APPLICATION OF TEMPORAL TEXTURE FEATURES TO AUTOMATED ANALYSIS OF PROTEIN SUBCELLULAR LOCATIONS IN TIME SERIES FLUORESCENCE MICROSCOPE IMAGES

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ABSTRACT

Protein subcellular locations, as an important property of proteins, are commonly learned using fluorescence microscopy. Previous work by our group has shown that automated analysis of 2D and 3D static images can recognize all major subcellular patterns in fluorescence micrographs, and that automated methods can be used to distinguish patterns that are subtly different. Since many proteins are in constant movement within the cell, we extended our studies to time series images, which contain both spatial and temporal information. In this paper, we present the application of a set of temporal texture features, which do not require predefining objects for tracking, to the classification of subcellular location patterns. We demonstrate that these features successfully captured new information contained in the time domain by evaluating the accuracy of automated classification of a data set of five proteins with similar location patterns.

1. INTRODUCTION

Proteomics requires the discovery of every characteristic of all proteins, including the subcellular location. A protein's location indicates its environment and possible function, and therefore it is one of the key properties that need to be learned. A common way to identify a protein's subcellular location is to label it with fluorescent dye, take microscope images and then make decisions by human visual inspection. Our group has developed computer programs that can replace this last step. The automated approach is more objective and sensitive than visual examination, and single cell 2D and 3D images of major subcellular patterns can be classified with accuracy over 90% [1-3]. We have also grouped proteins by their similarity to build subcellular location trees [3]. All the machine learning and statistical tools we have assembled are based on features that extracted from static images. We now expand our study to time series images, which contain additional information in the time domain. Many proteins are in constant movement. For example, many membrane proteins are transporters that carry molecules into or out of the cell, and cytoskeletal proteins change their patterns during the cell cycle. In order to completely understand a protein's behavior within the cell, analyzing time series images will be essential.

The challenge of studying protein movement is the difficulty of defining targets for tracking, which is the most intuitive way of studying movement. Proteins are often not grouped to form rigid objects, and blobs of fluorescence can merge or split. Previous computer vision studies have addressed motions with similar nature, for example, wavy water or flags blowing in the wind. Pioneering work by Nelson and Polana was published in 1992 [4], where the authors raise the concept of "temporal texture" to define such "complex and nonrigid" motion. They calculated the normal flow on each pixel to represent movement along the image gradient. Then they compared the directionality obtained from the flow field to uniform direction flow. Bouthemy and Fablet later adapted the Haralick co-occurrence texture for static images to movies [5]. Ngo, Pong and Zhang developed a series of features based on temporal slices and utilized co-occurrence matrices as well [6]. To our knowledge, temporal texture features have not previously been applied to fluorescence microscope images. Given their simplicity and generality compared with approaches based on more complicated models to study protein movement, we describe here the application of simple co-occurrence temporal textures to the classification of time-series images of protein distributions.

2. METHODS

2.1. Image Acquisition

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A number of NIH 3T3 cell lines expressing different proteins fused with green fluorescent protein (GFP) by CD-tagged have been described previously [7]. Five of these lines were selected for this study. Cells were plated on glass-bottom culture dishes 48 h before imaging. The imaging system consists of a LaserPhysics Reliant 100s 488 Argon laser, a Yokogawa CSU10 Confocal Scanner Unit, and an Olympus IX50 microscope with a 60x 1.4NA objective. Images were collected with a Roper Scientific/ Photometrics CoolSnap HQ Cooled CCD camera. The resulting images have 1280 x 1024 pixels in one slice and the distance between neighboring pixels was 0.11 micron. For each cell, 15 slices were taken to form a 3D stack, where the distance of two neighboring pixels in the z direction was 0.5 micron. The exposure time for each slice was 3s, so the time interval between two 3D stacks was approximately 45 seconds (ignoring time for image transfer and storage). Each movie consists of 7-34 time points, depending on the extent of fluorescence photobleaching.

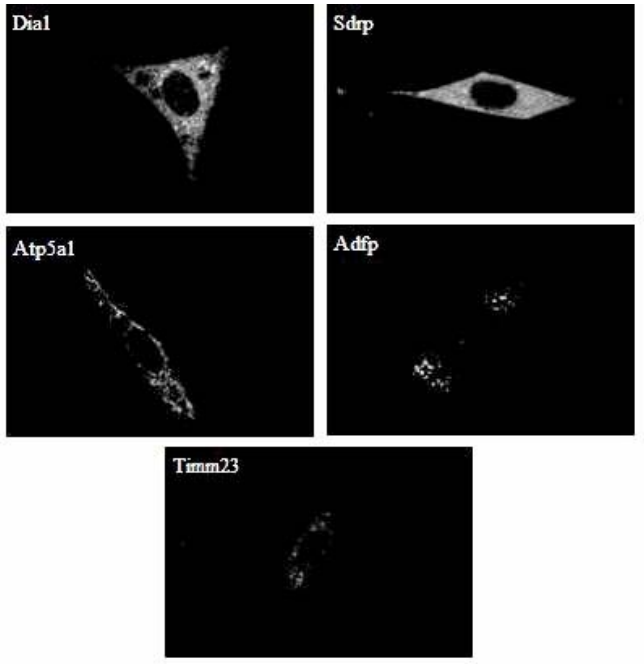

Fig. 1. Sample fluorescence microscope images showing the distribution of five GFP-tagged proteins. Each image is a 2D slice from the first time point of a 4D (3D plus time) series.

We collected 3D time series images of five proteins: cytochrome b-5 reductase, diaphorase 1 (Dia1), serum deprivation response protein (Sdrp), ATP synthase (Atp5a1), adipose differentiation related protein (adfp) and ADP-ATP translocase 23 (Timm23). 20-51 movies were taken for each cell line. Figure 1 illustrates their 2D static patterns. All of the five proteins are distributed outside the nucleus. Dia1 and Sdrp have both even distribution in the cytoplasm and bright spot-like vesicles. Atp5a1, Adfp and Timm23 have only a punctate pattern coming from vesicles or mitochondria.

2.2. Feature Calculation for Static Images

To perform machine learning or statistical analysis, we represent each image using numerical features. We have extensively described sets of such features appropriate for analysis of subcellular patterns in static images, and have defined a number of sets of these Subcellular Location Features (SLFs). These include morphological features, edge features, geometric features, DNA features, Haralick texture features and others [8]. A detailed description of each feature and feature set is available at http://murphylab.web.cmu.edu/services/SLF.

2.3. Feature Extraction for Time Series Images

The temporal texture features we used on time series images were inspired by the Haralick texture features, which capture the intensity correlation of neighboring pixels in *space* [9]. The co-occurrence based temporal texture features capture the value correlation of neighboring pixels in *time*. The starting point for calculation of Haralick features is the generation of a gray-level co-occurrence matrix that captures the correlation (or lack of correlation) between the gray levels of adjacent pixels.

This is an NxN matrix, where N is the number of gray levels (e.g., 256). The element in row *i* and column *j* of the matrix is the frequency that a pixel that has value *i* in the image at time point 1 has a value *j* in the same position in the image at time point 2.

For proteins that show no movement from t1 to t2, the two images at the two time points are the same, and the co-occurrence matrix will only have non-zero values on the diagonal. Different protein movement patterns can be expected to result in different co-occurrence matrices.

Given the co-occurrence matrix for a pair of images separated by a certain time interval, we calculate 13 statistics described by Haralick [9] (we use only 13 of the statistics originally described by Haralick due to computational instability with the 14^{th}). These features are then compiled across all images separated by that interval, and we use the mean and variance across the series as the final features of the image. This process was carried out for spacings of 45s (adjacent time points), 90s (every other time point), 135, 180s, and 225s. The result is 13*2*5 =130 features in total.

As discussed above, temporal texture features based on co-occurrence matrices have been previously described. It is therefore worth noting the differences between these approaches and ours. Bouthemy and Fablet [5] built the co-occurrence matrix using the magnitude of normal flow rather than directly on intensity, and Ngo, et al. [6] built the co-occurrence matrix on x-t and y-t temporal slices and only

calculated two statistics (smoothness and contrast) from the each matrix.

2.4. Automated Classification

We seek to determine how well the patterns of the five GFP-tagged proteins can be distinguished using either static or temporal features. Before feeding the features into a classifier, we used Stepwise Discriminant Analysis (SDA) [10] to select the features that have the best power to discriminate between the classes. Feature selection is necessary because features that confound the classes can reduce the classifier's ability to learn the real differences. The SDA algorithm has been tested against other feature selection methods and proved to perform the best in our previous subcellular classification work [11]. Once a set of features was selected by SDA, it was used to train Support Vector Machine (SVM) classifiers. An SVM is a generalized linear classifier that transforms the features into a new feature space using kernel functions. In the new feature space, a linear decision boundary can be drawn to separate classes. SVM will find the maximum margin hyperplane in the feature space to insure the minimal prediction error. We used the max-win strategy to deal with multiple classes, and 10-fold cross validation to evaluate the classification accuracy.

3. EXPERIMENTAL RESULTS

To test the ability of temporal features to distinguish similar patterns, we collected images of five cell lines that express GFP-tagged proteins with similar location patterns. The choice was based on results of cluster analysis of static 3D images of 90 tagged cell lines [_insert Chen 2005 ref]. The five lines chosen are all contained within two adjacent clusters found by this analysis.

In order to demonstrate that temporal texture features capture useful extra information in the time domain, we did classification experiments using static features and using static features plus temporal texture features. Forty two 3D static features (SLF11) were calculated for the 3D stack at the first time point and 84 2D static features (SLF7) were calculated on the center slice of the first stack. SDA selected 10 features from the 126 static features. Six of them are 2D haralick texture features, two are 2D morphological features and two are 3D edge and morphological features. This suggests that 2D static patterns are more useful in distinguishing the five classes. Using 10-fold cross validation to train and test SVMs, we obtained an average accuracy of 75.32% (Table 2). The confusion matrix shows that Dia1 is confused with Sdpr, and only 15% can be correctly recognized. The other 4 proteins can be corrected predicted with above 70% accuracy.

We next added the 130 temporal texture features, and repeated the same procedure. SDA selected eight features, four of which were temporal texture features (which included the top two as ranked by SDA) and four of which were 2D Haralick texture features. Surprisingly, no 3D static features were selected. This suggests that for our data set, the temporal texture features have captured all the useful information that the 3D static features provided. It demonstrates an important fact that temporal texture features can depend not only on the movement pattern, but also on the static pattern of the carrier of the movement. Let us imagine that we cut out a small hole on a piece of paper, lay the paper on top of a picture, and slide the picture underneath. Depending on what is on the picture, what we observe will be very different. If the picture is of a clear sky, we will see no changes; if the picture is of a sky full of stars, we will see black and white shifts. The same idea applies when we build the temporal co-occurrence matrix; we analyze the pixel value changes in each position. How the value changes over time depends on the values in the neighborhood, as well as the movement itself.

True Class	Prediction by Classifier				
	Dia1	Sdpr	Atp5a1	Adfp	Timm23
Dia1	15	75	0	0	10
Sdpr	9	83	0	0	9
Atp5a1	0	10	85	0	5
Adfp	0	0	2	94	4
Timm23	0	0	8	20	73

Table 2. Confusion matrix for classification of five protein patterns using 2D and 3D static features. The overall average accuracy is 75.32%.

True Class	Prediction by Classifier				
	Dia1	Sdpr	Atp5a1	Adfp	Timm23
Dia1	50	35	5	0	10
Sdpr	9	87	0	4	0
Atp5a1	0	5	95	0	0
Adfp	2	0	2	92	4
Timm23	0	5	0	8	88

Table 3. Confusion matrix for classification using 2D and 3D static features plus temporal texture features. The overall average accuracy is 85.06%.

Using the eight selected features to train and test an SVM classifier (Table 3), we obtained an average accuracy of 85.06%, 9.74% better than using only static features. Recognition accuracy of Dia1 is increased from 15% to 50%. Timm23 also has a 15% increase in recognition accuracy.

We also repeated the same procedure for the temporal features without combining them with static features. SDA selected seven features from the 130 temporal texture features, four of which are among the six temporal texture features that SDA selected from the mixture of static and temporal features. The overall classification accuracy with

True Class	Prediction by Classifier				
	Dia1	Sdpr	Atp5a1	Adfp	Timm23
Dia1	35	30	5	0	30
Sdpr	0	57	0	0	43
Atp5a1	5	0	75	10	10
Adfp	0	0	4	92	4
Timm23	5	3	3	10	80

Table 4. Confusion matrix for classification using only temporal texture features. The overall average accuracy is 74.03%.

just the seven temporal features was 74.03% (Table 4). The result shows that temporal texture features themselves capture a significant amount of information, at least about the variation in pattern between these five proteins. The prediction accuracy for three of the proteins is above 70%. Summarizing the results in Tables 2-4, we can see that the static features alone give 75% accuracy, the temporal texture features alone giving 74% accuracy, and the combination gives 85% accuracy. While it is clear that much information contained in the two feature sets is overlapped, there is sufficient new information in the combination to reduce the error rate by 40% (from 25% to 15%).

4. CONCLUSIONS

Time series images provide a important dimension of information for protein subcellular distribution. We applied the concept of temporal texture from the computer vision field to our protein fluorescence images. We used co-occurrence based temporal texture features as a numeric description of movement characteristics. The features are defined on a pixel basis, and require no predefined entity for tracking. By combining the temporal texture features with static features, we improved the classification accuracy for a five protein data set and therefore demonstrated the value of using time series images and temporal texture features to better understand protein subcellular distributions. The co-occurrence based temporal texture features we used are powerful features that capture not only temporal but also spatial information and they can provide high classification accuracy by themselves.

5. ACKNOWLEDGMENTS

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6. REFERENCES

[1] M. V. Boland and R. F. Murphy, "A Neural Network Classifier Capable of Recognizing the Patterns of all Major Subcellular Structures in Fluorescence Microscope Images of HeLa Cells," *Bioinformatics*, vol. 17, pp. 1213-1223, 2001.

[2] M. Velliste and R. F. Murphy, "Automated Determination of Protein Subcellular Locations from 3D Fluorescence Microscope Images," *Proc 2002 IEEE Intl Symp Biomedl Imag*, pp. 867-870, 2002.

[3] X. Chen, M. Velliste, S. Weinstein, J. W. Jarvik, and R. F. Murphy, "Location proteomics - Building subcellular location trees from high resolution 3D fluorescence microscope images of randomly-tagged proteins," *Proc. SPIE*, vol. 4962, pp. 298-306, 2003.

[4] R. C. Nelson and R. Polana, "Qualitative recognition of motion using temporal texture," *CVGIP: Image Understanding*, vol. 56, pp. 78-89, 1992.

[5] P. Bouthemy and R. Fablet, "Motion characterization from temporal cooccurrences of local motion-based measures for video indexing," *Proc. Int. Conf. Pattern Recognition*, vol. 1, pp. 905-908, 1998.

[6] C. W. Ngo, T. C. Pong, and H. J. Zhang, "Motion retrieval by temporal slices analysis," *Proc. Int. Conf. Pattern Recognition*, vol. 4, pp. 64-67, 2002.

[7] J. W. Jarvik, G. W. Fisher, C. Shi, L. Hennen, C. Hauser, S. Adler, and P. B. Berget, "In vivo functional proteomics: Mammalian genome annotation using CD-tagging," *BioTechniques*, vol. 33, pp. 852-867, 2002.

[8] Y. Hu and R. F. Murphy, "Automated interpretation of subcellular patterns from immunofluorescence microscopy," *J Immunol Meth*, vol. 290, pp. 93-105, 2004.

[9] R. M. Haralick, "Statistical and structural approaches to texture," *Proc. IEEE*, vol. 67, pp. 786-804, 1979.

[10] R. I. Jennrich, "Stepwise discriminant analysis," in *Statistical Methods for Digital Computers*, vol. 3, K. Enslein, A. Ralston, and H. S. Wilf, Eds. New York: John Wiley & Sons, 1977, pp. 77-95.

[11] K. Huang, M. Velliste, and R. F. Murphy, "Feature Reduction for Improved Recognition of Subcellular Location Patterns in Fluorescence Microscope Images," *Proc SPIE*, vol. 4962, pp. 307-318, 2003.