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UTILIZATION OF TWO-DIMENSIONAL FAST FOURIER TRANSFORM AND POWER SPECTRAL ANALYSIS FOR ASSESSMENT OF EARLY DEGENERATION OF ARTICULAR CARTILAGE

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ABSTRACT

Degeneration of articular cartilage begins from deterioration of the collagen fibres in the superficial zone. Standard histology using 2D imaging technique is often used to determine the microstructure of collagen fibres and the physiological functions of articular cartilage. However, information of the 3D collageneous structure in the cartilage could be lost and misinterpreted in 2D observations. In contrast, confocal microscopy permits studying the 3D internal structure of bulk articular cartilage with minimal physical disturbing. Using fibre optic laser scanning confocal microscopy, a 3D histology has been previously developed to visualize the collagen matrix in the superficial zone by means of identifying the early arthritic changes in articular cartilage. In this study, we characterized the collagen orientation in the superficial zone of normal cartilage, the cartilage with surface disruption and fibrillated cartilage using Fast Fourier transforms and power

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spectral analysis techniques. Thus, we have established an objective method for assessing the early pathology changes in the articular cartilage.

Keywords: Fast Fourier transforms; Power spectral analysis; Collagen orientation; Confocal microscopy.

INTRODUCTION

The structure of the collagen fibres within articular cartilage (AC) reflects the biomechanical and physiological states of the tissue.[2,5,7,11,14,16,19,22,25] Early osteoarthritis is related to erosion of the articular surface[17,19–21,23] as a result of disruption of the collagen matrix and deterioration of the collage fibres in the superficial zone.[11–13] Therefore, standard histology using visualization of the architecture of the collagen fibres is frequently used to study the pathology of AC. However, this method often involves using 2D microscopy to observe the 3D collagen matrix. Therefore, some important information about the 3D collagen structure could be lost and misinterpreted.[30] For stereological study, traditional methods involve physically sectioning the cartilage into hundreds of slices and dehydrating the tissue. These processes cause unknown artifacts since cartilage is sensitive to any physical invasion and dehydration.[3]

Confocal microscopy permits studying the internal structure of bulk biological tissue while the tissue is not compromised of dehydration and physical dissection. It also offers higher resolution and image contrast than any conventional optical microscopy. The advantages of confocal microscopy have allowed to study the 3D structure of purified type I collagen[29] and the cell migration on 3D synthetic type I collagen matrix.[9,10] Using a 3D imaging technique developed,[31] we previously studied the collagen orientation in the superficial zone in relation to the physiological status of the AC. It has been found that the 3D collagen structure in the superficial zone was sensitive to alterations related to age and disruptions of the articular surface. More specifically, the collagen fibres in the superficial zone of normal cartilage had a complex 3D architecture: the interwoven collagen bundles near the articular surface were connected as a collagen network and laid over the collagen fibres which were predominantly oriented in a spatially oblique direction to the AC surface. This collagen structure in normal cartilage is believed to promote the integrity and durability of the cartilage. During aging, the interwoven collagen fibres near the articular surface gradually diminished from the collagen matrix while the obliquely orientated collagen fibres immediately underlying started to change to align perpendicularly, to the AC surface (approximately 50% of the aged cartilage specimens showed that the collagen fibres in the superficial zone had perpendicular orientation). Disruption of articular surface altered the collagen fibres in the superficial zone to be orientated predominantly in a direction perpendicular to the AC surface. Moreover, fibrillated cartilage has a distinctive macro appearance and abnormal collagen orientation.

As our 3D imaging technique developed in the previous study[31] requires visual inspections and human's interpretations, which are often subjective and time consuming. We have introduced an objective method using computer imaging analysis technique to identify the changes of the collagen orientation in AC.

Fourier transform and power spectral analyses are popular methods for characterizing the surface texture of engineering surface. Previously,

Fourier transform has been used to objectively study the collagen orientation in tendon 1, in cataractous lenses,[26,27] in skin[28] and in ligament scars.[6,18] More recently, Xia and Elder[32] quantified collagen orientation by calculating the eclipse of the spectral distribution of the collagen fibres in Fourier domain. However, these previous studies are mainly focused on TEM images.

Based on use of laser scanning confocal microscopy (LSCM), Peng and Kirk[24] applied Fast Fourier Transform (FFT) and power spectral analysis (PSA) to study the texture properties of 3D topographies of the surfaces of wear debris, and demonstrated the usefulness of using the techniques for effectively indicating the texture pattern of some typical 3D surfaces, i.e. isotropy or anisotropy.

In the present study, we attempts to apply 2D Fourier transforms and power spectral analysis on the fibre optic laser scanning confocal microscopy to identify the collagen orientation in the superficial zone of normal cartilage, aged cartilage, the cartilage with the surface disruption and fibrillated cartilage, and to explore an objective method to detect the early arthritic articular cartilage.

MATERIALS AND METHODS
Sample Preparation

Thirty-six cartilage specimens were selected from five cartilage groups with different physiological states:

1. Ten normal cartilage specimens were obtained from five femoral condyles and five femoral heads of approximately two-year-old cows within 24 hours of slaughter.
2. Ten aged cartilage specimens, which showed a generally glossy surfaces and few macroscopic arthritic signs, were obtained from five femoral heads of cadavers aged from 40 to 60 years old.
3. Eleven cartilage specimens without the most superficial layers were obtained by physically peeling off the semitransparent membrane covering the surface of AC of normal bovine cartilage.
4. Ten early cartilage specimens (with clearly cartilaginous appearances) were obtained from regions of ten arthritic femoral heads with slightly matte surfaces.
5. Six fibrillated cartilage specimens were harvested from arthritic human femoral heads removed during joint replacement surgery.

The specimens were fixed in 10% buffed formalin solution (BFS) for 24 hours. They were then immersed in 0.2% Phosphomolybdic acid solution for 24 hours at 4°, and stained by 1 g/L Picrosirius red for 72 hours. After this, the specimens were washed with 9 g/L saline solution and put into specially designed specimen dishes for imaging. During the imaging procedure, the cartilage specimens were kept in a hydrated state.

3D Image Acquisition and Analysis Techniques

Utilizing a fibre optic laser scanning confocal microscope (FOCM, Optiscan Pty Ltd, Australia), a digital image stack containing a series of 2D images of the collagen fibres in the superficial zone (approximately 60 to 80 μm depth from the surface) was acquired. An Olympus PlanApo 60 X / 1.4 oil immersion lens and a reflectance channel utilizing combined laser wavelengths of 488 nm and 514 nm were used. The optical step size was set as 0.541 or 0.689 μm.

Using computer software VoxBlast (VayTek, Inc, USA), the image stack was reconstructed into a 3D image for visual inspections. Utilizing a computer program F900e (Optiscan Pty Ltd, Australia), the

image stack was also reconstructed into a maximum brightness image (MBI) which is an image representing a view of all of the data in the 3D sequence as if all of the 2D optical sections were combined into a single image containing only in-focus information (Manual of FOCM, Optiscan Pty Ltd, Australia). Using a developed computer image analysis program[24] based on Optimas 6.5 (Media Cybernetics, USA), FFT and PSA were applied to a region of interest (ROI) in the MBI which is big enough to present an over all collagen orientation in the superficial zone in order to characterize the predominant collagen orientation, and determine the physiological and biomechanical states of AC. The details of FFT and PSA methods used are presented below.

Fast Fourier Transform (FFT)

Fast Fourier transform methods can be used to study a surface characteristic (such as isotropic and anisotropic) by transforming the spatial information of the surface to be power spectra in Fourier space, then examining the distribution of the power spectra.[4]

A 2D Fourier transform is usually considered to be two successive one–dimensional (1D) transforms 8 and expressed as:

$$H(f_x, f_y) = \int_{-\infty}^{\infty} \exp(-i2\pi y f_y)$$
$$\times \left[\int_{-\infty}^{\infty} h(x, y) \exp[-i2\pi x f_x dx] dy \right]. \quad (1)$$

In the Euclidean coordinate system, the function $h(x, y)$ stands for a continuous surface variable with x, y. In signal processing, $h(x, y)$ is a 2D signal representing the surface height at position (x, y) while f_x and f_y are the frequency coordinated in direction x and y respectively. For computing purposes, $h(x, y)$ in Eq. (1) is assumed to be sampled with sampling intervals K_x in the x direction and K_y in the y direction, and the finite numbers of

sampling points are M and N in the x and y directions respectively. Thus, $h(x, y)$ can be expressed as discrete $h(pK_x, qK_y)$, where $p = 0, 1, \ldots, N - 1$ and $q = 0, 1, \ldots, M - 1$. Analogous to the 1D FFT, the discrete 2D FFT for computing purposes can be expressed as two successive 1D FFTs[8]:

$$H(n/NK_x, m/MK_y)$$
$$= \sum_{q=0}^{M-1} Z(n/NK_x, qK_y) \exp(-i2\pi mq/M) \quad (2)$$

where $Z(n/NK_x, qK_y) = \sum_{p=0}^{N-1} h(pK_x, qK_y) \exp(-i2\pi np/N)$; and $n = 0, 1, \ldots, N$; $m = 0, 1, \ldots, M - 1$.

Therefore, the process of 2D FFT becomes N 1D FFTs for the collagen surface data along the x direction followed by M 1D FFTs along the y direction. More specifically, the FFT plots of the orientation of the collagen fibres in the superficial zone of AC were obtained in a two stage process using Eq. (2). In the first stage, data points were transformed using a 1D FFT, row by row, to obtain an intermediate transform $Z(n/NK_x, qK_y)$. In the second stage, the 1D FFT was processed again to the intermediate transform, column by column, to get the last 2D FFT $H(n/NK_x, m/MK_y)$.

The ''energy'' distribution of a FFT indicates directional characteristics of a surface, i.e. anisotropic or isotropic. If the collagen fibres have an oblique orientation to the AC surface, the main ''energy'' distribution of a 2D FFT is concentrated along the direction perpendicular to the predominant orientation of the collagen fibres in MBI.

Power Spectral Analysis (PSA)

Power spectral analysis also coverts information about a surface in spatial domain into frequency domain, and then enhances and recognizes the distribution of the power spectral density (PSD) or angular spectra or radial spectra in the frequency domain to identify isotropic or anisotropic characteristics of the surface.[8]

The PSD denoted as $P(f_x, f_y)$ can be expressed in the form of a Fourier transform for computing[8,24]:

$$P(f_x, f_y) = \lim_{Lx, Ly \to \infty} \frac{1}{4LxLy} \left| \int_0^{Lx} \int_0^{Ly} h(x, y) \right.$$

$$\left. \times \exp\left(-i2\pi(xf_x + yf_y)dxdy\right) \right|^2 \quad (3)$$

where Lx and Ly are the lengths of a continuous surface in x- and y-directions respectively. Then, the discrete power spectral density for computation purposes is approximated as[24]:

$$P(n/NKx, m/MKy)$$

$$= \frac{1}{MNKxKy} |H(n/NKx, m/MKy)|^2, \quad (4)$$

where $n = 0, 1, \ldots, N - 1; m = 0, 1, \ldots, M - 1$.

Frequently, a PSD $P(fx, fy)$ in a Cartesian coordinate system is further transformed to a corresponding angular spectrum $P_a(\theta)$ in a polar coordinate system with θ ranging from $0°$ to $179°$.[8,24] Thus, the isotropic or anisotropic features of the surface can be identified through studying the angular spectrum $P_a(\theta)$:

$$P_a(\theta) = \int_0^{R(\theta)} P(f_r, \theta)df_r \quad (5)$$

where $f_r = \sqrt{f_x^2 + f_y^2}$, $\theta = arctg \frac{f_y}{f_x}$, $0° \leq \theta \leq 179°$, and $R(\theta) = \frac{1}{2\sqrt{(K_x \cos \theta)^2 + (K_y \sin \theta)^2}}$.

The advantage of the angular spectrum is that it offers information about the distribution of power spectral densities in different directions from $0°$ to $360°$ in a polar coordinate system. Usually, an isotropic surface has a uniform distribution of power spectral densities in all directions while an anisotropic surface directional distribution of power spectral densities. However, since a sampling area is divided into two orthogonal directions, artificial frequency may be introduced in the two orthogonal frequency axes.[8] For example, in position $0°$ and $90°$, the angular spectrum may exhibit peaks for an isotropic surface. This is called Gibbs phenomenon.[8]

RESULTS

Figure 1(a) shows the 3D collagen matrix in the superficial zone of normal articular cartilage. The collagen fibres were predominantly align in a spatially oblique direction to the AC surface despite some randomly interwoven collagen bundles presented near the articular surface [they could be seen more clearly when they were observed separately from the obliquely orientated collagen fibres underlying, as shown in Fig. 1(b)]. Thus, these collagen fibres appeared orientated predominantly in a direction parallel to the AC surface in an MBI which is analogous to a 2D observation, as shown in Fig. 1(c). Therefore, as shown in Fig. 1(d), the 3D plot of the FFT analysis performed in the MBI demonstrated clearly a directional "energy" distribution, which is perpendicular to the predominant orientation of the collagen fibres in Fig. 1(c). The angular spectral densities have a peak at approximately $44°$, as shown in Fig. 1(e). This indicates that the collagen fibres in the superficial zone were orientated predominantly in a direction approximately $44°$ to the x-axis in Fig. 1(c).

In the aged cartilage, the interwoven collagen bundles near the articular surface gradually disappeared, as shown in Fig. 2(a). However, approximately fifty percent of the specimens showed that the collagen matrix in the superficial zone had little or slight disruptions and the collagen fibres were arranged predominantly in a spatially oblique direction to the AC surface, as shown in Fig. 2(a). These collagen fibres appeared orientated predominantly in a direction parallel to the AC surface in the MBI, as shown in Fig. 2(b). Thus, as shown in Fig. 2(c), the 3D plot of FFT performed in the MBI consequently exhibited a directional spectrum distribution, which was perpendicular to the predominant orientation of the collagen fibres in Fig. 2(b). The angular spectrum also had peaks in a range of $108°$ to $134°$, as shown in Fig. 2(d). The indicates the collagen fibres in the
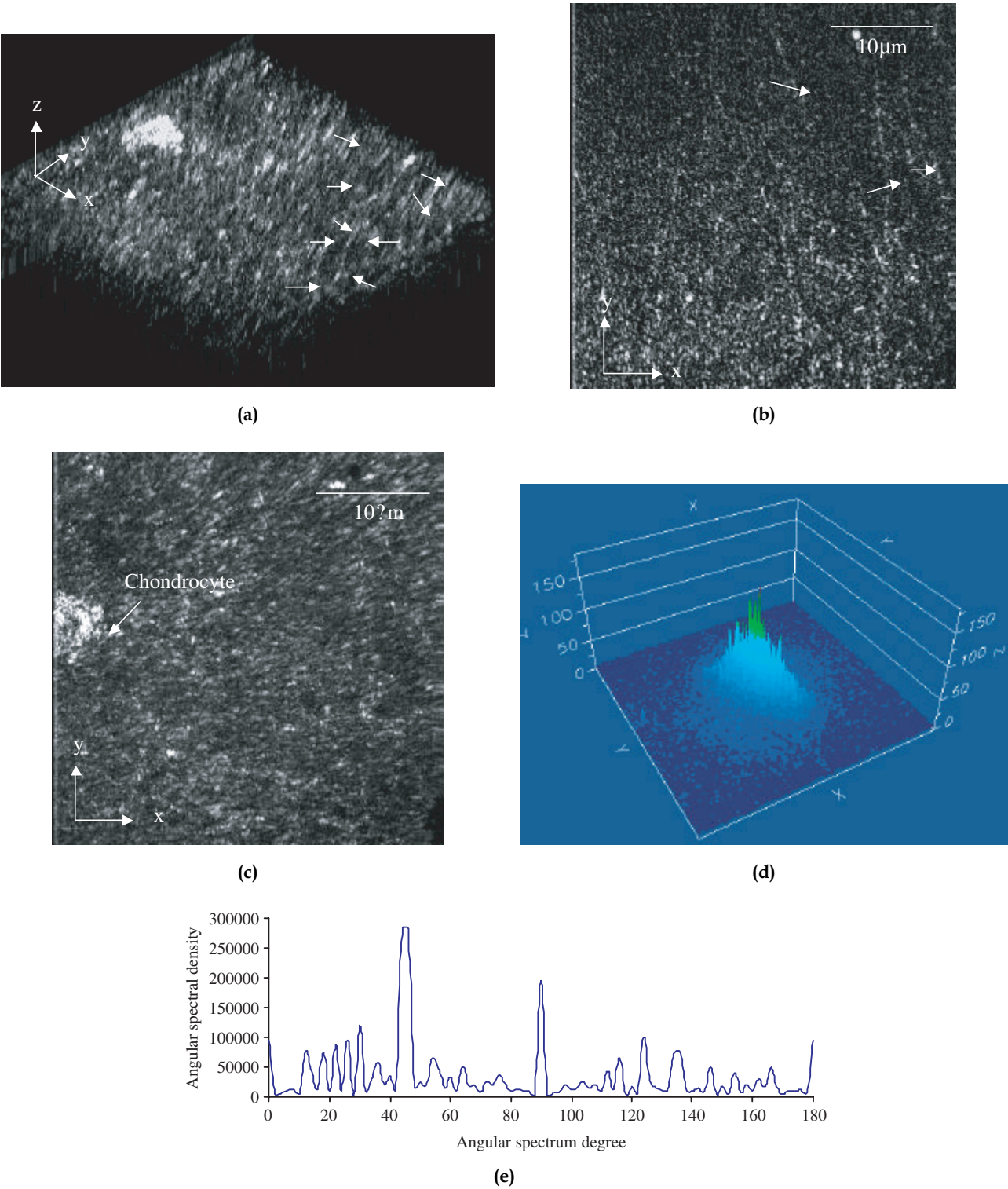
(a)

(b)

(c)

(d)

(e)

Fig. 1 The collagen fibres in the superficial zone of normal articular cartilage had a complex structure. (**a**) 3D image of the collagen fibres in the superficial zone of normal cartilage. (**b**) MBI of the collagen fibres in the most superficial layer corresponding to the semitransparent membrane which can be physically peeled off. (**c**) MBI of the collagen fibres in the superficial zone show the collagen fibres were predominantly orientated in a direction parallel to the articular surface. (**d**) 3D plot of FFT performed on the MBI. (**e**) angular spectrum of the collagen fibres.
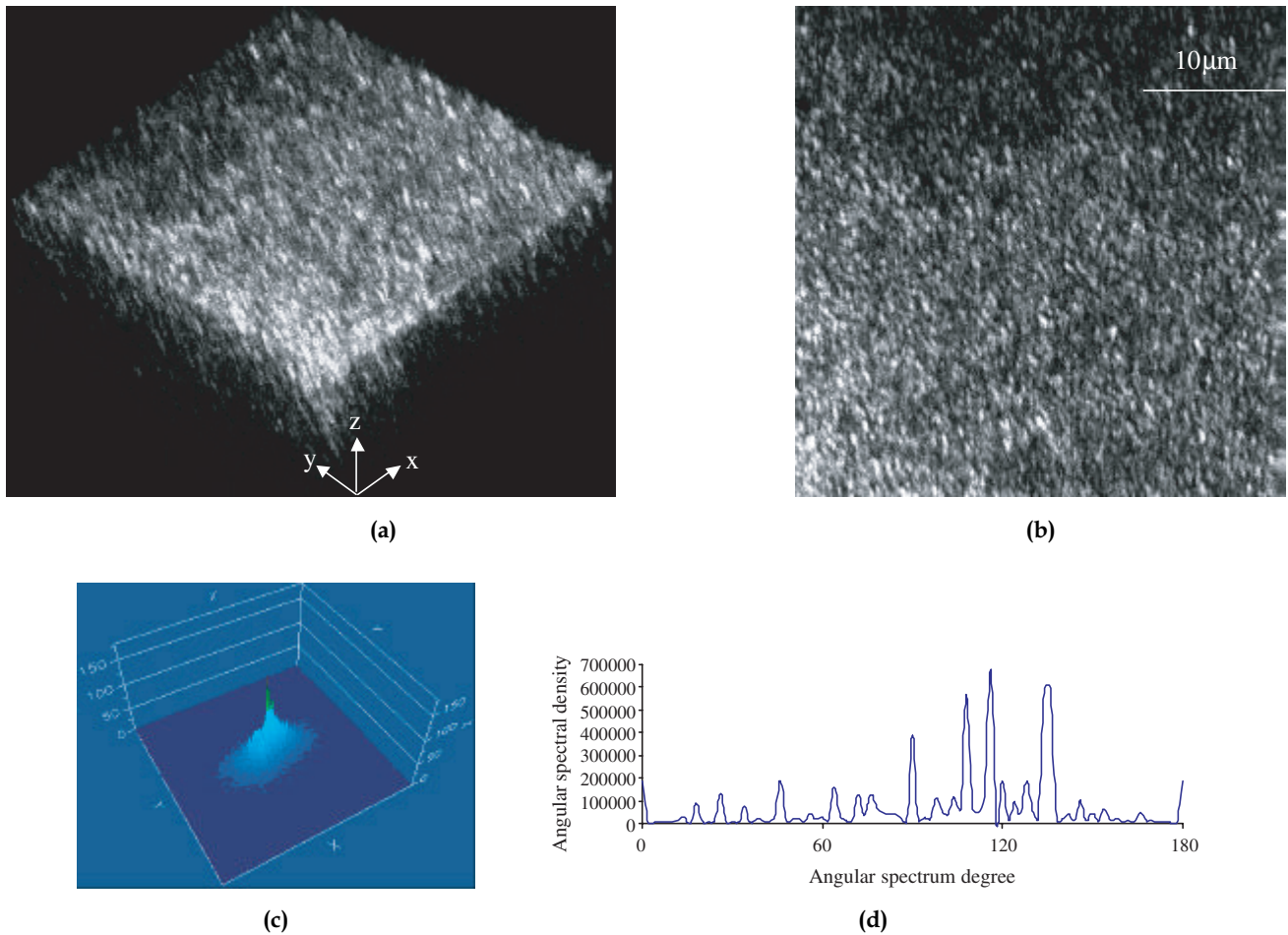
(a)

(b)

(c)

(d)

Fig. 2   Although the interwoven collagen bundles near the articular surface of the aged cartilage (cadaveric cartilage) gradually dismissed, approximately fifty percent of the specimens showed that the collagen fibres in the superficial zone were oriented predominantly in a direction oblique to the articular surface. (**a**) 3D image of the collagen fibres in the superficial zone. (**b**) MBI of the collagen fibres. (**c**) 3D plot of FFT performed on the MBI. (**d**) angular spectrum of the collagen fibres.

superficial zone aligned predominantly in 108° to 134° to the *x*-axis in the MBI, as shown in Fig. 2(c). In another fifty percent of the (aged) specimens, the collagen matrix in the superficial zone was more seriously deteriorated, and the fibres appeared orientated predominantly in a direction perpendicular to the AC surface, as shown in Fig. 3(a). Therefore, the collagen fibres presented an isotropic distribution in the MBI, as shown in Fig. 3(b). FFT performed on the MBI demonstrated an even spectral distribution, as shown in Fig. 3(c). The angular spectral analysis correspondingly indicated an isotropic

distribution of the power spectral densities from 0° to 360°, as shown in Fig. 3(d).

Physically peeling off the semitransparent membrane from the surface of normal AC altered the orientation of collagen fibres in the superficial zone of the cartilage [shown in Fig. 4(a)] to resemble that of the collagen fibres of the aged cartilage shown in Fig. 3 and that of the collagen in the cartilage with a slightly matt surface [shown in Fig. 5(a)]. The collagen fibres in these cartilage groups were featured to be orientated perpendicularly to the AC surface. Therefore, the collagen fibres appeared a uniform
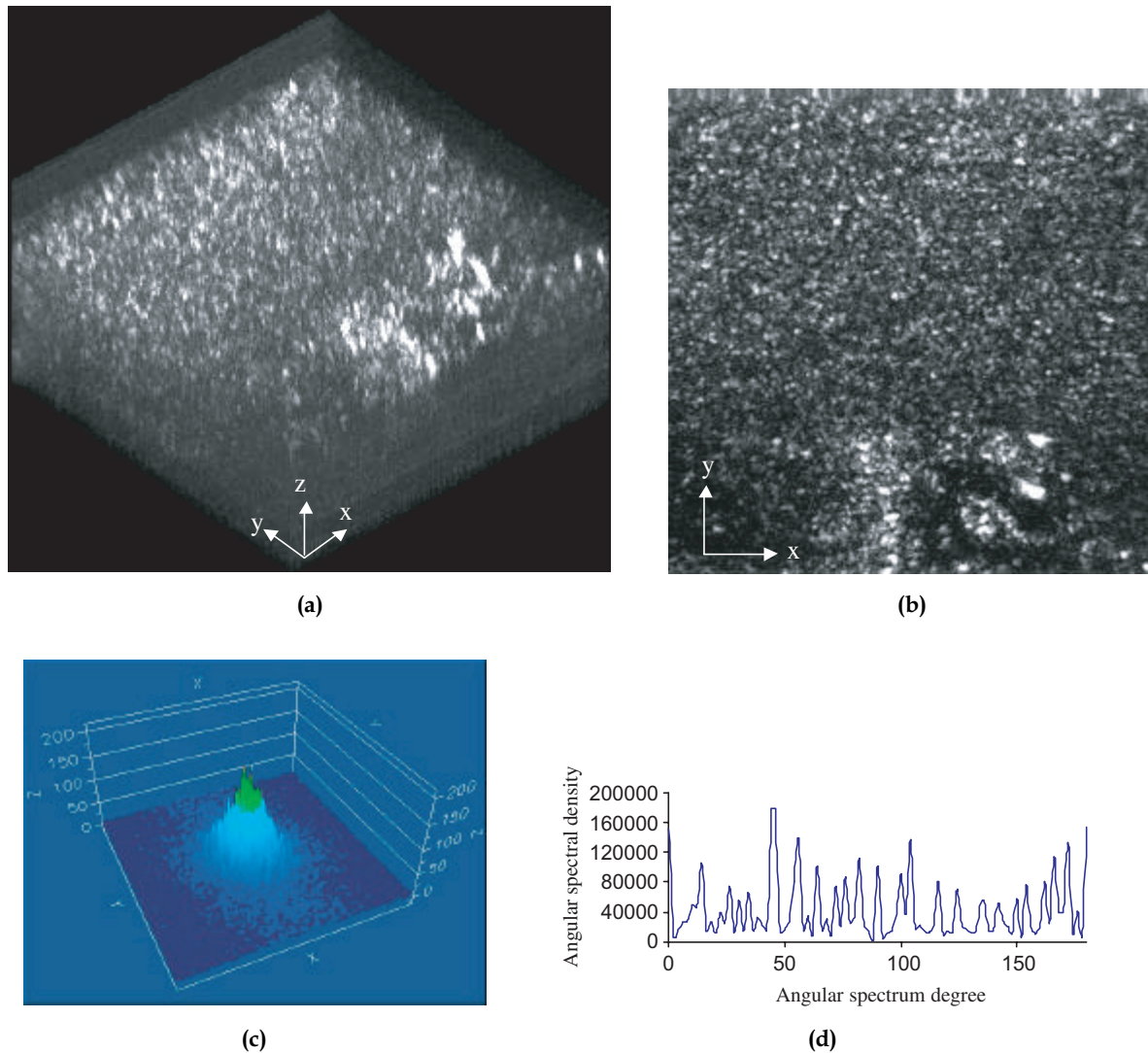
(a)

(b)

(c)

(d)

Fig. 3   In approximately fifty percent of the aged cartilage specimens (cadaveric cartilage), the collagen fibres in the superficial zone were orientated predominantly in a direction perpendicular to the AC surface. (**a**) 3D image of the collagen matrix in the superficial zone. (**b**) MBI. (**c**) 3D plot of FFT performed on the MBI. (**d**) angular spectrum performed in the MBI.

surface texture in the MBIs, as shown in Figs. 4(b) and 5(b). Consequently, the FFT performed in the MBIs exhibited an even spectrum distribution, as shown in Figs. 4(c) and 5(c). The angular spectra also showed even distributions of the power spectral densities from 0° to 360°, as shown in Figs. 4(d) and 5(d) respectively, except a peak at 90° which will be discussed later.

Fibrillated cartilage not only had a distinctive macroscopic appearance but also contained a microscopically abnormal collagen matrix, as shown in Figs. 6(a) and (b). However, the abnormal orientation of the collagen fibres in this type of cartilage could not be clearly indicated by FFT and power (angular) spectral analysis, as shown 6(c) and (d). However, a quick visual inspection is sufficient to distinguish
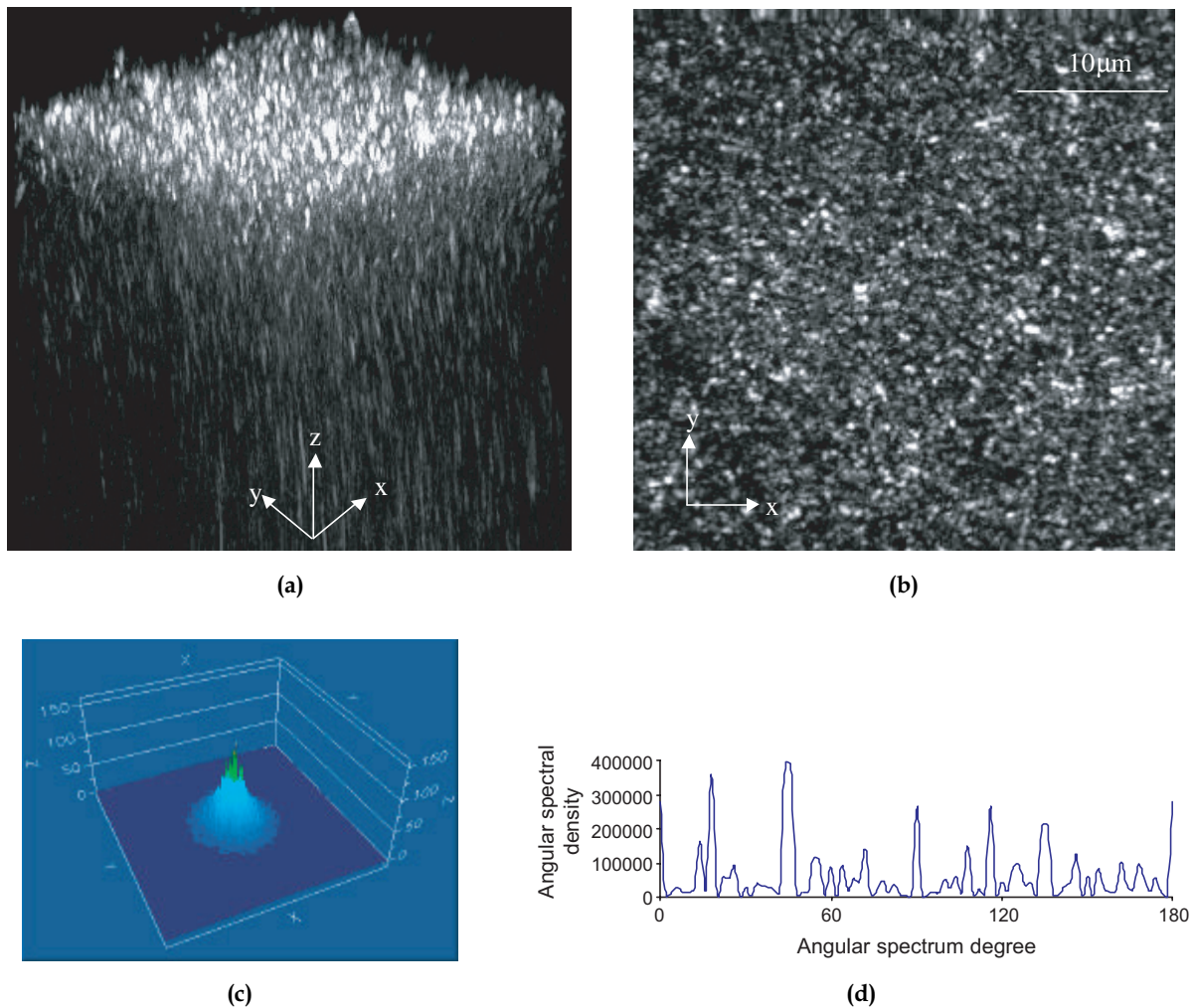
(a)

(b)

(c)

(d)

Fig. 4   The collagen fibres in the superficial zone of the cartilage from which the semitransparent membrane physically was peeled off aligned predominantly in a direction perpendicular to the AC surface. (**a**) 3D image of the collagen matrix in the superficial zone. (**b**) MBI. (**c**) 3D plot of FFT performed on the MBI. (**d**) The angular spectrum performed in the MBI.

this cartilage from the early degenerated cartilage.

DISCUSSION

In present study, FFT and PSA were utilized to characterize the collagen orientation in the superficial zone by means of objective identifying the early pathological changes in AC. This is because there is a distinctively difference in the collagen orientation between normal cartilage and cartilage with surface disruption, and this difference can be effectively distinguished using FFT and PSA techniques. However, before applying computer image analysis, it is essential to obtain images which contain reliable information about the collagen matrix. LSCM has advantages to image the 3D collagen structure in the superficial zone with minimal artifacts related to tissue sectioning and dehydration. Also, reflection is an intrinsic property of a material. The reflectance mode used for the image acquisition in this study
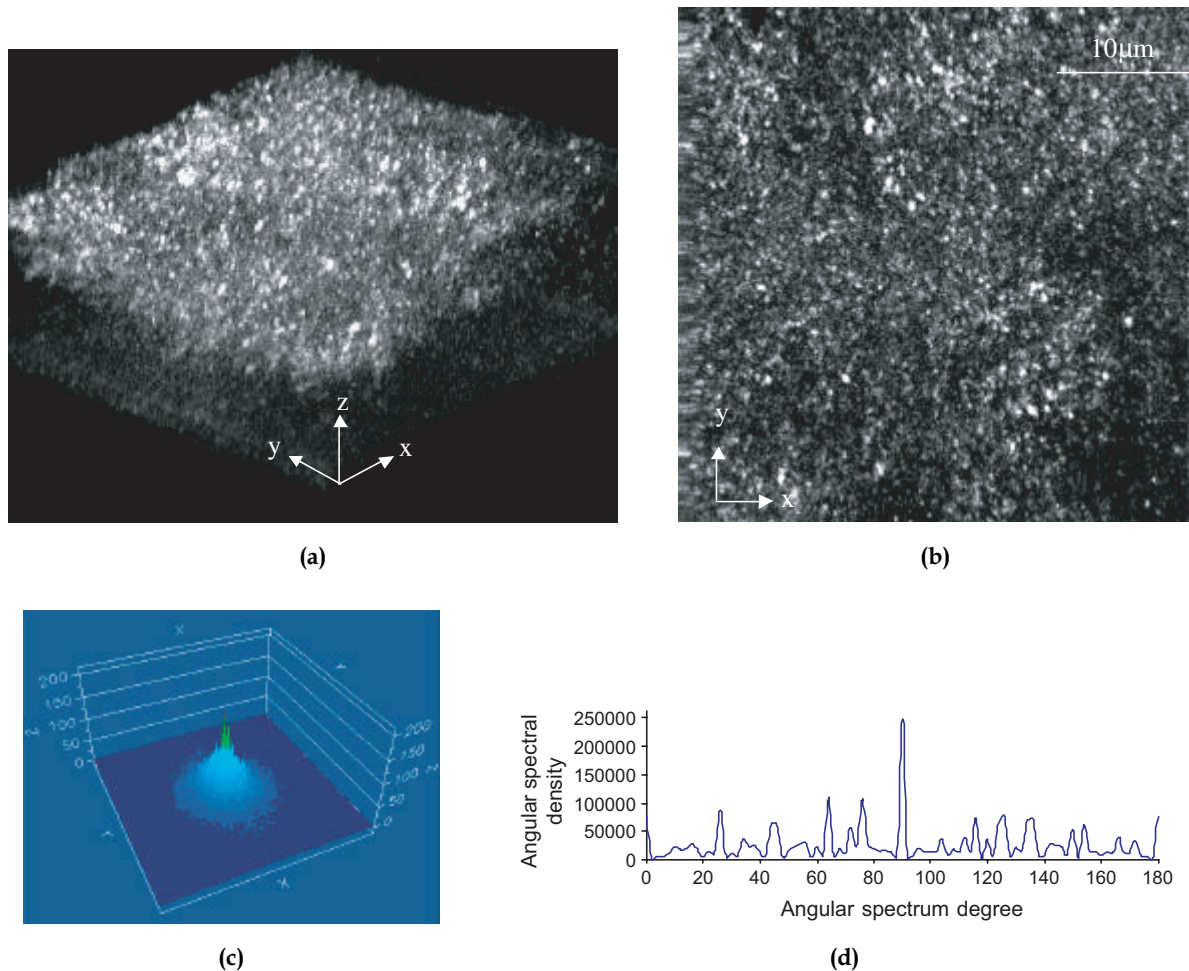
(a)

10μm

(b)

(c)

(d)

Fig. 5 The collagen matrix in the superficial zone of the cartilage obtained from the region of the arthritic cartilage showing a slightly matte surface appeared deteriorated, and the collagen fibres there aligned predominantly in a direction perpendicular to the AC surface. (**a**) 3D image of the collagen matrix. (**b**) MBI. (**c**) 3D plot of FFT performed on the MBI. (**d**) angular spectrum of the collagen fibres.

causes very little photobleaching in comparison with a fluorescent mode. Therefore, the images in this study are relatively representative of the collagen structure in the cartilage.

Since the collagen fibres in the superficial zone have a 3D structure,[30] which is difficult to be characterized using the 2D FFT and PSA, in this study, the FFT and angular spectral analysis were performed on the MBIs of the collagen fibres, and the collagen orientation revealed by the FFT and angular spectrum is related to the MBIs. Nevertheless, this does not affect to use the FFT

and angular spectral analysis to indicate a general collagen orientation in the superficial zone of the cartilage with different physiological status. This is because if the collagen fibres in the cartilage (e.g. normal or some aged cartilage which appeared normal) are orientated spatially in a predominantly oblique direction to the AC surface, they will appear directional or anisotropic in the MBIs. Elsewhere, if the collagen fibres in the cartilage (most the cartilage with surface discruption) align perpendicularly to the AC surface, they will appear uniform or isotropic in the
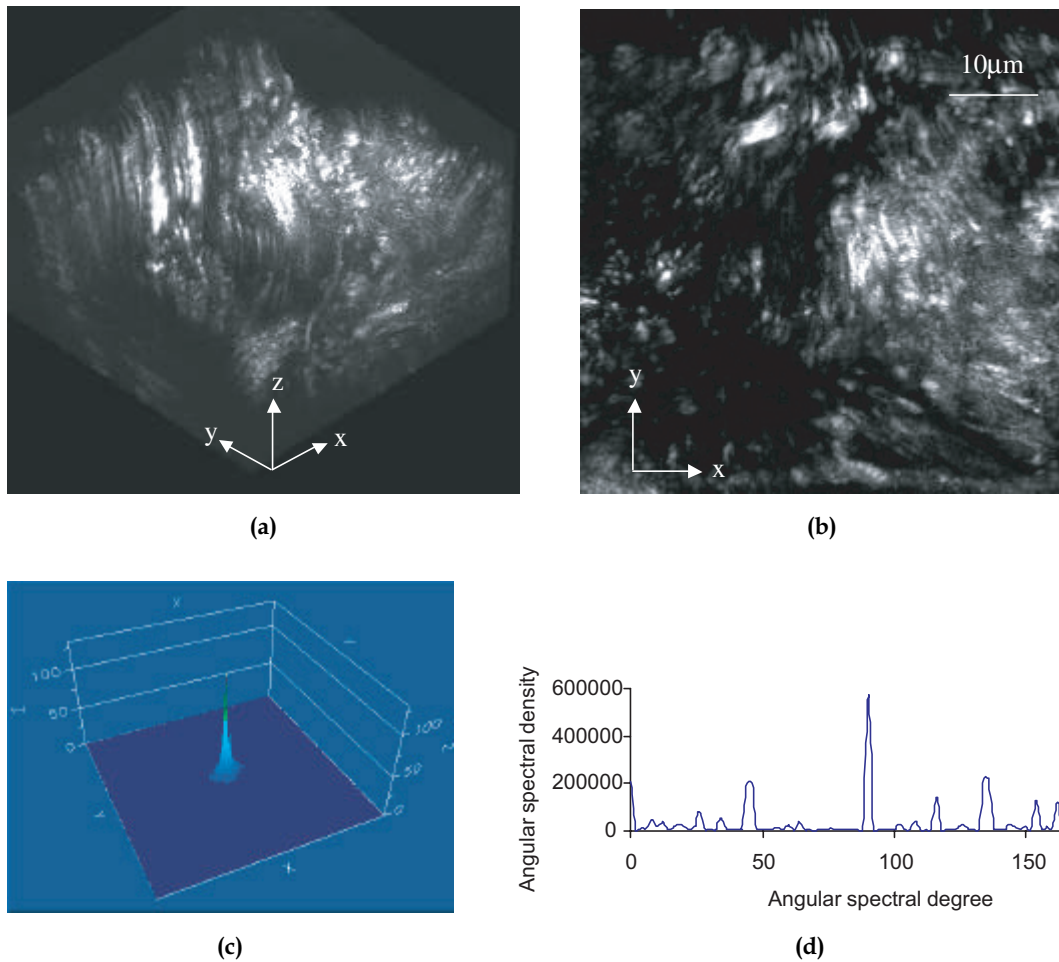
(a)

(b)

(c)

(d)

Fig. 6 Macroscopically appeared fibrillated cartilage had abnormally oriented collagen fibres. (**a**) 3D image. (**b**) MBI. (**c**) 3D plot of FFT performed on the MBI. (**d**) angular spectrum of the collagen fibres.

MBIs. However, FFT and PSA cannot be used to clearly distinguish the normal cartilage from some aged cartilage which appears normal (e.g. Figs. 1 and 2).

Furthermore, in comparing to the FFT, the angular spectrum offers more information about the predominant orientation of the collagen fibres in the superficial zone.[24] For example, in Fig. 1(e), a peak at 44° in the angular spectral graph indicates that the majority of the collagen fibres align approximately 44° to the x-axis in Fig. 1(c).

Since the orientation of collagen fibres is very complex and can be slightly different, the angular spectrum exhibits successive peaks in a range of

degrees. For example, in Fig. 2(d), the peaks are spanned from 108° to 134°. This means that the collagen fibres were predominantly orientated in a range of 108° to 134° with respect to the x-axis in Fig. 2(c). This is consistent with the collagen orientations shown in Fig. 2(b).

Moreover, the peak in the angular spectrum generally indicates the predominant orientation of the collagen fibres. However, a ninety-degree peak can be either an indication that the collagen fibres align predominantly along the y-axis direction in the MBIs or a result of "Gibbs phenomenon" due to discontinuity of Fourier series in two orthogonal directions 8. The 90° peak in

Fig. 5(d) appeared to be the "Gibbs phenomenon" as the fibres appeared "evenly distributed" in Fig. 5(b).

Furthermore, the collagen matrix in the superficial zone of normal cartilage contains randomly interwoven collagen bundles and the collagen fibres present typical periodic collagen bands. When analyzing the predominant orientation of the collagen fibres using the FFT and angular spectrum analyses, the orientations of the collagen bundles and collagen bands will also be taken into account in the energy spectrum. Therefore, the FFT of normal cartilage presented some small spectral distributions, as shown in Figs. 1(d) and (e).

In this study, the abnormal orientation of the collagen fibres in the fibrillated cartilage cannot be indicated using the FFT and angular spectra analysis. However, the fibrillated cartilage has distinctive macroscopic appearance from other types of cartilage investigated in this study, a simply direct macroscopic inspection is sufficient to distinguish it.

In conclusion, LSCM allows studying the microstructure of biological tissue with minimal physical destruction to the tissue. It also offers a higher image resolution and contrast than traditional optical microscopy while is much less expensive than electron microscopy. In particular, the characteristics of the FOCM used in this study have allowed studying the subsurface structure of articular cartilage *in vivo*.[15] Thus, the methodology presented in this study has a clinical potential for being developed to objectively identifying the early pathological changes in AC.

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