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Characterizing the Mechanical Properties of Bone with the Assistance of Digital Light Microscopy

Abstract

Micro-indentation analysis of cross-sectioned bone samples has the potential to unravel differences in bone quality and fracture mechanisms. Since bone consists of a spongy structure, it is imperative to control the position of indentations using high-resolution imaging, ensuring every indent is exclusively located in mineralized material. Electron microscopy is commonly employed for inspection, but this is time consuming and image quality is limited due to out-of-focus problems. At the University Medical Center Hamburg-Eppendorf, Dr. Björn Busse and his group have instead employed the Olympus DSX500i inverted digital light microscope to streamline micro-indentation testing. Fast and efficient, this system enhances image quality to confirm the exact localization of the indentations.

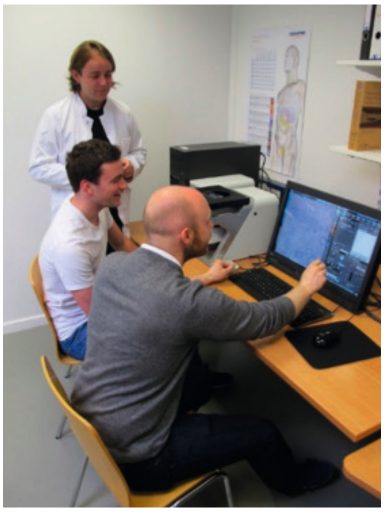
Micro-indentation is a popular approach for determining the mechanical properties of a range of materials. At the Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf (Germany), Dr. Björn Busse and his research group are employing this technique to investigate the mechanical properties of bone. Bone has a complex hierarchical structure, the properties of which are relevant for human diseases such as osteoporosis.

An important step during micro-indentation is the retrospective analysis of the indentations in order to ensure that only the bone matrix was subject to indentation, rather than voids, pores or the polymer used for embedding the samples. This was previously achieved using Electron Microscopy (EM), which involves time-consuming sample preparation. To improve the efficiency of the micro-indentation process, Dr. Busse has employed high-resolution digital light microscopy as an alternative to EM, with the Olympus DSX500i inverted digital light microscope. With 13x zoom optics, the combination of high resolution with operating simplicity vastly improves the speed and efficiency of the micro-indentation process, and presents further avenues of research – investigating the properties of cracks and their propagation that occur adjacent to indentations.

These capabilities also offer possibilities throughout a vast field of applications - as advanced micro-indentation techniques translate to researching materials through all sectors of material science.

At a Glance

- Testing mechanical properties of bone with micro-indentation is vital for understanding human diseases such as osteoporosis
- High-resolution imaging confirms indentations occur in mineralized material
- Electron microscopy is time consuming
- The Olympus DSX500i inverted digital light microscope streamlines micro-indentation testing



Visualizing micro-indentation testing of bone in the laboratory. (From back to front) Dr. Busse with medical students Mr. Kilian Stockhausen, M.Sc. and Mr. Christoph Riedel, B.Sc. operate the Olympus DSX500i system via the touchscreen interface..

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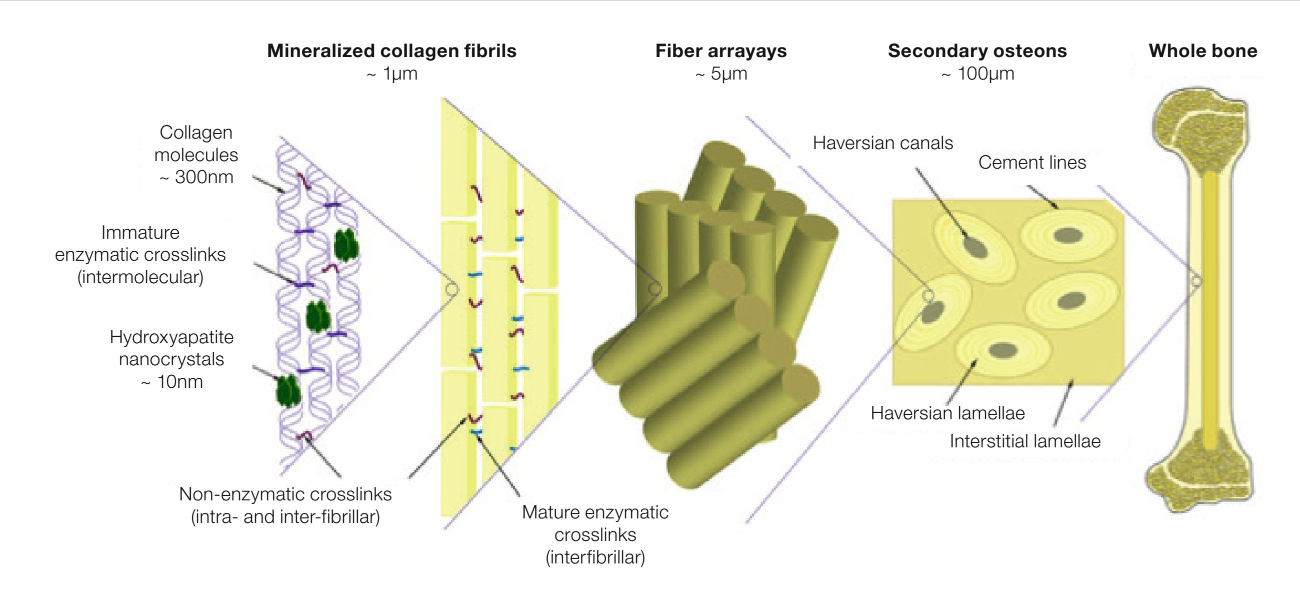

Figure 1: Human bone is a complex hierarchically organized composite material. [2]

The Complex Structure of Bone in Health and Disease

Bone consists of several hierarchical levels, from the whole bone structure, over secondary osteons (the basic structural unit in bone) and down to the individual collagen molecules (Figure 1). This complex structure is upset in a number of diseases, for example osteoporosis.

“Looking back 20 years, it was initially thought that the primary contributing factor of osteoporosis was low bone mass, leading to increased fracture risk. However, we’ve since found something different,” explains Dr. Busse: “Now we know it is not only bone loss, but also the quality of bone that is significant.” Paget’s disease of bone is another research focus of Dr. Busse’s group, and is characterized by a high rate of bone turnover in addition to bowing, deformity, cracking and pain. The orientation of structures also has implications for the bone’s resistance to fracture. [1]

With bone structure being increasingly recognized to play an important role in human disease, understanding the properties of bone is a priority.

Understanding the Properties of Bone

The assessment of patient’s bone samples using standard brightfield microscopy is a common method to quantify bone formation, bone resorption and bone remodeling. However, from a mechanical viewpoint this does not provide sufficient information.

Scanning electron microscopy (SEM) and backscattered EM provide a quantification of the dominant mineral component in bone: hydroxyapatite. However, bone is not purely formed of this material, but also contains other components such as the collagenous matrix that provides the bone with its inherent flexibility. For these components, other techniques including Raman spectroscopy and Fourier transform infrared spectroscopy can be employed. Discussing the various approaches for bone analysis, Dr. Busse comments: “We have a variety of experimental methods to extract information from the bone tissue, which helps guide surgeons and physicians towards the best treatment decisions. But in the end, an in vivo tool to measure the mechanical properties of bone is still missing.”

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Measuring Mechanical Properties with Micro-indentation

Mechanical properties of bone can be investigated using a micro-indentation device on plastic-embedded bone samples. Once a probe is impressed into the material at a known load, a variety of mechanical properties can be calculated from the indentation depth and the corresponding load. “This is comparable to a classical nano-indenter (Figure 2), but instead of a diamond tip it has a metallic probe, and we apply cyclic loading,” says Dr. Busse.

The indentations are measured from multiple locations across the bone sample to provide a more accurate representation. The indentation depth increases with each cycle, and a load over displacement curve is created from the data.

Bone is a composite material, formed from a mix of mineral crystals, collagen fibers and fibrils. This gives rise to a range of key properties providing toughness mechanisms that could be helpful under different loading conditions.
Dr. Busse explains: “Once this material is deformed and the energy is absorbed, this information is stored within the indentations, and our aim is to try and uncover this.” From the micro-indentation testing such information comes in many forms including:

- First cycle indentation distance – the indentation depth of the first loading cycle
- Total indentation distance – the maximum indentation depth during the last loading cycle
- Loading and unloading slope – the force/displacement plot shows dynamic information
- Energy dissipation – measuring the material’s response to indentation, this reflects elasticity

Figure 2: Medical nano-indenter *for analysis of mechanical properties of bone used by the team of Dr. Busse*

“But this leads us to the next hurdle to overcome.”
Porous structures in bone, e.g. reflected by vascular channels in the cortical shell of the bone or the spongy network inside the bone, become infiltrated by plastic when the biopsy samples are embedded for preservation. When these samples are ground and polished, a true bone surface is exposed on top. However, there are also regions of plastic. “So we were looking for some kind of tool to verify that we hit the bone, not the plastic. In the past we used SEM, but this is labor-intensive in terms of sample preparation, and we were looking for a faster and easier way of checking we indented the correct region.”

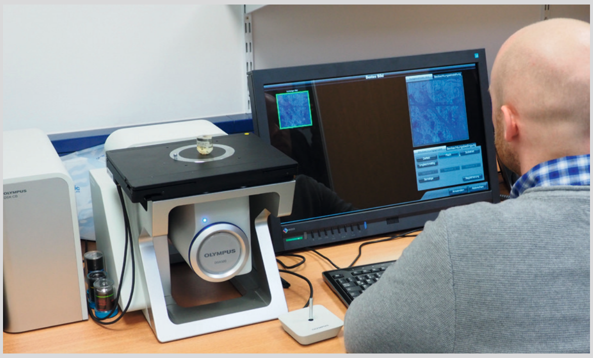

Figure 3: DSX500i in use. *The Best Image function allows Christoph Riedel to quickly find the best possible settings to reveal the relevant details.*

Improved Efficiency with Digital Light Microscopy

Medical student Christoph Riedel, B.Sc. explains the process of analyzing indentation samples with EM: “Samples need to be coated, then we put them into the microscope and apply the vacuum. This all takes time.” Since adopting the Olympus DSX500i into their laboratory, the speed and efficiency of sample analysis were improved. “Now with the DSX500i, we just put the sample on and go. The system is very easy to use, with everything controlled using the mouse, and we find this far easier than a standard light microscope.”

“The software is easy to handle, and we benefitted from the full system introduction we received from Olympus,” says Christoph. There is both a beginner mode that helps the user to find the best possible settings, as well as an expert mode that allows the user choose every setting. “Initially it takes some time to adjust the optimum settings, but once you have the settings you can save these and apply them for further images.” Multiple indentations are made on each bone specimen, and using the DSX500i (Figure 3), it can be determined quickly how many indentations have hit bone, helping the researchers decide if more indentations are required. In other cases, EM and digital light microscopy also present complementary approaches, with the backscattered EM providing additional information on mineral composition.

However, the EM does not provide quantitative information regarding the depth of the indent. “With the DSX we also get depth information. If the bone tissue is softer and has a lower mineral content or is harder and more brittle, this information is reflected in the depth of the indents.”

The Olympus DSX500i incorporates a variety of digital capabilities to facilitate the complete industrial and material science microscopy workflow. This has proved beneficial for Dr. Busse and his group. For example, the Stitching Function vastly expands the field of view, with hundreds of individual capture areas automatically assembled into a single wide-field 3D image, which can then be rotated, expanded and reduced on the digital screen view (Figure 4). As Christoph found, “I used the Stitching Function to provide a representative overview, locating the indentations and to know where I am on my sample. We also used the Stitching Function for other projects, to get an overview of bigger structures.”
Dr. Busse adds: “In our research we encounter a lot of different specimens, like implants or different bone sections, etc. To adapt to different sample conditions, the Best Image Function proved useful to speed up the process of finding the most appropriate image settings.”

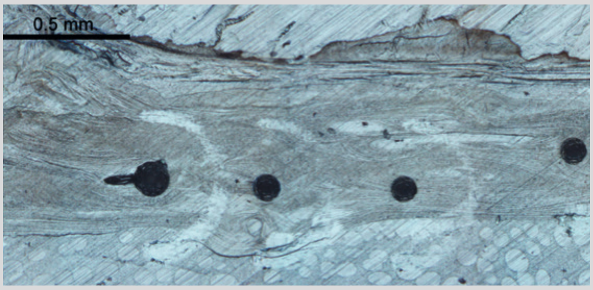

Figure 4: Four micro-indentations in cortical bone. *Image was taken using the Stitching Function of the DSX500i.*

The Best Image Function provides an on-screen display of multiple capture settings, allowing the user to pick the most insightful imaging technique. “When we want to see abrasion particles or edges, we often use the Darkfield imaging, which can provide better results for specimens of low contrast (Figure 5). For other specimens we used the DIC [differential interference contrast] mode, as this really highlights the scratches on certain surfaces.”

In addition, flexibility in sample analysis is facilitated by the inverted microscope platform.

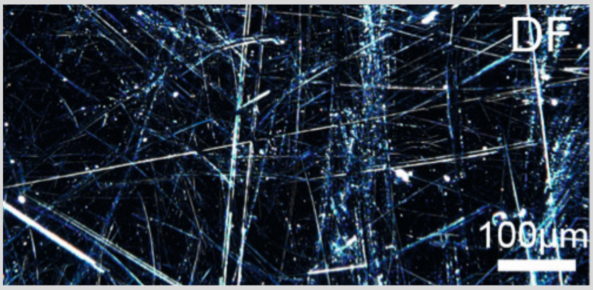

Figure 5: Surface of a hip joint implant after explantation. *Image taken using the Darkfield mode of the DSX500i.*

Flexibility in Sample Format with an Inverted Microscope

Biopsy samples can come in a range of different shapes and sizes, and an inverted system provides the flexibility to analyze such samples. “We are not restricted to a specific sample size or shape. While a typical bone biopsy size from the iliac crest is approximately 2.5 cm long x 2 cm thick, including the plastic embedding, we also perform experimental research on bones of different dimensions. We have collaborations with the Department of Forensic Medicine, so sometimes, for example, we have human samples from the femur. Complete cross sections from the femur can be 3-4 cm in diameter, and even samples of this dimension can be handled by the DSX500i.”

An additional potential application is implant analysis. “After a fracture, patients usually need an implant to fix the fracture. This is a complex procedure, and we are interested in the interface between the metal and the bone.” In general, these implants remain in the body for approximately 15-17 years, before another loss in bone mass can be observed and the insertion of a new implant is required. “We analyze these retrieval parts thoroughly, since they offer crucial information on why the implant may have failed and how it can be optimized in the future.”

The Future of Bone Material Research

There are many future avenues to explore with digital light microscopy (see Text Box). A recent study from Dr. Busse’s group looked at treatment-naive osteoporotic bone in comparison to bone treated with anti-resorptive agents as well as healthy bone. [3] Classical indents were made in bone samples using the micro-indentation device, which interestingly triggered the propagation of cracks at the boundary of the indents. “You can experimentally induce these changes, which may change from disease to disease. This is something we would like to look into in the future, as we believe we could gain additional information using digital light microscopy, especially with 3D analysis and measurement of the indent depth.”

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Summary

In summary, the process of micro-indentation material analysis has been streamlined with quick and efficient assessment of indentation location using the Olympus DSX500i, which can translate to a variety of material science and industrial inspection applications. Moreover, a range of sample shapes and sizes can be analyzed on the inverted platform, and now the release of the new DSX510i introduces several new features such as the ability to fit two objectives instead of just one. This allows researchers to switch seamlessly between the 10x and 40x magnifications, inspecting the sample at a higher resolution with minimal effort. With a host of digital capabilities this technology presents many opportunities to drive research into the mechanical properties of bone.

Broadening the Horizons of Material Research into Bone

Bringing together researchers from around the campus for an interactive exchange of information, Olympus provided a full workshop at the University Medical Center Hamburg-Eppendorf. Dr. Busse invited other groups working in similar fields who were interested in the possibilities of digital light microscopy in bone research. The specialist team from Olympus was also present.

One interesting application explored was from a colleague of Dr. Busse working with mice, who wished to image the whole jaw and teeth. With the interface between the teeth, the bone and the jawbone, this large structure has a complex morphology, while the jaw itself has a round shape. This is therefore challenging to image, and macro-photo images are not ideal, since they lack detail. With the ability to image large samples in high resolution, 3D imaging and analysis using the Olympus DSX500i allowed the teeth and the jaw bone to be imaged at the same time, and a direct comparison therefore made between two experimental groups.

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