Classification and recognition of texture collagen obtaining by multiphoton microscope with neural network analysis

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Abstract  Second harmonic generation microscopy (SHGM) was used to monitor the process of chronological aging skin in vivo. The collagen structures of mice model with different ages were obtained using SHGM. Then, texture feature with contrast, correlation and entropy were extracted and analysed using the grey level co-occurrence matrix. At last, the neural network tool of Matlab was applied to train the texture of collagen in different statues during the aging process. And the simulation of mice collagen texture was carried out. The results indicated that the classification accuracy reach 85%. Results demonstrated that the proposed approach effectively detected the target object in the collagen texture image during the chronological aging process and the analysis tool based on neural network applied the skin of classification and feature extraction method is feasible.

1. Introduction
Skin aging is an important issue in dermatology and cosmetic science. It involves chronological aging and photoaging process [1]. Chronological aging is the natural aging process induced by the passage of time. In terms of skin health, chronological aging causes visible changes that appear in skin with time, usually starting around the age of 25 years. The degeneration of collagen is a major factor in age-related dermal alteration. Chronological aging occurs as the natural regenerative processes begin to slow; a slower turnover of the surface skin cells follows [2]. Currently, the process of cutaneous aging and its underlying molecular mechanism and biochemical have attracted worldwide attention [1, 3-4]. A second harmonic generation microscope (SHGM) [5] is a powerful tool has been applied on the study of biological tissues [6-8]. SHGM is a nondestructive imaging tool that enables the clear visualization of non-centrosymmetric structural proteins, such as myosin, thyroid tissue, and collagen, without fixation, sectioning, dehydration, and using exogenous dyes or stains [8]. The age-related structural and morphological changes in collagen result in optical parameter alterations, causing an optical contrast that can be detected in vivo using SHGM. Many researchers have paid increased attention to structural and morphological changes. However, they mainly focused on qualitative analyses of the altered skin structure [9].

Texture refers to the properties that represent the surface or structure of an object and is defined as something consisting of mutually related elements. Texture analysis is an important and useful method for
processing visual information. The spatial distribution of texture gray levels is a function of spatial variation in pixel intensities. It has a number of perceived qualities that play an important role in describing texture. This spatial distribution is useful in a variety of applications and has been the subject of intense studies by many researchers [10]. Therefore, quantitatively characterizing skin collagen, which has complex geometrical and local optical properties, is an important but difficult task.

Till now, few studies had extracted quantitative texture information from SHG images to evaluate collagen alteration during the aging process in vivo. This study aims to evaluate collagen texture using SHG images combined with mathematical processing to assess the chronological aging stages. SHGM was used to obtain collagen morphology during the chronological process in vivo. A quantitative analysis was established throughout the gray level co-occurrence matrix (GLCM) [10-12] between collagen alteration and aging progression to determine aging characteristics. The results show that combined SHG and GLCM is feasible to determine the main impact on age-related skin. It is important to understand the process of aging process and establish preventive regimens that would slow aging progression.

2. Materials and methods

2.1 Animal model

Forty matured Kunming mice with 8, 16, 50, and 60 weeks old, respectively, were selected as animal models in this study and were provided by the Animal Center of Fujian Medical University. All studies involving mouse tissues were approved by the Institutional Animal Care and Use Committee at Fujian Normal University. The mice were kept in a humid environment at a constant temperature and a 12 h light/dark cycle. They were fed a standard diet and given water. Before the experiment, each mouse was anaesthetized using intraperitoneal phenobarbital injection, and their dorsal hair was removed [13]. The mouse dorsal skin was stabilized in a certain fold chamber, covered with a cover slip, and observed by SHGM.

2.2 Second harmonic generation microscopy system

SHGM system used in this study has been described previously [8, 13]. Briefly, it is a commercialized technique based on the combination of an inverted microscopy with Axiovert 200 microscope of Zeiss LSM510 META laser scanning microscopy and a Coherent Mira 900-f mode-locked femtosecond Ti:sapphire laser (110 fs, 76 MHz), with tunable wavelength ranging 700 nm to 980 nm. The excitation wavelength at 850 nm was employed and the average output power was limited at 10 mW irradiated on the sample spot. An objective with Plan-Neofluar×10 (NA=0.3) was used to detect the SHG signal. All images are of 512×512 pixels with a size of 921 μm × 921 μm. SHG signal was detected at 425 nm in center with bandwidth of 20 nm, which was generated and collected at the sample focal plane.

2.3 Gray level co-occurrence estimate analysis

The images of collagen structure were processed by GLCM, which was reprogrammed and computed using Matlab 7.0. Spatial gray level co-occurrence estimates image properties related to second-order statistics, which is defined as the likelihood of observing a pair of gray values occurring at the endpoints of a dipole with a random length placed in the image at a random location and orientation. Haralick [14] suggested the use of GLCM, which has become one of the most well-known and widely used texture features. The greatest advantages of GLCM are its computational ease and powerful features. The G×G
GLCM Pd for a displacement vector \( d = (dx, dy) \) is defined as follows. The entry \((i, j)\) of \( P \) is the occurrence number of the gray level pairs \( i \) and \( j \), which are a distance \( d \) apart.

\[
P(i, j, d, \theta) = \left\{ \left[ \left( x, y \right), \left( x + \Delta x, y + \Delta y \right) \right] \mid f(x, y) = i, f(x + \Delta x, y + \Delta y) = j; \right\}
\]

First, an original texture image \( D \) is requantized into an image \( G \) with reduced number of gray levels \( N_g \), with a typical value of either 16 or 32. Then, GLCM is computed from \( G \) by scanning the intensity of each pixel and its neighbor, defined by displacement \( d \) and angle \( \theta \). \( d \) can take a value of \( 1, 2, 3, \ldots, n \), whereas \( \theta \) is limited to \( 0^\circ, 45^\circ, 90^\circ, \) and \( 135^\circ \). The \( P(i, j | d, \theta) \) of GLCM is the second-order joint probability density function \( P \) of the gray level pairs in the image for each element in the co-occurrence matrix, which is obtained by dividing each element with \( N_g \). Finally, scalar secondary features are extracted from this co-occurrence matrix [10]. The common statistical features used in this study were contrast, correlation, and entropy, which were described following.

The contrast feature, which measures intensity contrast as a function of pixel distance, is related to the collagen structure with detached fibrils [14].

\[
f_i = \sum_{j} P(i, j)^2 \tag{2}
\]

Contrast shows the definition and the degree of texture depth of the groove pattern of the image. The value of contrast signifies the distribution of the matrix pixel. Matrix pixel distribution is uniform in the large contrast value, and vice versa.

Correlation is the process of passing the mark \( w \) by the image matrix \( f_{correlation} \) in the manner proposed by [14].

\[
f_{correlation} = \frac{\sum \sum ijP_d(i, j) - \mu_x\mu_y}{\sigma_x^2\sigma_y^2} \tag{3}
\]

where

\[
\mu_x = \sum_i \sum_j P_d(i, j) \quad \mu_y = \sum_j \sum_i P_d(i, j)
\]

\[
\sigma_x^2 = \sum_i (i - \mu_x^2) \sum_j P_d(i, j) \quad \sigma_y^2 = \sum_j (j - \mu_y^2) \sum_i P_d(i, j)
\]

\( \mu_x, \mu_y \) are mean values and \( \sigma_x^2, \sigma_y^2 \) are variances.

The correlation of the images reflects the similarity on a direction of the image texture area and is the linear correlation measure of the local gray level in the image. This correlation is a measure of the dependence between two different pixel values. In particular, the correlation feature indicates a relation of intensity measure as a function of pixel distance, which is used to assess the similarity of matrix pixel horizontally and vertically.

Entropy is the complexity feature of an image matrix, and it mainly reflects the texture grayscale randomness distribution of the image. Entropy is a measure of the amount of information in an image and is defined as follows [14]:
\[ f_{\text{entropy}} = \sum \sum P_d(i, j) \log_2 P_d(i, j) \]  \hspace{1cm} (4)

When the image has many fine textures, the number of \( P(i, j) \) is approximately equal, and thus the value of entropy is larger, and vice versa. When the image has less number of fine textures, the number \( P(i, j) \) differs greatly, resulting in a smaller entropy value of the images.

These features were obtained using a combination of different displacements and angles. The calculated displacements of numerical parameters ranged from 0 to 60 pixels in the horizontal direction of each image.

Ten images from region of interest, with an area of 921 \( \mu m \times 921 \mu m \) in each stage of each mouse, were selected for quantitative analysis. The experimental results were analyzed by statistic test with SPSS 15.0 software (SPSS Inc., USA). Statistical significances of the data in different types ages were evaluated by T-test which was used to determine whether there were any significant differences between the means of two independent groups. The differences were considered statistically significant when the \( P \) values obtained from T-test analysis were less than 0.05.

3. Results and discussions

3.1 Collagen structure

Figure 1 the morphological structure of collagen obtained by SHGM. The images are 512\times512 pixels with an area of 921 \( \mu m \times 921 \mu m \), scale bar=100\( \mu m \). (a) The 8 weeks skin; (b) the 60 weeks skin.

As skin at various ages has different epidermis thicknesses and optical parameters [15], collagen under the epidermis of different skin types may stay in different skin layers, and it may result in false analysis. To better determine the characteristics of collagen, different sections of collagen from different depths of the dermis layer with the strongest collagen intensity were investigated. This process guarantees obtain the best layer of collagen in different skin types. Figure. 1 partially shows the SHG images that visualize the collagen structure status at different depths where the SHG signals are the strongest in different aging processes.
Figure 2 the (a) and (b) are the gray images of mice skin of 8 and 60 weeks; The (a1) and (b1) are the make binary images; the (a2) and (b2) are the voronoi processing images.

Then, the images were processed to perceptual intuition. Band-pass-filter was used to improve the visualizing degree. The results were displayed in Figure 2. The (a) and (b) are the gray images of mice skin of 8 and 60 weeks, respectively; The (a1) and (b1) are the make binary images; the (a2) and (b2) are the voronoi processing images. From the voronoi processing images, we could see that the quantity of polygonal in normal skin were much more that other states of chorological skin. The areas of polygonal were uniformity size in 60 weeks skin. With age, the quantity of polygonal decreased, and the size difference of areas were very big.
3.2 texture feature

The parameter of contrast extract from SHG imaging using GLCM method. The results of skin contrast at various ages are shown in Figure 3. From the principle, if the contrast value sharply increases as the pixel distance, the collagen matrix is distinct. While, if this value remains constant along with the pixel distance, then the collagen matrix has a less defined fibrillar contrast structure.

![Figure 3](image)

Figure 3 the normalized value of contrast with pixel distance in different age skin of images extract by GLCM.

From figure 3, the contrast value in 8-week increased sharply with the pixel distance, similar to that in 16 weeks. In the 50- and 60-week skin textures, the value gradually increased as the pixel distance increased. At a distance of 20 pixels, the contrast value in a young skin slowly increased, and the chronological aging skin became stable and the values fluctuated less. Therefore, the contrast at 20 pixels is defined as the collagen contrast in this study. The results show that the contrast value in young skin was larger than that in chronological aging skin. The data were statistically significant in two types of skin groups. This strong significance showed that the collagen intensity in young skin was higher than that in chronological aging skin. Moreover, the result indicated that a loss of fine structure led to less contrast in collagen fibrils. The collagen texture of young skin was distinct with a great difference in the pixel matrix, whereas collagen texture was obscure with a homogeneous pixel matrix in aging skin. Therefore, contrast values may be used to determine the intensity of collagen during the chronological aging process.

The relationship between correlation features and pixel distance in skin at different ages is shown in Figure 4. As far as our study is concerned, if the correlation value gradually decreases as the pixel distance increases, the collagen matrix is distinct. Conversely, when this correlation value remains at a constant value with the pixel distance, then the collagen matrix has less defined fibrillar correlation structure.
The values of correlation that decreased along with the pixel in all ages are shown in Figure 3. At a distance of 20 pixels, the correlation values became stable and remained steady. Thus, the correlation number at 20 pixels is defined as the collagen correlativity in this study. This definition of correlation indicates that a loss of fine structure can result from great collagen correlativity. Thus, the collagen fibril correlation sharply decreased with distance in aging skin, implying that the texture was obscured and had a big matrix at this skin state. Moreover, the collagen correlation value in chronological aging skin was larger than that in young skin, indicating that aging skin collagen texture had faint fibrils and a uniform pixel matrix. The value of collagen correlation was steady with distance in young skin, indicating that the collagen texture was distinct with a great difference in pixel matrix. Thus, the value of collagen correlation could be used to quantitatively estimate the similarity among various skin types. The extent of the similarity depended on the correlation value.

Figure 4 the normalized value of correlation with the pixel distance at various age skin gotten by GLCM.

Figure 5 the normalized value of entropy with pixel distance in different age skin from SHG image obtained by GLCM.
The entropy values in skin at different ages are shown in Figure 5. The entropy values were also stable at a distance of 20 pixels. Thus, these values of entropy are also defined as the collagen entropy in this study. Collagen entropy increased as distance in both young and chronological aging skin. However, the entropy value in young skin was greater than that in aging skin. This result indicated that the young skin collagen texture had many fine textures and linear fibrils, whereas the aging skin had a less fine texture. Thus, texture was complex in young skin and simple in chronological aging skin. Therefore, entropy value may be used to characterize the complexity of skin.

3.3 Classification skin type by neural network

Table 1 The network training results of mice skin images

| Serial number | Theory output | Actual output | Classification |
|---------------|---------------|---------------|----------------|
| \( a_1 \)     | 0 0 0         | 0.005 0.011 0.039 | younger        |
| \( a_2 \)     | 1 0 0         | 1.007 0.012 -0.045 | older          |

The texture features extracted from the gray level co-occurrence matrix were classified by the neural network tool [16, 17]. First, to establish the feature that is confirmed by the known statue. Let the feature vector of skin texture as \( X = (\text{CON}, \text{COR}, \text{ENT}) \), that is \( X = (X_1, X_2, X_3) \). Then input the normalization data in the NNtool. The output of NNtool is \( Y = (Y_1, Y_2, Y_3) \), the output result means the skin statue. Using the neural network toolbox of matlab6.5, setting the relation parameters, and then training the network and classification the skin texture features. The training sample is about fifty, and the prediction samples about fifty, with the results of classification as shown in table 1.

The theory output were set 0, 0, 0 corresponding CON, COR and ENT in younger skin texture feature. And the actual outputs were 0.005, 0.011, and 0.039 of these parameters. And the theory output were set 1, 0, 0 corresponding CON, COR and ENT in older skin texture feature. And the actual outputs were 1.007, 0.012, and -0.045 of these parameters. It means that the third parameter entropy is a litter big in two statues. The results indicated that the classification accuracy reach 85%. The result shows that the neural network tool of Matlab was applied to train the texture of collagen in different statues during the aging process is feasible.

4. Conclusions

In conclusions, GLCM was used to characterize the images of the texture features of skin at various ages obtained from SHGM. Three main parameters, namely, contrast, correlation, and entropy, were investigated to determine the intensity, similarity, and complexity of the skin during the chronological aging process, respectively. The results obtained from GLCM showed the distinct features of young skin, which were reflected through the great difference in the pixel matrix of the collagen. The collagen fibril contained abundant microgrooves in young skin, indicating complexity in terms of collagen texture distribution. By contrast, in chronological aging skin, the image of the collagen was blurred and had a uniform pixel matrix. The fine collagen decreased and the thick texture increased, indicating that the
collagen distribution had a more definite orientation. The neural network tool of Matlab was applied to train the texture of collagen in the two statues of skin and the classification accuracy reach 85%. the results indicated that the approach effectively detected the target object in the collagen texture image during the chronological aging process and the analysis tool based on neural network applied the skin of classification and feature extraction method is feasible.

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References

[1] Farage M A, Miller K W, Elsner P and Maibach H I 2007 Structural characteristics of the aging skin: a review Cutaneous and ocular toxicology 26 343-57
[2] Fisher G J, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S and Voorhees J J 2002 Mechanisms of photoaging and chronological skin aging Archives of dermatology 138 1462-70
[3] Chung J H, Seo J Y, Choi H R, Lee M K, Youn C S, Rhie G-e, Cho K H, Kim K H, Park K C and Eun H C 2001 Modulation of skin collagen metabolism in aged and photoaged human skin in vivo Journal of Investigative Dermatology 117 1218-24
[4] Cua A, Wilhelm K-P and Maibach H 1999 Elastic properties of human skin: relation to age, sex, and anatomical region Archives of Dermatological Research 282 283-8
[5] Denk W, Strickler J H and Webb W W 1990 Two-photon laser scanning fluorescence microscopy Science 248 73-6
[6] Jiang X, Chen S, Chen J, Zhu X, Zheng L, Zhuo S and Wang D 2011 Monitoring process of human keloid formation based on second harmonic generation imaging Laser Physics 21 1661-4
[7] Perry S W, Burke R M and Brown E B 2012 Two-photon and second harmonic microscopy in clinical and translational cancer research Annals of biomedical engineering 40 277-91
[8] Zhuo S, Wu G, Chen J, Zhu X and Xie S 2012 Label-free imaging of goblet cells as a marker for differentiating colonic polyps by multiphoton microscopy Laser Physics Letters 9 465
[9] Koehler M J, Hahn S, Preller A, Elsner P, Ziener M, Bauer A, König K, Bückle R, Fluhr J W and Kaatz M 2008 Morphological skin ageing criteria by multiphoton laser scanning tomography: non-invasive in vivo scoring of the dermal fibre network Experimental dermatology 17 519-23
[10] Honeycutt C F and Plotnick R 2008 Image analysis techniques and gray-level co-occurrence matrices (GLCM) for calculating bioturbation indices and characterizing biogenic sedimentary structures Computers & Geosciences 34 1461-72
[11] Clausi D A 2002 An analysis of co-occurrence texture statistics as a function of grey level quantization Canadian Journal of remote sensing 28 45-62
[12] Soh L-K and Tsatsoulis C 1999 Texture analysis of SAR sea ice imagery using gray level co-occurrence matrices Geoscience and Remote Sensing, IEEE Transactions on 37 780-95
[13] Wu S, Li H, Yang H, Zhang X, Li Z and Xu S 2011 Quantitative analysis on collagen morphology in aging skin based on multiphoton microscopy Journal of biomedical optics 16 040502–3
[14] Haralick R M, Shanmugam K and Dinstein I H 1973 Textural features for image classification Systems, Man and Cybernetics, IEEE Transactions on 6 160-21
[15] Wu S, Li H, Zhang X and Li Z 2013 Optical features for chronological aging and photoaging skin by optical coherence tomography Lasers in medical science 28 445-50
[16] Ripley B D 1996 Pattern recognition and neural networks: Cambridge university press
[17] Yoshida T and Omatsu S 2000 Pattern recognition with neural networks. In: Geoscience and Remote Sensing Symposium, 2000. IGARSS 2000. IEEE 2000 International: (IEEE) pp 699-701