Mid Term Report for ESA-Project

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"2D and 3D Quantification of Bone Structure and its Changes in Microgravity Condition by Measures of Complexity"

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This report describes the research activities of the project “2D and 3D Quantification of Bone Structure and its Changes in Microgravity Condition by Measures of Complexity” for the period of time after the beginning of the project (signed contract received on December 20, 2000) until the end of December 2001.

Motto

WG suggested at the project board meeting on February 12, 2001, to follow the success of Pioneer 10 as a shinning example for this project.

1. First Task: Computer Set-Up at FUB

The proposed computer, Onyx RE2, needed to work with large data sets, was offered at a prohibitive high cost. After consultations with SUN, Hewlett-Packard, IBM, and experts from our partner ZIB, we came to the conclusion to order three computers where each has a specific task instead of buying a large computer that can everything. These consultations took place in December 2000.

We received the best offer from HP, negotiated the price, and ordered the equipment (PC workstation for image data visualization, Unix workstation for computing of acquired data, Linux server for data storage and archive). The computers were delivered between December 2000 and the end of January 2001. The machines were in working order at our first meeting on February 12, 2001. Due to missing small parts, an early broken tape drive, and a broken CPU, all machines were fully operational in May/June 2001. The Unix-Workstation and the Linux-Server were completely installed at that time. Although the PC-Workstation was fully integrated in the network of the set-up, the complete installation was finished on September 7, 2001. This delay was due to a special ordered volume-rendering engine. The computer set-up was completed in all its aspects on September 7, 2001.

The funds to purchase the three computers and their peripherals were supplied by our industrial partner Siemens AG. The payment was made in full after FUB confirmed complete delivery. Thereby, the financial commitment of Siemens AG was fulfilled to the full term of the contract on schedule.

The Amira framework, a 3D visualization and quantification software package developed by our partner ZIB, is installed on the PC-Workstation since January 2001, on the Unix-Workstation since February 2001, and has been up-dated constantly. The Amira-framework is installed on a Unix-

Workstation located within the Institute of Physics at the University of Potsdam since September 2001.

Tests of the workability of the Amira framework on micro-CT data (FUB) were performed before the signature of the contract. When the first human tibia biopsy data sets became available in July 2001, they were processed on the Amira framework. We found no problem in regards of handling the amount of data.

When large biopsy cylinders (4 cm long with a 7 mm diameter) were scanned on the micro-CT (FUB), it became obvious that these probes cannot be processed with one acquisition. It was decided to scan the long probes in two separate sets of data.
The partner ZIB developed a procedure to merge the two separate data sets. Therefore, we are able to work with large merged micro-CT data sets.

This manifold task was part of the first milestone (3D milestone 1st year, 12th month), formulated as: "Fully installed and complete workable set-up of the Onyx computer, including fully workable Amira framework on micro-CT data sets."
We consider this point of the milestone to be achieved, although we changed the computer set-up as described above.

In order to fulfill this first task, the partners FUB (AB, MG, PS, WG), ZIB, and Siemens AG were fully involved and worked together without even minor problems.

2. Collection of Specimens and Their Preparation

FUB (WG, MG) collected from 30 human cadavers 6 bone specimens each (lumbar spine, proximal femur, calcaneus, distal tibia, distal radius, midshaft humerus) in February 2001. These specimens remain at FUB for the duration of the project. The collection of specimens was made possible by a co-operation with the Institute of Anatomy (Prof. Bogusch) of the Humboldt-University in Berlin and with a co-operation with the Institute of Anatomy (Prof. Graf) of the Free University Berlin.
The cadavers were 20 females (age 57 – 98 years) (peripheral bones taken from the left side: 13, from the right: 7) and 10 males (age 60 – 94 years) (peripheral bones taken from the left side: 3, from the right: 7).
We proposed to use the resources of the Dept. of Pathology at FUB but were unable to utilize it, due to limited resources at that institution.

It is known that bone specimens obtained from formaldehyde-prepared cadavers contain air in the bone marrow region. A procedure to extract the air permanently had to be found.
The experimental set-up to extract air from the specimens was finished on March 23. It includes an excicator, vacuum pump, manometer, and all the appropriate connections. Formaldehyde/alcohol solution, plastic welder, and plastic bags were needed as well. The equipment and material was purchased for this project.

Vacuum extraction tests were performed. The results of the vacuum tests led to the conclusion: the bone specimens have to be in the formaldehyde solution for 2 hours to warrant a mostly airless bone marrow space. The pressure within the vacuum system has to be maintained at 80 mbar for 2 hours. The vacuum system has to be left airtight over night.
Results of plastic bag welding experimentation: the plastic bags remain airtight after welding. The handling is not easy due to the formaldehyde solution. The fluid, when carefully handled, does not influence the welding. However, the toxic fumes make the procedure difficult.

The experimentation and air-tightness was checked with a pQCT-scanner at FUB.

All 180 specimens were airtight and packaged by November 2001. They are kept in a refrigerator.

This task was achieved by FUB and the non-project co-operation partners mentioned above. The task was completed without problems. We consider the experimentations and its problems usual scientific work.

3. Imaging of 180 Bone Specimens and BMD Measurements

3.1 pQCT Imaging

A Standard Operational Procedure (SOP) for the bone mineral density measurement (BMD) and imaging of each skeletal region by peripheral quantitative computed tomography (pQCT) was written by WG. The scanning required the invention of new table fixations, a new table construction, and the invention of positioning support material. All necessary requirements were obtained through the help of the Technical Department at FUB.
The Center of Bone and Muscle Research (formerly Osteoporosis Research Group) at FUB provided a pQCT-scanner Stratec XCT 2000 for scanning 30 radii. This was performed in April 2001. Due to the workload on this scanner, the scanner was no longer available in May. An identical scanner was ordered from Stratec Medizintechnik GmbH in Pforzheim, Germany. The additional pQCT-scanner did not provide the same image quality we expected to achieve while we imaged 30 tibiae. Therefore, extensive phantom tests were performed on the second scanner. By arrangement with Stratec Medizintechnik GmbH, WG brought the second scanner back to the company and watched the up-grading of the scanner at the factory. When the factory-intern tests seemed to be perfect, additional tests to confirm our requirements were performed. However, the acquisition time was long and the heat generation at the x-ray tube seemed to be too high for a safe performance of the scanner. The engineers at Stratec determined that an additional cooling fan would be necessary to run the scanner safely. The fan was screwed onto the cover of the scanner, so that a particularly built pQCT-scanner resulted from our image requirements. Additionally, a software break was implemented to stop the machine after 2 hours of operation.
It was agreed to pay a monthly leasing fee for this special scanner to the company.

All images and all BMD-measurements of all specimens from all skeletal regions were performed on this special pQCT-scanner. The slice thickness was 1 mm; the x-y-pixel size was set to 0.2 x 0.2 mm. This set-up was maintained for all bones throughout the study. It required re-scanning of all tibiae and radii.

Slice location definition:

1. Distal Radius: 4% of the entire radius length has been subtracted from the most dense part of the distal radial joint surface. That location was used for the slice location. The individual lengths of the radii were unknown. We assumed an average length for females of 240 mm and for males of 275 mm. This location is similar to patient examinations.
2. Proximal tibia: 17 mm distal of the most dense part of the medial joint surface. This location is in the area of a potential surgical biopsy site.
3. Lumbar vertebral body L3: center of the vertebral body in the transaxial direction.
4. Femoral neck: The slice was taken at the thinnest diameter of the femoral neck (center of the neck) and perpendicular to the main axis of the femoral neck.
    - A second slice was taken in the area between the femoral neck and the femoral head. The angle to the main axis was determined on a radiograph.
5. Calcaneus: The slice was taken perpendicular to the anatomical length axis. The location was 20 % of that length subtracted from the dorsal end.
    - A second slice was taken at 30 % location.
6. Midshaft humerus: The slice was taken perpendicular to the axis at the middle of the bone. The center was determined on a radiograph.

The task was finished on December 6, 2001.

3.2 Phantom Tests Performed on pQCT

Experiments on resolution were performed at FUB. The phantom tests showed a improved signal/noise ratio, excellent detail resolution, and very good contrast. Therefore, certain actions (noise reduction procedure) as discussed at the project board meeting in July 2001, were not necessary to perform. In addition, the images were compared with the results of two scans acquired on a pQCT-scanner (Densiscan) by Scanco Medical AG. The image quality obtained on the special scanner by Stratec Medizintechnik GmbH was superior. It is assumed that a better image quality cannot be obtained on any commercial pQCT-scanner at this point in time.

3.3 BMD-Measurement of Lumbar Spines

All L3 vertebral bodies, and when L3 was fractured either L2 or L4, of the 30 lumbar spines were examined on the pQCT-scanner first.
Secondly, exactly the same vertebral bodies were scanned on a CT-scanner Somatom Plus S in QCT-mode. This procedure was performed in two different x-y-resolutions: 0.182 x 0.182 mm and 0.323 x 0.323 mm. The first relates to a high-resolution mode, while the latter is the resolution obtained on patient examinations. In both resolution modes, slice thicknesses of 1, 2, 4, 8, and 10 mm were obtained at the midvertebral transaxial location of the vertebral body. The lumbar spines were not moved or touched between each of the ten scans.
The data will be used for BMD-measurements. The slices will be used in a sub-study that will explore the dependency of the measures of complexity on slice thickness, noise levels, and resolutions.

This task was finished in November 2001.

3.4 QCT of the Femoral Neck

Although our initial proposal envisioned the scanning of the femoral neck on a CT-scanner, the developer of the software to measure the BMD of the femoral neck by QCT stopped further development. This is a result of a decreased market demand. DF approached the developer (Institute of Medical Physics at the University of Erlangen, Germany) about the situation but a solution was not available.
It was concluded at the project board meeting on July 5, 2001, that the project will not insist on QCT-scanning of the femoral neck. The progress of the project is more important, in particular, the development of the complexity measures in 2D and 3D than the development of refining tools to assess the BMD of the femoral neck. Therefore, the femoral necks will be imaged and scanned for BMD measurement on pQCT only. It is understood that this procedure is for experimental purposes only and cannot be transferred to patient examinations. However, the BMD derived from these images will be reliable. The pQCT-images can be used for the development of the 2D measures of complexity without restrictions.

3.5 Radiography

Radiographs in two views were taken from all 30 tibiae. In addition, radiographs were taken from all 30 proximal femora in one view, from all lumbar spines in two views, from all calcanei in two views, and from all humeri in one view.
The radiographs were necessary to define a repeatable slice location for the pQCT-scanner and for the correct positioning of the bones on the examination table of the pQCT-scanner.
All tibia biopsies were x-rayed in mammography technique and in two views. The images will serve for identification purpose if the adhered labels fail.

All radiographic procedures were finished in November 2001.

The issues of points 3.1-3.5 involved FUB (WG, PS, DF) and the industrial partner Scanco Medical AG as well as the non-project co-operation partner Stratec Medizintechnik GmbH. All problems were solved without delays. Point 3.4 led to a change of the proposed research process.

4. Image Size and Measures of Complexity

It was found at FUB that the pixel size of human vertebral body images obtained from CT-scanners has an influence on measures of complexity. This influence is of a magnitude which cannot be ignored. Therefore, in preparation for 2D measurements on bones from different skeletal sites, the problem of pixel size influence on measures of complexity had to be studied, defined, and a solution to be found.

4.1 Scientific Solution, February 2001

In January 2001, WG scanned two lumbar specimens (since the specimens for this project were not collected at that time, non-prepared spines from a different project were used) on the CT-scanner Somatom Plus S at FUB. The L3 vertebrae were chosen. The midvertebral slice in 1 mm slice thickness was repeatedly scanned at exactly the same location. However, the scans differed in magnification (zoom size). The zoom ranged from 1.7 to 3.2 in 0.1 steps. Additional scans in 4.0 and 5.0 zoom were obtained on one specimen.

These CT scans were used by PS to develop a correction procedure to account for different zoom factors. The normalized mean edge led to a correction of the static-dynamic coefficient (an encoding parameter). Moreover, a change of the “block of symbols” was needed as well and was directly coupled to zoom factor changes.
Another approach was taken at ZIB (SP, CH). Several resampling algorithms were tested and the results were statistically tested at UoP (AZ). The calculation of errors for measures of complexity showed that a windowed sinc algorithm in its specialized form as Lanczos alg. leads to acceptable errors in the range of 1 to 2 %.

Another potential problem was explored by ZIB and UoP: Influence of location shift of bone material within the pixel matrix. The errors due to shift are acceptable, the range is between 1 and 3.5%.

The results were limited at that time because the spine samples had air in their marrow spaces and it did not simulate the behavior of trabecular bone accurate enough. The experimentation, however, showed already the direction in which we had to go in order to find a solution. As soon as the specimens for this project were collected and prepared, the experiments were repeated.

4.2 Scientific Solution, July 2001

Further experimentations with new samples without air in the marrow space reinforced the direction already taken. The Lanczos algorithm kernel 20 was able to give excellent results (stable measures of complexity) within a magnification range of 1.9 to 2.6. This is the range that can be expected in practice. The error of four measures of complexity obtained from resampled images within this magnification range is less than 5%.
Downsampled images showed the least remaining influence on the results of the measures of complexity, whereas upsampling is object dependent. The object we used, human vertebral bones, may not be an example for all bone regions.

The geometrical correction algorithm developed by PS can be seen in principle as an alternative to the resampling algorithm of Lanczos. Several parameters need to be changed when using the geometrical correction, whereas the resampling algorithm works automatically all over the image. A similar automatic procedure for the correction algorithm can be written, however, the changes made to the preprocessing procedure could lead to difficulties to compare results obtained from images with different magnification factors. In addition, the geometrical alterations may alter the original bone structure in unforeseeable directions. Therefore, the decision was made to use the Lanczos algorithm kernel 20 to resample CT-images taken at different magnification factors.

4.3 Conclusion

1. Whenever possible a standardization of CT-image acquisition should be achieved when specimens or patients are scanned: zoom factor 2.2 or FOV 100.

2. Since the strict application of standardization is for practical reasons in the hands of radiological technicians a guarantee that the specific zoom factor or the FOV will be implemented cannot not be assured. An alternative method to compare measures of complexity calculated from images taken at different zoom factors was needed.
3. The Lanczos algorithm kernel 20 is the best resampling algorithm for vertebral trabecular bone within a magnification range of 1.9 to 2.6. Using this algorithm to reach the standardized zoom factor 2.2 as a preprocessing step will lead to comparable results when a different magnification was used than 2.2.
4. The error introduced by the resampling algorithm is below 5%, whereas non-preprocessed images with different magnifications produce complete different non-comparable results.
5. The location shift of resampled pixel information introduces a negligible error of less than 3.5%.
6. The alternatively developed geometrical correction algorithm may be used in the future to detect problems with magnification factors from other skeletal regions.
7. At a later stage of the project, the magnification factor issue will be further explored on models.

The points 4.1-4.3 involved very close team work by FUB (PS, WG), ZIB (SP, CH), UoP (AZ, JK), and discussions with the industrial partner Siemens AG.

5. Measures of Complexity for 2D Images Obtained at Different Skeletal Locations

The technique consists of three main stages which are executed in the following order:

1. Image preprocessing

   It entails a standardized segmentation of an area of interest (bone) from the rest of the CT-image and split the area of interest into three data sets: entire, trabecular, and cortical bone.

2. Image encoding

   It simplifies the image and substitutes every gray-scale value of every pixel by a symbol. Thereby the level and dynamics of the x-ray attenuation in the vicinity of the considered pixel are taking into account.

3. Quantitative assessment

   The quantification of the bone architecture by measures of complexity requires the symbol-encoded image from step 2.

5.1 Image Preprocessing

The procedure to segment the bone area from the connective or soft tissue and formaldehyde filled plastic bag has been described in detail in our publications prior to this project.
However, further refinement of the algorithm to select the region of interest (ROI) in a vertebral body was necessary. Some specimens have a complete spinal canal and the dorsal appendices in place, whereas others were cut off in the middle of the pedicles. An algorithm was necessary to standardize the ROI obtained from both sorts of vertebral bodies.
It was accomplished by PS (FUB) in October 2001.

5.2 Image Encoding

After the ROI is segmented, the image is encoded by symbols before their architecture is quantified by measures of complexity. This reduces the amount of gray-shades, whereas the pixel resolution itself is maintained. Two encoding parameters must be specified
static-dynamic threshold e_{ds},
marrow threshold a_m.

The value of a_m was found experimentally. These parameters depend on the attenuation levels which are a representation of the underlying materials. The levels of the encoding parameters must be appropriate to cover fat tissue, formaldehyde, bone, and soft marrow tissue. The following values of the encoding parameters are used in this study: e_{ds} = 80 pQCT values, a_m = 275 pQCT values.

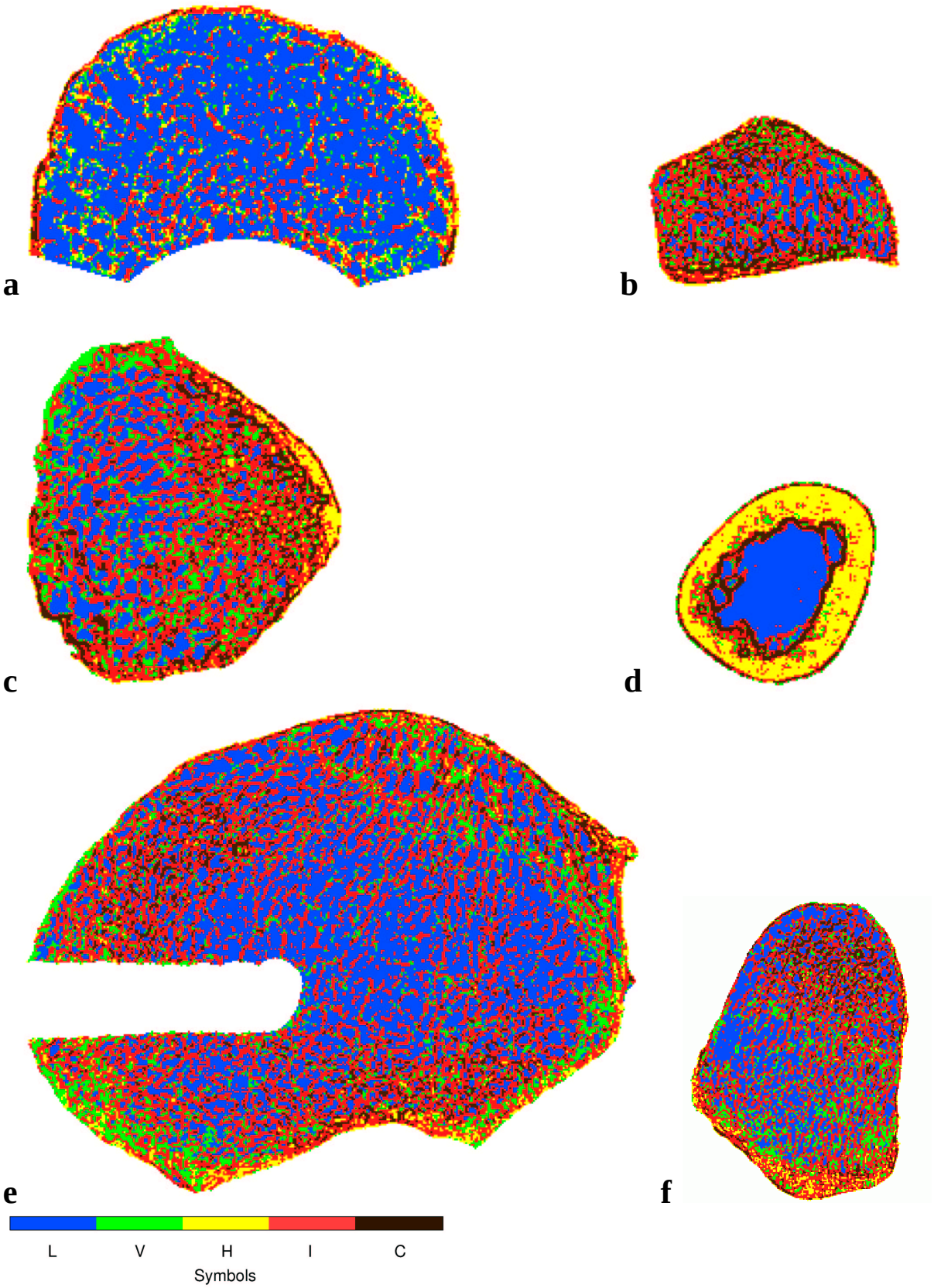

Fig. 1: Symbol-encoded high-resolution pQCT-images of one subject (59-99) at six different skeletal locations: a) vertebral body L3, b) distal radius, c) femoral neck, d) midshaft humerus, e) proximal tibia, f) calcaneus. The symbol annotation is derived from a landscape terminology, symbolizing the undulations of a x-ray attenuation curve through an image. L = lake, V = valley, H = highland, I = incline, C = cliff.

These values are fixed for all different skeletal locations: lumbar vertebral body, femoral neck, proximal tibia, distal radius, calcaneus, midshaft humerus.

An example of symbol-encoded high-resolution pQCT-images from 6 different skeletal locations of one subject (59-99) is shown in Figure 1.

5.3 Quantitative Assessment

The complete analysis of all 2D data is due as an milestone at the end of May 2002. Therefore, the presented data at this point is preliminary and was not subject to rigorous scientific scrutinizing. However, only minor corrections are expected.

We use six different measures to quantify different aspects of the bone architecture. Those measures were introduced in prior publications: Structure Complexity Index SCI, Structure Disorder Index SDI, Trabecular Network Index TNI, Index of Global Ensemble IGE, Size of Maximal L-block, and Size of Average L-block.

The calculation of complexity measures from bones of different sizes requires an appropriate width of a moving window N that collects and analyzes local statistics (block size). An optimal window width which includes enough structural elements for a small bone like the radius is unacceptable narrow for a large bone like the tibia where a narrow window could hardly includes one trabecula. The opposite situation can be found as well: a window optimized to analyze the local structures of a tibia could include the entire image of a radius. This problem must be considered for the global purpose of studying 2D bone architecture. There are two interrelated approaches to analyze the structure of the bone at different skeletal location:

- Optimization of the distinction between normal and pathological structures for every skeletal location separately. - This approach can be aimed to find an optimal location from the view point of diagnostics, and to establish an optimal set of structural measures to detect the pathological changes in this most sensitive location as early and precise as possible. This task requires that the parameters which control the image encoding and calculation of complexity measures need to be set differently and optimized for every particular skeletal site.

- Comparison of the structural composition and dynamics of structural changes in different skeletal sites in general. – Such an approach would concentrate on differences and similarities in the behavior of the architecture of different bones of a human body rather than on the precise distinction between normal and pathological changes of each individual site. For this purpose, it would be important to use the very same fixed set of all parameters governing the image encoding and measures of complexity calculation while processing different skeletal sites.

At this stage of the project we focus more on the second approach and use a fixed set of controlling parameters to process the images from all skeletal locations.
The width of the window that collects the local statistics has been set to be a compromise and to be appropriate to analyze both small and large bones. All processed skeletal sites were analyzed using the width of the window of N=35 pixels.

So far, we are using bone structure versus bone density diagrams to study the architecture. Since the pQCT-scanner is always calibrated before the image acquisition and the relation between the pQCT values and BMD has been found experimentally, it is possible to calculate the BMD from exactly the same area which is used for the calculation of complexity measures. This is the first time that we are able to use BMD obtained from specified ROIs. We call this parameter *calculated BMD* to distinguish it from the measured BMD which is provided by the scanner software and relates to a slightly different area of interest.

Figures 2 and 3 show the structural measures of complexity versus calculated BMD for each skeletal location. Figure 2 concentrates on trabecular bone, figure 3 on the entire bone (that is cortical and trabecular bone together). We are coming more and more to the conclusion that the trabecular bone

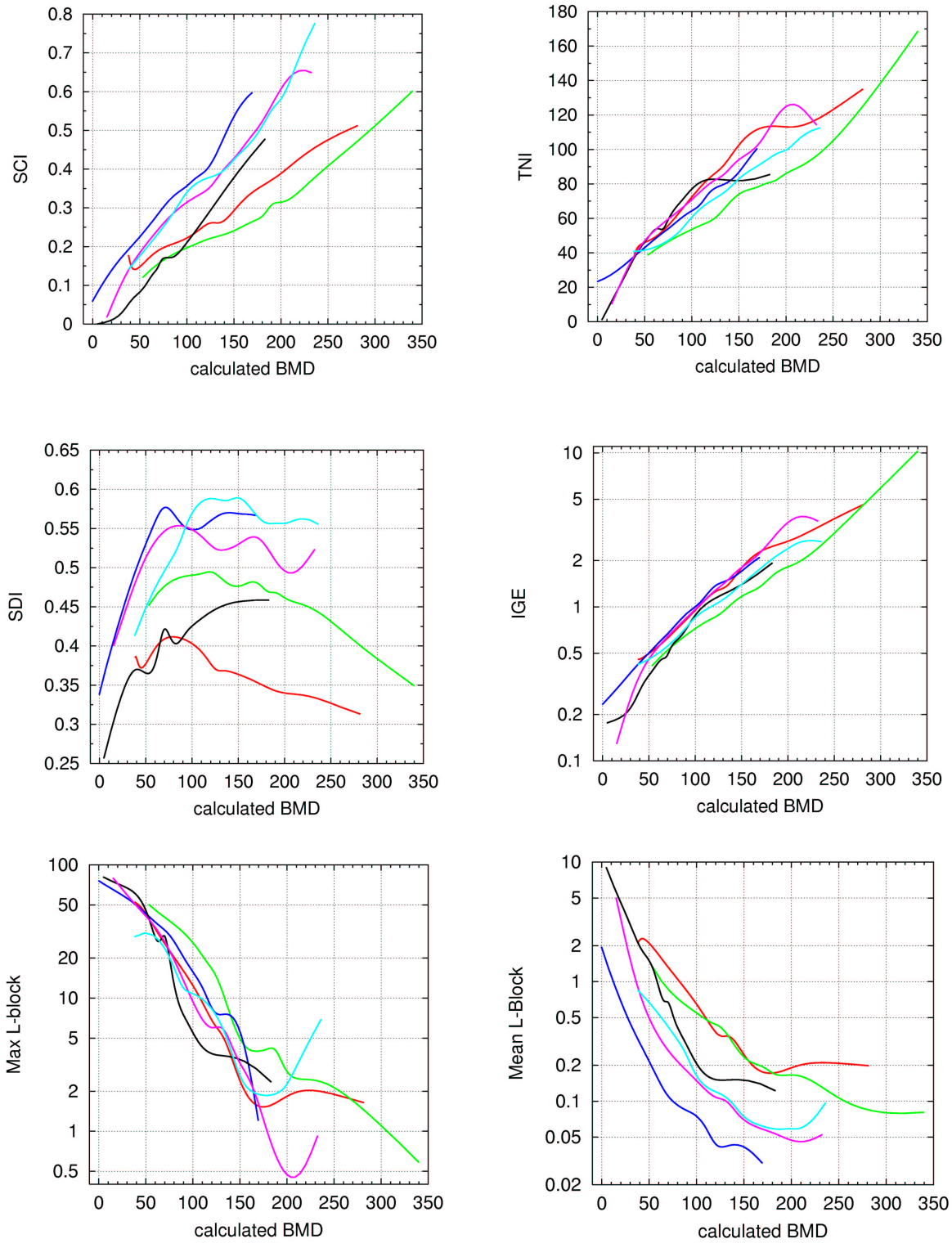

Fig. 2: Measures of complexity versus calculated BMD. The measures of complexity as well as the BMD are derived from the segmented trabecular bone images of 6 skeletal regions: radius, tibia, vertebral body L3, femoral neck, calcaneus, femoral head/neck border.

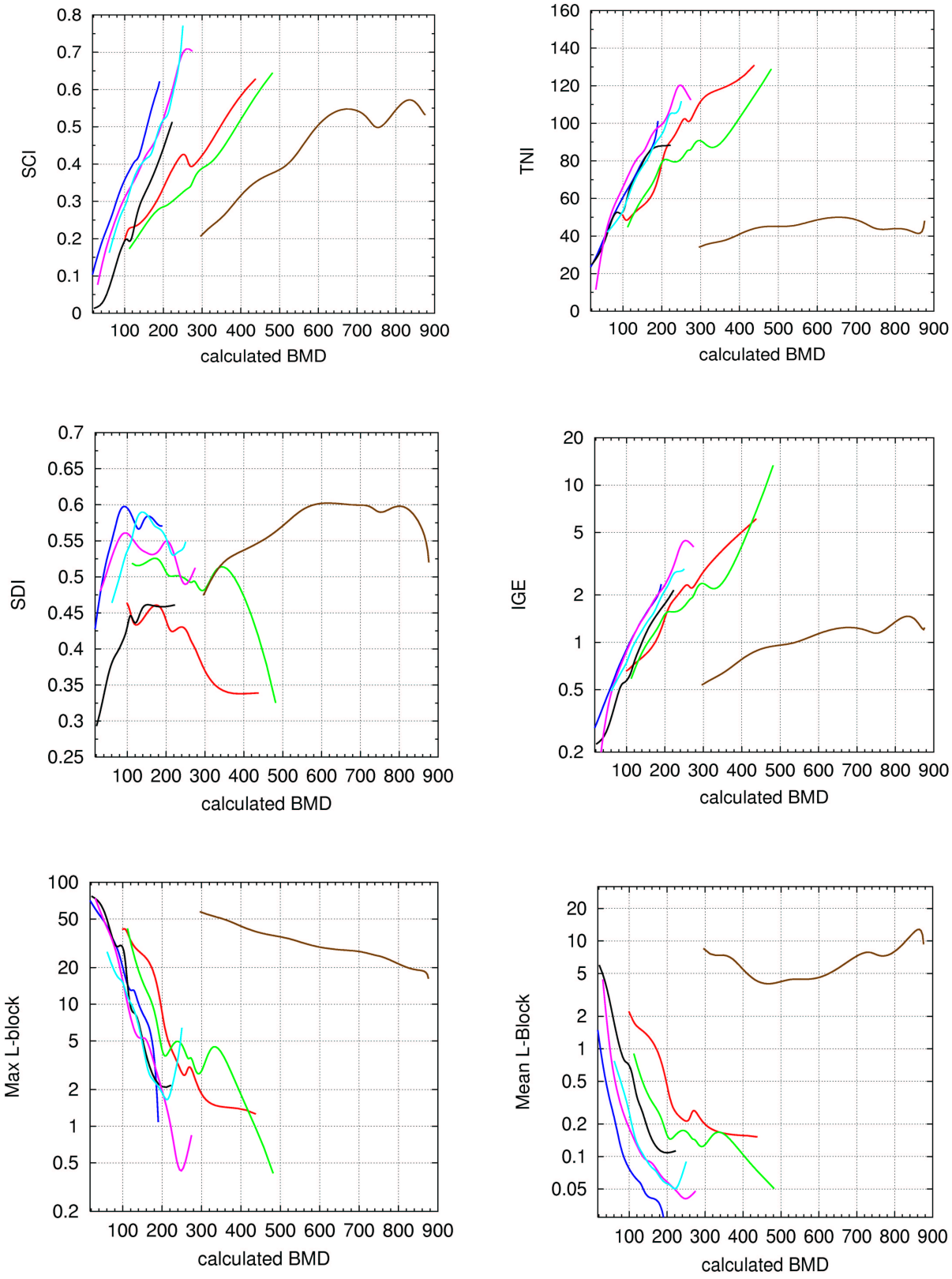

Fig. 3: Measures of complexity versus calculated BMD. The measures of complexity as well as the BMD are derived from the entire bone images (trabecular and cortical bone) of 7 skeletal regions: radius, tibia, vertebral body L3, femoral neck, calcaneus, femoral head/neck border, humerus.

cannot be prudently studied only by itself when we want to quantify the architecture of the bone. Even the most sophisticated cortical/trabecular separation procedure is somehow arbitrarily. The cortical bone belongs to the architecture of the bone as much as the trabecular bone does. The distinction between different skeletal locales is much more pronounced when we study the entire bone instead of the trabecular part of the bone only.

The data fitting curves are Bézier curves with the following characteristics: parametric curve of degree *n*, *n* is the number of data points, the end points are connected to the data curves. We experimented with other forms of presentations before we concluded that Bézier curves are the best approximation tools for our set of data.

The interpretation of the diagrams in figures 2 and 3 leads to the following preliminary statements:

- Skeletal changes can be distinguished from another by both their density expressed by BMD and by their architecture assessed by structural complexity measures.

- The structural organization of the bone is related to the skeletal location. When we consider all the bones from different skeletal sites at a value of BMD of 150 mg/cm³, for example, the complexity of the femoral neck architecture (SCI) is minimal at this value of the BMD. The complexity of a radius is higher by 30 %. The complexity of the vertebra is 60% higher, and the complexity of a tibia exceeds the complexity of the femoral neck by 130%.

- The Structure Disorder Index SDI indicates the differences in orderliness of the bone architecture. The femoral head/neck border region got the most disordered trabecular bone structure. The radius, whose SDI is 40% less then the SDI of the tibia, is characterized by the most ordered structure.

- The trabecular parts of bones from different skeletal locations have different complexities and different degrees of disorder despite that they have the same BMD value. The analysis of the entire bone confirms this conclusion, see figure 3.

- The rate of change of the SCI varies for each location. The results suggest that the same amount of bone loss expressed by BMD will cause a much more dramatic decay of the structural complexity in the tibia than in the femoral neck or the radius. A qualitatively similar behavior is also observed by the analysis of the entire bone.

- TNI and IGE exhibit a similar behavior with less distinction between different skeletal locations.

- The midshaft humerus, as a mostly cortical bone, does not show differences in TNI, as expected. The IGE does not vary much either, a suggestion that the global architecture of this bone is stable. That is what we would expect from a cortical bone. However, the local dynamical complexity (SCI) increases and seems to find a saturation, even in cortical bone.

- Despite the humerus as a sole cortical bone, it appears that the calcaneus, the tibia, and the area between femoral head and neck behave similar. The radius and the femoral neck, both bones have a larger cortical content, behave different.

- The proximal tibia is an excellent peripheral bone region to study architectural changes in bones. The fast changes over a limited change in BMD makes it a good candidate for examinations.

- Further scrutinized analysis of the data is necessary in order to come to solid conclusions.

The final analysis of the 2D data is due with the next milestone at the end of May 2002.

The points 5.1-5.3 involved mostly FUB, in particular PS and WG.

6. Bone Modeling

Although the timeline for the execution of this research program calls for model applications at the end of the second year, we were in need to find a bone model in order to test certain behaviors of the measures of complexity.
The results of the modeling experiments in 2D lead to the following conclusions:

- Measures of complexity are very sensitive in the detection of local defects and local elements which appear invisible to the eye.
- Measures of complexity are more efficient than BMD.
- Measures of complexity are equally good in the detection of order or disorder.
- Measures of complexity are very sensitive to the appearance of disorder.
- Measures of complexity change quickly during the evolution of change in a regular or random way.
- Measures of complexity can differentiate between regular or random changes.

The model is still work in progress and there are many more aspects which we like to study.

This point involved UoP (AZ, JK) since May 2001.

7. Acquiring Bone Biopsies from Human Tibiae Specimens

Bone biopsies are needed to check the 3D development of the trabecular network and its changes in microgravity condition. It was proposed to use tibia bone as a most accessible site for harvesting trabecular bone.
The biopsies were taken at the medial side, 14 mm distal of the tibia plateau. This is the surgical site for biopsies.

The biopsies were taken with surgical compressed air equipment. The coring drill is coded with diamonds on the outside as well as on inside.
All biopsies were acquired with the utmost care and precision. The biopsies came out of the tibiae within the coring drill. A pushpin of the exact size as the inner diameter of the core of the drill was lightly applied to press the biopsy out of the coring. The biopsy slid out of the coring drill directly into a 70% alcohol filled plastic tube. None of the biopsies were handled with human fingers. The care was essential in order to achieve an artifact free trabecular bone biopsy.
All biopsies have a diameter of 7 mm. The length of the biopsies varies between 2 and 4 cm. There were 3 biopsies which provided biopsy cylinders of 1 cm length only. The bone cylinder broke while the cylinder was taken out of the tibia. The longer part of the biopsy remained inside the bone.

All 30 tibia biopsies were sent to UoA (JT) in the originally labeled plastic tubes.

The entire procedure on all 30 biopsies was carried out by WG (FUB) with the help of a study nurse.

The procedure took place in June 2001.

8. Preparation of the Tibia Biopsies for μ-CT Scanning

UoA received all 30 tibia biopsies in July 2001. Due to vacations in the lab, the preparation was started immediately but took longer then expected. Other problems occurred when the embedding in methylmetacrylate took place in plastic cups. Too much material was needed so that the curing went fast at a high temperature. This caused air-bubbling. The air bubbles were hold in place.
A few embedded biopsies were sent to FUB to test the possibility to use them for scanning. MG at FUB cut the surplus material. It was found that the material around the air bubbles was soft and not hardened. Except for 3 biopsies the embedded material was sent back to UoA. JT was able to correct the procedure and resolve the methylmetacrylate with chloroform. That took 4 days per specimen.
A new procedure was set up. The process involves the following steps:

- Wash out of formaldehyde by ethanol; takes 3 weeks.
- The plastic cups were replaced with glass tubes of an inner diameter of 14 mm.
- The amount of methylmetacrylate was dramatically reduced.
- The curing was performed in vacuum condition so that potential air bubbles were sucked away.
- The hardening took place in a refrigerator, takes 2 weeks. It was found that water cooling would reduce this amount of time.
- Only a small batch was handled at a time (5-10 biopsies) for better control of the process.
- The glass test tubes were smashed after hardening.

The embedded biopsies were sent back to FUB.

This point was carried out by JT (UoA) in close consultation with FUB (MG, WG).

The procedure started in July and was finished by the end of November 2001.

9. 3D Scanning of Tibia Biopsies

µ-CT machines are available for this project at FUB and at Scanco Medical in Switzerland. At the end of year one, 9 biopsies were scanned at FUB. These biopsies were used to find the best acquisition parameters. Questions like

- What would be the best resolution?
- Can we use this resolution for all biopsies as a standard?
- Can the biopsies be scanned in one or two scans?
- If two scans are necessary, due to the length of the biopsy, can we stack the scans together without loosing information?
- Can the biopsies be arranged on the examination table without any wobbling?
- Do we need to manipulate the plastic material to fit all biopsies in a standardized parameter protocol?
- Does the plastic material influence the outcome?
- Do air bubbles influence the visualization of the trabecular bone?
- Do air bubbles disturb the calculation of measures of complexity?

needed to be answered before any systematic scanning of the biopsies could begin.

- While we scanned the earlier preparations with a voxel size resolution of 25 µm and 30 µm, the set-up of 14 mm thick cylinders led to a small decrease of the resolution. The appropriate resolution is 32 µm for the size of the cylinder. However, the resolution can be improved when the plastic material surrounding the biopsies would be reduced. A standardized protocol has been established. If it would be necessary in the progress of the project to acquire a higher resolution of the biopsies, it can be achieved by reducing the plastic material down to a diameter of 8 mm. Thereby, the geometry of the µ-CT-scanner at FUB would allow an increase of the resolution to about 18 µm.
- Most of the biopsies need to be scanned in two sections, due to their length. The two scans can be stacked on top of each other. There is no loss of data information in that process.
- The biopsies within that 14 mm cylinder can be safely arranged on the table without wobbling.
- The plastic material does not influence the data of the trabecular bone. The analysis did not show artifacts or increase of noise due the plastic material.
- Miniscule air bubbles within the trabecular network do not influence the visualization.
- The symbol encoding of the space between the trabeculae will be changed as a prerequisite to assess the measures of complexities. The air bubbles do not prohibit the symbol-encoding procedure. The air bubbles do not compromise the results of the measurements of complexity.

Three biopsies were send to Scanco Medical in the summer. We received the data at the end of December 2001.

The comparison between the results of the scanner at FUB and the scanner from Scanco Medical AG is in process.

This task started in September 2001 and is in progress. It involved FUB (MG, WG), UoA (JT), ZIB (SP), and the industrial partner Scanco Medical AG. The task had problems due to delays.

10. Damage Assessment and Identification of Potential Artifacts in Bone Biopsies

There are at least two fundamental different ways to acquire bone biopsies: manually or with a power-driven drill. The manual procedure is some kind of drilling into the bone by turning a coring drill by hand. That particular coring drill has teeth at its end. A side effect is that those teeth can bend and the biopsy material gets spiral cuts around the cylinder. In addition, the outer parts of the biopsy can have broken trabeculae, due to the force of the hand pressing and rotating the coring drill into the bone. Thereby, the manual procedure has a high potential for artifacts that would influence the appearance of the trabeculae within the biopsy, and therefore, would have effects on any type of quantification as well. We decided against the manual procedure in April 2001.

The second approach requires a drill driven by compressed air. The drill is a typical surgical AO instrument and is found in all surgical departments. In co-operation with the Department of Trauma Surgery of the University Hospital Benjamin Franklin at FUB, we borrowed the necessary equipment to run preliminary tests. The procedure was performed under fluoroscopic conditions in the Department of Radiology of FUB. The coring drill has very small diamonds attached on the inside as well as on the outside. The cylinders acquired with such a coring drill are smooth on the outside and do not have any spiral cuts. Trabeculae at the outer range of the biopsy are not broken but sharply cut by the diamonds. The utmost care and the best possible precision were taken for the procedure. Visual inspections of the bone biopsies led to the conclusion that the material is completely intact.

The micro-CT-scans of the first biopsies confirmed our visual observation that there was no damage to the trabeculae at the very edge of the biopsies. All trabeculae were cut with a clear sharp edge.

However, we found bone dust, due to the cutting diamonds inside the coring drill, in the outer parts of the biopsies. These bone particles are very small, max. 2 µm in diameter. They are located in the soft tissue between the trabeculae and around the entire circumference of the biopsies. The dust particles reach as deep as 0.7 mm into the probes.
The partner at UoA, having the most experience with bone biopsies, reports that drilling residues in bone biopsies are unavoidable. Furthermore, the amount of drilling residues found in the tibia biopsies is much less than the amount of drilling residues found in e.g. iliac crest biopsies. The reason for that is that the bone marrow consists almost exclusively of fat in the tibial metaphysis whereas it is haemopoetic in the iliac crest. During the embedding of the tibial biopsies, some of the fatty marrow is resolved in the ethanol used in the embedding process and a substantial amount of the drilling residues is thereby “washed out”.
The drilling residue is easily identifiable and the standard method in bone histomorphometry for removal of drilling residues is to edit out these particles with image editing software. The partner at UoA has used this method during the last 7 years. Whereas in micro-CT studies, the standard for removal of drilling residue is to consider only a sub--area of the volume by excluding a small zone of bone tissue that is nearest to the edge of the biopsy. Both methods for removal of the drilling residues are valid and widely used.

In addition, we found very small dense particles in the outer edge of the biopsies. At this point in time, we are not able to identify those extremely small particles. They may be small diamonds from the coring drill. Histological sectioning will probably be able to identify this particles.

Since the bone dust and high-dense particles are visualized by micro-CT, they would influence the symbol-encoding procedure used for measures of complexity. A procedure to cut the area off in which

these particles are found was needed. ZIB (SP) developed within the Amira framework a procedure to determine and to select a defined volume of interest (VOI) within the volume of the biopsy. Cutting off a ring in which the particles are found may lead to a volume that can be safely used for complexity analysis. The reduction of the size of the biopsy will probably not affect the results of the complexity analysis. The biopsy is already a small sample of a larger structure (the tibial metaphysis) and the results will therefore always only be a representative for the entire tibial metaphysis and the skeleton *per se*.

It has been found that it is not possible to make all bone sections acquired by histomorphometry and µ-CT 100% identical. During sectioning of the histological samples, the knife of the microtome will stretch the very thin (10 µm) plastic embedded sections in the direction parallel to the microtome knife. This stretching may not even be uniform over the length of the section. However, this effect is so small that it should not significantly influence the histomorphometric measurements. During the mounting of the thin plastic sections onto microscope slides, there is a possibility of creation of wrinkles in the sections and there is a risk for the sections to be damaged. This means that not all of the histological sections are of such high quality that they can be used for comparison with the micro-CT sections.
In order to perform histomorphometry the sections must be segmented into bone and marrow. This is carried out through a threshold filtering process. For technical reasons of different gray-scales in histomorphometry and µ-CT, it is not possible to use exactly the same level for the threshold filtering in the histological samples and the micro-CT sections.

At the project board meeting on December 18, 2001, the team decided to carry out a test series of a few additional tibia biopsies to study influences of virtual cuts (for removal of saw dust close to the edge) on complexity measures and on histomorphometry. The technique of editing the sections in histomorphometry could also be transferred to µ-CT by erasing isolated particles of small diameters. Influences of this technique have to be studied as well. The studies will begin as soon as other biopsies become available at FUB.

A disadvantage of the micro-CT scanner is its inability to detect osteoid (i.e. bone tissue that is not mineralized yet). We have chosen a staining method for the histomorphometric procedure that stains the bone dark blue and stains the osteoid tissue very light blue. Consequently, this should not be a source of discrepancy between the histological sections and the micro-CT sections. It should be noted though, that the amount of osteoid tissue is very low in normal individuals.

The damage assessment of bone biopsies revealed no damage when biopsies were taken with power-driven surgical coring drills, other artifacts were investigated and solutions were found, and the histomorphometric examination of these biopsies does not foresee any hindrance or problematic results. This manifold task was part of the first milestone (3D milestone 1st year, 12th month), formulated as:
“Controlled experimentation on damage assessment and other potential artifact sources on bone biopsies examined by micro-CT and histomorphometry, and its solutions for image processing set-up.”
We consider this point of the milestone to be achieved.

This task involved FUB (MG, WG), ZIB (SP, CH), and UoA (JT). The first results were produced in June, the conclusions were reached in December 2001.

11. Appropriateness to Use µ-CT Data Sets for 3D Measurements of Complexity

In order to investigate the usefulness of micro-CT data sets for 3D measurements of complexity, we needed to find ways how to deal with large amounts of data, and how to transfer our ideas of 2D complexity measurements into the 3D space.
UoP (AZ) and ZIB (SP) achieved a workable transfer of symbolic dynamics and symbol-encoding from 2D to 3D. The method of transformation is valid and it is appropriate to use micro-CT data sets

for the measurements of complexity as proposed. Figures 4 - 6 show a small part of a biopsy encoded with symbols. It is visible that each voxel of the volume is encoded.

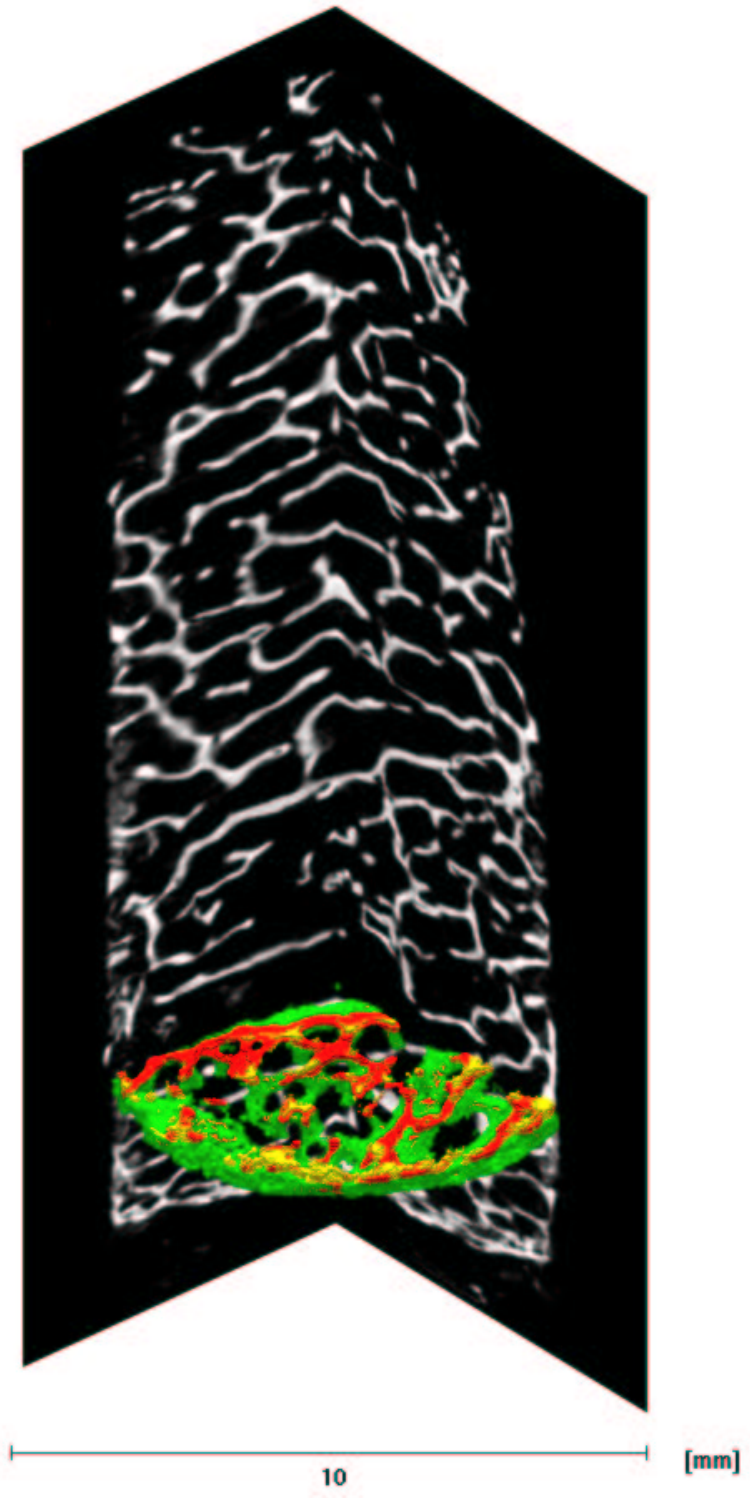

Figure 4: Tibia bone biopsy data set acquired by µ-CT. Sagittal and coronal plane reconstructions are shown in gray-scale. A transaxial symbol-encoded slice is seen in the lower part. The thin cortical tibia bone from the medial side of the tibia is at the bottom of the image.

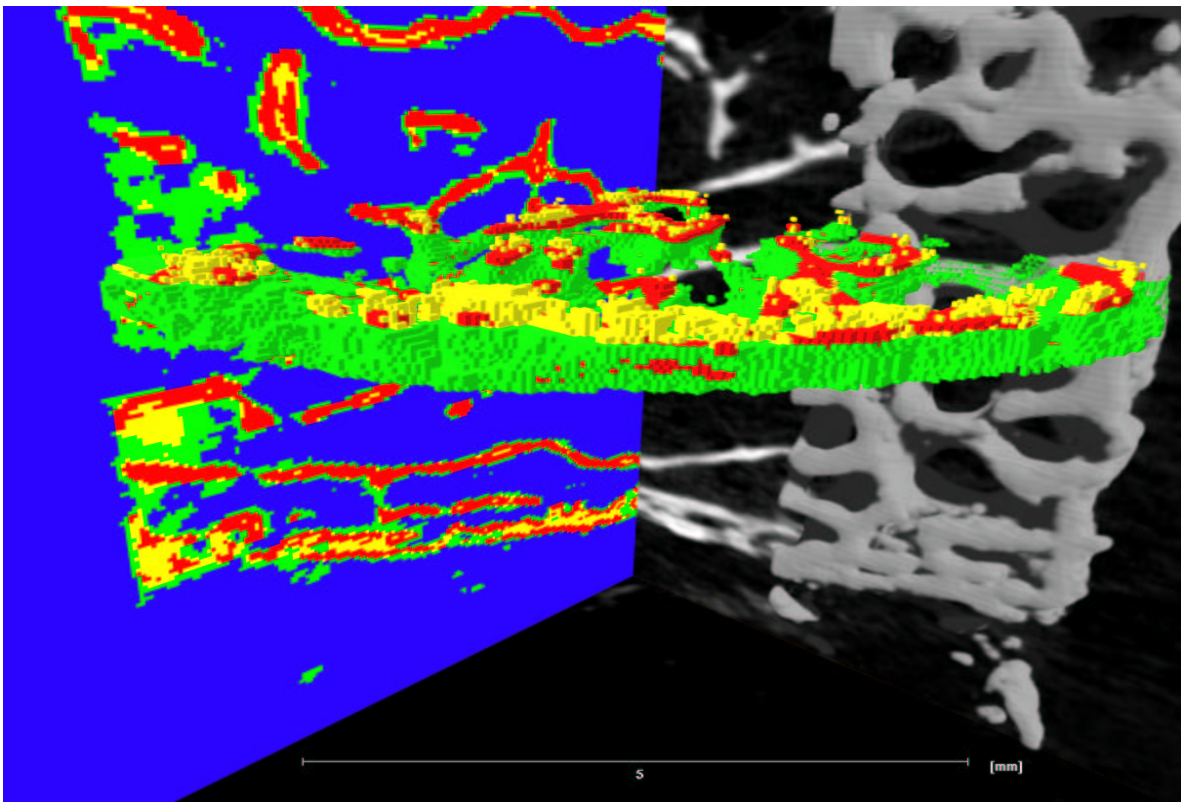

Figure 5: Similar area as shown at the bottom of figure 4. The left plane is a 2D sagittal reconstruction of symbol-encoded trabeculae, the right plane represents 2D and 3D reconstructions based on X-ray attenuation in the coronal direction. The transaxial fragment of the volume shows symbol-encoded trabeculae. Note the denser parts on both sides at the outer contour inside the biopsy. They represent bone dust within the marrow space due to the drilling extraction of the biopsy.

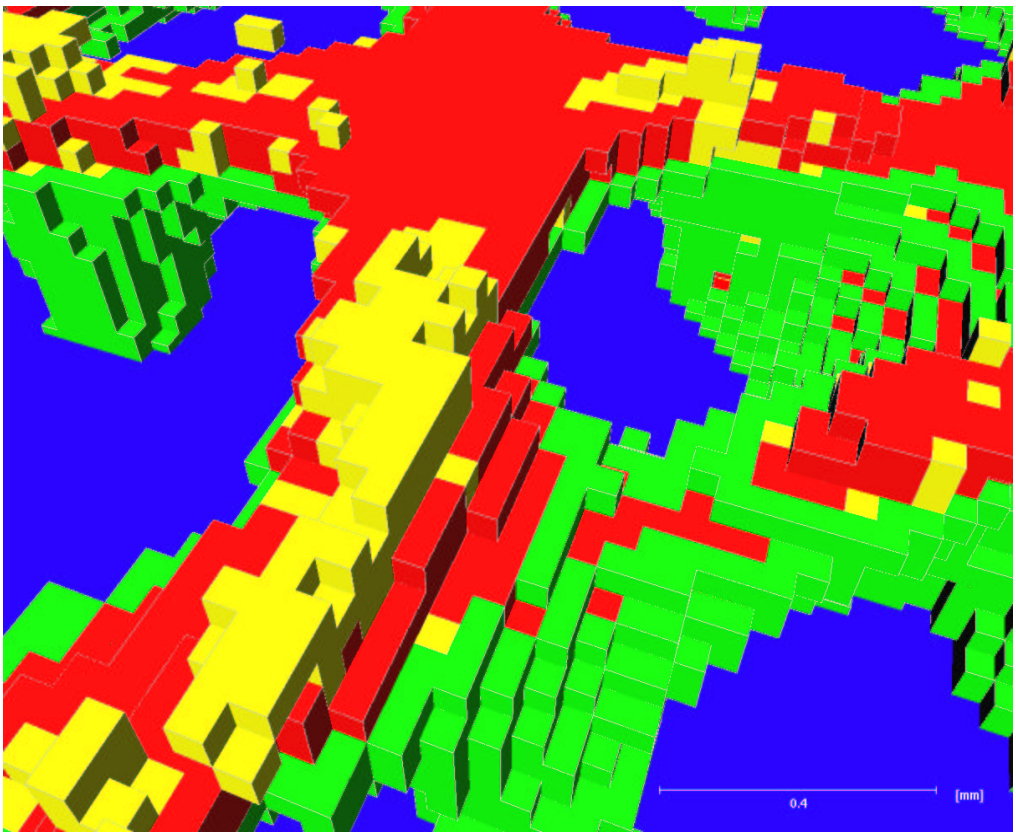

Figure 6: Magnification of a 3D symbol-encoded trabecular knot found within the transaxial fragment of the volume as shown in fig. 5. The resolution as well as the differentiation of the symbols becomes visible. The blue areas represent marrow space.

The symbol-encoding procedure revealed that the configuration of the data from two different micro-CTs (FUB and Scanco Medical) leads to two different edge definitions that will result in slightly different numeric results of complexity. This is a technical problem related to micro-CT. A solution to the problem is a phantom calibration of the micro-CT-scanners involved.

The other way of the approach to develop 3D measurements of complexity was a reduction of the amount of data to its essential structural configuration. When that would be achieved, a set of measures of complexity can be developed. ZIB (SP) developed a method of skeletonization of the trabecular network including a color-coding procedure, so that the original information is still kept within the reduced data set.

The visualization algorithms and its additional tools are constantly improved by ZIB and further developed when needed. Improvements that are specific to the project or in an early stage and are not included in the official Amira 2.3 release:

- Minor changes in the resampling module for investigating resampling artifacts (February – June 2001);
- Better automatic alignment of two image volumes. Needed to compare image data from different acquisitions (March, November 2001);
- Special import function for the micro CT data (September 2001);
- Modules to handle datasets that do not fit into main memory (started in August 2001);
- Module that allows to merge several blocks to one large data set with seamless transitions (September 2001);
- Histogram Module that allows to restrict the calculation to a volume of interest (October 2001);
- Begin of development for modules to calculate 3D measures of complexity (started in October 2001);
- Begin of development for skeletonization to a central surface (started in October 2001).
- General improvements in Amira 2.3 (August 2001) that are relevant for the project:
  - Improvements in the calculation of distance maps.
  - New features for interactive image segmentation. Useful for selecting volumes of interest.
  - Improvements and new tools for measurement (lengths and angles). These tools allow interactive measurement on any 3D object.
  - Slice aligner: new tool for manual and automatic alignment of 2D slices in a 3D image stack (facilitates reconstruction of geometric models from mechanical/histological cross sections, tutorial and demo included). Will be useful when the first histological slices from Aarhus arrive.
  - New options for resampling: specify voxel size of output data set; take dimensions and/or resolution of output from reference object.
  - New module ApplyTransform (resamples transformed 3D image onto new grid with identity transformation, resamples 3D image onto a new grid oriented as defined by a slicing module).
  - Volume rendering: support for palette textures and SGI/HP color table extension (less memory consumption, improved performance), real-time selection of subvolumes via a tabbox dragger (can be activated using the command "Voltex showBox 1"), much less flicker when rotating objects in 2D texture mode.
  - Better support for transparencies in slicing modules such as OrthoSlice and ObliqueSlice by use of OpenGL's alpha test function.

This manifold task was part of the first milestone (3D milestone 1st year, 12th month), formulated as: “Results on the appropriateness to use micro-CT data sets for 3D measurements of complexity, and potential revisions on the reconstruction (micro-CT) or visualization algorithms (Amira).”
We consider this point of the milestone to be achieved.

This task involved ZIB (SP), UoP (AZ), FUB (MG), and the industrial partner Scanco Medical AG. It started at the beginning of the project.

12. Skeletonization of the Trabecular Network and Other Visualization Tools as a Basis for Structural Quantification

Based on the previous topic, the Amira platform as a visualization tool has grown in specific directions. One direction is the improvement of the visualization of large 3D data sets as well as 2D images. The other direction is the development and incorporation of quantification tools. Both directions are essential for this project. In addition, the development of the platform has to follow the demands of imaging requirements as well as the development of the quantification measures. Up to this point in time, the team worked perfectly together to achieve the required goals. The main work load is on the team at ZIB (SP, CH) in close consultation with FUB (PS, WG, MG) and UoP (AZ, JK).

The amount of data derived from the bone biopsies is large. A procedure which preserves the geometrical arrangement of the trabecular network and reduces the amount of data was developed. A skeletonization method reduces the trabeculae to a central plane. This plane is one voxel thick. In order to preserve the local thickness of the trabeculae, the skeleton received a continuous color code. Figure 7 and 8 show an example.

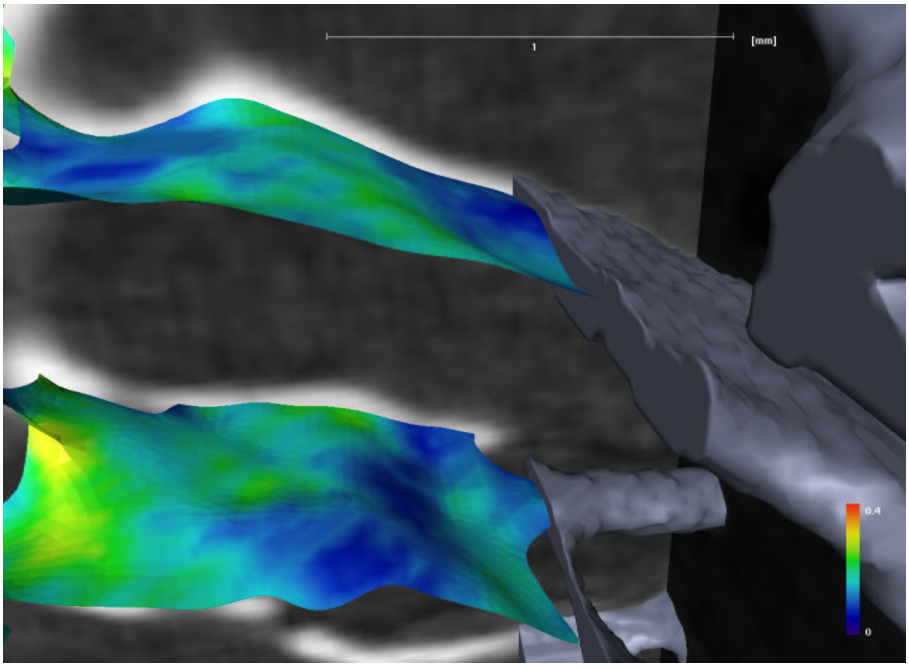

Figure 7: Detail of a color-coded skeletonization in comparison with 2D (background) and 3D reconstructions (right) of a tibia bone biopsy. The skeleton itself is located in the geometrical center of the trabeculae. This can be seen at the border to a 2D sagittal plane on the left, and on the right in comparison to a 3D coronal reconstruction. A color and length scale is provided at the lower right.

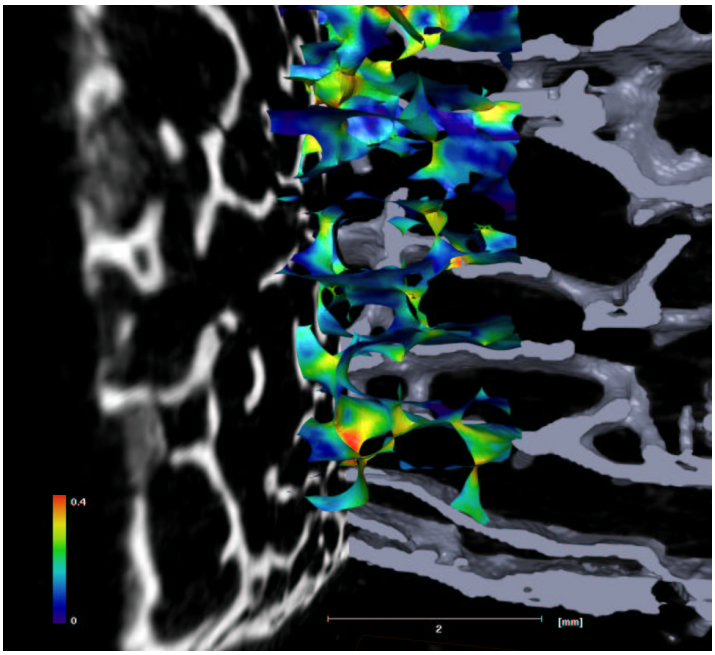

Figure 8: Visualization of the color-coded skeleton of the trabecular network within the lower part of the tibia biopsy as shown in fig. 4.

Other tasks included the provision of an alignment procedure for two stacked data sets from μ-CTed bone biopsies. The result was a development of an automatic alignment procedure.

The dust and probably diamond particles in the outer layers of the bone biopsies required a procedure which eliminates these particles. A particular volume of interest was created that can cut away structures that contain particles.

The development of these tools started after the availability of the bone biopsy data sets in September 2001. It is still work in progress.

13. Potential Directions to Apply Measures of Complexity to Quantify the 3D Structure of Bone

The team developed a strategy to develop measures for the quantification of the bone structure based on 3D bone biopsy data sets. Involved are ZIB (SP, CH), UoP (AZ, JK), and FUB (PS, WG, MG).
At this point in time there are four potential directions:

1. Entropy of the x-ray attenuation
2. Entropy of the skeleton data
3. Entropy of the geometrical arrangement of the bone in 3D
4. Measures of complexity derived from symbol-encoded data

All directions have been explored. Thorough investigation is necessary to decide which direction will be preferred.

14. Coordination between Team Members

The team members were in close contact with each other during the entire time of this report. Numerous ad hoc meetings took place, 2 times per month on average. These meetings were held either at FUB, UoP, or occasionally at ZIB. These meetings were necessary to facilitate coordination between the partners, to streamline research activities, to make decisions based on preliminary test results, and to exchange ideas. These meetings were attended by the team members concerned with time sensitive research activities: WG, PS, AZ, SP, MG, JK, DF, CH.
The frequency of communication between FUB, UoP, and ZIB increased over time. There is now a minimum of one telephone or email conversation between the team members of these three partners per day.
The communication between FUB and UoA has been telephone and internet contact. The frequency started to increase when UoA received the first bone biopsy samples, as expected, in the beginning of the 7. month of this research project program. Any specific research problem is addressed immediately, although the personal contact is limited to the project board meetings. One team member from UoA, LM, is ill since the middle of 2001. The project's activities are not limited by it, and LM is always available for comments and recommendations.

The coordination between FUB and the industrial partners Scanco Medical AG and Siemens AG has been excellent. Siemens delivered its promised financial part on time. The representative from Siemens, KK, made valuable comments and suggestions to the research activities during project board meetings.
The contact with Scanco Medical developed as promised. Bone biopsies were delivered to Scanco Medical and the team received 3D μ-CT-data from these biopsies. The communication by telephone or email with Scanco Medical (BK and colleagues) increased during the last months of the reporting time.
Both industrial partners have contributed very valuable resources and services to this project so far. The scientific partners are available for exchange.

The international cooperation between German, Danish, and Swiss partners led already to results which could not be obtainable if this project would have been based on national resources alone.

The objectives of the MAP program within the realm of this project could be enhanced by increased involvement of the pharmaceutical industry. Connections have been made as well as a substantial financial contribution by Roche Pharmaceuticals. It can be envisioned that the association with Roche Pharmaceuticals will be important for further developments within a drug-based prevention program of bone loss under microgravity conditions.

The team members of FUB are always on search for the best available image quality for the assessment of bone architecture. It is imaginable that this team or other partners will find ways and access to better radiological machines or procedures leading to improved image quality and, therefore, to more precise quantifications of the bone's structure. It that respect, programmatic adaptations could be highly possible.

15. Publications During the Time of the Report

15.1 Publications Related to the Project Containing Data from Previous Studies

1. The paper "Bone Architecture Assessment with Measures of Complexity" was published in Acta Astronautica 49, 171-178, 2001.
   ABSTRACT
   Architectural changes in trabecular bone by osteoporosis were utilized as a model for the changes which probably occur in human bone while exposed to microgravity conditions. Although there are many concerns about microgravity-induced bone loss, little is known about the impact of microgravity on the three-dimensional architecture of the skeleton [1]. 50 (level L3) and 57 (level L4) vertebral bones harvested from human cadavers were investigated by computed tomography (CT) and quantified in terms of bone mineral density (BMD). Based on the symbol-encoded transformed CT-images, five measures of complexity were developed which quantify the structural composition of the trabecular bone. This quantification determines the bone architecture as a whole. Depending on the specific measure of complexity and its relation to BMD, a 5-10% change of BMD is related to a 5-90% change in structural composition. The method requires a non-invasive CT-procedure of the lumbar spine resulting in a radiation exposure of about 30 µSv effective dose [2]. The technique is useful for the evaluation of the bone status of space-flying personnel as well as for patients on ground.

2. Oral presentation at Experimental Chaos Conference 2001 by W. Gowin. The presentation's title was "Bones and Nonlinear Dynamics – The Quantification of Architecture". A full paper was submitted to the publishing committee and is in print at this point in time.
   ABSTRACT
   In order to quantify the internal architecture of a bone in a holistic manner based on radiological images, the methodology of nonlinear dynamics was applied. Image processing algorithms, an expansion of symbolic dynamics, and five measures of complexity were introduced to quantify the trabecular part of human lumbar vertebral bodies. Healthy vertebral bones have a complex and ordered architecture with a high degree of spatiodynamics. Pathology changes the architecture significantly and can be quantified by measures of complexity.

15.1 Publications Containing Data from this Research Project

3. An abstract by the title "Regional Structural Skeletal Discordance Assessed by Measures of Complexity" (by W. Gowin, P. Saparin, D. Felsenberg, J. Kurths, A. Zaikin, S. Prohaska, H.-C. Hege) has been submitted to the World Congress on Osteoporosis, Lisbon, May 2002. It has been accepted as a poster.
   REGIONAL STRUCTURAL SKELETAL DISCORDANCE ASSESSED BY MEASURES OF COMPLEXITY
   Aims: The aim of the study was to compare the structural composition of trabecular bone as well as of the whole bone at six different skeletal regions. The bones' composition was evaluated by measures of complexity.

Methods: Bone specimens of thirty human cadavers were examined. The distal radius, the proximal tibia, the vertebral body L3, the femoral neck, the calcaneus, and the midshaft of the humerus were scanned in high resolution mode (20 x 20 micro-m) in 1mm slice thickness on a XCT-2000 scanner (Stratec, Germany). The acquired images were numerically segmented into trabecular and cortical bone areas using previously described preprocessing techniques. Symbolic dynamics were applied to the images in order to receive simplified and easy to analyze symbol-encoded images. After this image-processing procedure, the segmented data sets were evaluated by five different measures of complexity. The BMD was measured as well.
Results: The numerical architectural complexity at each skeletal region is different. Although there is a interpersonal variation affecting all skeletal regions, the variation from region to region is greater. The highest interpersonal variation was found in the calcaneus.
Conclusions: The discordance of architectural composition at different skeletal regions can be accurately calculated by measures of complexity. The architectural composition of bones depends among other factors on genetic programming as well as on biomechanical load conditions. Despite of the regional discordance, the bone structure at all six skeletal regions follows the same general rule: Loss of bone density occurs simultaneously with a rapid decreasing structural complexity. This transition is accompanied by an increased degree of disorder within the bone architecture at intermediate bone densities. The high interpersonal variation of structure within the calcaneus is evidence of the difficulties to standardize quantitative measurements at this skeletal site.

4. Three abstracts have been submitted for the 8th European Symposium on Life Sciences Research in Space and the 23rd Annual International Gravitational Physiology Meeting to be held in Stockholm from June 2-7, 2002. All three abstracts have been accepted as oral presentations.
    a. “Visual Analysis of Trabecular Bone Structure” by S. Prohaska, H.-C. Hege, M. Giehl, W. Gowin
    We explore within the framework of a MAP-Project the structural deterioration of human bone tissue in osteoporosis as a model for bone loss under microgravity conditions. With μCT scanners it is possible to acquire high resolution image data of trabecular bone biopsies (hundreds up to thousands of voxels in each dimension). Beneath the task to assess these data quantitatively it is important to visualize them to literally see the structure as well as its variations and changes. This might lead to a better understanding of the modeling and remodeling processes involved.

    We present visualization techniques that can be used interactively on state-of-the-art PCs and demonstrate how the frontier can be pushed further. Volume rendering and surface rendering of the trabecular bone are useful to get an overview at reduced resolution or for viewing small parts of the structure. A fast rendering of the whole biopsy at high resolution is not possible. Another problem is that the rendered images are very complex due to the huge number of trabeculae. Structures near to the virtual camera hide the rest and make it difficult to grasp the whole architecture. Identifying the structural important elements (rods and plates) of the real trabecular network leads to a significant reduction in the number of graphical primitives to be displayed. To reach this goal, a skeletonization process is applied to create the central surface. After triangulation interactive frame rates are possible even for large structures. Furthermore, the visual complexity is reduced. It is easier to conceive overall aspects as well as details of the architecture. Local measures like trabecular thickness or averaged CT attenuation can be color coded onto the surface to focus on different aspects of the trabecular network.

    All the methods are integrated in a common framework that allows to use them simultaneously in one viewer. In the same way oblique slices through the original data and interactive tools for measuring 3D distances and grey value profiles are available. This software environment facilitates the development of new structural measures

through the visual support that helps to generate new ideas. Prototype modules for bone analysis can easily be integrated through a program interface.

b. "Bone Modeling and Structural measures of Complexity" by A. Zaikin, J. Kurths, P. Saparin, W. Gowin
Absence of the gravitation leads to a loss of bone mass and to the development of osteoporosis. It is important to note that this process entails not only changes in bone mineral density (BMD) but crucial transformations of the bone structure. As a consequence, the measurements of the BMD alone as routinely performed in clinical settings, do not adequately reflect the deterioration of the bone. To analyze architectural changes a technique [1] has been proposed which is based on symbol encoding of the bone image and the consequent calculation of a set of structural complexity measures to characterize different aspects of the architectural complexity. The application of these sensitive methods have resulted in typical dependencies between structural measures of complexity (SMC) and BMD.

The aim of this project is to test the sensitivity and powerfulness of SMC with simulated test objects, whose compositions are similar to the architecture of human trabecular bones. We checked how SMC reflect the local and global ordering and disordering processes, whether SMC are sensitive to detect local defects and whether SMC can be used to detect hidden ordered structures within a globally disordered object. Our investigations on simulated structures show that applications of SMC seem to provide additional information on bone architecture, and therefore, could be useful for the structural quantification of real human bones. They could provide powerful and sensitive quantifications of the bone structure additionally to the conventional measurement of BMD.

Another direction of the investigation is the adaptation of bone modeling algorithms in order to reproduce the dependencies between SMC and BMD. It will be verified by experimental measurements. We study different modeling algorithms which describe the loss of the bone mass and look for possible mechanisms causing the changes in structure observed experimentally.
[1] P.I. Saparin, W. Gowin, J. Kurths, and D. Felsenberg, Phys. Rev. E 58, 6449 (1998).

c. "Comparison of Bone Loss with Changes of Bone Architecture at Six Different Skeletal Sites Using Measures of Complexity" by P. Saparin, W. Gowin, D. Felsenberg
We explore within the framework of a MAP-Project the structural deterioration of human bone tissue in osteoporosis as a model for bone loss under microgravity conditions. We apply measures of complexity to assess quantitatively the structural composition of bone tissue at six different skeletal locations. The complexity of the bone architecture is analyzed and compared with each other at the different locations.

Eight high-resolution 2D CT-images (pixel size 0.2 x 0.2 mm, slice thickness 1 mm) were acquired from skeletal regions of 30 human cadavers: proximal tibia, vertebral body L3, distal radius, midshaft humerus, femoral neck, femoral region between neck and head, and calcaneus at two different locations.

Our technique consists of three stages: image segmentation, encoding of the image by symbols, and quantification of symbol-encoded structure by measures of complexity. For each skeletal site an originally developed image segmentation procedure provides two standardized regions of interest: the trabecular bone and the entire bone. At the next stage, the segmented image was encoded using a mixture of 3 static and 2 dynamical symbols. The spatial arrangement of symbols representing the bone structural composition is assessed by six structural parameters based on measures of

complexity and symbolic dynamics: Structure Complexity Index expresses of the complexity and homogeneity of the architecture; Structure Disorder Index assesses the degree of order or disorder within the architecture; Trabecular Net Index informs about the richness of the trabeculae and its interactions with each other; Index of Global Ensemble evaluates the dynamics of the assembly of structural elements; Size of maximal L-block and Average Size of L-block measure the bone element replacement by marrow tissue. In addition, for every region Bone Mineral Density was calculated.

We analyzed the relation between the amount of bone mass and the quantification of bone architecture by using bone density vs. bone structure diagrams. It was found that the structural composition responds to a loss of bone mass differently and with a different rate at each skeletal site. It was shown quantitatively that for the same amount of bone density the complexity of the resulting bone structure and the degree of disorder within its architecture does depend on the skeletal location. The proposed technique is able to quantify to a high degree the structural loss of the bone tissue and may help to diagnose and to monitor changes in bone structure of the space-flying personnel as well as of patients on Earth.

16. Brief Review of Activities for the Second Year

1. Finishing of the development of preprocessing algorithms for 2D measures of complexity for six different skeletal sites.
2. Evaluation and comparison of all 2D data derived from 180 bone specimens (30 per each skeletal site) = Milestone 2.
3. Beginning of collection of patient 2D data.
    a. Archival data can be used. They are available at FUB without delay.
4. Development of patient data preprocessing for 2D measurements.
5. Collection of 20 selected patients and measurements at 5 different skeletal sites each – 2D data processing and evaluation.
    a. Planning of this study at FUB is in its final stages. It is expected that a study with hemiplegia patients can start in April 2002.
6. Continuation of development of 3D preprocessing tools, preliminary data sampling on 3D quantification by measures of complexity.
    a. Ideas and its technical implications have been explored already.
    b. Work load distribution will be determined after the Mid-Term Report Meeting.
7. Refinement of biopsy assessment by micro-CT.
    a. Comparison study of the μ-CT scanners is already on its way.
8. Application of 3D encoding to a model, study of typical 3D bone composition, application of measures of complexity in 3D models.
    a. The study of models in 2D has already started.
    b. UoP will develop and study 3D models.

These activities will be executed according to our Timeline of Execution of the Research Program in its revised version from Oct. 10, 2000.