Chance and limit of imaging of articular cartilage in vitro in healthy and arthritic joints - DEI (Diffraction Enhanced Imaging) in comparison with MRI, CT and ultrasound

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ABSTRACT:

Description of purpose: Treatment of osteoarthritis in stages of reversible disease requires high resolution visualization of early cartilage damage and of subchondral bone. Here, DEI (Diffraction Enhanced Imaging) is compared to MRI, computed X-ray tomography (CT) and ultrasound (UI) in its ability to detect early degeneration of articular cartilage. In contrast to conventional absorptive X-ray examination where cartilage is poorly visible DEI captures cartilage by detection of selected refraction.
Methods: Human femoral heads were investigated by macroscopic inspection, conventional X-ray examination, DEI, MRI, CT, UI and histology. DEI is an imaging technique applying a monochromatic parallel synchrotron X-ray beam. Image features were verified by histology.
Results: DEI, MRI and ultrasound lead to interpretable images of cartilage. Of all techniques, DEI provided highest image resolution revealing the structural tissue architecture. MRI needs a very long exposure time (more than 5 hours) to achieve comparable quality. Application of ultrasound is limited because of joint geometry and, at high sound frequency, the necessity of close contact between cartilage and transducer. DEI is an experimental technique which needs synchrotron radiation.
Conclusion: DEI is a very promising imaging technique for visualization of cartilage and bone. It may serve as an excellent analytical tool for experimental studies. Our pictures show a part of future of optimised techniques for imaging. Synchrotron based DEI may lead the way towards optimisation of improved techniques for imaging. Upon development of adequate small scale X-ray sources, DEI will also be an important supplementation for medical imaging.

Keywords: DEI (Diffraction Enhanced Imaging), cartilage imaging, X-ray imaging, comparison DEI with MRI, CT and ultrasound

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1. INTRODUCTION

Currently, osteoarthritis is usually only detected at far advanced stages of the disease, with cartilage as the primarily affect joint tissue being widely destroyed. Lack of imaging quality in practically all medical imaging technologies with respect to articular cartilage is the prime reason for this unfortunate situation. The x-ray images in figure 1 highlight this problem with an example from a patient with hip osteoarthritis and femoral head necrosis. Treatment of osteoarthritis in stages of reversible disease would therefore require high resolution visualization of early cartilage damage and of subchondral bone. In contrast to conventional absorptive X-ray examination diffraction enhanced imaging (DEI)[1-3] captures cartilage by detection of refraction [4-6]. Previous work from our group indicated DEI as a particularly well suited method to detect the edges of joint cartilage and also to find structural damage within diseased cartilage [4-6]. It is however necessary to compare DEI with standard imaging techniques under experimental conditions to search for features of cartilage defects presented by each method of imaging at stages of the disease where cure might still be a feasible goal.

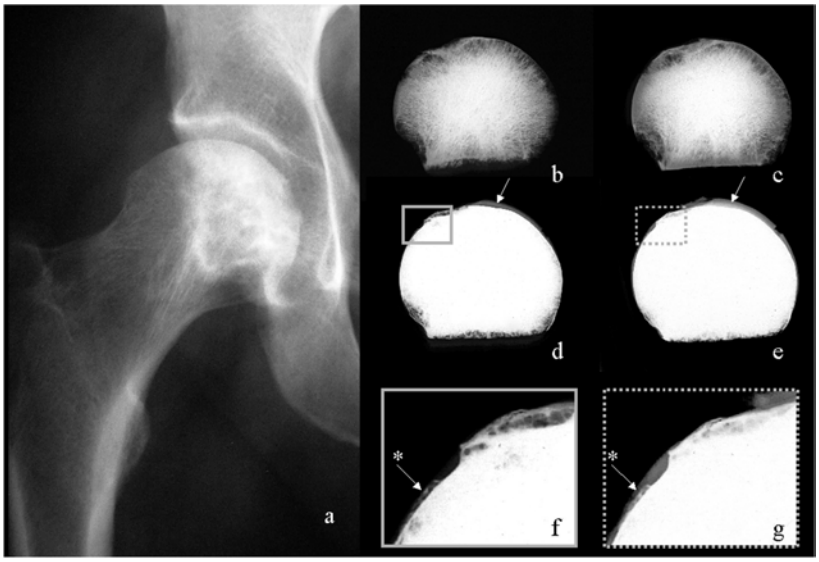


Figure 1: (a) Conventional absorptive X-ray examination, cartilage is invisible. (b-g), experimental conventional absorptive X-ray examination of the same femoral head. (b) 40kV, 18mAs, (c) 38kV, 12.5mAs, (d) 24kV, 31mAs, (e) 24kV, 18mAs, (f) detail from (d), (g) detail from (e). Only conditions with experimental kV and mAs selected allow to visualize cartilage but the surface and the cartilage height is still not clearly defined (see arrows). The asterisk/arrow shows a nascent osteophyte.

2. METHODS

Human femoral heads from patients with hip osteoarthritis or necrosis of the femural head obtained through joint replacement surgery were investigated by macroscopic inspection, conventional X-ray examination and CT, DEI, MRI, ultrasound (UI) and histology. DEI is an imaging technique applying a monochromatic parallel synchrotron X-ray beam. Description of the setup is shown in figure 2. Different images are collected characterized by defined angular refraction within the sample. DEI allows the the separation of scattered from non-scattered X-rays behind the imaged object with help of a so called analyzer crystal installed between sample and detector. The rocking curve describes the amount of intensity which passes the analyzer without sample at a given tuning angle. Therefore 100% (the “top” position) characterizes the intensity maximum as the analyzer is optimally tuned, lower percentages (50%, 15 %, etc.) reflect crystal positions tuned to the slopes of the rocking curve, with “+” and “-“ refering to the first and second slope. In the so-called “top position”, non-refracted radiation which is leaving the tissue exactly parallel to the

entering beam will pass the analyzer and will be detected whereas the refracted part will be rejected, and therefore, leads to a contrast enhancement. This so detected image is called "top image" (fig. 3). From the slope positions, the "+" or "-" images will be recorded. These images are characterized by the specific amount of radiation refracted from the object at a given slope and will be used to calculate the so called refraction image (Figure 3).

Conventional X-ray images were created by using a GE mammograph. *UI* was performed applying a high resolution commercially available device used in dermatology at 20 MHz and conventional 7,5 and 10 MHz devices (Siemens). *MRI* s were performed on a T5NT (Philips) with surface coil, at sequences T1 SPIR TFE TE 22, TR 95, Flip 50 and at T1 FFE MTL TE 22, TR 80, Flip 50 (slice thickness 1.2 mm). *Conventional CT* was performed on a Siemens Somatom Balance with 120kV generating 1 mm slices. Further conditions are specified in the corresponding figure legends. *Histology* was performed on 5 µm sections from decalcified and paraffin embedded select segments from the femoral head after imaging. The sections were stained by the azan-based Masson-Goldner procedure to discriminate bone and soft tissue, alternatively by safranin O/light green to visualize the composition of articular cartilage.

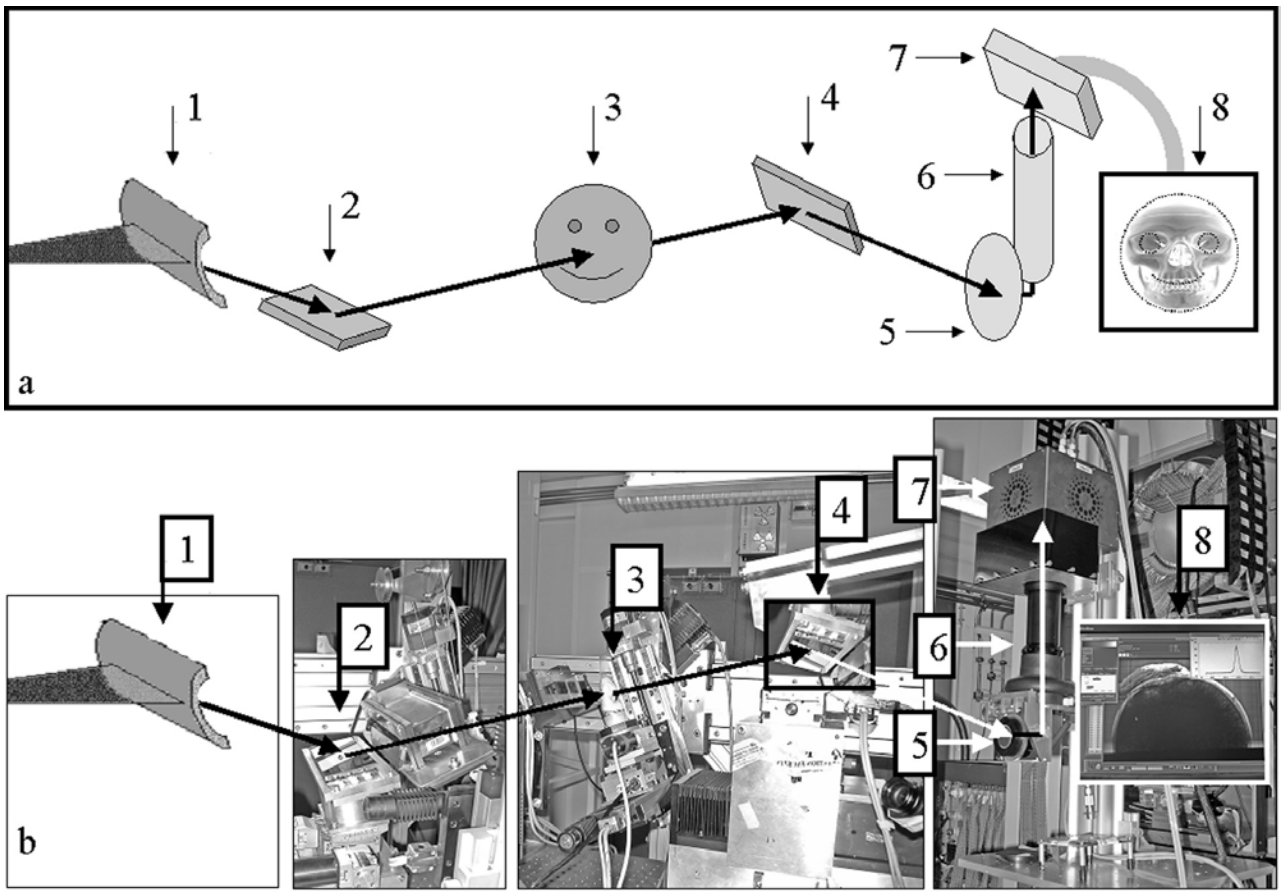


Figure 2: Schematic representation of DEI: (a) Experimental set-up with synchrotron radiation source. The arrows symbolize the direction of the X-ray beam. Defined-angle X-rays will pass the accordingly tuned analyzer (4) to the detector. (b) The experimental set-up (pictures taken at ID-17 beamline, ESRF) with schematic Laue crystal (1), path of monochromatic X-ray-beam through the secondary monochromator (2), the specimen (3), the analyzer crystal (4), scintillator (5) and fiberoptical image transfer (6) to the frelon camera detector (7). Computer screen with display (inset at position 8) of (+15%)-image of femoral head. to detect parallel beam position and to generate a top image. Diffraction images are created by detuning for example to 15% as in fig. 3. Experiments took place at HASYLAB, DESY Hamburg, Germany, and at beamline ID17, ESRF Grenoble, France.

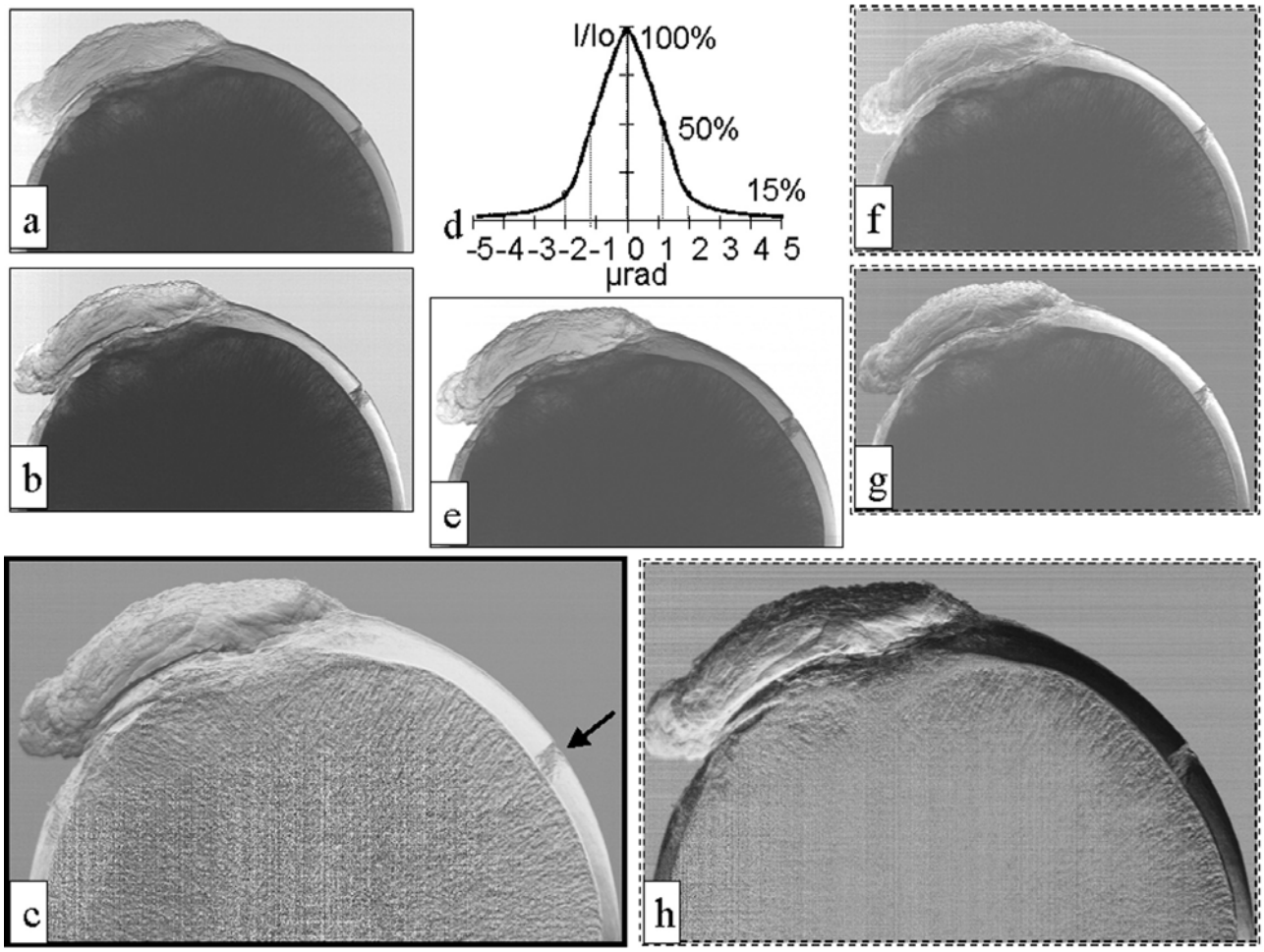


Figure 3: DEI of a femoral head. The figure depicts images obtained at the top, 15% and 50% positions of the rocking curve (d, schematic) and their conversion into refraction images (a) + 50%, (b) –50%, (c) refraction image at 50%, (f) +15%, (g) –15%, (h) refraction image at 15% , (e) top-image. The images are optimized to detect cartilage and ligament. The arrow points to an artificial defect also analyzed in fig. 5**.**

3. RESULTS AND CONCLUSIONS

DEI , MRI, CT, X-ray imaging and ultrasound lead to interpretable images of cartilage when used under idealized experimental conditions. Of all techniques, DEI provided highest image resolution revealing the structural tissue architecture with the least amount of false signals. MRI needs a very long exposure time to achieve comparable quality. Application of ultrasound is limited because of joint geometry and, at high sound frequency, the necessity of close contact between cartilage and transducer. DEI is an experimental technique which needs synchrotron radiation.

The clear presentation of the cartilage surface is imperative for unequivocal diagnosis of the state of disease of the joint. Obviously, this interface is only clearly defined in DEI (figs. 4-10). Cartilage changes its absorptive properties (due to water and fat content) gradually towards the joint space, with density coming close to that of the joint gap fluid, resulting in ambiguous visualization of cartilage borders in conventional X-ray, CT and MR imaging. CT and conventional X-ray imaging produce no clear borders of cartilage, when

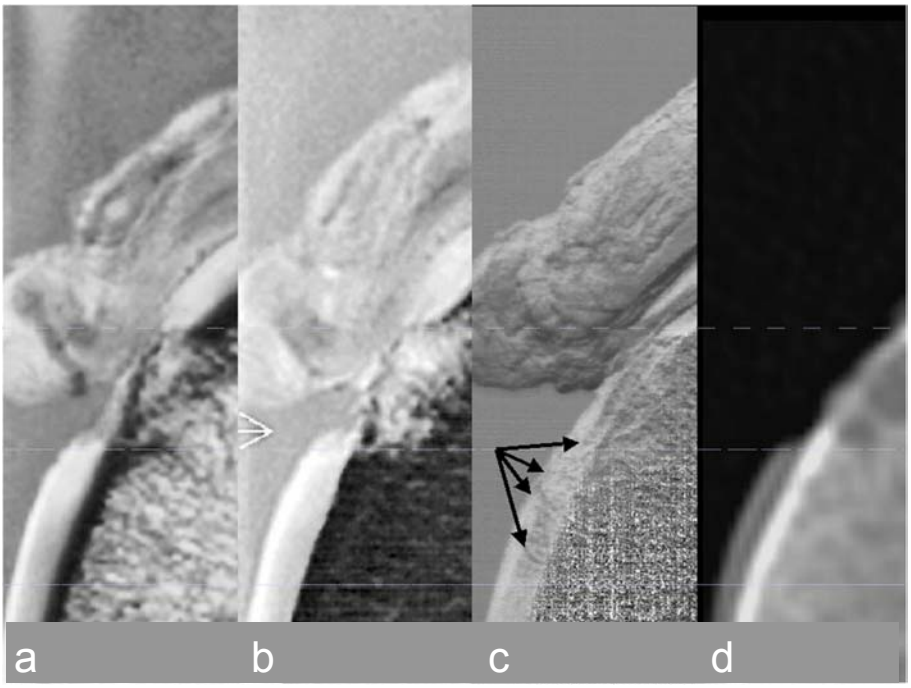


Figure 4: Comparison of MRI, DEI and CT from an arthritic lesion of the femoral head also shown in fig. 5b: (a, b) experimental MRI with >5 hours of examination, (c) refraction image by 50%, (d) conventional CT (130kV 60mA, window 1500 level 400). The horizontal dotted lines are for better orientation. Note the black arrows in the DEI image (c) showing cartilage damage. Despite (c) is a projection image structural damage of cartilage is displayed that is invisible in the corresponding MRI and CT slices. Images have been optimized for presentation of soft tissue.

using the typical physical parameters of orthopaedic radiology, since the density of cartilage is too low. In MRI, either by generating fat or water -based images, the layered structure of cartilage forces a compromise that either obscures the border to the bone or the border to the joint space. The MRI-imaging of cartilage will only generate good results after excessively long exposure times and optimal arrangement of the presented image layer (fig. 7). If the layer is, as in our example (fig. 10), not in the median cross section the cartilage border will be at an oblique angle producing very blunt signals (partial volume effect).

DEI, in contrast, is specifically based on depicting material edges rather than absorption of matter. Therefore, DEI generates images that most closely resemble the appearance of anatomical and histological tissue structures[8]. Even though the physical basis for the generation of the images differs drastically from the otherwise commonly used techniques, image interpretation is therefore facilitated for physicians.

The images presented here suggest that diffraction analysis of articular cartilage depicts prominently gross structural failures such as clefts, gaps, surface roughness, and so on. However, the clear discrimination from the superficial zone of articular cartilage and the cartilage below the tidemark (fig. 9) points to additional properties of DEI. The orientation of collagen fibers in the superficial zone is horizontal to the tissue surface, whereas below, collagen orientation is vertical down to the bone[6]. Thus, the refractive signal might come from collagen fiber diffraction rather than from macroscopic edges or density fluctuations[7]. Similarly, the tidemark just above the bone is formed by a transition to a mineralized cartilage with different molecular content with respect to collagens and proteoglycans. Also, aside from tissue density alterations, fine structures such as macromolecular organization and highly diffractive microcrystal deposits may contribute

to the image. Further studies in the imaging properties of such suprastructures have to be carried in more detail in order to extend our knowledge concerning diffraction enhanced image interpretation of complex organic composites.

Color coding is a widespread mean of image enhancement and may provide significant advantages for a viewer[9]. However, the greater dynamic range gained at the same time sacrifices spatial resolution[10]. In other words, only a method with extreme resolution like DEI will permit color coding of tissue images without significant loss of structural information. This is particularly true in bone, where DEI enhances the appearance of the trabecular network[11].

DEI is a novel imaging technique for visualization of cartilage and bone. Upon development of adequate X-ray sources, DEI will be an important supplement for medical imaging. The results of our study promise significant improvements in image analysis utilizing such optimised techniques. Precondition for using DEI in medical practice is its disposal independent of synchrotron radiation. Steps towards that goal are optimization of imaging detectors and development of mobile high energy radiation sources.

REFERENCES

1. L.D. Chapman, W. Thomlinson, R.E. Johnston, D. Washburn, E. Pisano and N. Gmür, "Diffraction enhanced x-ray imaging", *Phys. Med. Biol.* **42,** pp. 2015-2025, 1997.
2. M. Z. Kiss, Sayers, D. E., Zhong, Z. Parham, C. and E. D. Pisano, "Improved image contrast using diffraction enhanced imaging", *Phys. Med. Biol*., **49,** pp. 3427-3439, 2004.
3. T. Dill, W. R. Dix, C. W. Hamm, M. Jung, W. Kupper, M. Lohman, B. Reime, and R. Ventura, "Intravenous coronary angiography with synchrotron radiation", *Eur. J. Phys.* **19,** pp. 499-511, 1998.
4. J. Mollenhauer, M. Aurich, Z. Zhong, C. Muehleman, A.A. Cole, M. Hasnah, O. Oltulu, K.E. Kuettner, A. Margulis, and L.D. Chapman, "Diffraction enhanced x-ray imaging of articular cartilage", *Osteoarthritis Cartilage*. **10**, pp. 163-171, 2002.
5. C. Muehleman, S. Majumdar, A. Sema-Issever, F. Arfelli, R.H. Menk, L. Rigon, G. Heitner, B. Reime, J. Metge, A. Wagner, K.E. Kuettner and J. Mollenhauer, "X-ray detection of structural orientation in human articular cartilage", *Osteoarthritis Cartilage* **12**, 97-105, 2004.
6. C. Muehleman, L.D. Chapman, K.E. Kuettner, J. Rieff, J.A. Mollenhauer, K. Masuda, and Z. Zhong, "Radiography of rabbit articular cartilage with diffraction-enhanced imaging", *Anat. Rec.* **272A**, pp. 392-397, 2003.
7. J. Mollenhauer, M. Aurich, C. Muehleman, G. Khelashvilli and T.C. Irving, "X-ray diffraction of the molecular substructure of human articular cartilage", *Connect. Tissue. Res.* **44,** pp. 201-207, 2003.
8. S. Majumdar, A. Sema-Issever, A. Burghardt, J. Lotz, F. Arfelli, L. Rigo, G. Heitner and R. H. Menk, "Diffraction enhanced imaging of articular cartilage and comparison with micro computed tomographyof the underlying bone stucture", *Eur. Radiol*., **14**, pp. 1440-1448, 2004.
9. E.A. Krupinski, "Practical Applications of Perceptual Research", in *Handbook of medical imaging*, Jacob Beutel, H. L. Kundel and R. L. van Metter, eds. Volume 1, 895-929, SPIE - The International Society for Optical Engineering, Bellington, 2000.
10. H. Levkowitz, and G. T. Herman, "Color Scales for Image Data", *IEEE. Comp. Graphics. and Applic.* **12**, pp. 72-80, 1992.
11. C. Muehleman, D. R. Sumner and Z. Zhong, "Refraction effects of diffraction-enhanced radiographic imaging a new look at bone", *J. Am. Podiatr. Med. Assoc*., **94**, pp. 433-435, 2004.

12. 6. COLOR PLATE

Fig. 5: Cartilage imaging with DEI, UI and CT: (a) UI with 13.5mHz, (b) photograph, boxes indicate the sectors analyzed, (c) UI with 13.5mHz, (d) DEI 50% refraction image, (e) UI with 20mHz (dermatological), (f) and (g) layers from CT 130kV, 60mA (window 1500 level 400), (h) UI with 20mHz. Boxed 1 indicates surface of bone and boxed 2 indicate the surface of cartilage. Asterisks point to the artefact made by a knife. Note in (h) an UI-signal (boxed 3) not related to a real structure. It is an effect of varying speed of sound in air and solid matter. The real mineralization front border has no dent (compare to the CT-layer in (g)). The effect in (c) is not as strong because of the lower frequency applied.

Fig. 6: Comparison of CT to DEI-CT: (a) conventional CT (130kV, 60mA, window 1500 level 400), (b) corresponding to (a) color coded DEI-CT generated from the images in (c, d, e) (50 keV, 0.25 mm slice), (c) DEI -50% (d) DEI top image, (e) DEI +50%. The pink and yellow arrows point to the very strong demarcation of the surface of the cartilage and bone, respectively in DEI. The border of cartilage and bone in conventional CT is not as clearly defined (white arrows).

Fig. 7: Comparison MRI, UI, CT and DEI-CT: (a-c) Experimental MRI with 5.5 hours of exposure, (d) circumferential reconstruction of segmental UI with 13.5mHz, (e) conventional CT with false color contrast for better optical visualization of cartilage (130kV, 60mA, window 1500, level 400), (f) color coded DEI-CT (as in fig. 6). All images show the approximately same slice of the femoral head segment as illustrated by red line in the inset. The yellow arrows point to damaged cartilage and the pink asterisks indicate the knife artefact.

Fig. 8: Color-coded DEI-CT segment (0.25 mm slice, as in fig. 6) compared to 5 µm thick microscopic histology sections. The picture shows a comparison of normal-looking cartilage from two selected sites. Note that the damaged trabecular meshwork of the bone to the left and the fine trabecular meshwork to the right are equally well imaged in DEI as in microscopy. Also, the height of the cartilage in histology matches the imaged geometry from DEI.

Fig. 9: A comparison of structural details obtained from DEI-CT and from histology of a healthy segment from the femural head. (a) DEI-CT slice, refraction image. The blue boxe indicates the magnified region in (b), the white box the safranin O/ light green - stained histological section in (c). Safranin O (red) stains for regions with high proteoglycan content, light green (blue) contrasts regions with low proteoglycan content. Note that DEI is capable to separate the intensely red stained mineralized cartilage from the unmineralized cartilage. In addition, the superficial layer cartilage of the joint surface, stained in blue, is also discriminated.

Fig. 10: Histological correlation of a structural arthritic defect visualized by DEI: (a) Colour coded DEI, magnified from (b). (c) Microscopic section of same region as in (a), Masson-Goldner stained (red: mineralized tissue, blue: unmineralized cartilage). Compare the histological representation of the cartilage damage zone within the DE image.

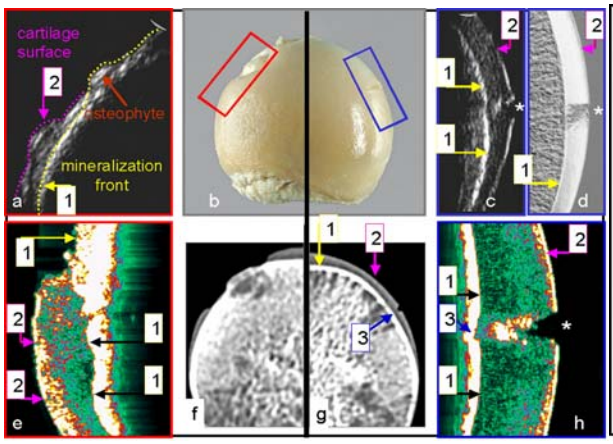
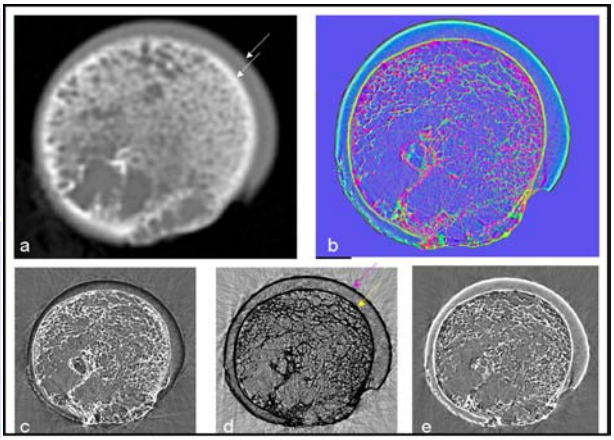


Figure 5



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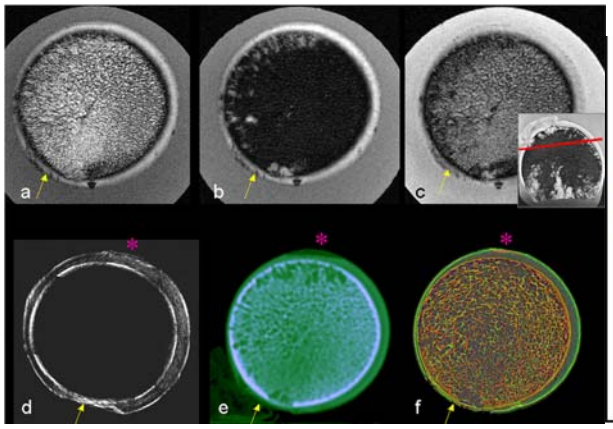

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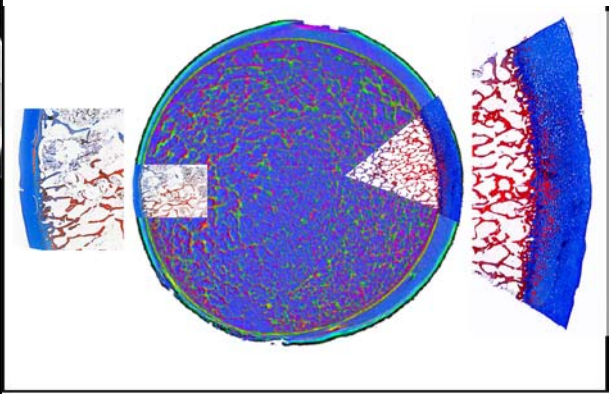

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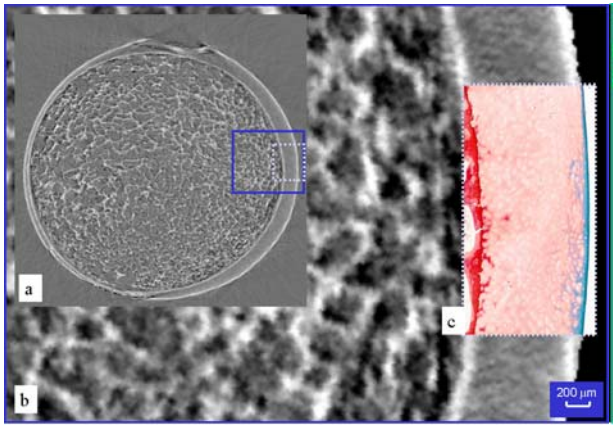

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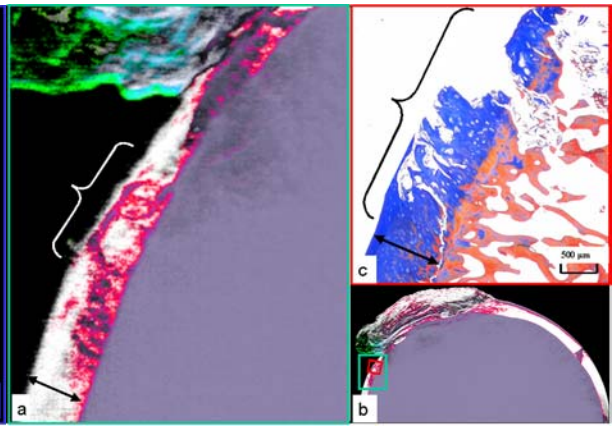

Figure 10