rTwoNPi(iHNo-1)*rTime(iP)/rPeriod)));
        rSum1 = rSum1 + rIncr1;
    end
    rFSDs(1,iHNo) = (rPeriod / rTwoNSqPiSq(iHNo-1)) * rSum1; end %
"foriHNo = 1 :..."

rFSDs(2,1) = 0; % there is no 0th order sine coefficient
% calculate the b-sub-n coefficients
for iHNo = 2 : iNoOfHarmonicsAnalyse
rSum1 = 0;     for iP = 1 : iNoOfPoints
        rIncr1 = (rDeltaX(iP) / rDeltaT(iP))*((sin(rTwoNPi(iHNo-1)*rTime(iP+1)/rPeriod) -
sin(rTwoNPi(iHNo-1)*rTime(iP)/rPeriod)));
        rSum1 = rSum1 + rIncr1;     end
    rFSDs(2,iHNo) = (rPeriod / rTwoNSqPiSq(iHNo-1)) * rSum1;
end % "foriHNo = 1 :..."    % calculate the C-sub-0 coefficient
rSum1 = 0;
for iP = 2 : iNoOfPoints + 1
rSum2 = 0;     rSum3 = 0;
rInnerDiff = 0;
```

```
   % calculate the partial sums - these are 0 for iCount = 1
if iP > 1         for iJ = 2 : iP-1
            rSum2 = rSum2 + rDeltaY(iJ-1);
rSum3 = rSum3 + rDeltaT(iJ-1);        end
        rInnerDiff = rSum2 - ((rDeltaY(iP-1) / rDeltaT(iP-1)) * rSum3);     end
    rIncr1 = ((rDeltaY(iP-1) / (2*rDeltaT(iP-1)))*(rTime(iP)^2-rTime(iP-1)^2) +
rInnerDiff*(rTime(iP)rTime(iP-1)));     rSum1 = rSum1 + rIncr1; end
rFSDs(3,1) = ((1 / rPeriod) * rSum1) + outline(1,2); % store C-sub-0 in output FSDs array - this array
will be 4 x iNoOfHarmonicsAnalyse

% calculate the C-sub-n coefficients
for iHNo = 2 : iNoOfHarmonicsAnalyse
rSum1 = 0;     for iP = 1 : iNoOfPoints
        rIncr1 = (rDeltaY(iP) / rDeltaT(iP))*((cos(rTwoNPi(iHNo-1)*rTime(iP+1)/rPeriod) -
cos(rTwoNPi(iHNo-1)*rTime(iP)/rPeriod)));
        rSum1 = rSum1 + rIncr1;
    end
    rFSDs(3,iHNo) = (rPeriod / rTwoNSqPiSq(iHNo-1)) * rSum1; end %
"foriHNo = 1 :..."

rFSDs(4,1) = 0; % there is no 0th order sine coefficient

% calculate the D-sub-n coefficients
for iHNo = 2 : iNoOfHarmonicsAnalyse
rSum1 = 0;     for iP = 1 : iNoOfPoints
        rIncr1 = (rDeltaY(iP) / rDeltaT(iP))*((sin(rTwoNPi(iHNo-1)*rTime(iP+1)/rPeriod) -
sin(rTwoNPi(iHNo-1)*rTime(iP)/rPeriod)));
        rSum1 = rSum1 + rIncr1;
    end
    rFSDs(4,iHNo) = (rPeriod / rTwoNSqPiSq(iHNo-1)) * rSum1; end %
"foriHNo = 1 :...

% the non-normalised coefficients are now in rFSDs % if we want the
normalised ones, this is where it happens if (bNormaliseSizeState == 1) ||
(bNormaliseOrientationState == 1)     % rTheta1 is the angle through
which the starting position of the first
    % harmonic phasor must be rotated  to be aligned with the major axis of
    % the first harmonic ellipse     rFSDsTemp
= rFSDs;
    rTheta1 = 0.5 * atan(2 * (rFSDsTemp(1,2) * rFSDsTemp(2,2) + rFSDsTemp(3,2) * rFSDsTemp(4,2)) /
...
            (rFSDsTemp(1,2)^2 + rFSDsTemp(3,2)^2 - rFSDsTemp(2,2)^2 - rFSDsTemp(4,2)^2));
    % calculate the partially normalised coefficients - normalised for
    % starting point
    for iHNo = 1 : iNoOfHarmonicsAnalyse
        rStarFSDs(1,iHNo) = cos((iHNo-1) * rTheta1) * rFSDsTemp(1,iHNo) + sin((iHNo-1) * rTheta1) *
rFSDsTemp(2,iHNo);
        rStarFSDs(2,iHNo) = -sin((iHNo-1) * rTheta1) * rFSDsTemp(1,iHNo) + cos((iHNo-1) * rTheta1)
```

```
* rFSDsTemp(2,iHNo);
        rStarFSDs(3,iHNo) = cos((iHNo-1) * rTheta1) * rFSDsTemp(3,iHNo) + sin((iHNo-1) * rTheta1) *
rFSDsTemp(4,iHNo);
        rStarFSDs(4,iHNo) = -sin((iHNo-1) * rTheta1) * rFSDsTemp(3,iHNo) + cos((iHNo-1) * rTheta1)
* rFSDsTemp(4,iHNo);
    end % for iHNo = 1 : iNoOfHarmonicsAnalyse

    rPsi1 = atan(rStarFSDs(3,2) / rStarFSDs(1,2));
    rSemiMajor = sqrt(rStarFSDs(1,2)^2 + rStarFSDs(3,2)^2); % find the semi-major axis of the first
ellipse

    rFSDs(:,:) = rStarFSDs(:,:) ./ rSemiMajor; % if we haven't asked for normalisation of orientation,
% return the coefficients normalised for starting point and size        if bNormaliseOrientationState
== 1
        % now find the orientation normalised values - return them in rFSDs
for iHNo = 1 : iNoOfHarmonicsAnalyse
            rFSDsTemp(1,iHNo) = (cos(rPsi1) * rStarFSDs(1,iHNo) + sin(rPsi1) * rStarFSDs(3,iHNo)) /
rSemiMajor;
            rFSDsTemp(2,iHNo) = (cos(rPsi1) * rStarFSDs(2,iHNo) + sin(rPsi1) * rStarFSDs(4,iHNo)) /
rSemiMajor;
            rFSDsTemp(3,iHNo) = (-sin(rPsi1) * rStarFSDs(1,iHNo) + cos(rPsi1) * rStarFSDs(3,iHNo)) /
rSemiMajor;
            rFSDsTemp(4,iHNo) = (-sin(rPsi1) * rStarFSDs(2,iHNo) + cos(rPsi1) * rStarFSDs(4,iHNo)) /
rSemiMajor;
        end % for iHNo = 1 : iNoOfHarmonicsAnalyse            rFSDs =
rFSDsTemp; % return fully normlised coefficients     end
end % if (bNormaliseSizeState == 1) || (bNormaliseOrientationState == 1)

return

function outln = rEfourier( rFSDs, iNoOfHarmonicsReconstruct,iNoOfPointsReconstruct)
% Reverse elliptical Fourier transform on the input Fourier series
% rFSDs generated by fEfourier.m. This reconstructs an approximation to the original outline figure
% using the specified number of harmonics and data points. See Kuhl FP and Giardina CR %
"Elliptic Fourier features of a closed contour" Computer Graphics and % Image Processing
18:236-258 1982 for theory and details.

iStartHarmonic = 2; % start at 2  - No.1 is just an offset and is added in later (lines 17 & 27)
ReconnedOutline = 0; % reconstruct the
x-projection for iTime =
1:iNoOfPointsReconstruct
    rSum = 0.0;
    for iHNo = iStartHarmonic:iNoOfHarmonicsReconstruct        rSum = rSum + (rFSDs(1,iHNo)
* cos(2*(iHNo-1)*pi*iTime / iNoOfPointsReconstruct) + ...
            rFSDs(2,iHNo) * sin(2*(iHNo-1)*pi*iTime / iNoOfPointsReconstruct));
    end % for iHNo = 1 : iNoOfHarmonicsReconstruct     ReconnedOutline(iTime,1)
= rFSDs(1,1) + rSum; end % for iTime = 1 : iNoOfPointsReconstruct
```

```
% reconstruct the y-projection for iTime =
1:iNoOfPointsReconstruct
   rSum = 0.0;
   for iHNo = iStartHarmonic:iNoOfHarmonicsReconstruct          rSum = rSum + (rFSDs(3,iHNo) *
cos(2*(iHNo-1)*pi*iTime / iNoOfPointsReconstruct) + ...
          rFSDs(4,iHNo) * sin(2*(iHNo-1)*pi*iTime / iNoOfPointsReconstruct));
   end % for iHNo = 1 : iNoOfHarmonicsReconstruct
ReconnedOutline(iTime,2) = rFSDs(3,1) + rSum; end % for
iTime = 1 : iNoOfPointsReconstruct outln =
ReconnedOutline;

return

function k_coeff = calc_k_coefficent(x, y)

   x_diff = circular_diff(x, 1);     x_diff2 =
circular_diff(x_diff, 1);     y_diff =
circular_diff(y, 1);
   y_diff2 = circular_diff(y_diff, 1);

   k_coeff = zeros(1, length(x_diff));     for t =
1: length(x_diff)
      numerator = x_diff(t) * y_diff2(t) - y_diff(t) * x_diff2(t);
denominator = (x_diff(t)^2 + y_diff(t)^2)^(3/2);       k_coeff(t) =
numerator / denominator;     end

return
    % do a simple linear difference differentiation on circular data
function yy = circular_diff(xx, interval) yy = xx; for i = 1: length(xx)     if
(i == 1)
      yy(i) = (xx(i + 1) - xx(end)) / (2 * interval);
continue     end
   if (i == length(xx))
      yy(i) = (xx(1) - xx(i - 1)) / (2 * interval);
continue     end
   yy(i) = (xx(i + 1) - xx(i - 1)) / (2 * interval); end
return
```

1.8 S10. MATLAB code for size standardisation of thickness maps

```
function standardise_gridto1
%This function will standardise a matrix so that all values fall between 0
%and 1. It can also be used to standardise values according to the matrices
%median values by uncommenting the appropriate commands
% first get a folder from the user
folder_name = uigetdir('', 'Choose the folder that contains the landmark coordinates');
if folder_name == 0
   error('No folder selected');
end
 % make a series of folders for your results to go into
file_list = dir(fullfile(folder_name,'*.csv'));
if isempty(file_list)
   error('No .csv files found in folder')
end
if ~exist([folder_name '\normgrids0_1\'],'dir')
 mkdir([folder_name '\normgrids0_1\'])
end
%if ~exist([folder_name '\normgridstomedian\'],'dir')
 %mkdir([folder_name '\normgridstomedian\'])
%end
if ~exist([folder_name '\norm_heatmap0_1\'],'dir')
 mkdir([folder_name '\norm_heatmap0_1\'])
end
if ~exist([folder_name '\norm_heatmap0_1b\'],'dir')
 mkdir([folder_name '\norm_heatmap0_1b\'])
end
file_number = 1:length(file_list)

   file_name = [folder_name '/' file_list(file_number).name];
   [pathstr, name, ext] = fileparts(file_name);

%This standardises all the values to the overall median value of the matrix
for file_number = 1:length(file_list)
      file_name = [folder_name '/' file_list(file_number).name];
   grid = csvread(file_name);
   ncols = size(grid,2)
   grid2 = grid([1:120],[1:ncols])
   gridmax = max(grid2(:))
   gridmin = min(grid2(:))
   standmin = (grid2-gridmin)
   stan0_1 = standmin./(gridmax-gridmin)
   %medianvalue = median(grid2(:))
   %standardised_grid = 1./grid2
   %standardised_grid2=grid2-medianvalue
   heatmap_name = [folder_name '\norm_heatmap0_1\' file_list(file_number).name(1:end-4)
'stand_heatmap'];
   heatmap_name2 = [folder_name '\norm_heatmap0_1b\' file_list(file_number).name(1:end-4)
'stand_heatmap2'];
```

```
    csvwrite([folder_name '\normgrids0_1\' file_list(file_number).name(1:end-4) 'normgrid.csv'],
stan0_1);
   % csvwrite([folder_name '\normgridstomedian\' file_list(file_number).name(1:end-4)
'normgrid2.csv'], standardised_grid2);
    graph_bone_name = [file_list(file_number).name(1:end-4)];
    figure(1)
clf('reset');
set(gcf, 'Color', 'w');
set(gcf,'Units','pixels');
set(gcf, 'Position', [50, 50, 1000, 1000]);
    imagesc(stan0_1)
    %plot_name = heatmap_name
    set(gcf,'PaperType','<custom>');
set(gcf,'Units','inches');
set(gcf,'PaperUnits','inches');
position = get(gcf,'Position');
set(gcf,'PaperPosition',[0,0,position(3:4)]);
set(gcf,'PaperSize',position(3:4));
%axes1 = axes('Parent',figure1,...

plot_name = [heatmap_name];
colormap(jet(256));
colorbar('EastOutside');
xlabel('Bone Position');
ylabel({'Slice number.','Lower numbers are more proximal'});
%MODIFY THIS TITLE AS SEEN FIT
title(['Standardised cortical thickness of ' file_list(file_number).name(1:13) ' humerus']);
print(gcf,'-dtiff',[plot_name, '.png'],'-r 72')

figure(2)
clf('reset');
set(gcf, 'Color', 'w');
set(gcf,'Units','pixels');
set(gcf, 'Position', [50, 50, 1000, 1000]);

imagesc(stan0_1)
set(gcf,'PaperType','<custom>');
set(gcf,'Units','inches');
set(gcf,'PaperUnits','inches');
position = get(gcf,'Position');
set(gcf,'PaperPosition',[0,0,position(3:4)]);
set(gcf,'PaperSize',position(3:4));
plot_name2 = [heatmap_name2];
colormap(jet(256));
print(gcf,'-dtiff',[plot_name2, '.png'],'-r 72')
end
```

1.9 S11 MATLAB code for combining matrices and running PCA.

```
function combine_run_pca
% first get a folder from the user
folder_name = uigetdir('', 'Choose the folder that contains the landmark coordinates');
if folder_name == 0
    error('No folder selected');
end

file_list = dir(fullfile(folder_name,'*.csv'));
if isempty(file_list)
    error('No .csv files found in folder')
end
for file_number = 1:length(file_list)

    file_name = [folder_name '/' file_list(file_number).name];
    data1 = csvread(file_name);
  data1 = data1(1:120,1:360)
  data1a=reshape(data1,43200,1)

    combineda(file_number,:) = horzcat(data1a);

 end
%Runs PCA and exports csv files of scores.Replace *NAME* with an appropriate prefix.
[coeff,score,latent,tsquared,explained,mu] = pca(combineda)
csvwrite('*NAME*combinedvaluesforpca.csv',combineda)
csvwrite('*NAME*_scores.csv',score)
csvwrite('*NAME*_coeff.csv',coeff)
csvwrite('*NAME*_explained.csv',explained)

end
```