

# ANALYSIS OF CONFOCAL MICROSCOPE IMAGES FROM RETINA DETACHMENT EXPERIMENTS USING TEXTURE BASES FEATURES

*Zhiqiang Bi, B.S. Manjunath\**

Department of Electrical and Computer Engineering and Center for Bio-Image Informatics,  
University of California,  
Santa Barbara, CA 93106, U.S.A.  
{zb26,manj}@ece.ucsb.edu

## ABSTRACT

Retinal detachment, the separation of retina from retinal pigmented epithelium, causes changes to many types of cells in retinal tissue. A successful modeling of these changes can help to understand the undergoing biological processes and to devise more effective therapies to heal injured retinal tissue and eventually to recover the vision. In this paper, we first apply image processing techniques to extract texture based feature vectors using Gabor filters from confocal microscope images taken from retinal detachment experiments. We then correlate the image features with biological meta data using Chi square test. We study the distribution of images in a reduced feature space and classify images into biological classes labeled by experimental conditions. Results show that our texture feature vector clearly capture the biological experimental conditions under which the images are acquired, therefore they can be used in recognizing and analyzing biological patterns in images, as wells as content based retrieval in biological image database.

## 1. INTRODUCTION

Retinal detachment, the separation of retina from the retinal pigmented epithelium caused by eye injury, has been studied extensively for decades [1]. After retinal detachment, many types of cellular changes occur inside the retina tissue, such as the degeneration of the light sensitive photoreceptor outer segment, the growth of neurite into subretinal space, etc. These changes significantly affect the structure, state and function of the retinal tissues, thus may cause the loss of vision. In order to devise therapies for retinal detachment, extensive research has been done to model these cellular changes and to study how the changes can be recovered by various treatments. The goal is to, after various treatments, reattach the retina and recover cells to normal states, and therefore to return the vision.

Retinal detachment experiments are usually performed on animals that have retina similar to that of humans, such as

cats, mice, rabbits, or squirrels. Microscope images of retinal tissue are taken at various time points after retina is manually detached, typically 1 hour, 1 day, 3 days, or 21 days after detachment, as shown in Fig.1. These images show the distribution of proteins that are labeled by various antibodies. Traditional study in this field usually deals with a small number of retinal tissues and their microscope images. After decades of research and experiments, thousands of retinal images under many different experimental conditions have been collected, which, together with associated biological meta data, make it possible to build an overall model that helps us gain deeper understanding of the biological processes during the retinal detachment. However, it also becomes a great challenge to efficiently manage, process, and analyze the huge amount of scientific information.

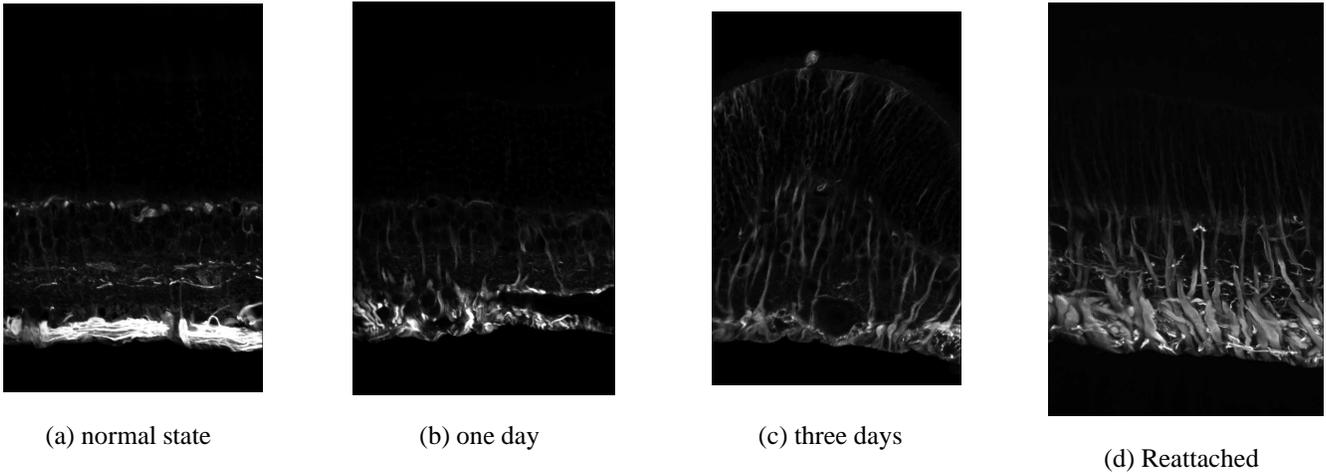
In this paper, we apply image processing techniques to extract numerical features based on image textures, and then using this feature vector as representation of the image, we statistically correlate the image features to the biological parameters, such as experimental conditions. We then study the behavior of various groups of images taken under different experimental conditions and perform classification between these images classes.

## 2. IMAGE PROCESSING AND MODELING

### 2.1. Data set

Our image collection contains over 700 microscopic images from retina detachment experiments. Each image represents the distribution of certain proteins labeled by corresponding antibodies. These protein distributions change dramatically after the retinal detachment and demonstrate various characteristics at various stages of the detachment. Therefore, observations of protein distribution changes can provide some insights about the status of certain cells and the state of the retina tissue. The microscope images represent retinal tissue under different experimental conditions, such as normal tissues, retinal tissues after one day of detachment, three days after detachment, or reattached retina tissue after treatments

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**Fig. 1.** The protein GFAP distribution of retinal tissues changes significantly from normal tissues, to one day after detachment, three days after detachment and reattached state.

(see Fig.1). The antibodies are primarily used for labeling the proteins and they have no effects on the biological status of the cells, and the experimental conditions are the main cause of the changes in the images. The antibodies and experimental conditions are the biological parameters in this study. Our goal is the find the statistical correlations between biological parameters and the features extracted from the images, and to learn whether our image features can separate various groups of images, and therefore can be used as basis for biological modeling.

## 2.2. Feature extraction

We extract texture features from images in the following steps: At first, Gabor filters [2] are applied to the images, and from each pixel in the image we obtain a 30 dimensional feature vector (5 scales, 6 orientations). Next, we cluster similar pixels together according to their Gabor filter outputs, which means pixels in each cluster demonstrate similar texture structure in their neighborhood. Clustering is done using K-means algorithm and we group the pixels in twenty cluster in this study. After the clustering, we replace each pixel with its cluster label to obtain a texture map. Finally, we compute the histogram of these texture cluster labels and use this histogram as a feature vector to represent the original image in our study.

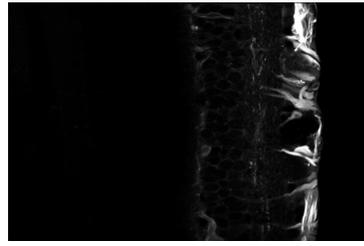
The reason for using this texture based features to characterize these images is that retinal tissues contain several distinctive layers, such as inner or outer nuclear layer (INL and ONL), and each layer shows one or more texture structures, so the histogram of the texture classes roughly correspond to the weight of each layer in the tissue. There is some related work that extracts texture features from retina images [3], but their goal is to use the feature for similarity search in image

database, while our emphasis is to find relationship between biological parameters and image features. We choose Gabor filters because they have been shown to work well in a wide range of applications, such as texture classifications, segmentations, and similarity search problems[3, 4, 5].

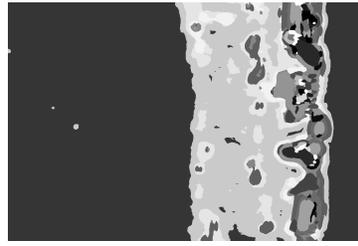
Fig.2 shows the original image of the protein vimentin distribution, the texture map obtained from the original image and the histogram of each texture cluster for both normal retina tissue and retina tissues 3 days after detachment. In the normal state as show in Fig.2(a), the protein vimentin was orderly distributed with a concentrated layer at the end region of Müller cells. Fig.2(b) shows the texture map of the image. The histogram of the texture clusters in Fig.2(c) shows two big peaks representing the background and the texture of a layer of retina tissue, which is called Outer Nuclear Layer (ONL). Fig.2(d,e,f) show the vimentin distribution, texture map and histogram of texture for a detached retinal tissue. We find in Fig.2(d) that there are some “hairy” structure growing from the originally concentrated end region. This is known as the intermediate filament cytoskeleton growth from the end region into the outer retina region. This effect, caused by retina detachment also reflects in the texture map and histogram, Fig.2(e, f). The histogram shows more signals, which means that the protein distribution now contains more significant texture classes due to unorderly growth. The interpretation of the texture histogram and its various peaks is not a topic of this paper, but it will be an interesting research subject in the future.

## 3. FEATURES EVALUATION AND THEIR APPLICATION IN IMAGE CLASSIFICATION

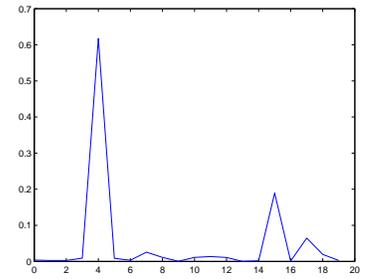
In this section, we perform a Chi-square statistical test to study if the texture histogram features can separate the im-



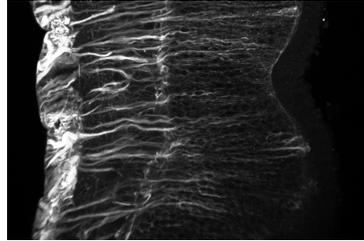
(a) Vimentin distribution of normal retinal tissue



(b) Texture map



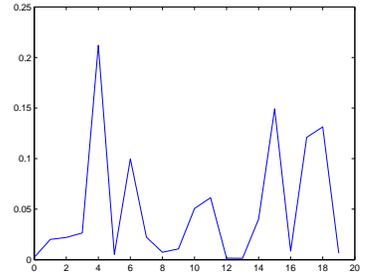
(c) Histogram of texture classes.



(d) 3 days after detachment



(e) Texture map



(f) histogram of texture classes

**Fig. 2.** The protein vimentin distribution of retinal tissues changes significantly from normal tissues, to tissues one day after detachment, or tissues three days after detachment. In the normal state, the distribution is more orderly and concentrated, and texture histogram shows two major peaks. After detachment, a lot of growth appears and texture histogram shows many peaks.

ages of normal retina and retina after detachment. We also use linear discriminant analysis (LDA) [6] to visualize the image distribution in reduced feature space to study how protein distribution images evolve under different experimental conditions.

### 3.1. Chi-square test

In statistics, Chi-square tests are usually performed to test if tabulated data are sampled from two different distributions. In order to find if the images feature of the normal tissue and tissue of detached retina can be statistically separated, we first take the mean of these two types of feature vectors, and obtain two mean vectors  $f$  and  $\bar{f}$ , correspondingly, which are substituted into the Chi-square test formula

$$\chi^2 = \sum_{i=1}^k \frac{(f_i - \bar{f}_i)^2}{(f_i + \bar{f}_i)/2} \quad (1)$$

and the results are show in Table 1

From the Chi-square test results, we find that distributions of all four proteins in our tests, CD44, GFAP, vimentin and peanut agglutinin, exceeds the cutoff value, which means there is a statistically significant change in the texture feature space from normal state to detached state, therefore we conclude that these two kinds of images can be distinguished with Chi-square test with high statistical confidence.

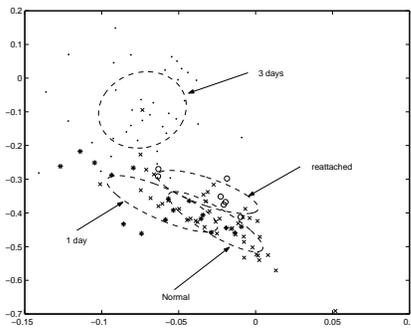
Antibody	CD44	GFAP	vimentin	pea. aggl.
$\chi^2$	41	32	48.6	41.2

**Table 1.** Chi-square test results show that in the four protein categories, we find that the normal retina tissues and retina tissues after detachment can be distinguished with our texture features with a 95% confidence statistically. The cutoff  $\chi^2$  score for 19 degree of freedom is 30.15.

### 3.2. Dimensionality reduction

Although the texture histogram feature can separate normal and detached retina images, it is hard to visualize the distribution of the images in this feature space because of its high dimensionality (20 in our case). We therefore use linear discriminant analysis (LDA) to reduce the dimensionality to two so we can visualize the image distribution and study their behavior under various conditions. We consider images of retinal tissues from four types of experimental conditions, normal state, detached for one day, detached for three days, and reattached retina. Fig.1 shows the sample images of GFAP distribution from these four states.

Fig.3 shows the separation of the four classes of images in a reduced two-dimensional space obtained by LDA. We find in this two dimensional space that images of retina after one day of detachment move away from the normal im-



**Fig. 3.** GFAP distributions of retinal tissue under four experimental conditions are shown in this reduced two dimensional space obtained from LDA. After 1 day detachment, the images move slightly in this space, but they still overlap significantly with the images under normal state. After 3 days, a clear separation appears. The images for reattached retina move back closer to the normal state.

ages, although these two groups remain mainly overlapped, which indicate some slight changes occur during the first day of the detachment. After three days of detachment, the images change significantly, so in the feature space, image distribution shows greater deviation from the normal images such that they can be mostly separated. Then after the reattachment, the images change back closer to normal images. These observations agree nicely with biological expectation, and more significantly, these biological trends are clearly captured in our feature space (Fig.3) even after we reduce the dimensionality to two.

LDA also allows us to compute numerically the separation between these classes of images. We first estimate the mean and covariance matrices of the four classes, with which we classify testing image set consisting of 37 normal images, 17 one-day images, 39 three-day images and 7 reattached images. The classification results are listed in Table 2

	Normal	One day	Three days	Reattached
Normal	0.923	0	0.077	0
One day	0.027	0.919	0.055	0
Three days	0.024	0.118	0.641	0
Reattached	0	0	0	1

**Table 2.** Classification results using LDA shows clear separation between different classes of images.

The classification results as shown in Table 2 indicate clear separation of the four classes in the feature space, which means that texture histogram is well suited for classification confocal retina images from different experimental conditions. It provides numerical measure of how retina images deviate from the normal state, thus helps scientists to quantitatively model the biological processes of retina detachment.

## 4. CONCLUSION

In this paper, we introduced a feature vector based on texture to represent the confocal microscope images. We find this feature vector shows strong statistical correlations to biological conditions, and it can capture the difference between retinas from normal state to detached state with high statistical confidence. Classification results also show that using the texture based feature vector we can identify whether images are of normal or detached retina with high accuracy. These results mean that our texture features successfully capture the biological background information, the experimental conditions that caused the retinal changes, therefore are useful in various areas, such as numerical modeling the retina detachment process, similarity search in an image database of retina tissues. In the future, we will perform feature selection and find which feature components response most to certain biological conditions, and start building an overall model that can simulate the biological processes in retina.

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