DISTINGUISHING SECOND HARMONIC GENERATION IMAGES OF MOUSE PRETERM LABOR VIA WAVELET-BASED TEXTURE FEATURES

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ABSTRACT

This paper presents an image processing system for detecting mouse preterm labor using Second Harmonic Generation (SHG) microscopy images. Two classes of SHG images are considered: normal pregnant cervix and premature cervical remodeling induced by Mifepristone. Among the commonly used texture features in image processing, wavelet-based texture features together with previously utilized image features for SHG microscopy of artificial collagen gels are identified to form an effective set of features for distinguishing the two classes of images. The results obtained indicate that correct detection rates above 98% are achievable.

Index Terms – Preterm labor bio imaging application, second harmonic generation imaging, image texture features, wavelet-based texture features

1. INTRODUCTION

Delivery of a healthy baby requires that childbirth be initiated at just the right time in pregnancy. Annually, approximately 500,000 babies in the United States (12.5%) are born prematurely and daily twelve infants die due to complications associated with prematurity [1]. Costs for care of these infants approach $26 billion a year [1]. Despite extensive research, in 50% of preterm births the cause is unknown and onset of premature labor is unexpected. Development of accurate methods to detect impending preterm labor is one key to reducing the rate of prematurity.

In preparation for birth, collagen fibers that are the main structural component of the cervix undergo progressive changes in strength, shape and organization, leading to increased cervical flexibility in women as well as in animal models such as the mouse [2]. A clinical imaging method that could detect these changes would have tremendous potential for staging pregnancy and predicting preterm birth. An exciting new laser-based technology for non-invasive imaging of collagen, called Second Harmonic Generation (SHG) imaging [3], has the potential to make this possible in the near future. SHG imaging is a non-linear optical method that detects an intrinsic signal from non-centrosymmetric structures with submicrometer resolution. In unstained biological tissues, collagen type I is the predominant source of SHG signals. The method thus has great potential for visualizing collagen matrix organization in live animals by endoscopy.

As a step towards detecting preterm labor by endoscopy, this paper presents an image processing system for identifying changes in features of cervical collagen structure in SHG images obtained from mice treated with Mifepristone (RU486), a progesterone receptor antagonist that induces preterm labor within 15-18 hours after treatment [4]. The mouse has a 19 day pregnancy and in this study images were obtained by a SHG microscope from mouse cervices at gestation day 15 ± Mifepristone treatment for 13 hours to induce premature modeling. These two classes of images exhibit texture differences in their collagen ultrastructure. Figure 1 illustrates sample SHG images of a mouse cervix corresponding to untreated day 15 and day 15 Mifepristone treated.

In studies of artificial collagen gels, several image features based on pore and fiber characteristics were extracted from SHG images [5]. In this work, we present an image processing framework to distinguish Mifepristone treated samples from untreated samples. A rich collection of image texture features are examined to provide the most effective ones for this application.

Section 2 provides an overview of the texture features considered. In section 3, the selection, combination, and classification of these features are discussed. The results obtained and their discussion is presented in section 4 and the conclusion and future work in section 5.

2. OVERVIEW OF TEXTURE FEATURES

Image texture features have been extensively studied in image processing. Among various texture features, the most notable ones have been derived based on co-occurrence...
matrix [6], granulometry [7], and wavelets [8], [9], [10]. In this work, these features are analyzed to see which ones are most effective for this application. It is useful to provide an overview of these features.

Co-occurrence matrix-based or commonly known as Haralick features reflect statistical properties in an image. They are often computed for four spatial directions 0°, 45°, 90° and 135°, and then averaged to take into consideration all spatial orientations. The main texture features in this group include image contrast, homogeneity, entropy, and correlation. In our case, we have computed these features for interpixel distances of 5, 10 and 15 resulting in a total of $3 \times 4 = 12$ co-occurrence matrix-based texture features.

Granulometry-based texture features reflect morphological characteristics in an image. To construct a gray-level granulometry, one begins with a primitive structuring element $g$ and a sequence of structuring elements $g_1, g_2, g_3, ...$ constructed in this manner $g_i = g_{i-1} \oplus g, g_i = g_{i-1} \oplus g \oplus g, ...$, where $\oplus$ denotes gray-level dilation. The sequence of opened images $\{f \ast g_i\}$ for a gray-level image $f$ forms an image sequence of decreasing sizes. The normalized size distribution of this sequence can be regarded as a probability distribution function and its derivative as a probability mass function known as pattern spectrum. The moments of pattern spectrum denote granulometry-based texture features. Here, four moments (mean, variance, skewness and kurtosis) are examined for this application.

Geometrically, openings probe image topography from below. Similarly, one can probe an image from above, which means performing a sequence of morphological closing operations. Based on two commonly used structuring elements of square and disc, a total of $4 \times 2 \times 2 = 16$ granulometry-based texture features are thus considered here.

A group of texture features that have been widely used in medical image processing is derived from wavelets. Expressed in a compact form, wavelet transform of an image can be computed by using a filterbank as follows [11]:

$$L_n (x,y) = [H \ast [H \ast L_0]_{1,2}]_{1,2} (x,y)$$  \hspace{1cm} (1) 

$$D_{n1} (x,y) = [H \ast [G \ast L_0]_{1,2}]_{1,2} (x,y)$$  \hspace{1cm} (2) 

$$D_{n2} (x,y) = [G \ast [H \ast L_0]_{1,2}]_{1,2} (x,y)$$  \hspace{1cm} (3) 

$$D_{n3} (x,y) = [G \ast [G \ast L_0]_{1,2}]_{1,2} (x,y)$$  \hspace{1cm} (4)

where $\ast$ denotes the convolution operator, $\downarrow_{1,2}$ subsampling along rows $x$ and columns $y$, $L_0 = f(x,y)$ the original image, and $H$ and $G$ represent a lowpass and a bandpass filter, respectively; $L_n$ image is obtained by lowpass filtering and is referred to as the low resolution image at scale $n$, and $D_n$ images are obtained by bandpass filtering along a specific direction and are referred to as the detail images. This way the original image $f$ gets represented by a set of four subimages at several scales. In other words, across $d$ scales, $\{L_{d}, D_{d}\}_{d=1,2,3}$ Subimages are obtained providing a multiscale representation of the image $f$.

Wavelet-based texture features are defined based on the normalized energy $E$ of the above subimages containing $M$ wavelet coefficients as follows [9]:

$$E_0 = \frac{1}{M} \sum_{x,y} L_n^2 (x,y)$$  \hspace{1cm} (5)

$$E_{ni} = \frac{1}{M} \sum_{x,y} D_{ni}^2 (x,y)$$  \hspace{1cm} (6)

Basically, the wavelet-based texture features reflect the distribution of energy across different frequencies and orientations.

In this work, 18 types of wavelets that are available in the Matlab Wavelet Toolbox are used with a scale depth $d=2$. Experimentally one can see that higher scale depths matrix [6], granulometry [7], and wavelets [8], [9], [10]. In this work, these features are analyzed to see which ones are most effective for this application. It is useful to provide an overview of these features.

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beyond two do not provide additional information for the SHG images under consideration. That is to say per wavelet, based on the four wavelet-based texture features $E_0$ and $\{E_{ni}\}_{i=1,2,3}$, a total of $18 \times 4 = 72$ features are examined.

3. FEATURE SELECTION, COMBINATION AND CLASSIFICATION

To identify effective texture features among the above total of $12+16+72=100$ features for distinguishing Mifepristone treated from untreated SHG images, the Bhattacharyya distance measure is used [12]. This measure provides how far apart the means of image classes are and at the same time how compact the spread of each image class is. Per feature, this measure provides a degree of overlap between the distributions of the two image classes. It is found that neither co-occurrence nor granulometry-based texture features provide adequate discriminatory power for distinguishing these two classes of images. Figure 2 shows the high degree of overlap between the distributions of a sample co-occurrence matrix-based texture feature and a sample granulometry-based texture feature. Other features in these groups exhibit similar outcomes.

However, among wavelet-based texture features, it is found that five of them generate adequate discriminatory power for distinguishing the two image classes. These features correspond to the wavelet energy $E_0$ for the five wavelets that are listed in Table 1 together with their corresponding Bhattacharyya distances. In this table, $N$ refers to the order, $N_d$ the order of decomposition and $N_r$ the order of reconstruction of the wavelet. Figure 3 shows that relative to co-occurrence-based and granulometry-based texture features, a much higher separation of the distributions is obtained when using these wavelet features.

For classification, four different types of Bayesian classifiers [12] consisting of linear, diagonally linear, quadratic, diagonally quadratic decision boundaries are considered. The quadratic classifier is found to produce the most separating decision boundary in terms of generating lowest misclassification rates.

Hence, the image processing system developed for this medical imaging application consists of a feature extraction module and a classification module. The five identified effective wavelet-based texture features are extracted automatically from SHG images and fed into a previously trained quadratic classifier. In addition to the wavelet features, four additional image features that were useful in [2] are extracted. These features include characteristic fiber diameter, pore size, pore count, and pore density. They denote collagen fiber diameter, and the size, number and spacing of regions in the image with no collagen.

**Table 1**

<table>
<thead>
<tr>
<th>Wavelet Type</th>
<th>Order</th>
<th>Bhattacharyya distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biorthogonal</td>
<td>$N_r=1, N_d=1$</td>
<td>0.37</td>
</tr>
<tr>
<td>Coiflet</td>
<td>$N=4$</td>
<td>0.35</td>
</tr>
<tr>
<td>Coiflet</td>
<td>$N=1$</td>
<td>0.26</td>
</tr>
<tr>
<td>Reverse Biorthogonal</td>
<td>$N_r=6, N_d=8$</td>
<td>0.25</td>
</tr>
<tr>
<td>Biorthogonal</td>
<td>$N_r=3, N_d=5$</td>
<td>0.23</td>
</tr>
</tbody>
</table>

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Fig. 2. Distributions of (a) co-occurrence matrix-based correlation feature with an offset distance of 15 pixels, (b) granulometry-based mean feature for opening using square structuring element.

Fig. 3. Distributions of the wavelet-based texture feature $E_0$ for the biorthogonal wavelet with $N_r=1$ and $N_d=1$. 
4. RESULTS AND DISCUSSION

Our dataset included two classes or sets of mouse SHG images, each containing 100 images of size 776 × 776 pixels with each pixel representing 0.3 micron. These images were obtained via a SHG microscope at the University of Texas Southwestern Medical Center. The first class included a preterm birth model of gestation day 15 Mifepristone (RU486) treated cervix SHG images. The second class corresponded to gestation day 15 control cervix SHG images.

The wavelet texture features in section 3 were computed and fed into a quadratic Bayesian classifier. For training the classifier, 75% of the images were randomly chosen. The remaining 25% were used for testing without any overlap with the training images. The training procedure was repeated 20 times, each time selecting a different random set of training images, and the classification results obtained were averaged. The classification was done in three different ways: (1) using the above five wavelet texture features, (2) using the four previously identified image features in [5], and (3) using the combined nine features. For (1), the average classification error was obtained to be 5.7%, whereas for (2), the average classification error was obtained to be 22.5%. For (3), however, the average classification error was reduced to 1.8%. Here it is worth mentioning that no improvement in the classification rates was observed when the dimensionality reduction techniques were utilized.

Table 2 provides a sample confusion matrix for the combined case noting that only one of the images out of 50 test images was misclassified when using the combined set of features. The results shown indicate that the utilization of wavelet-based texture features greatly increases the discriminatory power between the two classes of SHG images.

<table>
<thead>
<tr>
<th>True/Classified</th>
<th>Class 1</th>
<th>Class 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Class 2</td>
<td>1</td>
<td>26</td>
</tr>
</tbody>
</table>

5. CONCLUSION

This paper has provided the first attempt to identify effective texture features in mouse Second Harmonic Generation (SHG) images for the purpose of detecting changes in cervical collagen that precede onset of normal and preterm labor. The image processing framework discussed in this study has the potential for it to be used as part of a clinical tool to identify women at risk of preterm labor in human pregnancy at an earlier time point and with greater accuracy than currently possible when used in conjunction with the promising SHG imaging modality. More specifically, an image processing system has been devised to distinguish mouse preterm labor from normal pregnant SHG images. It is found that by using five wavelet-based texture features together with four previously identified image features, a high detection rate can be achieved. In our future work, we plan to examine SHG images throughout the normal 19-day pregnancy duration in mice and in other mouse models of prematurity cervical remodeling as well as evaluate human pregnant cervixes to identify clinical guidelines towards detecting preterm labor.

6. ACKNOWLEDGMENTS

This work was jointly supported by the Erik Jonsson School of Engineering and Computer Science at the University of Texas at Dallas and by a grant from The Hartwell Foundation to the University of Texas Southwestern Medical Center. Imaging was carried out in the UT Southwestern Live Cell Imaging Facility.

7. REFERENCES