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Articular Cartilage Surface Topography and Roughness in Frozen Tissue Samples Using Optical Coherence Microscopy

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Abstract

This paper describes a method to visualize the topography of the surface of cartilage. Optical Coherence Microscopy (OCM) technique has been used to get two dimensional images of frozen tissue samples of bovine articular cartilage. Optical Coherence Microscopy (OCM) is a combination of optical coherence tomography and confocal microscopy. Coherence gate from OCT and confocal gate from confocal microscopy can achieve higher resolution and deeper penetration depth. The precise three-dimensional topography of the cartilage surfaces has been obtained. The full-image roughness, for frozen samples has been obtained.

Key Words: Articular cartilage, surface topography, optical coherence microscopy.

Introduction

The materials classed as cartilage exist in various forms and perform a range of functions in the body. Depending on its composition, cartilage is classified as articular cartilage (also known as hyaline), fibrocartilage, or elastic cartilage. Elastic cartilage helps to maintain the shape of structures such as the ear and the trachea. In joints, cartilage functions as either a binder or a bearing surface between bones. The annulus fibrosus of the intervertebral disc is an example of a fibrocartilaginous joint with limited movement (an amphiarthrosis) [1].

In the freely moveable synovial joints (diarthroses) articular cartilage is the bearing surface that permits smooth motion between adjoining bony segments. Hip, knee, and elbow are examples of synovial joints. The mechanical behavior and function of the articular cartilage found in freely movable synovial (diarthroidal) joints.

In a typical synovial joint, the ends of opposing bones are covered with a thin layer of articular cartilage. On the medial femoral condyle of the knee, for example, the cartilage averages 0.41 mm in rabbit and 2.21 mm in humans [2]. Normal articular cartilage is white, and its surface is smooth and glistening. Cartilage is a neural, and in normal mature animals, it does not have a blood supply. The entire joint is enclosed in a fibrous tissue capsule, the inner surface of which is lined with the synovial membrane that secretes a fluid known as synovial fluid. A relatively small amount of fluid is present in a normal joint: less than 1 mL, which is less than one fifth of a teaspoon. Synovial fluid is clear to yellowish and is stringy. Overall, synovial fluid resembles egg white, and it is this resemblance that gives these joints their name, synovia, meaning "with egg."
Cartilage clearly performs a mechanical function. It provides a bearing surface with low friction and wear, and because of its compliance, it helps to distribute the loads between opposing bones in a synovial joint. If cartilage were a stiff material like bone, the contact stresses at a joint would be much higher, since the area of contact would be much smaller.

These mechanical functions alone would probably not be sufficient to justify an in-depth study of cartilage biomechanics. However, the apparent link between osteoarthrosis and mechanical factors in a joint adds a strong impetus for studying the mechanical behavior of articular cartilage. [2]

The surface roughness and physical characteristics of the superficial layer of cartilage play important roles in understanding the frictional properties and load bearing mechanisms in articulating joints. Several fluid film and boundary lubrication models for the frictional response of articular cartilage have been proposed. Fluid film lubrication, which includes hydrodynamic, elastohydrodynamic, and microelasto-hydrodynamic modes, requires a minimum fluid film thickness of three times the surface roughness of cartilage to remain viable [1,2].

Other lubrication models, such as weeping and boosted lubrication [3,4], are premised on the existence of peaks and valleys on the articular surface where synovial or cartilage interstitial fluid is trapped. Under loading conditions detrimental to fluid film lubrication, some form of boundary lubrication is also believed to exist [5]. Boundary lubrication theories assume that the boundary lubricant is contained in synovial fluid and is adsorbed onto the cartilage surface, or is synthesized by chondrocytes in the superficial zone [6]. The superficial layer of cartilage has been physically described as a highly viscous, electron dense, non-fibrous, superficial layer

between 0.3 and 1μm thick [6]. In order to evaluate the plausibility of these lubrication theories, the characteristics of uppermost layer(s) of cartilage must be further investigated.

Cartilage roughness, as determined using a variety of scanning microscope techniques, can indicate how well the material will perform under shear or friction. Other imaging techniques that look at the tissue as a whole can be used to evaluate not only the surface characteristics but also any breakdown of the tissue below the surface [7].

Friction in natural joints is described as EHL. This mode of lubrication is possible due to the elastic deformations of cartilage. The modulus of elasticity of cartilage is much smaller than the modulus of elasticity of materials such as PE, CoCrMo or ceramics. Locally acting pressure decreases the surface roughness of cartilage from about 0.07-0.45 μm when unloaded to about 0.01-0.03 μm under loading. The coefficient of friction of natural joints is about $\mu = 0.005\text{-}0.037$ and is very low compared to the coefficient of friction of CoCrMo on CoCrMo ($\mu = 0.22\text{-}0.27$) or CoCrMo on PE ($\mu = 0.06\text{-}0.08$) [8].

Furthermore, the effects of sample preparation and testing methods must be carefully considered. In this study, measurements of articular surface roughness were reported using Optical Coherence Microscopy (OCM). In vivo optical microscopic imaging techniques have recently emerged as important tools for the study of neurobiological development and pathophysiology. In particular, two-photon microscopy has proved to be a robust and highly flexible method for in vivo imaging in highly scattering tissue. However, two-photon imaging typically requires extrinsic dyes or contrast agents, and imaging depths are limited to a few hundred microns. Here we demonstrate Optical Coherence Microscopy (OCM) for in vivo imaging of neuronal cell bodies and cortical myelination up to depths of ~1.3 mm in the rat neocortex. Imaging does not require the administration of exogenous dyes or contrast agents, and is achieved through intrinsic scattering contrast and image processing alone. Furthermore, quantitative measurements of optical properties (index of refraction and attenuation coefficient) using OCM was demonstrated in vivo, in the cortex, and correlate these properties with laminar cellular architecture determined from the images. Lastly, we show that OCM enables direct visualization of cellular changes during cell depolarization and may therefore provide novel optical markers of cell viability.

Fourier Domain Optical Coherence Microscopy

Optical coherence microscopy is a combination of optical coherence tomography and confocal microscopy. Coherence gate from OCT and confocal gate from confocal microscopy can achieve higher resolution and deeper penetration depth.

Figure 1 shows the 870nm Fourier/spectral domain OCM microscope, built for in vivo imaging. The light source is a 3×1 Broadband SLD light source with a bandwidth of 190 nm. The axial (depth) resolution was 3.5 um in air with all 3 SLDs on and 6 um in air with first 2SLDs on. The power on the sample was about 3 mw and the sensitivity was 98 dB. A spectrometer with a 2048 pixel CCD line scan camera (Atmel Inc) working at 27,000 axial scans per second. Scanning was achieved by using a pair of high-performance galvanometers capable of high-speed linear raster scanning (Cambridge Technologies, 6215H). The scanners enabled image line rates of over 1 kHz using a triangle drive waveform and over 2.5 kHz with a sinusoidal waveform. The galvanometer mirrors had 3 mm aperture. The scan lens after the galvanometers was a near-infrared achromatic lens (Thorlabs) with 50 mm focal length and the tube lens was a near-infrared achromatic lens (Thorlabs) with 150 mm focal length. An air 10× /0.45 NA Plan-apochromat objective lens (Zeiss, Inc) achieved a lateral resolution of 2 microns ((full-width at half-maximum of the intensity profile) corresponding to a depth of focus of about 18 microns. The imaging field of view was approximately 1.2 mm by 1.2 mm. An identical reference arm was built to achieve ideal dispersion compensation.

Optical coherence microscopy (OCM), is used for imaging cartilage and allow the assessment of its quality. Spatial perception is considerably reduced when viewing the cartilage sample volume slice by slice or by multi-planar reconstruction. This makes reading of the data unnecessarily difficult and prolongs the examination time. Moreover, the articular cartilage is a curved structure. Thereby, reading of the thickness changes from a direct volume rendered or a reconstructed surface model is quite difficult.

Our approach to cartilage visualization deals with unfolding of the cartilage and depicting it as a height field. In comparison to direct volume rendering or surface reconstruction methods, the height field representation of the cartilage eliminates the complexity of the 3D shape of the cartilage. This allows the user to concentrate solely on the inspection of the cartilage thickness.

The height field representation of the cartilage offers several visualization modes for representing the thickness information: color

mapping, scaling, glyphs, iso-lines, etc. The entire cartilage is depicted at once, thus, giving an overview of the global thickness.

General surfaces cannot be flattened without some amount of distortion. The distortion can be reduced, or in some cases (e.g., developable surfaces) even eliminated by introducing cuts and seams.

Such operations split the surface and introduce discontinuities, thus, losing spatial relations.

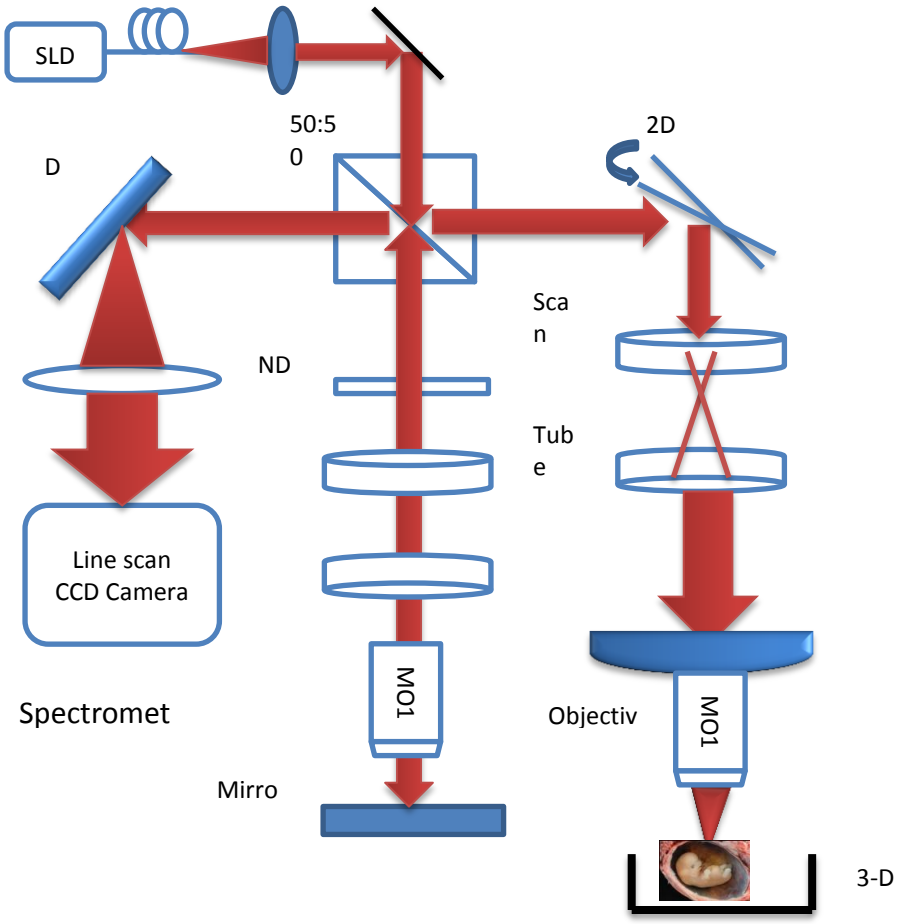

Figure 1: Schematic setup OCM microscope, built for *in vivo* imaging of chick and mouse embryonic heart.

Materials and methods

Sample preparation

Six samples of bovine humeral heads cartilages, for OCM (6×6×6 mm) and macroscopic measurements. Approximately 6 mm of the tissue for CFM and 0.5mm of the tissue for macroscale friction measurements were removed from the deep zone to remove remnants of subchondral bone and vascularized tissue and produce a surface parallel to the articular side, leaving the articular surface intact.

Two dimensional slices images

425 slices were gotten from OCM for each sample. Figure (2) show samples of these two dimensional images.

Volume images

The slices for each sample were emerged to get a three dimensional volume image, Figure (3) show samples of these three dimensional images.

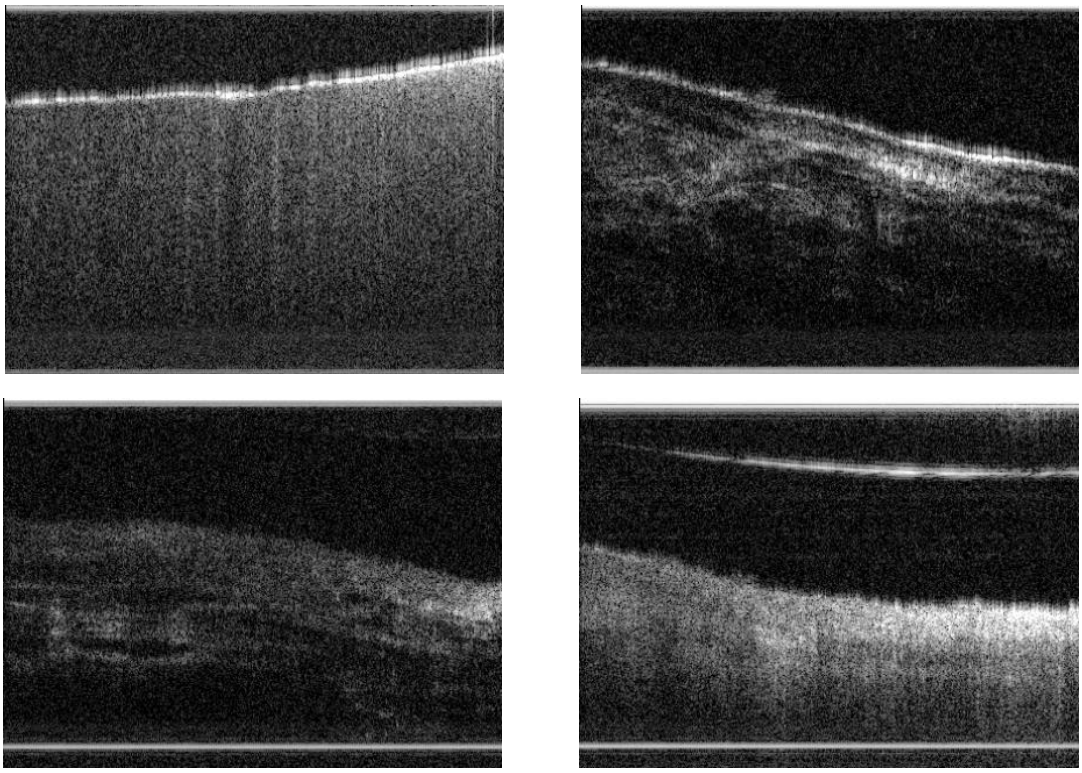

Figure 2: Two dimensional OCM surface images of articular cartilage

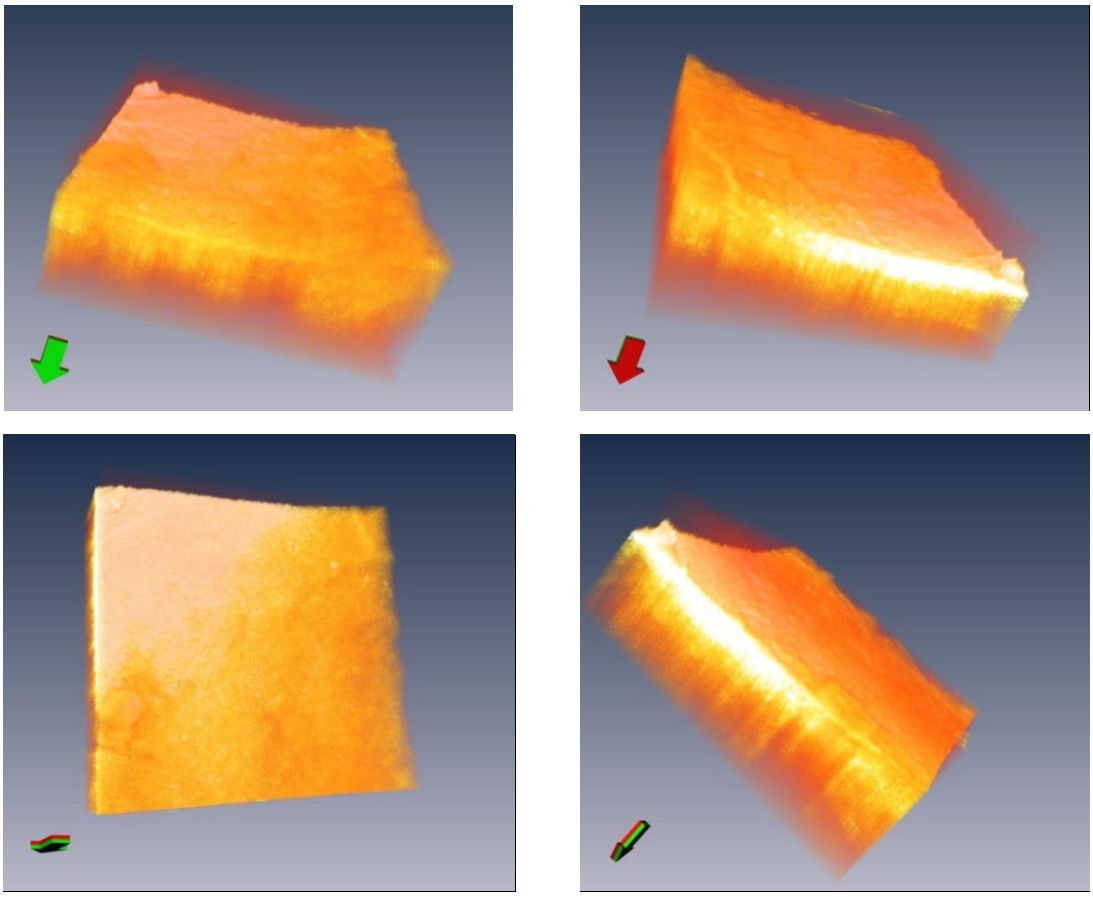

Figure 3: Three dimensional volume images

Surface structure analysis

Average surface roughness, $R_a = \frac{1}{n}\sum_{i=1}^{n}|z_i|$, the height deviation from the mean plane and n is the number of pixels in the region of interest [10], was measured for all height images using Digital Instruments Nanoscope III software. No pre-processing of the original image was performed. R_a was determined for the entire 425×6 mm image. Each image was also characterized according to its surface structure as described below. Surface roughness measurements were compared using AMIRA.

AMIRA is a powerful, multifaceted 3D software platform for visualizing, manipulating, and understanding data from computed tomography, microscopy, MRI, and many other imaging modalities.

With incredible speed and flexibility, AMIRA enables advanced 3D imaging workflows for specialists in research areas ranging from molecular and cellular biology to neuroscience and bioengineering.

Results and discussion

In this study, the roughness index of the cartilage surface was quantitatively described using the OCM surface imaging. The results of cartilage surface images measured by AMERA are shown in Figure (4). The cartilage thickness decreased in some cartilage samples. Previous studies reported consistent results that the proteoglycan content of articular cartilage changed first in the early osteoarthritis, decreasing gradually from the surface to the deeper layers with the degeneration [9, 10]. It has been well known that the earliest signs of osteoarthritis include the loss of proteoglycans in the superficial layer and the disruption of the superficial collagen network, leading to fibrillation in the surface and softening in the superficial tissue. The full-image roughness, Ra-425, for samples 1-6 was 2.923474, 4.629039, 3.504221, 2.875851, 3.775275, 4.771975 respectively. In engineering, the roughness index is mainly used to describe the small valley and pitch conditions on the material surface, as known as micro-roughness. Recently, it has been used to describe the roughness of the cartilage surface in the evaluation of cartilage degeneration. The results of the surface roughness calculation show that the OCM surface imaging of the cartilage surface is a good method compared with atomic force microscopy method [1]

Two limitations of this study require further investigations. First, the OCM surface imaging was only evaluated subjectively by two pathologists in this study. The quantitative analysis of changes in compositions and structural parameters such as PG content and cartilage thickness and the sequent study in their relationships to other parameters are needed. Secondly, the numbers of the samples with different osteoarthritis grades were uneven. Thus further studies with large number of samples are needed.

Figure 4: Two dimensional surface roughness (6×6 mm)

Conclusions

The past few years have seen a dramatic increase in the number of publications on cartilage and chondrocyte research. It was presented a method to visualize the thickness of curved thin objects. The approach has been illustrated on the visualization of articular cartilage. This is a structure where the detection of slight thickness changes is vital for diagnosis. It has been shown that unfolding of anatomic organs is promising since it enables the application of 2D visualization methods. Application of these methods is not possible on the curved reconstructed surfaces.

The above described work has been implemented as a part of a framework for cartilage visualization. It includes several linked views, which allow inspection of the articular cartilage with back coupling to the reconstructed surface as well as to the original slices.

Using the OCM surface images method with AMERA software was a good, simple, new, and reliable tool for measuring the surface topography of cartilage surfaces.

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طبوغرافيا وخشونة سطح غضروف مفصلي لعينات أنسجة مجمدة باستخدام طريقة التماسك البصري المجهري

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الخلاصة:

يوضح هذا البحث استخدام طريقة لتصوير تضاريس سطح الغضروف. حيث استخدمت تقنية التماسك البصري المجهري (OCM) للحصول على صور ثنائية الأبعاد من عينات لأنسجة مجمدة من غضروف بقري مفصلي.ان التماسك البصري المجهري (OCM) هو مزيج من التصوير المقطعي للتماسك البصري والفحص المجهري متحد البؤر. بوابة التماسك من طريقة التماسك البصري المجهري وبوابة مبائر من الفحص المجهري متحد البؤر يمكن تحقيق دقة أعلى وعمق أكبر للاختراق. تم الحصول على تضاريس دقيقة ثلاثية الأبعاد لسطح الغضروف. تم الحصول على صورة كاملة لخشونة سطح العينات المجمدة