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2 **Title:** Noncontact Ultrasound Elastomicroscopy: Potentials for Assessment for Articular
3 Cartilage

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9
10 **Abstract:** Research in elasticity imaging typically relies on 1 to 10 MHz ultrasound.
11 Elasticity imaging at these frequencies can provide strain maps with a resolution in the
12 order of millimeters, but this is not sufficient for applications to skin, articular cartilage,
13 or other fine structures. In this paper, we introduced two methods of noncontact
14 ultrasound elastomicroscopy for imaging the elasticity of biological soft tissues with high
15 resolutions. In the first system, the specimens were compressed using water jet
16 compression. A water jet was used to couple a focused 20 MHz ultrasound beam into the
17 specimen and meanwhile served as a “soft” indenter. Because there was no additional
18 attenuation when propagating from the ultrasound transducer to the specimen, the
19 ultrasound signal with high signal-to-noise ratio could be collected from the specimens
20 simultaneously with compressing process. The compression was achieved by adjusting
21 the water flow. The pressure measured inside the water pipe and that on the specimen
22 surface was calibrated. This system was easily to apply C-scan over sample surfaces.
23 Experiments on the phantoms showed that this water jet indentation method was reliable
24 to map the tissue stiffness distribution. Results of 1D and 2D scanning on phantoms with
25 different stiffness are reported. In the second system, we used osmotic pressure caused by
26 the ion concentration change in the bathing solutions for the articular cartilage to deform
27 them. When bovine articular cartilage specimens were immersed in solutions with
28 different salt concentration, a 50 MHz focused ultrasound beam was used to monitor the
29 dynamic swelling or shrinkage process. Results showed that the system could reliably
30 map the strain distribution induced by the osmotic loading. We extract intrinsic layered
31 material parameters of the articular cartilage using a triphasic model. In addition to
32 biological tissues, these systems have potential applications for the assessment of
33 bioengineered tissues, biomaterials with fine structures, or some engineering materials.
34 Further studies are necessary to fully realize the potentials of these two new methods.
35

36 **Keywords:** ultrasound, biomicroscopy, elasticity imaging, elastomicroscopy, water jet,
37 indentation, ultrasound indentation, osmotic pressure, triphasic model, articular cartilage.
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1 **INTRODUCTION**

2 Tissue elasticity is generally known to be associated with pathologic changes, such as
3 sclerous cancer, edema, degeneration, fibrosis and pressure sore [1-2]. Current research
4 in ultrasonic elasticity imaging of soft tissues typically relies on 1 to 10 MHz ultrasound
5 which is the frequency range used for most medical imaging applications [3]. The spatial
6 resolution obtained at these frequencies (on the order of millimeters) is not sufficient for
7 the study of very fine structures in tissues such as skin layers or articular cartilage [4-7].

8 Nanoindentation [8] has recently been used for the microscopic mechanical
9 assessment of biological tissues including bone [9], spinal fusion [10], and articular
10 cartilage [11-12]. However, nanoindentation can not provide mechanical properties of
11 tissues at different depths. The testing results are highly dependent on the surface
12 condition of the specimen [10]. Elasticity imaging based on optical coherence
13 tomography (OCT) has also been reported [13-14]. Optical beams are used to probe
14 tissues at different depths, thus images of the scattering intensity from sub-surface
15 structures of soft tissues are obtained to construct strain images [15-16].

16 High-frequency (20 to 100 MHz) B-mode imaging (ultrasound biomicroscopy) has
17 been widely used in recent years for the assessment of eye tissues, skin, blood vessel, and
18 articular cartilage with an axial resolution of approximately 100 to 20 μm [4].
19 Elastography of artery walls has been reported using intravascular ultrasound
20 backscattered signals (~ 30 MHz) obtained while cyclic blood pressure applied a
21 temporally varying loading source on the vessel [17-18]. In other tissues, requiring an
22 external compression source, attempts have been made to acquire high frequency (50
23 MHz) ultrasound signals while squeezing tissue through a slit in a compressor [6].

1 However, the slit introduces uncertainties in the mechanical boundary conditions on the
2 specimen and it's difficult to estimate the stress distribution. Fortin et al. [5] used two
3 parallel plates to compress the two sides of cartilage specimens and to collect 50 MHz
4 ultrasound from the open side. Using this configuration, the lateral tissue displacements
5 in one direction were mapped under an axial compression. Based on the ultrasound
6 indentation technique [19-22] using a probe with an in-series ultrasound transducer
7 (frequency ranged from 5 to 15 MHz) and a load sensor, Zheng and coworkers have
8 developed a number of systems for, mapping one-dimensional mechanical properties of
9 articular cartilage (AC) using high frequency ultrasound (20 to 50 MHz) [23-25]. The 2D
10 high-frequency ultrasound elasticity imaging has only been described in theory or using
11 computer simulation in the literature [26-27] because how to properly loading on the
12 tissue samples remains as a problem. In this paper, we introduce ultrasound
13 elastomicroscopy systems, which utilize noncontact loading techniques, one was a
14 mechanical loading using a water jet indentation device and the other was an osmotic
15 loading induced by changing the ion concentration of the bathing solution for the tissue.
16 In both systems, water served as a coupling medium, so that high-frequency focused
17 ultrasound beam could be used to monitor the tissue deformation at a microscopic level.
18 Using our ultrasound elastomicroscopy systems, the strain images at both ultrasound
19 propagation direction and orthogonal direction could be obtained. Furthermore, with the
20 estimated stress distribution, modulus images could also be derived. The system
21 architectures were described. Experimental results on both gel phantoms and bovine
22 articular cartilage were provided and discussed.

23

1 **METHODS**

2 *Ultrasound biomicroscopy for water indentation and osmosis loading*

3 A biomicroscopy system with a frequency range of 10 to 80 MHz was developed. It
4 was comprised of a pulser/receiver (Model 5601A, Panametrics, Waltham, MA, USA),
5 3D translating device (Parker Hannifin Corporation, Irvine, CA, USA), 500 MHz A/D
6 converter (Model CompuScope 8500PCI, Gage, Canada), PC and custom-developed
7 software. For the water jet indentation system, a bubbler was used to eject a water jet by
8 controlling the water flow (Figure 1). The diameter of the water ejecting nozzle was 1.94
9 mm. A 20 MHz focused ultrasound transducer with a focal length of 12.7 mm (GE
10 Panametrics, Inc., OH, USA) was fixed with the water ejector and the focused ultrasound
11 beam could propagate through the bubbler when it was full of water as the coupling
12 medium. The transducer and the bubbler were installed to a 3-D translating device which
13 was used to adjust the distance from the nozzle to the specimen surface and to perform 2-
14 D scanning over the tissue. During experiment, specimens were placed on a rigid
15 platform within a water container. A pressure sensor (EPB-C12, Entran Devices, Inc.,
16 Fairfield, NJ, USA) was used to measure the water pressure within the water pipe. A load
17 cell (ELFS-T3 mol/L, Entran Devices, Inc., Fairfield, NJ, USA) located under the
18 platform could monitor the overall force applied on the specimen. Both of them were
19 calibrated. The program was used to control the 3D translating device and collect,
20 process and display the ultrasound signal, together with the force and the pressure, in real
21 time during the indentation process. The movement of the transducer and the acquisition
22 of the A-mode ultrasound, force or pressure data were synchronized by the program. The
23 deformation of the specimen under water jet indentation was estimated from the

1 ultrasound echoes using a cross-correlation algorithm [24]. The modulus was calculated
2 from the water pressure and the deformation as well as the thickness.

3 For the setup of osmotic loading, a 50 MHz focused ultrasound transducer with a focal
4 length of 12.7 mm, and a focal zone diameter of 0.1 mm (Panametrics, Waltham, MA,
5 USA) and a specially designed container [28]. During the experiment, the saline solution
6 was used as the medium of ultrasound waves to penetrate into cartilage tissues. The
7 specimen was fixed at the bottom of the container and immersed in the saline solution.
8 Using the 3D translating device, the focal zone was first located in the middle of the
9 cartilage layer and then B-scan was conducted to image the cross-section of articular
10 cartilage.

11

12 *Phantom experiments*

13 Tissue-mimicking phantoms prepared for the experiments were designed as a simple
14 geometry that consisted of a stiff cylindrical inclusion inside a homogeneous background.
15 The phantoms were 25 mm in diameter and 5 mm in height. The stiff inclusion cylinders
16 were made of silicones and their diameter was 8 mm, while the backgrounds were made
17 of agar-water mixture with agar (Fisher Scientific Co. Fairlawn, NJ, USA) concentration
18 ranging from 10.0 g/L to 30.0 g/L. A total of seven phantoms, whose modulus contrast
19 (defined as the ratio of the modulus of the inclusion to that of the background) ranged
20 from 0.97 dB to 10.02 dB, were prepared for the C-scans.

21 During the water jet scan experiment, the phantom was placed on the platform and
22 slightly fixed to avoid the slip. An area of $12 \times 12 \text{ mm}^2$ was scanned with a step of 0.2
23 mm, i.e. a total of 3721 indentations were used to image the region of interest. The

1 pressure during a C-scan was maintained to be a constant. During each scan, the
2 temperature of the water was approximately 20°C. The scan time for each phantom was at
3 most 20 minutes. Typically, samples were preloaded with a pressure at 3 kPa and were
4 then scanned with a pressure no more than 20 kPa. For each sample, the maximal strain
5 was controlled within 5% and the materials were modeled as linear elastic materials
6 within this strain level. The ultrasound pulsed echoes reflected from the sample surface
7 and bottom were tracked to compose the deformation image. The compressive modulus
8 of each indentation site was estimated from the local stress and strain data with the
9 assumption that the Poisson's ratio of the inclusion and background material were the
10 same. In our measurement, the modulus contrast calculated from the modulus images
11 were compared to the actual values which were measured from the uniaxial compression.

12

13 *Experiments on articular cartilage*

14 Fresh mature bovine patellae without obvious lesions were obtained within five
15 hours slaughter and stored at -20°C condition until further experiments. The articular
16 cartilage specimen ($\varphi = 6.35$ mm) was cored out of the flat area of each cartilage-bone
17 slab using a metal punch. The thickness of the cartilage layer was 1.73 ± 0.42 mm (mean
18 \pm SD; n = 14). The specimen was thawed in physiological saline solution (0.15M NaCl)
19 at room temperature ($20^\circ\text{C} \pm 1^\circ\text{C}$) for three hours to be ready for the experiment. The
20 specimen was fixed at the bottom of the testing chamber on the platform and equi-
21 librated in physiological saline solution for one hour, and then hypertonic saline
22 solution (2 M NaCl) was immediately filled into the container after the 0.15 M saline
23 solution was removed. Sample was equilibrated for another one hour. Then the

1 solution was quickly changed back to the 0.15 M saline. As the ion concentrations inside
2 and outside the cartilage were different, a Donnan osmotic pressure on the cartilage was
3 generated. The dynamic deformation of the cartilage layer at different depths could be
4 observed in the ultrasound signals. The effects of the osmotic loading to the cartilage
5 specimens were monitored using the ultrasound biomicroscopy system in 1D and 2D.

6 In this study, the articular cartilage was modeled as a two-layer cylindrical
7 triphasic matrix based on the triphasic theory [29]. All swelling effects were assumed
8 to arise from the electrostatic interaction between negatively charged PGs and ions [30].
9 According to the depth-dependent strains of the cartilage obtained using the ultrasound
10 measurement and the depth-dependent contents of water and proteoglycan, the
11 mechanical and material parameters were predicted.

12

13 **RESULTS**

14 Figure 2 shows typical strain and modulus images of a phantom obtained under 4%
15 indentation level (the background's strain level). Figure 3 shows six strain images and
16 corresponding modulus images obtained from the six different phantoms with varying
17 inclusion/background modulus contrast. The modulus contrast was measured by
18 estimating the average modulus inside the inclusion and that inside the background. As
19 the scan area was typically $12 \times 12 \text{ mm}^2$ and the inclusion size was 8 mm, the average
20 modulus of the inclusion was obtained from a ROI of $3 \times 3 \text{ mm}^2$ at the center of the
21 inclusion and the average modulus of the background was obtained from the average
22 modulus in four ROIs ($2 \times 2 \text{ mm}^2$) at the four corners of the modulus image. The
23 measured modulus contrast values were compared to those obtained from uniaxial

1 compression test. It was found that there was a good agreement between the modulus
2 contrast measured from the modulus images and the actual modulus contrast with a
3 correlation coefficient ratio $r = 0.98$.

4 Figure 4 shows a type M-mode display of the 1D ultrasound monitoring of the
5 cartilage shrinkage induced by the change of the saline from 0.15 M to 2 M. It is obvious
6 that the cartilage surface moved towards the bone. Since the sound speed in cartilage
7 increased as salt gradually moved into the cartilage, the echo from the cartilage-bone
8 interface moved towards the transducer direction. We compensated the change of the
9 sound speed in the strain calculation assuming that the change of the sound speed was
10 uniform throughout the depth direction. The equilibrium swelling strains induced along
11 the depth direction were extracted from the RF ultrasound signals. We observed that the
12 equilibrium swelling strains were not uniform in the depth direction. The largest strain
13 was observed in the middle zone. It is found that the region near the bone has a relatively
14 higher modulus (24.5 ± 11.1 MPa) than the middle zone and the surface layer (7.0 ± 7.4
15 MPa and 3.0 ± 3.2 MPa, respectively).

16 Figure 5 shows the result of a type 2D scanning during the shrinkage process of the
17 cartilage induced by the change of saline from 0.15 M to 2 M. To analyze the distribution
18 of the movement of the interstitial tissue at different depth, a region of interest is outlined
19 by the dashed rectangle in the B-mode image shown in Figure 6a. The tissue
20 displacement images during the different periods are formed using the automatic
21 segmentation and 2D tracking method [25]. Figures 6b-d show the changes in the
22 distribution of the displacement of the tissue at different moments. They indicate that the
23 movement of the tissue is large during the beginning phase of the swelling and shrinkage

1 processes. As time going, the movements of tissues inside cartilage tend to be zero and
2 approach equilibrium.

3

4 **DISCUSSION AND CONCLUSION**

5 It is still difficult to directly image the strain and modulus distribution of soft tissues
6 at a microscopic level noninvasively. In this paper, we reported two new methods based
7 on ultrasound elastomicroscopy, which can provide strain images at both ultrasound
8 propagation direction and its orthogonal direction in a microscopy level. For the 20 MHz
9 ultrasound used in the water jet indentation, two cycles of damping period, and an
10 ultrasound speed of 1480 m/s [32], the theoretical axial and lateral resolutions were
11 approximately 58 μm and 0.44 mm, respectively [4]. For the 50 MHz ultrasound used in
12 the osmotic loading, the theoretical axial and lateral resolutions were approximately 35
13 μm and 90 μm , respectively.

14 The ultrasound water jet indentation system has shown its ability to image the strain
15 and modulus distribution by conducting C-scanning sequences with different pressures.
16 As demonstrated in this study, the modulus images obtained using the water indentation
17 system could be used to effectively identify the stiff inclusions in the phantoms. The
18 modulus contrast measured from the modulus image agreed very well with those obtained
19 using uniaxial compression tests. The contrast resolution of the measurement using this
20 ultrasound water indentation system is related to its ability to differentiate samples having
21 different values of modulus E . If the modulus contrast was defined as the smallest
22 difference of the modulus E between the two samples that was statistically significant, the
23 contrast could be obtained from a statistical analysis using a 95% as 4σ confidence

1 interval by assuming a normal distribution of the measured modulus E values. For a
2 σ value of 1.68 kPa computed from 35 independent measurement, the contrast resolution
3 was expected to be 6.7 kPa at a 0.05 level of significance. This contrast resolution may
4 satisfy the modulus imaging of most soft tissues [33]. The spatial resolution of the
5 measurements depended on the cross-sectional size and shape of the indenter, as well as
6 the connectivity between the stiff and soft region. In this study, a simple geometry of the
7 phantoms was designed by containing a uniform cylindrical stiff inclusion and a sharp
8 transition of the modulus was assumed at the inclusion/background interface. The
9 distance over which the transition occurred from 10% to 90% of the modulus values
10 between a stiff and a soft region was estimated as the spatial resolution of the system. For
11 the new ultrasound water indentation system with a water jet indenter of 1.94 mm in
12 diameter, the spatial resolution estimated from the modulus profiles was approximately
13 0.4 mm.

14 In this study, we have also demonstrated the potentials of using high frequency
15 ultrasound to monitor the swelling or shrinkage of articular cartilage induced by the
16 change of saline concentration in 1D and 2D. We have successfully used dynamic 2D
17 images of cartilage to extract the image of displacement distribution of the tissues. Since
18 no external compression is required to deform the cartilage, this method should have
19 potential for the cartilage assessment, particularly for small specimens. An improved
20 triphasic model was used to describe the 1D depth-dependent equilibrium swelling strain
21 and to extract the aggregated modulus of cartilage. Our results for bovine articular
22 cartilage showed that the swelling-induced strain was a function of the depth. This was
23 similar to the results of the canine and human cadaver cartilage [34, 35].

1 In spite of the potentials of the two new methods demonstrated by the
2 preliminary results of phantoms and cartilage specimens, a number of issues need to
3 be further investigated before these potentials can be fully realized. We only reported
4 the imaging of overall full-thickness elasticity of the phantoms, though it is possible
5 to map the elasticity distribution in the thickness direction using the ultrasound
6 signals scattered or reflected at different depths. Further investigations are necessary
7 to use real tissues or phantoms embedded with scatterers to demonstrate this potential.
8 On the other hand, the deformation of articular cartilage induced by osmosis loading
9 has been mapped along a cross-section, but we have not demonstrated the ability to
10 map the elasticity of the tissue in a C-scan. Furthermore, we have used triphasic
11 model to obtain layered moduli of cartilage using the osmotic loading, but we could
12 not map the modulus. Studies are being continued to fully realize the potentials of the
13 two techniques. Other issues such as the effect of depth-dependent ultrasound speed
14 in articular cartilage, the theoretical analysis of the interaction between the water jet
15 and the tissue, and the improvement of the signal-to-noise ratio of images are also
16 being investigated. Since both methods use water (it can be saline) as the medium to
17 couple ultrasound beam and to provide the disturbances to the tissue, it is potential to
18 combine these two techniques to provide a more comprehensive assessment for
19 tissues such as articular cartilage. During the swelling or shrinkage of tissues induced
20 by osmosis loading, water jet indentation can be used to continuously monitor the
21 stiffness change.

22 In summary, we combined high-frequency ultrasound with water jet
23 indentation and osmotic loading to image the mechanical properties of tissues. The

1 results of phantoms and articular cartilage specimens demonstrated the potentials of
2 the two approaches for mapping the elasticity of tissues in a high resolution. Further
3 studies are required to fully demonstrate their potentials for the tissue assessment.

4

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- 26
27

1 **Figure Captions:**

2 **Fig. 1.** Diagram of the ultrasound water jet indentation system. The water jet was
3 used as an indenter and focused high-frequency ultrasound was employed to monitor the
4 deformation of the soft tissue. The 3D translating device facilitated the system to easily
5 apply C-scan for the soft tissue. By applied different pressure during C-scan sequences,
6 the modulus image was obtained with the recorded pressure, deformation and thickness.

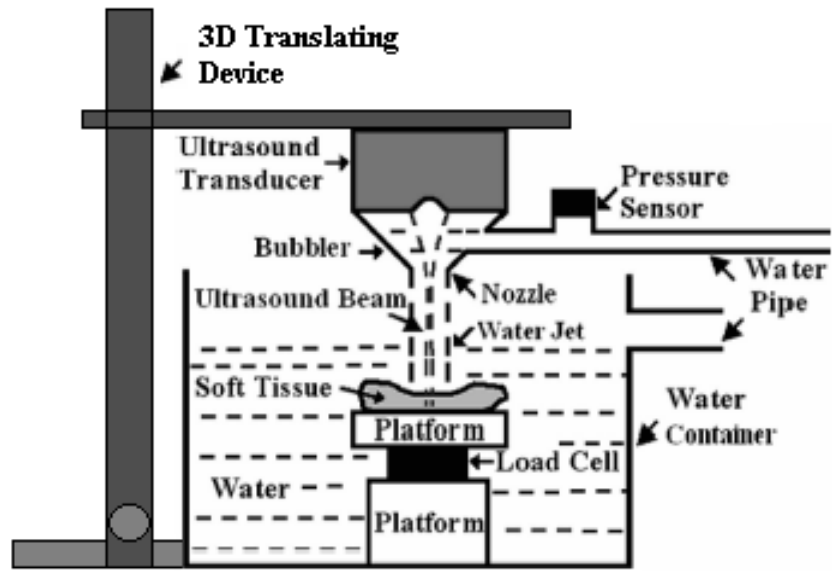
7 **Fig. 2.** Typical (a) strain and (b) modulus images of a phantom with a stiff inclusion
8 (the diameter of the inclusion was 8 mm) obtained under 4% indentation level
9 (background's strain level).

10 **Fig. 3.** Strain and corresponding modulus images obtained from the six different
11 phantoms of varying inclusion/background modulus contrast. The measured modulus
12 contrast was 9.61, 8.96, 7.71, 3.95, 1.32, 0.10 dB for the six phantom (CP1 to CP6),
13 respectively.

14 **Fig. 4.** A typical A-mode and M-mode display of the ultrasound signal collected from
15 the articular cartilage after the concentration was changed from 0.15 to 2 M. The images
16 were sampled at a rate of approximately 0.8 s per frame.

17 **Fig. 5.** (a) A B-mode ultrasound image of the cartilage cross-section. The grey levels
18 of the image linearly represent the amplitude of the RF signals. The images were sampled
19 at a rate of approximately 0.8 s per frame. The image of displacement distribution of the
20 region of interest indicated by the dashed rectangle (divided into 15×40 segments) in (a)
21 are calculated using the 2D a cross-correlation tracking method. The displacement
22 distribution of articular cartilage extracted from the 2D images obtained at (a) 2.5 min
23 and 4.2 min, (b) 5.8 min and 7.5 min, and (d) 10.8 min and 12.5 min. The grey levels of
24 images (b-c) represent the displacement value of the segments at two moments during the
25 shrinkage phase.

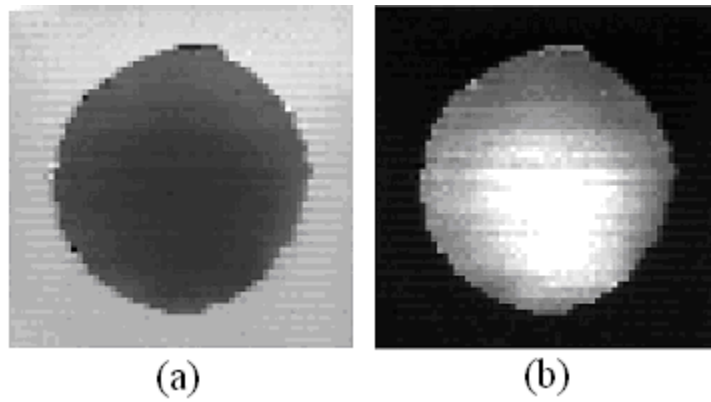
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1

2

Fig. 1.

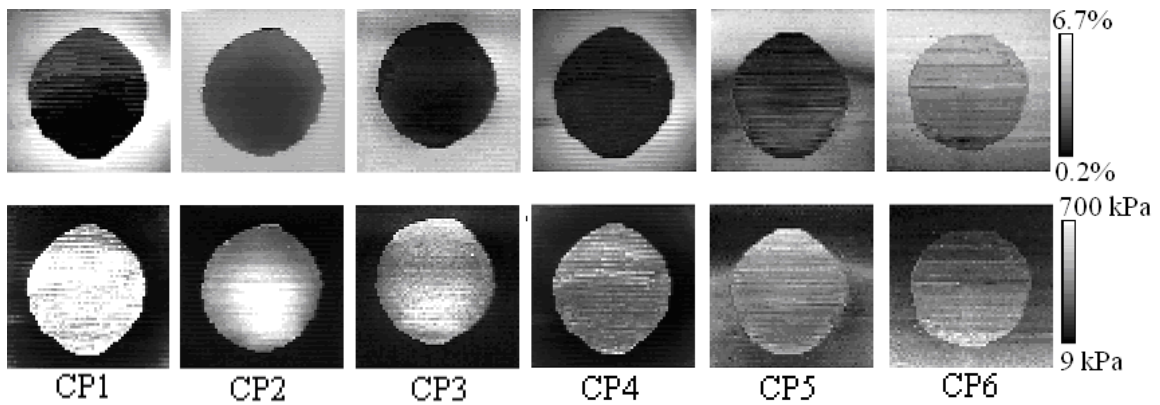


3

4

Fig. 2.

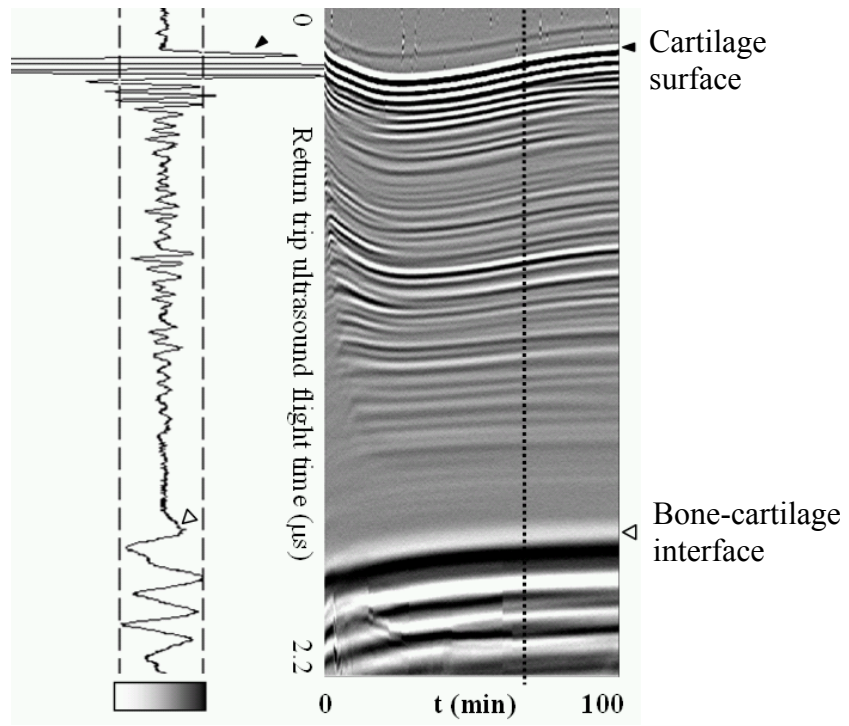
5



6

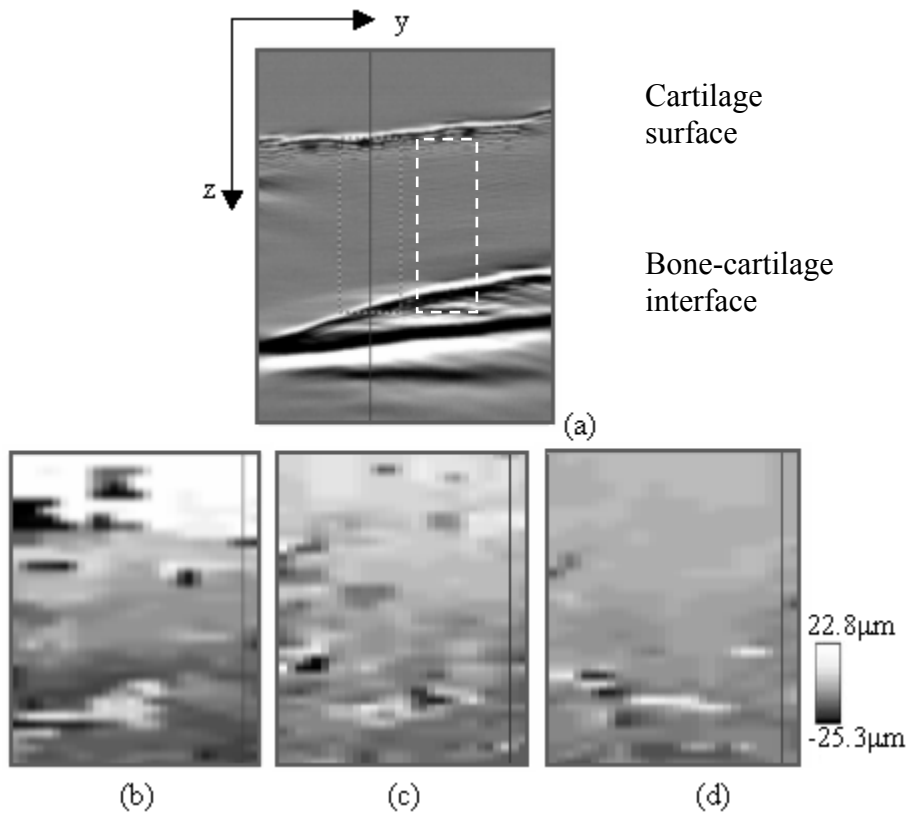
7

Fig. 3.



1
2
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Fig. 4.



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Fig. 5.