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An Approach to Differentiate Cell Painted ER and Cytoplasm Using Zernike Moment Descriptor and Multilayer Perceptron

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Abstract. Differentiation of cell organelle characteristics from microscopic images is a challenging task due to its intricate structural details. In this work, an attempt has been made to categorize Endoplasmic Reticulum (ER) and cytoplasm using orthogonal Zernike moments and Multilayer Perceptron (MLP). For this, Cell painted public source dataset comprising of ER and cytoplasm are considered. Zernike moments for different orders and repetition of the azimuthal angle are extracted to characterize the shape features. The extracted features are validated using MLP classifier for differentiating ER and cytoplasm. The prediction accuracy for variations in the number of hidden layers are evaluated. The experimental results show that the accuracy varies as the size of hidden layer increases. The extracted features with MLP achieved an accuracy of 85% with a hidden layer size of 5. The receiver operating characteristic curve (ROC) demonstrates the distinguishing power of MLP classifier with AUC=0.92. This study suggests that the proposed framework can be employed for analyzing the morphological variations of cell organelles due to chemical perturbations, genome variations and cytotoxic effects using the combination of Zernike shape descriptor and MLP. The orthogonality property of Zernike shape descriptor provides independent unique features which reduce redundancy and improve prediction accuracy for large datasets.

Keywords. Cell painting, zernike moment, multilayer perceptron

1. Introduction

Cell painting is an advanced fluorescent microscopy imaging technique that uses separate dyes for targeting specific cell organelles. This approach paints various cell compartments such as nuclei, Endoplasmic Reticulum (ER), cytoplasm, mitochondria and Golgi apparatus for profiling subtle patterns in cell structure [1]. Cell images can be quantitatively analyzed by capturing variations in the size, shape, texture and color [2]. Shape features are important biomarkers for the discrimination of cell organelles. An

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orthogonal Zernike moment can provide non redundant shape features for classification [3]. In this work, orthogonal Zernike moments are extracted and are fed to the Multilayer perceptron (MLP) algorithm for differentiating ER and cytoplasm. MLP classifier is suitable for large dataset with good classification performance [4].

2. Methodology

2.1 Image Dataset

For this study, Cell Painted images of Human U2OS cells are obtained from Broad Bioimage Benchmark Collection [5]. 3456 images of ER and 3456 images of cytoplasm in 16-bit grayscale TIF format with 1080 x 1080 pixels are considered.

2.2 Extraction of Zernike moment features

Zernike moments are set of complex polynomials comprised of orthogonal basis functions defined in a unit circle. Three basic steps are involved in the extraction of Zernike moments: computation of (i) Zernike radial polynomials (ii) Zernike orthogonal basis functions and (iii)Zernike moments [6].The real valued 1D radial polynomial $R_{k,j}$ is given by Eq.(1).

$$R_{k,j} = \sum_{m=0}^{(k-|j|)/2} (-1)^m \frac{(k-m)!}{m!\left(\frac{k+|j|}{2}-m\right)\left(\frac{k-|j|}{2}-m\right)} \rho^{k-2m} \quad (1)$$

Where k is a positive integer or zero that represents the radial polynomial order, j represents repetition of azimuthal angle. An image function $f(u,v)$ with size N x N is multiplied by a complex orthogonal basis function $V_{k,j}(\rho,\theta)$ given by Eq.2 .This mapping transforms the image function into a new feature vector space defined by a unit circle with polar coordinates ρ *and* θ. 2-D Zernike complex basis functions $V_{k,j}(\rho,\theta)$ defined within a unit circle are represented by Eq. (2)

$$V_{k,j}(\rho,\theta) = R_{k,j}(\rho)e^{ij\theta} \ , |\rho| \leq 1 \quad (2)$$

Where ρ is the vector length from origin to the (u, v) coordinates,θ is the angle between vector ρ and u-axis in counterclockwise direction. The complex Zernike moments of order k with repetition of azimuthal angle j are defined as

$$Z_{kj} = \frac{k+1}{\lambda_N} \sum_{u=0}^{N-1} \sum_{v=0}^{N-1} f(u,v)\, V^*_{k,j}(u,v) \quad (3)$$

λ_N is a normalization factor and $f(u,v)$ is the image function. The Zernike moments for different orders (k) and repetitions (j) are computed. 32 moment features that satisfies the given conditions: $3 \leq k \leq 10$, $|j| \leq k$, $k-|j| = 2p$ & $p \in N$ are extracted as shown in Table 1. These orthogonal moment features are independent and provide unique image information with no redundancy.

Table 1. Zernike moments for different lower orders (k) and repetitions (j)

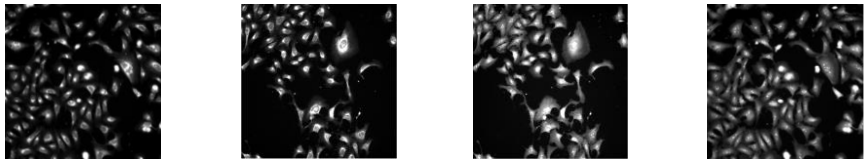
k (order)	3	4	5	6	7	8	9	10
j (repetitions)	1,3	0,2,4	1,3,5	0,2,4,6	1,3,5,7	0,2,4,6,8	1,3,5,7,9	0,2,4,6,8,10

2.3 Classification

The extracted Zernike features are fed to the MLP classifier. It is a feed-forward artificial neural network that consists of minimum of three layers: an input layer, a hidden layer and an output layer. Each layer consists of neurons and they are connected to the neurons in the next layer. The neurons are connected by weights and it uses backpropagation for training [7]. 70% and 30 % images are randomly selected for training and testing respectively.

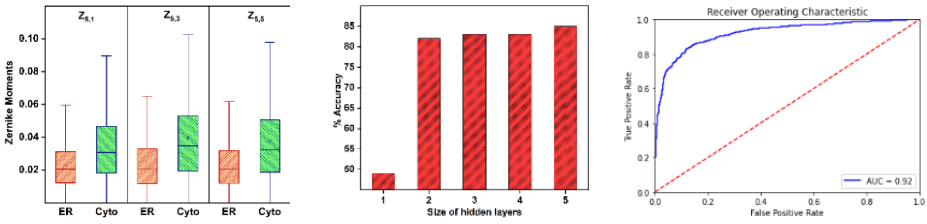
3. Results

The representative images of ER and cytoplasm are shown in Figure 1 (a and b) and (c and d) respectively. The morphological differences between these organelles cannot be identified on visual examination. Figure 2(a) shows the box plot containing the mean, median and interquartile range of representative Zernike moments of order 5 with repetitions1,3 and 5 for ER and cytoplasm. It is seen that the median and interquartile range of Zernike moments $Z_{5,1}$, $Z_{5,3}$ and $Z_{5,5}$ for ER is lower than that of cytoplasm indicating a significant variation in shape feature of the considered organelles. These statistical difference describes the ability of Zernike moments to capture unique image information to distinguish ER and cytoplasm.



(a) (b) (c) (d)

Figure1. Representative input images of (a) and (b) Endoplasmic Reticulum, (c) and (d) Cytoplasm



(a) (b) (c)

Figure 2. (a) Box plot of Zernike moments for different orders (k) and repetitions (j) (b) Variation in classification accuracy with different number of hidden layers (c) Receiver Operating Characteristics Curve

Figure 2(b) shows the variation in classification accuracies for different sizes of hidden layers. Classification accuracy of 85% is obtained with tuned parameters such as hidden layer size =5, activation='tanh' and maximum iteration=500.Figure 2(c) shows

the receiver operating characteristics (ROC) curve with AUC=0.92. This reveals the distinguishing capability of the multilayer Perceptron model (MLP) for the classification of ER and cytoplasm.

4. Discussion

The ER is an interconnected membranous structure located in the cytoplasm. The difficulty in differentiating this embedded organelle from cytoplasm is addressed in this study. From the obtained results it is observed that the significant morphological variations of ER and cytoplasm in the cell can be characterized by Zernike moments. These orthogonal independent moments can depict the unique shape information of these cell components necessary for accurate classification. The increase in number of hidden layers in the classifier network increases the number of neurons which can reduce the error function by updating the weights to produce more accurate output using MLP.

5. Conclusion

In this study, differentiation of ER and cytoplasm based on Zernike moment features and MLP are performed. The results demonstrate the suitability of Zernike orthogonal moments with multilayer perceptron in discriminating ER and cytoplasm. The independent and non-redundant Zernike moments provide unique shape information and with MLP achieved good classification accuracy of 85%. The proposed work has a clinical importance in identifying the morphological variations in cell organelles due to chemical perturbations, genome variations and cytotoxic effects which can further be utilized for accurate classification of cell organelles with minimal medical expertise.

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