NEW PARAMETER FOR ULTRA-SOUND ASSESSMENT OF CORTICAL BONE PROPERTIES

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Tiivistelmä

Opinnäytetyön tarkoituksena oli selvittää uuden mittausparametrin käyttöönottomahdollisuutta osteoporoosin diagnosointiin käytettävässä ultraäänimittauksessa. Aihe saatiin Bone Index Finland Oy:ltä, jonka tuotekehitykseen työ liittyy. Yrityksen kehittämällä USB-porttiin liitettävällä Bindex®-mittalaitteella voidaan luotettavasti seuloa ja diagnosoida osteoporoosia mittaamalla säären kuoriluun paksuutta. Tässä työssä tutkittavalla parametrilla voitaisiin mahdollisesti parantaa jo olemassa olevien parametrien diagnostista arvoa.

Tutkimuksia varten perehdyttiin laitteen ja ultraäänimittausten teoriataustaan aiheesta tehtyjen kliinisten tutkimusten sekä Janne Karjalaisen (FT) väitöskirjan avulla. Työn käytännön osuus tehtiin Itä-Suomen yliopiston laboratoriotiloissa mittamalla 43 ihmisluunäytettä yrityksen mittalaitteella. Vakiokokoisten näytteiden paksuus mitattiin kahdelta eri etäisyydeltä Bone Index Finlandin omalla asiakasohjelmistolla sekä tutkimusta varten tehdyllä mittausohjelmalla. Mittasignaaleja käsiteltiin Matlab-ohjelmalla digitaalisen signaalinkäsittelyn keinoin halutun parametrin esiin saamiseksi. Tulosten käsittelyä varten Matlabilla ohjelmoitiin graafinen käyttöliittymä, jolla voitiin käsitellä kaikki signaalit automaattisesti kerralla tai haluttaessa yksitellen. Uuden parametrin käytettävyys arvioitiin vertaamalla saatuja tuloksia luun muihin mitattuihin ominaisuuksiin.

Työn mittausosuus ja tulosten analysointi saatiin toteutettua onnistuneesti. Uusi parametri analysoitiin mitatuista ultraäänisignaaleista ja työssä arvioitiin sen kykyä ennustaa näytteiden muita ominaisuuksia. Tuloksia hyödynnetään tuotteen jatkokehityksessä, mutta parametrin käyttö mittauksessa vaatii vielä lisätutkimuksia.

Avainsanat			
diagnostic ultrasound, bone, osteoporosis, Bindex			

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Abstract

The purpose of this thesis was to introduce a new parameter to be used with an ultrasound measurement for osteoporosis diagnostics. The thesis was conducted for Bone Index Finland Ltd. and it is a relevant part of their product development. The company has designed a USB-connectable measuring device Bindex® for reliable screening and diagnosing of osteoporosis based on the thickness of the cortical shin bone. The new parameter might be able to add to the diagnostic value of the current parameters.

For background information about ultrasound and the measuring device, clinical studies and a doctoral dissertation by PhD. Janne Karjalainen on the matter were studied. The practical part of the thesis was conducted in the laboratory premises of the University of Eastern Finland. 43 human bone samples of a standardized size were measured using the Bindex® measuring device, the customer software and a specially tailored measuring program provided by Bone Index Finland. The signals acquired from the measurements were further analyzed with Matlab software using digital signal processing techniques to bring out the new parameter. A graphical user interface was also programmed using Matlab to enable the automated or manual processing of either single results or the entire sample group at once. The usability of the new parameter was evaluated by comparing the analyzed results to other measured properties of the bone samples.

Both the practical part and the analysis of the results were completed successfully. The new parameter was analyzed from the measured ultrasound signals and its ability to predict other properties of the measured samples was assessed. The use of the parameter in customer measurements needs further research, but the results of the thesis can still be used in the product development.

FOREWORD

This thesis was commissioned by Bone Index Finland Ltd., where the topic came up during my part-time job. The thesis took place from February to May 2014. The subject was interesting and the background information on osteoporosis and ultrasound technologies will most likely be of great use later on, as I hope to stay in the company to even further develop the measuring device.

I would like to express my thanks to my supervising teacher, Mr. Arto Toppinen and my instructor at Bone Index Finland, Janne Karjalainen , Technology Director, for their help and guidance. I am also grateful to the entire staff of Bone Index Finland for their support and new ideas brought up during the work. I want to thank my friends for bringing balance to the sometimes stressful studying.

Finally, I want to thank my wonderful, beloved wife, Riikka, and my two amazing children, Neeta and Hilla, for their never-ending love, support and patience during my studies and the long writing process. I also want to express my gratitude to my parents, Pekka and Päivi, and my parents-in-law, Pertti and Aulikki, for bearing more than their share of the load by helping with the little ones and basically just always being there for us.

Kuopio 3 June 2014
Timo Heiskanen

ABBREVIATIONS AND DEFINITIONS

AD-conversion = conversion of an analog signal to digital

AD-SOS = amplitude-dependent speed of sound

AIB = apparent integrated backscatter

BMD = bone mineral density

BUA = broadband ultrasound attenuation

BUB = broadband ultrasound backscatter

calcaneous = heel bone

cancellous bone = see *trabecular bone*

CE mark = Conformité Européenne, a mandatory conformity marking for all products sold in the European Economic Area

compact bone = see *cortical bone*

cortical bone = hard, thick outer layer of bone

Curie temperature = a temperature for all ferromagnetic materials in which their permanent magnetic properties are lost

DI = density index

distal = remote from the reference point (the midline of the body), cp. *proximal*

DPA = dual-photon absorptiometry

DXA =dual-energy X-ray absorptiometry

EMC = electro-magnetic compatibility, the ability of an electrical device to withstand and prevent electromagnetic disturbances caused to or by it

femur = thigh bone

goniometer = a device for precisely measuring or setting the angle of an object

GUI = graphical user interface

Hz = hertz, the unit of frequency (1 Hz = 1 cycle per second)

IEC = International Electrotechnical Commission

IRC = integrated reflection coefficient

PBS = phosphate buffered saline, a solution for preserving biological samples

phantom = a test platform or target for the calibration and functionality testing of ultrasonic probes

piezoelectric = a material in which a change in shape produces a voltage across it and vice versa

proximal = near to a reference point (the midline of the body), cp. *distal*

pulser = the part of an ultrasonic device that produces the electric excitation signal for the transducer

Q factor = quality factor, the ratio of the center frequency and the produced frequency bandwidth of a piezoelectric element

QCT = quantitative computed tomography

QUS = quantitative ultrasound

PE = pulse-echo

PZT = lead-zirconate-titanate, a piezoelectric material

SD = standard deviation

SNR = signal-to-noise ratio

SOS = speed of sound

SPA = single-photon absorptiometry

SPL = spatial pulse length

T-score = number of standard deviations below normal, used in BMD evaluation

tibia = shin bone

trabecular bone = the spongy, porous inner part of the bone between the cortical bone and bone marrow

TOF = time of flight

TT = through-transmission

ultrasound = sound inaudible to the human ear, frequencies of over 20 kHz

WHO = World Health Organization

CONTENTS

1   INTRODUCTION

Osteoporosis is an underdiagnosed condition that affects millions of people without their knowledge and causes hundreds of millions of euros worth of healthcare and insurance costs annually throughout the world. In osteoporosis bones become weaker and more fragile which leads to easier fracturing. Education on the subject, healthy living habits and effective screening at primary healthcare would prevent most of the fractures caused by osteoporosis that is too often only noticed after the first fracture.

The current DXA equipment for diagnosing osteoporosis can only be found in hospitals due to its large size and expensiveness. More portable and affordable solutions, both X-ray and ultrasound, have been introduced to the market, but they often vary in reliability and their results are not necessarily standardized, leaving them too open for interpretations. Efforts are made to standardize the results of devices from different manufacturers against the DXA measurements so that osteoporosis screening could be more widely conducted in primary healthcare too. The Finnish Current Care Guidelines (Käypä hoito) have just recently acknowledged an ultrasound measuring method for diagnosing osteoporosis, which is a step in the right direction.

In this thesis the possibility of utilizing a new measuring parameter for an ultrasound measuring device developed by Bone Index Finland Ltd. will be studied. Based on their research, the founders of the company believe the new parameter would bring more knowledge of the condition of the bone and add to the diagnostic value of the measurement. The parameter would be used in this kind of a device for the first time and the method of analysis may be patented. For this reason most of the analysis and the final results will be left out of this thesis. They may be presented later in a scientific publication after more extensive research.

The work will be conducted in three parts. First, the background of ultrasound measurements and osteoporosis will be studied using handbooks and studies on the matter. The Master's thesis and the doctoral dissertation by PhD. Janne Karjalainen, one of the founders of Bone Index Finland, will be utilized. Second, the practical laboratory work will be conducted at the University of Eastern Finland involving the measurement of human bone samples. Finally, the results will be analyzed with the help of Matlab® software and evaluated against other known properties of the bone.

2 BONE INDEX FINLAND LTD.

Bone Index Finland Ltd. was founded in the year 2011 in Kuopio, Finland. The company has developed a patented pocket-sized device, Bindex®, for the diagnosis of osteoporosis in primary healthcare. According to research an estimate of 75% of osteoporotic patients are left without a diagnosis and therefore treatment, because the measuring equipment for the currently used diagnostics method, axial DXA (Dual-energy X-ray Absorptiometry), is only found in special healthcare. Bone Index Finland wants to make diagnostics available for more patients in a cost-effective manner. (Bone Index Finland Ltd. 2014b)

The company was founded by Mr. Ossi Riekkinen (PhD, CEO) and Mr. Janne Karjalainen (PhD, Technology Director / CTO), who wrote their doctoral dissertations on diagnostic methods of osteoporosis. They both were a part of an osteoporosis research team at the University of Eastern Finland (former University of Kuopio), which has done research on the subject for 30 years. The idea of a commercial device that utilizes ultrasound in bone examination has been in development since the year 2006. (Vornanen 2014, 9)

Bone Index Finland has five employees in its operational staff (Vornanen 2014, 9). The company is currently looking for a partner to handle the global sales and distribution of Bindex. Their goal is to be the leading manufacturer of devices for osteoporosis diagnostics by the year 2016. The quality system of Bone Index Finland is ISO 13485 certified and their devices meet the requirements of the Medical Device Directive MDD 93/42/EEC (Bone Index Finland Ltd. 2014b).

3   PROPERTIES OF BONE

Bone is the ultimate biological material due to its mechanical strength and lightness. It serves many functions and works as the frame for other vital activities.

3.1   Functions and types of bone

The human musculoskeletal system (Figure 1) consists of bone, cartilage and striated muscle. Bones can be permanently knit together (e.g. the pelvic skeleton of an adult), or connected by connective tissue (e.g. the bones of an adult's skull), cartilage or fully mobile joints. Cartilage gives bones the ability to move slightly in relation to each other and it can be found for instance between the left and right pubic bone. Cartilage is softer and more flexible than bone and most of it can be found connected to bones e.g. as joint cartilage. It can also provide support and cover for soft tissues, such as in the nose or the bronchial tubes. (Nienstedt and Kallio 2006, 28-32)
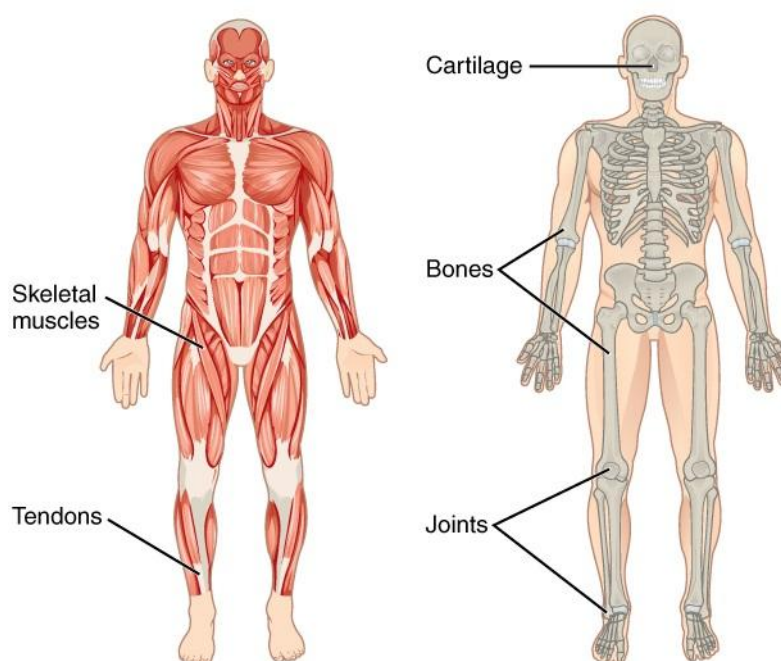

FIGURE 1. The muscular and skeletal system of the human body. (edited, original by OpenStax College 2014c)

The bony skeletal system of an adult is composed of 206 separate bones, which make up roughly 10% of the whole body weight (Luustoliitto 2013a). It acts as the primary supportive structure for the human body, protects softer tissues and inner organs and makes movement possible with the help of striated muscles attached to them (Nienstedt and Kallio 2006, 28). Blood cells, such as red blood cells, are produces in the center part of a bone, the bone marrow. The skeleton also has the ability to store vital minerals such as phosphorus and calcium (American Society for Bone and Mineral Research 2004a). On the other hand it can keep some potentially lethal substances, like lead, from entering the blood circulation. Bones also help maintain the acid-base balance of the human body and thus protect it from acidosis.

Bones of the human body can be subcategorized to 5 classes (see Figure 2). Long bones can be found e.g. in the limbs, such as the thigh bone (lat. *femur*) or the shin bone (lat. *tibia*). They are classified as having a shaft or *diaphysis* longer than it is wide, and two cartilage-covered growth plates or *epiphyses* at ends. Short bones are about equally long and wide bones whose purpose is to support other tissues and structures of the body, for example in the ankles or the wrist. The bones of the skull or the hip bone are an example of plate-like flat bones, which provide an attaching point for muscles and cover for inner organs. Flat bones also produce the most red blood cells in adults. Irregular bones are bones that cannot be clearly categorized by their appearance. Examples of irregular bones are the vertebrae or the lower jaw bone. Sesamoid bones can be found inside tendons, where they protect the tendon from strain. The knee cap, or *patella*, is a good example of a sesamoid bone. (TeachPE 2014b)
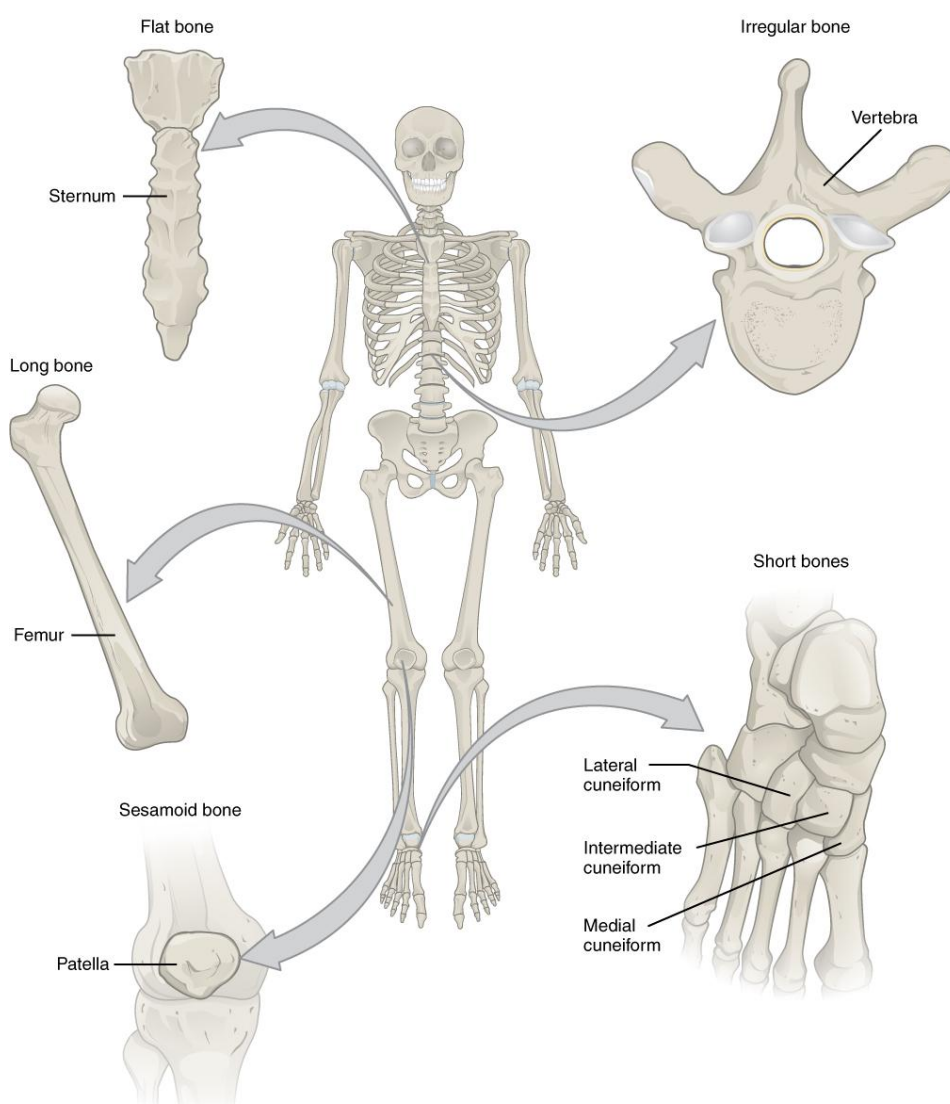

FIGURE 2. Classification of human bones. (OpenStax College 2014a)

3.2 Inner structure

The surface of bone is covered by *periosteum*, which is a layer of connective tissue (Nienstedt and Kallio 2006, 28). It provides nerves and blood vessels with access inside the bone. The bone tissue consists of bone cells and hard cell medium, which contains a lot of calcium. The tissue can be di-

vided into *cortical* (compact) and *trabecular* (cancellous) *bone* (American Society for Bone and Mineral Research 2004a). Solid cortical bone serves as the outer layer of bone to provide cover for the inner parts and to form the shaft of long bones. The spongy trabecular bone is found in the inner parts of bone and in the ends of long bones, and contains areas of cell medium as well as beams of compact bone. Trabecular bone is much more porous than cortical bone, but still it provides support to cortical bone with its well-oriented tissue matrix (TeachPE 2014a). The hollow center part of bone, the *medullary cavity*, is filled with bone marrow (Nienstedt and Kallio 2006, 29). Figure 3 shows the structure of a bone.

Long Bone

- Articular cartilage
- Epiphyseal line
- Spongy bone
- Medullary cavity
- Nutrient foramen
- Endosteum
- Periosteum
- Epiphysis
- Diaphysis
- Epiphysis
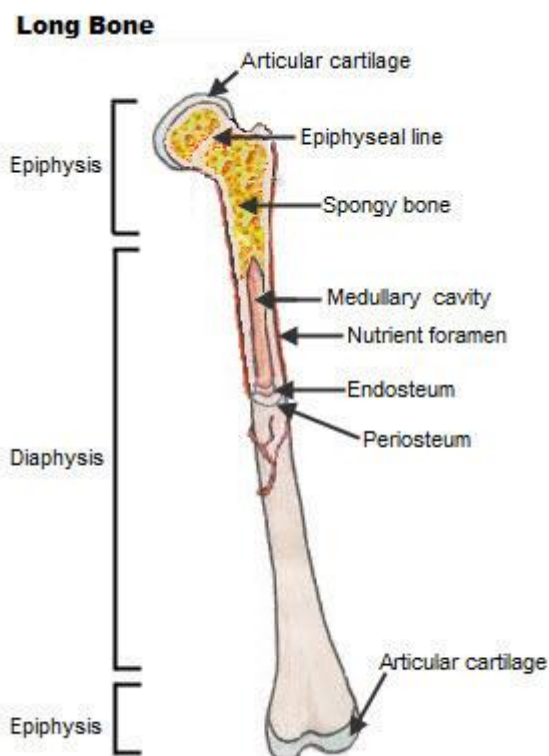- Articular cartilage

FIGURE 3. Structure of femur, a long bone. (U.S. National Institutes of Health 2014)

The bone tissue is constantly rebuilt and taken apart by the body's own cells. Types of bone cells are shown in Figure 4. *Osteoclasts* degrade old bone tissue, while *osteoblasts* generate new tissue right in their vicinity. Osteoclasts resorb the bone by secreting acids and enzymes to the surface of the bone. After completing its task, the osteoclast undergoes *apoptosis*, a programmed cell death. Osteoblasts are differentiated from bone marrow precursor cells. They produce the proteins needed for the organic part of the bone matrix and also take part in the mineralization of the bone. (American Society for Bone and Mineral Research 2004a)

Osteoblasts work in teams to make new bone tissue and some of them eventually get surrounded by the new tissue. These trapped cells further differentiate into osteocytes, which guide bone remodeling according to mechanical strain and repair damage caused by fatigue. Their exact purpose is not yet fully understood. Other osteoblasts differentiate into flat lining cells on the surface of the bone or undergo apoptosis. Lining cells can quickly release calcium into circulation in case of a low

blood calcium level. They also protect the bone from damaging or dissolving chemicals. (American Society for Bone and Mineral Research 2004a)
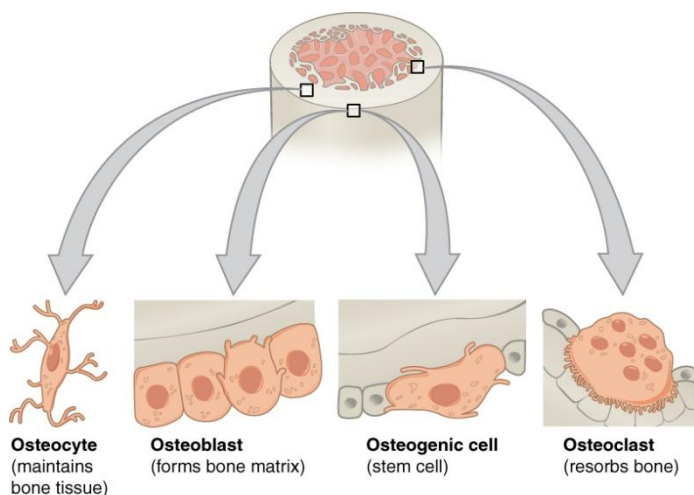

FIGURE 4. Bone cell types. Osteogenic cells are undifferentiated stem cells, which turn into osteo-blasts or fat cells. (OpenStax College 2014b)

Bones keep renewing throughout life. The renewal rate is higher in children and young adults, whose bones need to grow longer and thicker until of about 20 years of age. After reaching this peak mass the degradation and renewal rates of bone are balanced. The amount of bone tissue starts to diminish at about 50 years of age, when the degradation rate becomes higher than the re-newal. In women, menopause causes the degradation of bone to accelerate for 3-5 years, after which it normalizes. This is why osteoporosis is not as often found in men, whose bone degradation is more even and slower. (Luustoliitto 2013a)

4 OSTEOPOROSIS

4.1 Overview

With age, bones eventually become more brittle and the amount of bone mass decreases (Luustoliitto 2013b). This can be explained by the increased bone degeneration by osteoclasts compared to the rebuilding done by the osteoblasts. As a result, more bone is lost than gained and the inside of bone becomes more porous, as seen in Figure 5. Pores are filled with bone marrow or fat instead of bone tissue and its density is thus decreased (American Society for Bone and Mineral Research 2004b). A condition where the bone becomes abnormally brittle and the susceptibility to fracture increases significantly is called *osteoporosis*. The precursor of osteoporosis is called *osteopenia* in which the strength of bone has lowered, but not substantially (Luustoliitto 2013b).
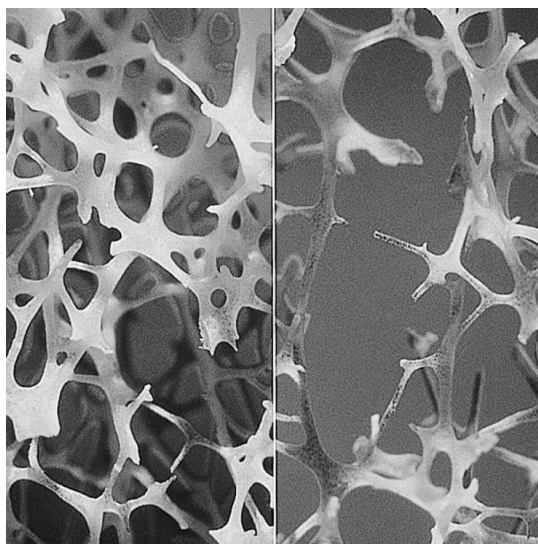

FIGURE 5. The structure of healthy bone (left) in comparison with osteoporotic bone. Beams of osteoporotic bone matrix can be seen to become thinner and the bone becomes more porous. (Wikimedia Commons 2012)

Osteoporosis can be divided into primary and secondary osteoporosis. In primary osteoporosis a single, clear reason for the condition cannot be identified. Primary osteoporosis can be diagnosed after menopause, due to aging, the decreased amount of sex hormones or a hereditary susceptibility. Secondary osteoporosis can be shown to have a cause, such as another medical condition (e.g. arthritis, diabetes requiring insulin treatment) or a medication, usually a long-term cortisone treatment. (Luustoliitto 2013b)

The greatest risk for osteoporosis is with women over 50 years old (Luustoliitto 2013b). The diminished production of estrogen during menopause accelerates the loss of bone mass in women more than in men of the same age. Despite its commonness among elderly people, osteoporosis can still be found in all ages and both sexes. Heredity dictates about 80% of a person's bone density and about 20% is determined by living habits, such as smoking, exercise or diet (American Society for Bone and Mineral Research 2004b). People with African heritage are less prone to develop osteopo-

rosis. Osteoporosis can be hard to diagnose, because it shows no early symptoms and can develop slowly over many years (Luustoliitto 2013b).

4.2   Osteoporotic fractures

Fractures caused by a low energy impact are often the first sign of osteoporosis, e.g. after falling from a height of less than 1 meter (Luustoliitto 2013b). The most common fractures happen in the spine, the hip or the wrists (American Society for Bone and Mineral Research 2004b). Spinal fractures can cause a person's shortening due to collapsed vertebrae (Figure 6) or changes in posture, especially in the form of increased curvature of the thoracic spine. They can also generate problems by blocking the full expansion of lungs or by increased heartburn. Osteoporosis is usually not painful until the bone fractures. Spinal fractures are not even necessarily painful, and 2/3 of fractured vertebrae do not cause pain.
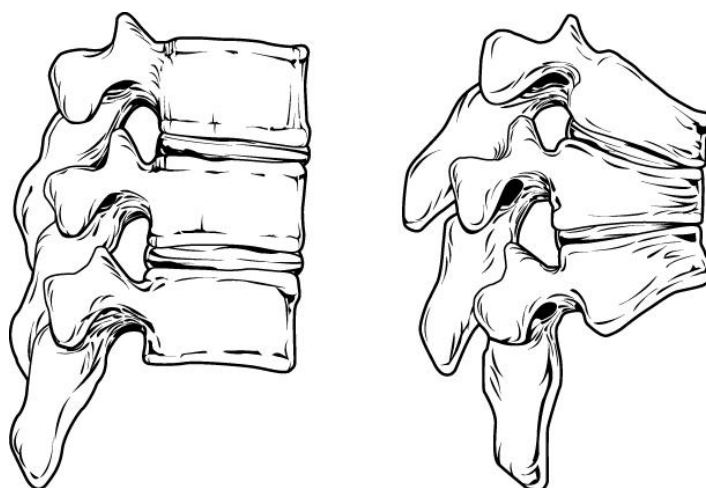

FIGURE 6. Healthy and osteoporotic thoracic vertebrae (respectively). The collapse of osteoporotic vertebrae due to lowered bone density and mass shows as curved posture and shortened body height. (edited, original by OpenStax College 2013)

In Finland fragile bones contribute to 35 000 to 40 000 fractures annually (Luustoliitto 2013b). The most common types are collapsed vertebrae, fractures in the wrist or the femoral neck. Hip fracture is probably the most dangerous type of osteoporotic fractures, as it often leads to a long treatment period in a hospital or a nursing home and to a permanently decreased ability to function. It is also the most common cause of accidental death in Finland among senior citizens over 70 years old. Additional factors that contribute to hip fractures are slimness, weak muscles, poor eyesight and balance and objects at a person's home which they can trip over (American Society for Bone and Mineral Research 2004b). A sideways fall is also six times more likely to cause a fractured hip than other directions.

It is estimated that of Finnish people over 50 years old about 40% of women and 14% of men will at some point fracture their wrist, vertebra or hip (Luustoliitto 2013b). Worldwide, Over 1/3 of women and 1/5 of men will suffer a fracture caused by osteoporosis, which means a new fracture

every three seconds (International Osteoporosis Foundation 2014b). There are no accurate numbers for the total number of osteoporotic patients, but an estimated 200 million people suffer from the condition.

4.3 Prevention and treatment

The pre-emptive measures for osteoporosis on a national level aim at preventing fractures. The most important objectives are sufficient intake of calcium and vitamin D, exercise throughout life, smoke-free living habits and the prevention of falls. In elderly people, the risk assessment usually happens through the evaluation of diet, medication and problems in everyday life hindering movement or causing danger. People with a grown risk for hip fractures may benefit from a special hip protective padding. (Käypä hoito 2014)

After a diagnosis for osteoporosis has been made, the same medication-free measures apply. The treatment of osteoporosis also aims at preventing fractures, specifically vertebra and hip fractures. Drugs can effectively be used only for a few years and their use is targeted to the elderly or those with a great risk for fractures. In case of an already occurred fracture, the treatment of pain and an early mobilization of the patient are key factors for success. (Käypä hoito 2014)

Drugs for osteoporosis can be divided into those affecting bone growth and hormone treatments. Medication affecting bone growth, such as bisfosfonates, teriparatide or denosumab, acts by preventing the resorbtion of bone by osteoclasts, by stimulating the generation of new bone by osteoblasts or both. Hormone treatments (estrogen for women, testosterone for men) restore the balance between the activity of osteoclasts and osteoblasts and thus increase bone density. Additional hormone treatment enhances bone condition for as long as it is used and the situation returns to normal quickly after stopping the medication. (Käypä hoito 2014)

4.4 Diagnostic methods

There are several methods for determining bone density and condition, none of which are perfect. Constant discussion on the reliability of different techniques takes place in the scientific community and healthcare. The use of ultrasound in bone diagnostics is discussed separately in Chapter 5 of this thesis.

4.4.1 Dual-Energy X-Ray Absorptiometry and Bone Mineral Density

Osteoporosis is most often diagnosed with a *bone mineral density* (BMD) test using DXA (*Dual energy X-ray Absorptiometry*, former DEXA) measurement, which has become the "gold standard" for BMD measurements. DXA measures the transmission of photons at two different energies from an X-ray source to determine the level of calcium in the bone. One of the energy peaks is absorbed more by soft tissue, the other more by bone. BMD can be determined by subtracting the soft tissue component from the results. Using DXA, the exposure time to radiation can be kept very low. The results are also accurate: the precision can be up to 1-2%. DXA can be used to monitor changes in

bone density for example to determine the effect of ongoing osteoporosis medication. (American Academy of Orthopaedic Surgeons 2007)

FIGURE 7. DXA equipment in use. The scanning arm moves over the test subject while he lies still on the table. (edited, original by University of Bristol 2014)

Former BMD measuring techniques used *single-* (SPA) or *dual-photon absorptiometry* (DPA), which measured transmission of a pencil beam through the patient (American Academy of Orthopaedic Surgeons 2007). The problem with photon absorptiometry was the eventual decay of their radioactive isotope, which lowers their precision and the ability to monitor changes in BMD. SPA and DPA are nevertheless accurate in predicting fracture risk. The first modern DXA scanners used a pencil beam, but newer fan or cone beams accompanied by a group of detectors instead of one and *c-arm technology* (as in Figure 7) have made measurements faster and easier (IAEA 2013, American Academy of Orthopaedic Surgeons 2007).

Despite its name the BMD value does not represent the actual mineral volume density but the amount of bone mass per defined area, i.e. areal density, given as g/cm^2 (Impivaara and Åstrand 2005). BMD measurement sites are chosen by their relevance and likelihood to fracture, and the most commonly used are the *proximal femur* (upper part of the thigh bone), the *lumbar spine* or both. Measurements of the femur are more reliable, since fracturing or collapsing of vertebrae or calcification of soft tissue near the lumbar vertebrae may cause errors to the density results. The BMD value of the proximal femur also gives a better correlation to the possibility of hip fractures compared to other sites. BMD is a very accurate means for predicting fracture risk (American Academy of Orthopaedic Surgeons 2007). It is even more accurate than predicting a stroke by blood pressure or a myocardial infarct by cholesterol levels.

Although DXA measurements are fairly reproducible, the differences in results are surprisingly great even with devices from the same manufacturer (Impivaara and Åstrand 2005). For this reason the World Health Organization (WHO) has defined BMD threshold values for post-menopausal women based on reference data collected from various DXA measurements of healthy young adults. Refer-

ence data has to be used, because results obtained from different measuring devices are not comparable. A patient is defined osteoporotic, if her BMD value is at least 2.5 standard deviations (SD) below the average for healthy reference population. The fracture risk can be considered to double for every SD below the average normal (American Academy of Orthopaedic Surgeons 2007). Results are usually given as a *T-score*, which is the number of SDs above or below normal. For instance, a T-score of -2.5 or lower is a sign of osteoporosis (Impivaara and Åstrand 2005). Table 1 shows the diagnostic criteria for different T-scores.

TABLE 1. Diagnostic criteria for osteoporosis based on BMD measurement. (International Osteoporosis Foundation 2014a)

Condition	BMD (T-score)
Normal	T-Score < -1
Osteopenia	-2.5 < T-Score < -1
Osteoporosis	T-Score < -2.5
Severe osteoporosis	T-Score < -2.5 and at least one fracture caused by fragility

The problem with DXA measurements is the amount of space taken by the equipment. The device is usually installed permanently to a specific room in a hospital, so patients need to be transported to the measurement location even from far away. DXA measurements and devices are also expensive and their number is limited especially in poorer countries. Their simultaneous use in research also decreases the amount of possible patients examined. In Finland, DXA measurements are currently conducted in special healthcare via a referral from a doctor. (Impivaara and Åstrand 2005)

4.4.2 Quantitative Computed Tomography

Quantitative Computed Tomography (QCT) can be conducted on a normal CT scanner, such as the one shown in Figure 8 (American Academy of Orthopaedic Surgeons 2007). It produces a 3D image of the skeleton or bone examined and tells the actual volumetric density of bone mineral. It can furthermore isolate an area from other tissues to leave out unnecessary or error-prone parts of the bone for the measurement so that only the density of trabecular bone is measured. The downside of QCT is that it is even more expensive than DXA and generates a radiation dose of about 10 times higher than a DXA measurement. In Finland QCT is currently in use only for research use (Käypä hoito 2014). The imaging is done from the wrist or vertebrae.

FIGURE 8. CT scanner. Quantitative computed tomography can be conducted on most commercial CT scanners. (Wikimedia Commons 2009)

Computed tomography measures the absorption of x-rays locally inside the measured object (Buschow, Cahn, Flemings, Ilschner, Kramer and Mahajan 2001, 6009). Image is formed from a two- or three-dimensional matrix, which is obtained by irradiating the object from several angles (at least 180°). The matrix is composed of volume elements or voxels. The proportional averaged attenuation from scattering or absorption in each voxel is represented as varying colors or shades in the final image (Ketcham 2012). A complete image is formed after the transmission profiles received from each angular view are convolved using a specific filter and projected back at the same angle. The principle of computed tomography can be seen in Figure 9.
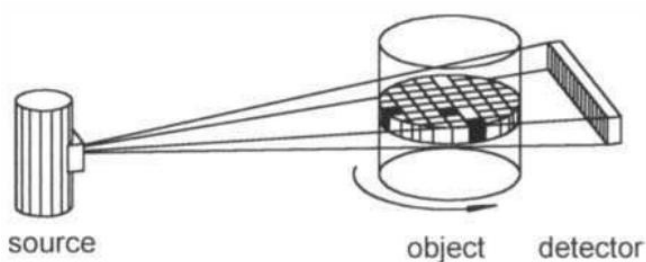

source   object   detector

FIGURE 9. Principle of QCT. In this example the source and detector are stationary while the object examined rotates. QCT can also be conducted using a moving source and detectors. (Buschow et al. 2001, 6009)

4.4.3 Peripheral measurements

Peripheral bone density testing means determining BMD values at peripheral sites of the body, such as the radial bone (*radius*) in the wrist or heel (*calcaneus*) (American Academy of Orthopaedic Surgeons 2007). Testing can usually be conducted on smaller, portable and less expensive devices, which makes it ideal for osteoporosis screening. Portable devices make it possible to measure patients, who otherwise would not be able to get to testing. Both peripheral DXA (pDXA) and ultra-

sound measurements are widely used to predict central bone density and fracture risk (Kröger 2014).

Instead of using the criteria for diagnosing osteoporosis by World Health Organization, several manufacturers have set their own so called osteoporosis equivalent threshold values. These values are usually set according to the classification of healthy and osteoporotic patients using DXA measurement. Threshold values give a 90% sensitivity and specificity in diagnosing osteoporosis, when all patients with their test results set between the thresholds are sent to DXA testing. Diagnosis for osteoporosis can be made and medication prescribed to patients who are tested to qualify below the lower threshold. If the thresholds are not used with peripheral testing, some of the patients with central osteoporosis may be left undiagnosed. (Kröger 2014)

The lack of studies on the effectiveness of medication started on the basis of a peripheral bone density test is currently the main reason for keeping DXA alongside the peripheral testing devices. Treatment can still be started with a peripheral test and thorough assessment of risk factors, if DXA measurement is out of reach. (Kröger 2014)

5   ULTRASOUND

Ultrasound is widely used in engineering and healthcare diagnostics. It can be used for imaging and measuring of distances or thicknesses, e.g. in human tissue. It is based on characteristic properties of materials, which affect the propagation and reflection of ultrasound waves transmitted to the target medium. Diagnostic ultrasound is a form of radiation since the energy of the wave is emitted from a source. However, the radiation is not ionizing, since sound is not a part of the electromagnetic spectrum. This makes diagnostics by ultrasound more widely usable than X-ray imaging. (Gibbs, Cole and Sassano 2009, 2)

5.1   Sound waves

Sound is an audible mechanical wave. A mechanical wave is a disturbance in a medium in which the particles of the medium vibrate around their original positions of equilibrium. A sound wave is caused by differences in pressure (i.e. the density of particles) in the medium propagating to the surroundings. The wave does not convey the actual medium particles as it propagates through the medium, but only energy brought into the system. The velocity of the wave is dependent on the properties of the medium. A mechanical wave is called *longitudinal* if the particles vibrate parallel to the direction of the wave. In a *transverse* wave the direction of particle vibration is perpendicular to the direction of the wave (see Figure 10). (Suvanto and Laajalehto 2008, 232-233, 277)
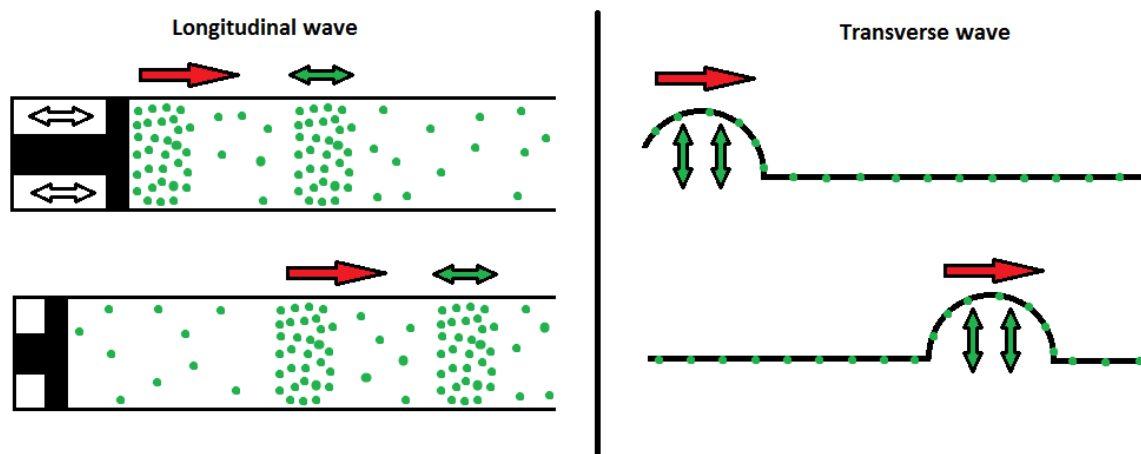
FIGURE 10. Mechanical waves. A longitudinal wave operates by compressing and dilating the medium and its particles, whereas a transverse wave does not affect the volume or the particle density of the medium. Green dots represent the particles, red arrow is the direction of the wave and green arrow is the vibrating direction of medium particles.

Ultrasound is a special category of sound with its frequency above the human hearing limit, i.e. greater than 20 kHz (Braun, Ewins and Rao 2002, 1437). Some applications use ultrasonic frequencies of up to several GHz. Ultrasound travels as a longitudinal pressure wave in a gas or liquid medium and as an elastic wave in a solid medium (Suvanto and Laajalehto 2008, 296). In gas or fluid, the advancing wave creates regions of *compression* and *rarefaction* in the medium as it moves

through it (Gill 2012, 7). In this case the properties of the wave are dictated by the compression stiffness and density of the medium.

In solids, an ultrasound wave can propagate as either a longitudinal (compression) wave or a transverse (shear) wave (Braun et al. 2002, 1437). A transverse wave does not affect the volume of the material, as the particle movement is perpendicular to the direction of the propagating wave. Transverse waves cause shear deformation of the material, which is an unwanted characteristic in buildings and mechanical parts, such as crankshafts (Suvanto and Laajalehto 2008, 239). Medical diagnostics are based on longitudinal waves propagating through tissues and body fluids. (Hoskins, Martin and Thrush 2010, 4)

5.2 Generation and measuring of ultrasound

At its simplest, ultrasound can be generated in the air with e.g. a whistle (Suvanto and Laajalehto 2008, 296). Most practical technological applications use ultrasound produced by *electrostriction* or *magnetostriction* in liquid or solid mediums. Electrostriction can generate ultrasound in the frequency range of 100 kHz to up to 150 MHz, whereas magnetostriction produces sound at lower frequencies (6-60 kHz). Since medical ultrasound mostly utilizes frequencies from 0.1 to 15 MHz (up to 40 MHz in research or special applications), magnetostriction is not used to produce diagnostic ultrasound (Hoskins et al. 2010, 7, NDT Resource Center 2007a).

In diagnostics, the ultrasound wave is generated and its echoes received by a *transducer* (see Figure 11), which converts electrical energy into mechanical vibrations and vice versa by the means of electrostriction and *piezoelectricity* (Gill 2012, 25). Electrostriction means the deformation of non-conductors or dielectrics when introduced to an electric field (Braun et al. 475). Piezoelectricity is a converse effect where mechanical strain induces a voltage to a crystal structured material, such as ceramics. The effects are related, and a material with such properties is commonly called *piezoelectric*. The cell length change (strain, *x*) of a material placed in an electric field is dependent on the *piezoelectric constant* of the material and the magnitude of the field:

$$x = dE \qquad\qquad [1]$$

where *d* is the piezoelectric constant and *E* is the electric field.

Ultrasonic diagnostic devices use a piezoelectric crystal with electrodes connected to its opposite faces, also known as the transducer element (Suvanto and Laajalehto 2008, 296, Azhari, 2010, 154). The thickness of the crystal varies according to changes in the electric field, which creates pressure in the medium. Ultrasound is then generated by pressure waves caused by the oscillating crystal. When an echo returns from the target to the transducer, it causes the crystal to oscillate, which induces a voltage in the electric circuit (Gill 2012, 25). The received signal is further amplified and AD-converted by the electronics, after which it is usually transferred to computer memory (Gibbs et al. 2009, 4). The data can be used to calculate distances in the target (e.g. organ placement or dimensions).
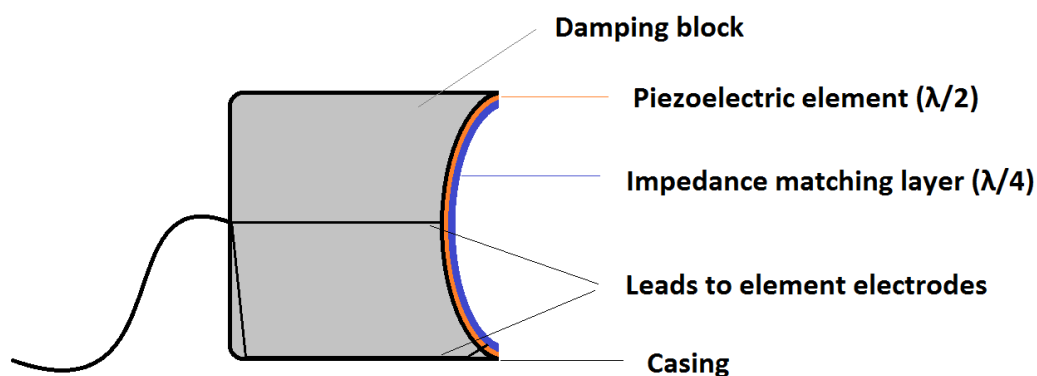
FIGURE 11. The structure of a focused transducer.

Most modern transducers utilize ceramic or composite crystals for their efficiency, meaning that the loss of energy by the conversion process is minimal (Gill 2012, 25). In composite crystal, ceramics and polymers are combined to reach a higher bandwidth than with plain ceramic crystals. Materials that are not naturally piezoelectric, such as a commonly used synthetic ceramic lead-zirconate-titanate (PZT), may gain piezoelectric properties through processing (Bushberg et al. 2012, 514-515). The material is first heated past its *Curie temperature*, while simultaneously subjecting it to an external voltage. The dipoles in the material align due to the applied voltage, which is then maintained until the material has cooled below its Curie temperature. After cooling, the dipoles have permanently aligned to their new state and compression in the material produces a voltage between its surfaces due two imbalanced charges. The cooled material is then worked into a desired shape. Quartz is a naturally occurring piezoelectric material, but it is fairly inefficient (Gill 2012, 25). This weakness can only be compensated by the use of higher power levels of the transmitted wave.

Pulse-echo ultrasound imaging (see chapter 5.5.1) mostly utilizes *resonance transducers*, in which the piezoelectric element vibrates at its natural resonance frequency, when excited by a short voltage pulse of about 1 µs. The pulse is transmitted and received at a certain *center frequency*, which is determined by the speed of sound in the element and its thickness. The element needs to be equal to half of the desired wavelength in thickness (λ/2) for it to resonate at its natural frequency. The frequency obtained is higher with thinner elements, whereas thicker elements produce lower frequencies. (Bushberg et al. 2012, 515)

Because the *acoustic impedances* (see 5.3) of piezoelectric materials and soft tissue are usually very unequal, impedance matching needs to be done using a *matching layer* (Azhari 2010, 516). Without the matching layer a significant portion of the energy transferred between the transducer element and the medium would turn into heat and lead to poor *signal-to-noise ratio* (SNR). All energy is transferred to the medium through the layer, when the thickness of the layer is a quarter of the used wavelength (λ/4) and its acoustic impedance (Z_{ml}) is in accordance with Equation 2:

$$Z_{ml} = \sqrt{Z_t * Z_m}$$
[2]

where Z_t and Z_m are the acoustic impedances of the transducer element and the medium, respectively (Gill 2012, 27).

A *damping block* is located in the back of the transducer element. Its purpose is to absorb ultrasound energy directed the wrong way, attenuate stray signals from the housing and dampen the vibration of the element. Dampening, or "*ring-down*", is needed to shorten the *spatial pulse length* (SPL) of the pulse to gain a better *axial resolution* (resolution parallel to the ultrasound beam). As a side effect, it increases the bandwidth of the pulse, as frequencies higher and lower than the center frequency are introduced when the natural resonance of the element is disturbed. The *Q factor* (Q for quality) describes the relationship between the center frequency and the produced frequency bandwidth of the element:

$$Q = \frac{f_0}{bandwidth} \qquad [3]$$

where f_0 is the center frequency. (Bushberg et al. 2012, 515-516)

Despite its name, the Q factor does not describe the actual quality of the element, but only its SPL and bandwidth characteristics. A transducer element with a low Q is only lightly damped and operates with a narrow bandwidth and a long SPL, whereas a high Q transducer is heavily damped and has a broad bandwidth and short SPL. A broad bandwidth is required for a high spatial resolution in imaging applications. Narrow bandwidth is needed e.g. in blood velocity measurements using Doppler instrumentation to gain all the information from small changes in echo frequencies. (Bushberg et al. 2012, 516)

An ultrasound wave can be focused to a *focal zone* by using a lens or a curved transducer element. The width of the focal zone is determined by the used wavelength and the dimensions of the element:

$$w = R\frac{\lambda}{r} \qquad [4]$$

where R is the radius curvature of the element, λ is the wavelength and r is the transducer radius. (Langton and Njeh 2004, 424)

5.3   Parameters of ultrasound propagation

The propagation of a sound wave is often expressed as a complex exponential harmonic function. The particle displacement in a longitudinal wave as a function of time is given by

$$u = Ae^{i(kx-\omega t)} \qquad [5]$$

where A is the displacement amplitude of the wave, k is the wavenumber, x is the location of the particle, t is time and $\omega = 2\pi f$ (angular frequency), where f is the frequency of the wave. The wavenumber is defined as:

$$k = \frac{\omega}{c} = \frac{2\pi}{\lambda} \qquad [6]$$

where c is the wave velocity and λ is the wavelength, defined as:

$$\lambda = \frac{c}{f} = \frac{2\pi c}{\omega}, \qquad [7]$$

The particle velocity can be acquired from the following equation using the particle displacement:

$$v = i\omega u \qquad [8]$$

The pressure of a compression wave is given by

$$\sigma = i\omega c \rho u = c\rho v \qquad [9]$$

where ρ is the density of the medium. (Braun et al. 2002, 1437-1438)

The acoustic impedance of a material is defined by its material constants (Giurgiutiu 2008, 137):

$$Z = \rho c \qquad [10]$$

Acoustic impedance is a characteristic property of each medium describing how much stress is required to direct at the medium particles for them to reach a given speed. In other words, it tells how much the medium resists the wave movement. Solid materials have one acoustic impedance for compression waves and another for shear waves, although the latter is not widely used (Braun et al. 2002, 1438).

Intensity indicates how much energy is transferred through a unit cross-sectional area per second, usually given as Wcm^{-2} or $mWcm^{-2}$ (Braun et al. 2002, 1438, Hoskins, Martin and Thrush 2010, 6). The cross-sectional area is perpendicular to the propagation of the wave. Intensity is dependent on the acoustic impedance of the medium and the pressure in the wave:

$$I = \frac{\sigma^2}{2Z} \qquad [11]$$

The speed of sound is an individual property of each medium and it is independent of the frequency used. The speed of a longitudinal wave in gas or fluid can be determined by:

$$c_l = \sqrt{\frac{K}{\rho}} \qquad [12]$$

where K is the bulk stiffness modulus of the medium and ρ is the density. (Braun et al. 1437)

In solids, the sound wave can propagate as a longitudinal, or a transverse wave. The speed of sound for compression waves (c_l) and shear waves (c_s) is calculated by the following equations:

$$c_l = \sqrt{\frac{E(1-v)}{\rho(1+v)(1-2v)}}$$

[13]

$$c_s = \sqrt{\frac{E}{2\rho(1+v)}}$$

[14]

where E is Young's modulus and v is Poisson's ratio. (Braun et al. 1437)

5.4 Interactions with tissue

Human tissue affects the propagation of ultrasound waves with several mechanisms, which cause the wave and the measured signal to *attenuate*. Attenuation describes the ratio between the input and output intensity of the ultrasound wave, usually given as decibels. It is calculated as:

$$attenuation = (10 * log\frac{I_1}{I_2})$$

[15]

where I_1 is the input ultrasound intensity and I_2 is the output intensity. (Gill 2012, 9)

The most significant types of attenuation are *absorption*, *reflection*, *refraction* and *scattering*.

5.4.1 Absorption

In absorption, some of the energy of a propagating ultrasound wave is lost into the medium, such as human tissue (Gibbs et al. 2009, 46). A part of absorption is caused by the characteristic resistance of the medium hindering the propagation of the wave. Because the medium (tissue) is never fully elastic, pressure variations introduce friction as particles move beside and against each other (Gill 2012, 9). Therefore energy is transformed into heat and lost, as in Figure 12.
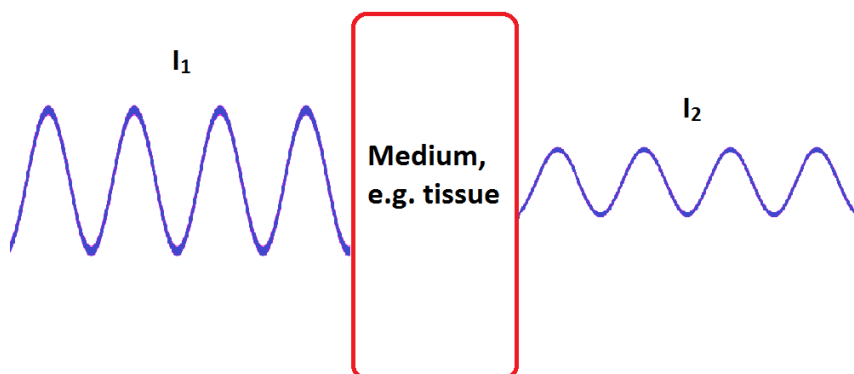

FIGURE 12. Absorption causes a part of the energy of the sound wave to be lost into the medium as heat.

Each material has its own *absorption coefficient*, which dictates the rate of absorption when acting as a medium. The absorption coefficient of bone is higher than that of soft tissue, which causes it to absorb a larger part of the energy of the ultrasound wave. Absorption coefficient is proportional to the frequency used: absorption coefficient increases with higher frequencies, leading to a higher rate of absorption and drop of intensity. Absorption is the main cause of attenuation of an ultrasound beam (Gibbs et al. 2009, 46)

5.4.2 Reflection

When a propagating ultrasound wave faces an interface that is larger than the wavelength of the sound, some of its energy is reflected back (Gibbs et al. 2009, 46). This reflection is known as an echo that can be registered by a measuring device. The rest of the energy of the wave continues into the new medium. The acoustic impedance mismatch of the two interfacing materials affects the amount of reflected energy. The reflected component is greater if the acoustic impedances are poorly matched and not of the same magnitude. Muscle-fat interface usually allows almost 99% of the intensity of the ultrasound wave to be transmitted onward, whereas in a muscle-air interface almost 100% is reflected (Bushberg, Seibert, Leidholdt Jr. and Boone 2012, 508). Air is a perfect reflector of ultrasound, making it necessary to use coupling gel at transducer-skin interface to avoid air pockets. See Equation 7 for the calculation of acoustic impedance. See Figure 13 for a graphical presentation of a reflection.
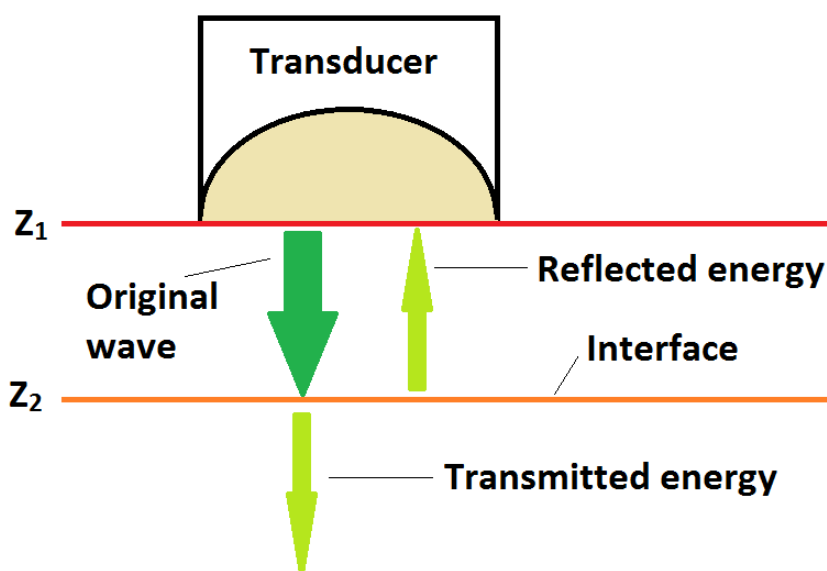

FIGURE 13. When a sound wave (dark green color) meets an interface, energy is reflected and transmitted. The proportions of reflected and transmitted energy (light green color) is determined by the acoustic impedance of the media and the angle of the beam.

The *reflection coefficient* of an interface shows the fraction of energy reflected and it can be calculated by:

$$R = \frac{I_r}{I_i} = \frac{(Z_2 - Z_1)^2}{(Z_2 + Z_1)^2}$$ [16]

where I_r and I_i are the reflected and incident intensities of sound waves, respectively, and Z_1 and Z_2 are the acoustic impedances of the first and second material, respectively.

If all of the energy in the sound wave is transmitted to the new medium, the reflection coefficient is 0 ($Z_1 = Z_2$). An interface where all of the energy is reflected receives an R of 1 (Z_1 and Z_2 are significantly unequal). (Gill 2012, 11)

The *transmission coefficient* of an interface can be determined by:

$$T = \frac{I_t}{I_i} = \frac{4 * Z_1 * Z_2}{(Z_2 + Z_1)^2}$$ [17]

where I_t is the transmitted intensity of the wave. (Gill 2012, 12)

It should be noted that in the previous equations the ultrasound beam is perpendicular to the medium interface. In case of a *specular (smooth) reflection* the incident angle is always equal to the reflected angle and thus, if the angle of incidence is not equal to 90°, the reflection is directed away from the sensor and not received. Also, if the surface measured is fairly crude and the wavelength of the ultrasound wave is close to or smaller than the size of dents or bumps in the surface, echoes may be scattered about and the measured reflected intensity is greatly reduced. This is known as a *non-specular (diffuse) reflection*. (Bushberg et al. 2012, 509)

5.4.3 Refraction

When the ultrasound wave arrives to the boundary of two materials with different propagation speeds for sound at a non-perpendicular angle, it refracts (changes direction) in the new medium, as in Figure 14. Although the speed of the wave may change in the new material, its frequency remains the same. The angle of refraction is dependent on the change of the speed of sound together with the angle of incidence. Their relationship is shown in *Snell's law*:

$$\frac{sin\theta_t}{sin\theta_i} = \frac{c_2}{c_1}$$ [18]

where θ_t and θ_i are the angles of transmission (refraction) and incidence, respectively, and c_1 and c_2 are the speeds of sound in the first and second medium in the path of the wave, respectively. (Bushberg et al. 509)
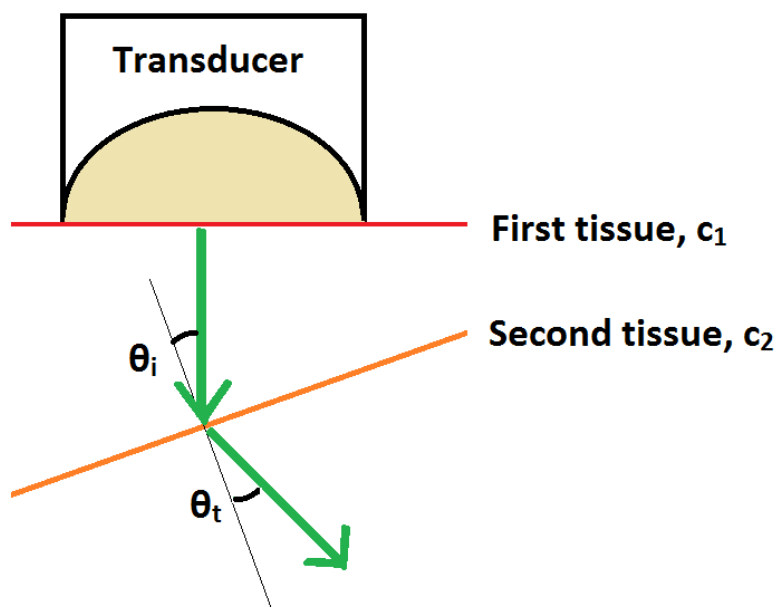
FIGURE 14. Refraction. The direction of propagation changes due to different propagation speeds of the two media.

If $c_2 > c_1$, the angle of refraction is greater than the incident angle and respectively, if $c_2 < c_1$, the refraction angle is smaller. In case of a perpendicular incidence angle or equal speeds of sound of the media, no refraction occurs. If the ultrasound beam hits the second medium at a *critical angle* (θ_c) and $c_2 > c_1$, the wave keeps propagating along the boundary of the media but does not transfer into it. Determining the critical angle is done by setting $\theta_t = 90°$ in Equation 18, which results in the following (Bushberg et al. 2012, 509-510):

$$sin\theta_c = \frac{c_1}{c_2}$$

[19]

Incident angles exceeding the critical angle result in *total reflection* of the ultrasound wave. Total reflection obeys the rules of a specular reflection, i.e. angle of incidence is equal to the angle of reflection. (Gill 2012, 15)

5.4.4 Scattering

If an object or interface is of about the same size as the wavelength of the ultrasound wave, e.g. red blood cells or capillaries, it acts as a non-specular reflector surface to the beam (Gill 2012, 12). As a consequence a part of the intensity of the wave is scattered in all directions, reducing the amplitude returning to the receiver (Bushberg et al. 510). Contrary to specular reflection, returning amplitudes caused by scattering are not greatly dictated by the beam direction, which means that they appear the same to the measuring device regardless of the angle of the transducer. Scattering is depicted in Figure 15.
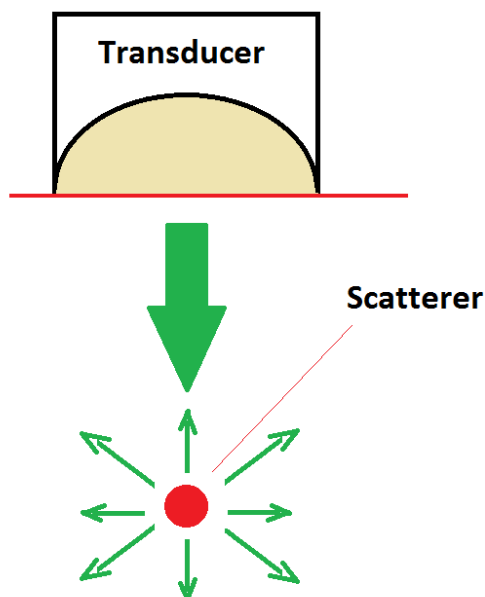
FIGURE 15. Scattering. The scatterer, presented as a red dot, scatters the ultrasound wave in all directions equally.

5.5 Quantitative ultrasound in bone densitometry

Quantitative ultrasound (QUS) is a form of medical ultrasonography, where quantitative variables of tissues, such as bone, are measured to evaluate their condition and properties (Baert, Knauth, Sartor and Grampp 2008, 163). This information can then be used in diagnosing illnesses or abnormalities of organs. QUS is therefore not primarily an imaging method, even though bone imaging can be done with ultrasound to some extent. Distinct advantages of quantitative ultrasound in the diagnostics of osteoporosis are its lower price and the fact that it doesn't produce ionizing radiation. Various techniques have been introduced to measure bone condition using ultrasound, but they are based on two main types (*transmission* and *pulse-echo*). Pulse-echo is a newer method in bone diagnostics and research has been made to more effectively utilize it due to its wider usability compared to peripheral transmission measurements.

5.5.1 Transmission technique

Transmission mode is used when the measured medium is significantly attenuating, e.g. cancellous bone (Langton and Njeh 2004, 426). In a transmission measurement a total of two transducers are used, from which one is for transmitting the pulse and the other for receiving. The transducers may be coaxially aligned, i.e. on the opposite sides of the measured bone facing each other (*through-transmission* or *transverse transmission* technique, TT), or *pseudo-reflection* aligned (*axial transmission*). In axial transmission the transducers are on the same side of the bone and the transmitted pulse travels along a thin layer just under the surface of the bone and is then received by the other transducer. With older devices a through-transmission measurement is usually made from the heel in a water bath for better coupling (Duck, Baker and Starritt 1998, 291). Newer dry contact systems can be used by applying ultrasound gel between the measuring pads of the device and the heel (Arden 2006).

The two parameters normally measured with all transmission techniques are *speed of sound* (SOS) and in through-transmission *broadband ultrasound attenuation* (BUA) (Duck et al. 1998, 291). Both parameters are reduced in an osteoporotic bone compared to a healthy one due to the decreased amount of bone (Arden 2006). BUA and SOS are sometimes combined using manufacturer-specific algorithms to produce new indexes that may enhance the readability and interpretation of results (Bonnick and Lewis 2013, 13).

Axial transmission technique enables a wider range of locations to be measured, since only one side of the bone is needed for the measurement. The velocity of the wave is calculated by the *time of flight* (TOF) and the distance of the transducers. Soft tissue has an effect on the propagation time of the wave and one of the biggest challenges concerning this technique. An array of transducers can minimize the effect of the soft tissue by using and monitoring multiple routes for the pulse. (Baert et al. 2008, 169)

SOS is based on the higher speed of ultrasound in bone than in water. When the speed of ultrasound in water is known, the SOS in calcaneus (expressed in m/s) can be determined by how much earlier the pulse arrives to the receiver through the heel, i.e. by the time difference Δt. The velocity in the subject (c_s) is acquired as follows:

$$c_s = \frac{d_s c_w}{d_s - c_w \Delta t}$$

[20]

where d_s is the heel width and c_w is the velocity of ultrasound in water. (Duck et al. 1998, 291)

Transverse transmission measurement of the cortical bone has its own special parameter *amplitude-dependent speed of sound* (AD-SOS) which is also calculated based on the transducer distance and TOF. The time of flight is measured from the start of the pulse to a point where the signal amplitude has reached a certain trigger level at the receiving end. Since speed of the pulse is lower in osteoporotic bone and its amplitude is decreased, the trigger level is reached later. This way AD-SOS may be more sensitive to changes in the bone than techniques that only measure the speed of the wave. However, AD-SOS is not suitable for all situations. In children the amplitude of the wave does not change during growth and it doesn't add to the diagnostic value of the results, but only to the possible error. (Baert et al. 2008, 168)

In BUA determination, a broadband ultrasound pulse (0.2 - 1 MHz) is transmitted through the heel in a water bath and compared to a reference pulse without the heel (Duck et al. 1998, 292-293). The signals are then Fourier transformed to gain the amplitudes in the frequency domain. Because bone attenuates higher frequencies more than the lower ones, attenuation curve is different for osteoporotic bone compared to that of a healthy bone (Baert et al. 2008, 166). BUA is the slope of the linear part of the attenuation curve versus the frequency and it is expressed as dB/MHz. The most significant sources of error for BUA are its negative dependency on the temperature of the measuring site and a possible oedema (accumulation of fluid under the skin or tissues).

5.5.2 Pulse-echo technique

Pulse-echo (PE) technique is mostly utilized in soft tissue imaging and it is the most common technique used with clinical ultrasound. It can also be used for investigating bones as a result of advanced measurement and calculation techniques. In PE measurement, the ultrasound pulse is generated and received by the same transducer on the skin surface. Coupling gel is used to eliminate air pockets. Tissue thickness can be determined by measuring the time it takes for the ultrasound wave to propagate and return to the transducer:

$$s = 2 * \frac{v}{\Delta t} \tag{21}$$

where v is the assumed velocity of the wave in the tissue and Δt is the time taken for the pulse to return. The multiplier 2 has to be added, because the wave travels the same distance twice in the measured time (from transducer to tissue and back). (Langton and Njeh 2004, 424)

A received pulse-echo signal has to be bordered to a desired *time window* for the calculation of parameters. In defining the suitable time window an echo from a perfect reflector, such as a metal plate, is used. The signal becomes longer in the presence of attenuating material between the transducer and the measured bone. This is caused by the low-pass filtering effect by the material, such as soft tissue. The change in signal shape has to be taken into account in measurements. The length of the pulse is also dependent on the center frequency and the width of the spectrum used. (Karjalainen 2011, 24)

The most commonly used parameters for pulse-echo measurements are *integrated reflection coefficient* (IRC), *apparent integrated backscatter* (AIB) and *broadband ultrasound backscatter* (BUB). As the names imply, they measure either the reflection at the interfaces or the backscattering of the ultrasound wave in the trabecular bone. BUB is the same as AIB, but it also includes an *attenuation compensation term β*. The time windows for AIB and BUB can be found in the measurement signal after the beginning IRC time window and they can be delayed to rule out the effect of the energy of the surface reflection. The equations for the three parameters are as follows:

$$IRC = \frac{1}{\Delta} f \int_{\Delta f} 20 (log_{10}(\frac{A_n(f)}{A_r(f)})) \tag{22}$$

$$AIB = \frac{1}{\Delta} f \int_{\Delta f} 20 (log_{10}(\frac{A_b(f)}{A_r(f)})) \tag{23}$$

$$BUB = \frac{1}{\Delta} f \int_{\Delta f} 20 (log_{10}(\frac{A_b(f)}{A_r(f)})) + \beta \tag{24}$$

where Δf is the used frequency band, β is the attenuation compensation term and $A(f)$ is the amplitude spectrum of the signal gated either at the reflection from the surface or backscatter window in

the bone, as indicated by the subscript: n is the sample, r is the perfect reflector and b is the backscatter window. (Karjalainen 2011, 24)

The attenuation of the signal inside trabecular bone is a problem to which no comprehensive solution has been found. The attenuation compensation term in BUB can be approximated if the attenuation coefficient and the speed of sound in trabecular bone are known using through-transmission measurement. Since AIB doesn't include any attenuation compensation, it is more easily and widely used, especially for *in vivo* measurements (Latin for "within the living", meaning the measurements are conducted on living test subjects). (Karjalainen 2011, 24-26)

6   BINDEX

Bindex[®] (Figure 16) is manufactured in Kuopio, Finland by Bone Index Finland and it is the first ever pocket-sized device for the screening and diagnosing of osteoporosis (Bone Index Finland Ltd. 2014a). It is Class IIa medical device that was CE approved in July 2013. The company is currently applying for an FDA (the United Stated Food and Drug Administration) approval for Bindex[®], making it available also for the American market. The device meets the Medical Device Safety standard IEC 60601-1 edition 3.0 and the Ultrasound Safety standards IEC 60601-2-37 edition 2.0 and IEC 62359 edition 2.0 (Bone Index Finland Ltd. 2013, 12).

FIGURE 16. The Bindex[®] measuring device. The pulser unit (bottom left) is connected to the USB port of a computer. The phantom (top left) can be used to calibrate and check the functionality of the device together with the probe (right). (Heiskanen 2014-04-24)

The Bindex[®] device is a pulse-echo measuring device that utilizes a focused ultrasound beam at the center frequency of 3.0 MHz (Bone Index Finland 2013, 10-12). The device consists of a USB-powered pulser and a measuring probe connected by an EMC shielded cable. The pulser produces the electrical pulse that is then transformed into mechanical energy (the ultrasound pulse) in the transducer of the probe. The transducer is located behind a concave silicon delay line. The function of a delay line is to add to the distance traveled by the ultrasound wave and thus to the time it takes for the echo to return (NDT Resource Center 2007b). This is necessary in high precision applications, such as measuring thicknesses near the surface of the examined object. The added delay gives the transducer enough time to switch to receiving mode after sending the pulse, which enhances the resolution of the device.

The weight of the whole device is 126 g, and the physical dimensions are 23 x 61 x 92 mm (height x width x length) for the pulser and 28 x 87 mm (diameter x height) for the probe. It operates at a normal USB voltage of 5 V. The operating and storage temperature is +10 to + 40 °C. The Bindex[®] software can be operated on a Windows Vista or Windows 7 operating system. It requires a total of

340 MB of hard disk space and an additional 1,3 MB for every patient. (Bone Index Finland 2013, 11-13)

The device is calibrated before every measurement by keeping the clean probe in the air while the strength of an echo from the surface of the delay line is measured. The functionality of the Bindex® device should also be validated weekly by conducting a *phantom measurement*, in which the thickness of a standardized target (known as a *phantom*, see Figure 11) is measured. The device is in condition if the received thickness also between given thresholds. (Bone Index Finland 2013, 31-32)

The measurement is made from the upper shaft of tibia (the shin bone) by placing the probe on the right location together with ultrasound coupling gel. The location can be found using a measuring stick provided by Bone Index Finland. The Bindex® device needs to be connected to a computer with the measuring software installed to conduct a measurement. The device measures the thickness of the cortical bone, after which the Bindex® software calculates a special *Density Index* (DI), which is a strong predictor of osteoporosis. The DI is an estimation of the bone mineral density the patient would get in a DXA measurement and it is given as g/cm^2. The results are also shown on a color scale for easy readability. The result sheet of the software can be seen in Figure 17. Based on the DI, the patients' probability of osteoporosis is determined to be one of the three categories found in Table 2. The measurement can be conducted on anyone, but the diagnostic criteria are currently clinically validated only for Caucasian women of over 50 years of age. The result is not a diagnosis by itself, as it always needs to be evaluated by a medical doctor first. (Bone Index Finland Ltd. 2013, 9, 37)
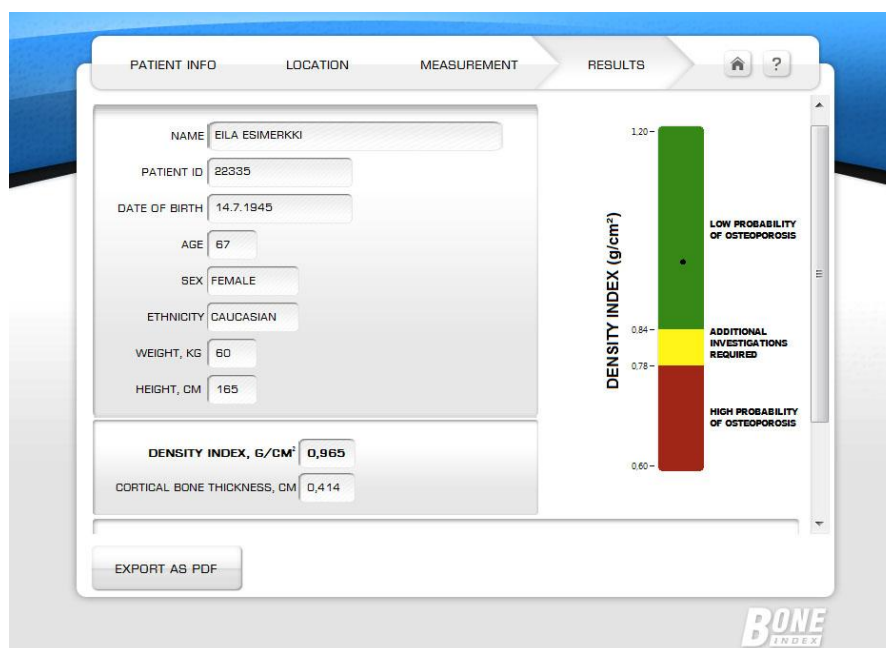

FIGURE 17. The result sheet of Bindex® software. The Density Index is shown as a black dot on the color scale. (Bone Index Finland Ltd. 2014a)

TABLE 2. The result interpretation of Bindex® software. (Bone Index Finland Ltd. 2013, 37)

Density Index (g/cm^2)	Color code	Probability of osteoporosis
over 0,84	Green	Very low / healthy
0,78-0,84	Yellow	Additional investigations required (DXA measurement)
under 0,78	Red	Very high / osteoporotic

The Bindex® measurement has also been proven to be significantly cost-effective in primary healthcare. Because of the device high sensitivity and specificity thresholds of 90%, only roughly 30% of patients are left in the yellow zone of the color scale and need to be further investigated and directed to a DXA scan. In a recent study, Bindex® measurement saved an average of 230€per screen compared to a traditional DXA scan. The current cost of a DXA scan with result interpretation and travel costs was estimated to be 423€, so the costs were reduced by nearly 50%. (Asseburg, Riekkinen, Karjalainen, Kröger and Soini 2013)

7   CONDUCTED MEASUREMENTS

The practical part of this thesis was conducted in the laboratory premises of the Department of Applied Physics in the University of Eastern Finland. The measurements took place between March 19 and 26.

Bone Index Finland had a sample set of 43 human bone samples from the tibial cortex. The thickness of the samples was standardized to 2.0 mm, while their length and width varied from 3.5 to 13 mm. In most cases the shape was fairly square-like, and the sides were 4-5 mm long and wide. The samples were stored frozen in phosphate buffered saline solution (PBS) as seen in Figure 18. PBS is an isotonic biological buffer solution that maintains the cell osmolarity of the sample, i.e. it maintains the cell structure by simulating the conditions in the human body (Medicago AB 2010). Several samples could be stored in the same cryogenic tube, as some samples were taken from the same bone. All the samples could be identified by a number marked on the surface of the sample.

FIGURE 18. The measured bone samples frozen in cryogenic tubes. (Heiskanen 2014-03-20)

The samples had to be thawed before proceeding. This was done by placing the cryogenic tubes in a cold water bath, where they were kept for 30-40 minutes (see Figure 19). The work was divided over several days, as there was only enough time to measure the contents of 3-5 tubes in one day. In the waiting time, distilled water was acquired and the vacuum pump needed for the next step of the measurement was turned on to warm it up.

FIGURE 19. Cryogenic sample tubes in a cold water bath. Cold water melts the ice without causing the bone samples to break because of a sudden rise in temperature. (Heiskanen 2014-03-20)

After the thawing process, air had to be removed from the samples and distilled water. As mentioned earlier in the thesis, liquid-air interface reflects practically all of the ultrasound energy transmitted. Air bubbles in the samples or the measuring medium could result in false readings or otherwise disturb the measurements. Bone samples were moved into separate plastic cups with distilled water. Each cup contained all the samples from the same bone (Figure 20). The cups were then placed in a vacuum chamber (Figure 21), which operates with the previously mentioned vacuum pump. The air inside the chamber is removed in less than a minute, resulting in a zero-bar air pressure. Because liquids can hold smaller amounts of gases the lower the atmospheric pressure drops, air bubbles begin to form and escape the distilled water (see Figure 22). The samples and the water used were kept in the vacuum chamber for two hours.
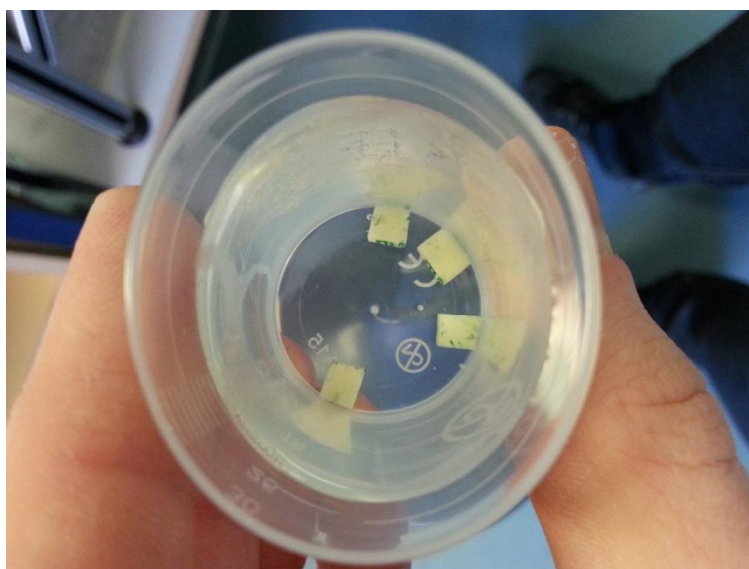

FIGURE 20. Sample cup with all the samples from one bone. The samples varied in size, but were equally thick. (Heiskanen 2014-03-26)

FIGURE 21. Distilled water for measurements and small sample cups in the vacuum chamber. (Heiskanen 2014-03-25)

FIGURE 22. Vacuum chamber in use. Air bubbles can be seen to form in the water when the air pressure is lowered below the normal atmosphere. (Heiskanen 2014-03-25)

After the air removal distilled water was added to the measuring tank, which sat on a two-axis *goniometer* (Figure 23). The goniometer is a device with which the angle of a desired object (the tank) can be set very precisely using micrometer gauges. Water was siphoned into the tank to eliminate any air bubbles that may form as a cause of pouring the water (Figure 24). The measuring probe needed to be able to move up and down to the two measuring locations, so roughly two thirds of the tank was filled with water.
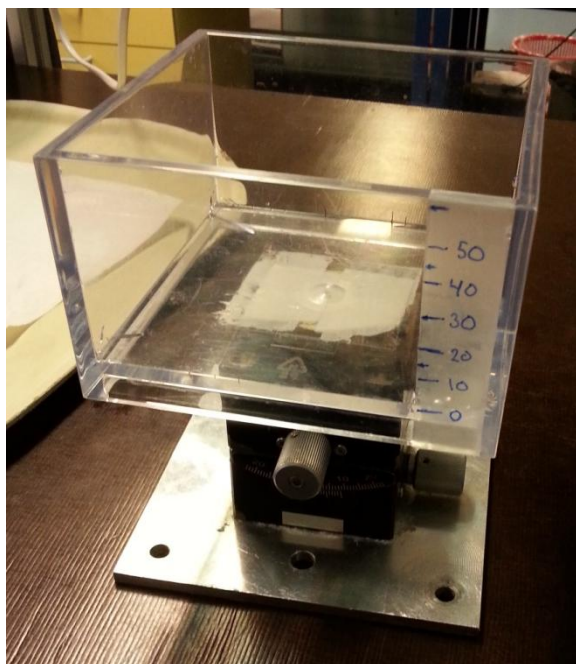
FIGURE 23. The measuring water tank on top of the goniometric stand. (Heiskanen 2014-03-21)
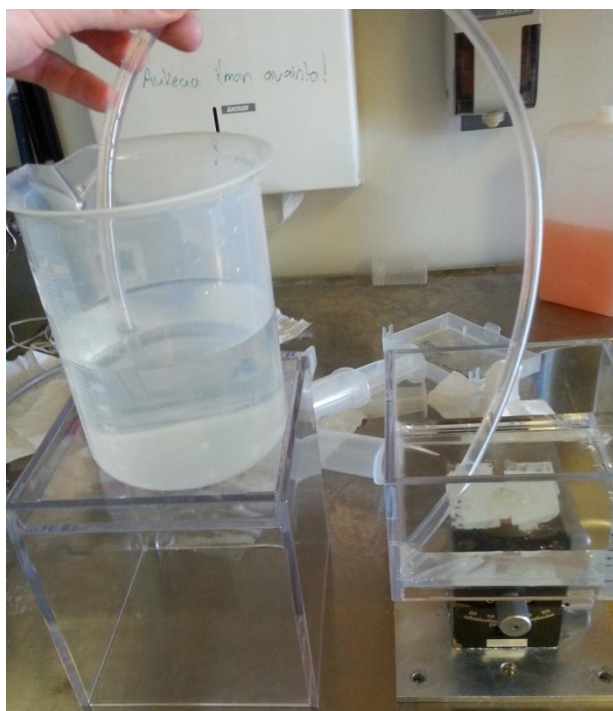

FIGURE 24. Siphoning of the distilled water into the measuring tank. (Heiskanen 2014-03-25)

For the measurement, a customized version of the Bindex® device was used with the probe cover left out (Figure 25). The probe could be more easily attached and set to a correct angle, i.e. perpendicularly to the bottom of the water tank. The probe was securely attached with adhesive tape to the motor-controlled arm of the UltraPAC, an ultrasonic imaging and research system. A metallic platform was placed in the water tank to reflect the remainder of the ultrasound energy at the sample-platform interface. Without it, reflections from the bottom of the tank and all its interfaces would have been received. The setup can be seen in Figure 26.

FIGURE 25. The research device. The curved probe cover was left out for a more robust attachment to the motor arm. (Heiskanen 2014-03-20)
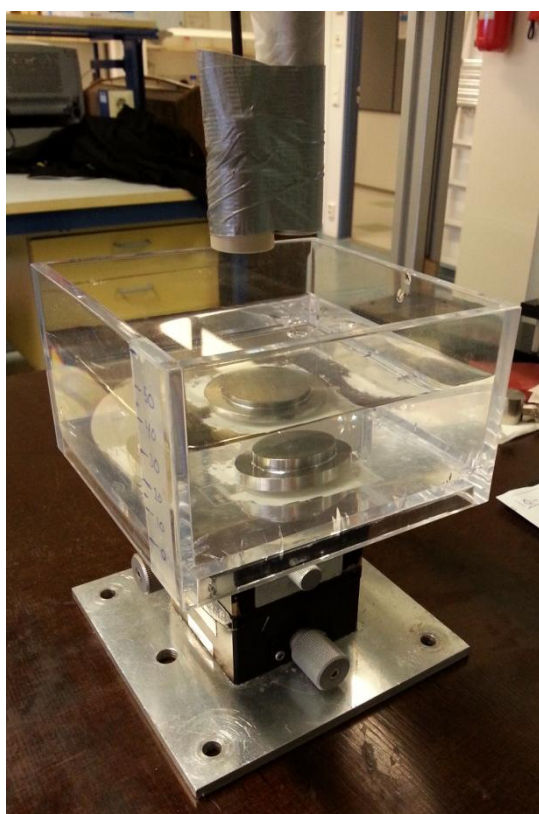

FIGURE 26. The measurement setup ready for the calibration. The probe is attached to the motor arm and the metallic platform can be seen at the bottom of the tank. (Heiskanen 2014-03-21)

Before the actual measurements, the tank had to be leveled for the angle of the ultrasound beam to be as perpendicular to the sample surface as possible. This was accomplished by submerging the probe into the water and moving the stand carefully so that the probe was located at the corner of the tank. The measurement using the research software was started and the time of flight of the ul-trasound pulse was observed in the software. The platform was then carefully moved so that the

probe scanned the floor of a certain side of the tank all the way to the other corner, where the TOF was read again.

If the TOF in the second corner was lower, it was closer to the probe, which meant that the tank was tilted. A higher reading meant that the corner was lower than the previous one. The goniometer screws were used to adjust the angle of the tank and the side scan was done again. This was repeated until the TOF stayed the same for the entire length of the side, i.e. the measured axis was horizontally leveled. The procedure was then repeated to the other axis of the tank.

When the probe is submerged into the water, the concave head of the probe gathers a small amount of air underneath it. The air had to be removed after every submersion using a small brush as seen in Figure 27.

§
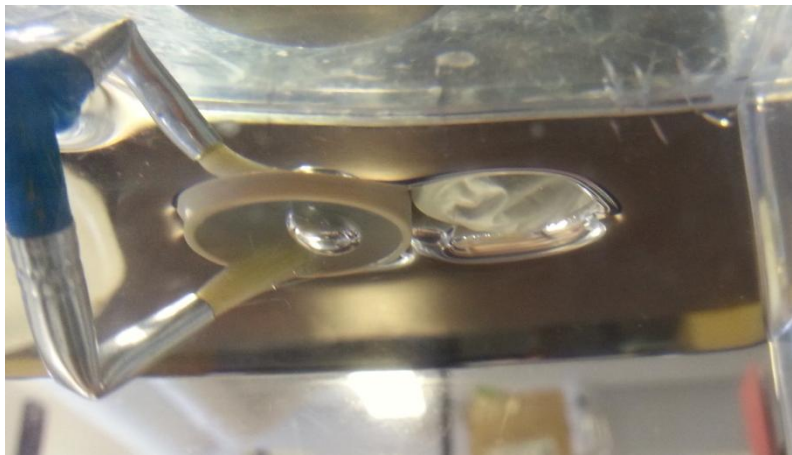
FIGURE 27. The air removal brush for the probe head. The tip of the brush was at an angle to ease its use in small spaces. (Heiskanen 2014-03-21)

After the leveling, the bone samples could be added to the tank. This was done by carefully lowering the one small sample cup at a time into the measuring tank and then pouring the samples underwater to the bottom of the tank. The samples had to be kept underwater for the entire time to keep any air from getting into their pores. Samples from only the same bone were kept in the tank at a time to keep them from mixing up. One sample was moved onto the metal platform for the measurement using a pair of tweezers. The harder *periosteal* (outer) surface of the bone was identified by the markings on the sample and faced up, leaving the softer and more porous *endosteal* (inner) surface against the metal plate. A sample ready to be measured can be seen in Figure 28.

FIGURE 28. The measuring setup. A sample is placed on the metal platform while two other samples wait at the side. (Heiskanen 2014-03-21)

After the initial setup, the distance of the probe was adjusted using the motor drive of the UltraPAC system. The controller software (Figure 29) enabled very small adjustments to the placement of the probe, making it possible to make changes as small as 0,02 mm. Observing the TOF value in the test software, the distances for the measurements were adjusted so that the first TOF was 48 µs and the second one 42 µs. The placement of the goniometric stand was adjusted so that the signal shown in the test software was of the right shape for a measurement, i.e. the bone was facing the probe correctly and the signal was strong enough. This could be determined by the peak sizes and shapes in the software. Signals with two distinct, fairly sharp peaks were accepted, of which the second peak was smaller or almost the same size as the first.
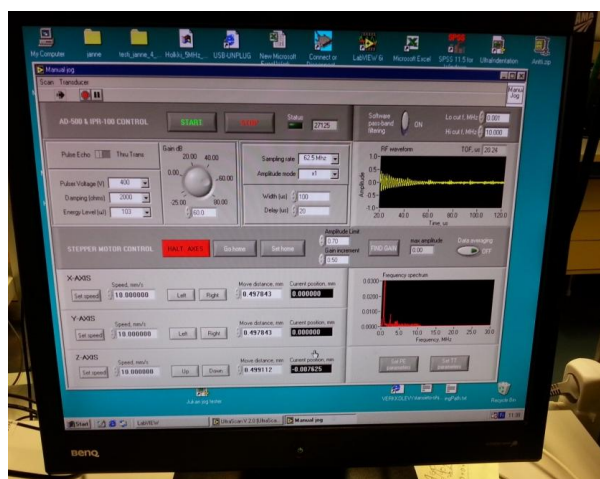

FIGURE 29. The control software for the UltraPAC. (Heiskanen 2014-03-21)

After the TOF was adjusted correctly, a Bindex® software measurement was made. The software measures the thickness of the bone based on the echoes from the outer and inner surfaces of the bone sample. The software cannot conduct the measurement without the calibration of the probe in air, so the probe had to be raised from the water tank and water wiped from the tip of the probe between measurements. UltraPAC allowed the equal horizontal movement of the probe to be made

both ways, so the TOF value stayed very close to the originally adjusted. The Bindex® software does not show the TOF to the user, so the test software had to be run to check the exact value after every measurement. At the same time the ultrasound signal was saved for further analysis. Figure 30 shows the test computer and Bindex® device and software in use.
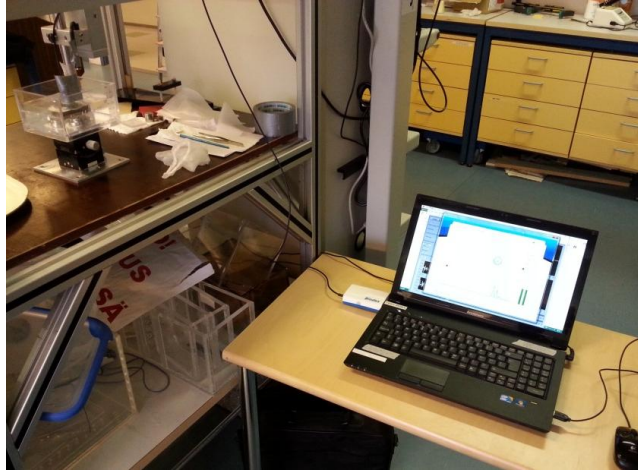

FIGURE 30. The measuring setup from afar. Bindex® software can be running on the laptop screen. (Heiskanen 2014-03-21)

After the test signal was saved, the probe was moved to the second location. Adjustments were made as needed, the device was calibrated again and the sample thickness measured and the test signal stored to the computer. If the measurement could not be made due an insufficient signal, the probe was lowered 0,5 mm at a time until the signal was accepted. The final accepted TOF was then written down and the signal saved. When the two measurements were made to a sample, a new one was moved to its place. After all the samples of one bone were measured, they were raised from the tank using a pair of tweezers, dried gently using a paper towel and their dimensions measured using an electronic slide caliper (Figure 31). The thickness of the bone was measured from four locations along the samples axis and the average calculated.

FIGURE 31. Electronic slide caliper used in the measurement of the sample dimensions. (Heiskanen 2014-03-25)

After the measurements, the bone samples were returned to the cryogenic tubes with PBS solution and frozen again.

8   ANALYSIS OF RESULTS

The analysis of the measured signals was done using Matlab® software by The MathWorks Inc. It is a complete programming and calculation environment meant for e.g. data analysis, simulations and algorithm development (The MathWorks, Inc. 2014b). Matlab® uses its own language that resembles C/C++, but has plenty of its own functions made especially for easy calculation. It is very useful in signal processing thanks to its separate DSP toolboxes, which contain many useful aids for the manipulation of signals. Matlab® has a built-in graphics capability, which allows data plotting to be made and customized. It also has an graphical user interface (GUI) building tool, which is fairly easy to use even for a beginner. A custom GUI was built in this thesis for easier and faster analysis of the results.

The program made for this thesis consisted of four basic functions or callbacks. "load_Callback" handled the loading and reloading of test files either as single files or the entire file folder at a time. It also enabled the program to move on to the other functions and created the Excel sheet at the end of a successful analysis. "Plot_Graphs" was used to separate a wanted section of the signal for further inspection and the plotting of graphs. In "Transform" the majority of signal processing was conducted and it returned the wanted results to "load_Callback". "reload_Callback" was only used if the manipulated signal was needed to be reset back to its original form.

For each measurement the test software produced a text file which contained the measuring delay (time from the start of the ultrasound pulse), the gain value and the measured amplitudes of the signal at intervals determined by the sampling frequency. One file at a time was opened in Matlab® and the signal vector plotted. A Hilbert transform was then taken of the signal to bring out the peaks of the first and second echo received from the sample. As a result of the transform a complex helical sequence is returned (The MathWorks, Inc. 2014a). This *analytical signal* has a real part (the original data) and an imaginary part, which is a 90° phase-shifted version of the original signal. The Hilbert transformed signal has all the same amplitudes and frequencies as the original and its phase is dependent on the phase of the original signal.

The signal was then manipulated using the functions allowed by Matlab® and viewed in different domains. The precise methods cannot be described here, as they are confidential. At the end of the analysis the results were copied to an Excel sheet, where they could be more easily viewed and further processed as a whole. The graphical user interface greatly enhanced the usability of the analysis program as plots could more easily be seen at one glance and the needed parameters could be printed out to a certain location on the screen. Roughly 550 code lines were written to produce the final version of the program. A part of the GUI can be seen in Figure 32.
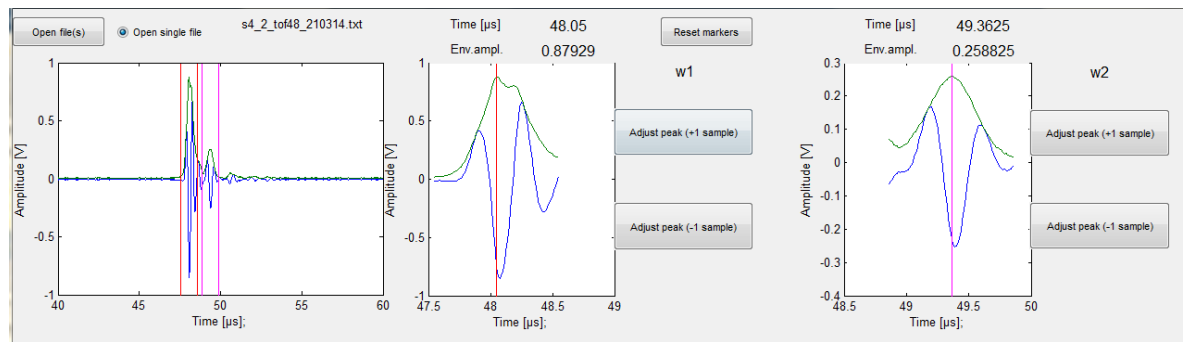
FIGURE 32. A part of the graphical user interface created with Matlab®. The Hilbert envelope is plotted around the signal and the echo peaks have been separated from the original signal.

9   RESULT ASSESSMENT AND CONCLUSIONS

Some possible sources of error in the measurements could be identified. One of the most likely ones is the non-perpendicular angle of the probe when compared to the surface of the bone sample. This may occur if the attaching of the probe with the adhesive tape has failed or if the setting of the goniometric stand is incorrect. This error could be minimized by checking the angle of the stand at the beginning of each day and any time the goniometric screws may have moved. The tape was firmly attached, leaving no loose parts to it.

Another problem may arise from the probe calibration procedure. Since the probe had to be raised from the water bath before every measurement, the distance setting checked from the test software may have changed after lowering the probe back into the water. This could be controlled by making the initial setting with the test software, calibrating and measuring with the Bindex® software, and then checking the TOF value from the test software again. In the majority of cases the TOF value was within 50 ns of the value of the first setting, which is acceptable. The motor arm was also moved in a single motion out of and into the tank to minimize the error.

As the deeper analysis of the signals is related to the product development of Bone Index Finland, the results cannot be published in this thesis. The measurements were conducted successfully and the analysis of sample data was thorough. The new parameter could be analyzed from the ultrasound signals and its reliability was assessed by comparing it to other properties of the bone samples. However, the parameter was found not suitable for patient measurements at the moment, but further studies will take place on the matter. Should the parameter prove useful in further measurements, the company will most likely apply for a patent on its use in ultrasound diagnostics.

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