ABSTRACT

We present a non-invasive, non-destructive automatable image-based methodology for classifying human embryonic stem cell (hESC) colonies. In contrast to differentiated colonies, pluripotent colonies contain homogeneous tight textures, thus allowing a statistical analysis of the coefficients obtained from a wavelet-based texture decomposition to discriminate between the colonies. Similarly, borders of undifferentiated cell colonies are sharp, and circular, while those of differentiated colonies are not. We confine our description in this paper to texture analysis, which relies on a parametric and non-parametric hierarchical statistical classification. Parametric classification relies on probability models for texture wavelet coefficients, while non-parametric classification makes use of Support Vector machines. Preliminary implementation using a truth set yielded a 96% rate of successful colony classification between distant classes, while for intermediate classes of colonies, with mixed population, the success rate was at least 86%. The texture analysis was also validated using individual egg cell images.

1. INTRODUCTION

Background: The successful culture of human embryonic stem cells (hESC) at the University of Wisconsin in 1998 opened the door for serious research into the potential medical benefits of these unique cells. The leading disease targets for embryonic stem cell therapy include Parkinson’s disease [1], diabetes [2], and heart disease [3]. In order for hESC to be viable for such research, they must remain undifferentiated throughout the culturing process. To ensure this normality, the stem cell colonies undergo a continuous quality control process where they are monitored and tested for signs of degradation and differentiation. The current state of the art for this quality control process involves either the performance of invasive tests, destructive tests, or direct visual inspection by an expert stem cell microscopist. In the biological test approach, the resulting destruction of the inspected colonies is a significant drawback. The visual approach has limited reproducibility, is subject to variability among culture technicians, and quickly becomes impractical considering the large number of colonies needed for research and therapeutic purposes.

Classification Attributes: Figure 1 shows examples of an undifferentiated and a differentiated stem cell colony. Notice that the undifferentiated colony in Figure 1 (a),(c) exhibits a tight, homogeneous texture with a clear, generally circular border. In contrast, the differentiated colony in Figure 1(b),(d) has a rather heterogeneous textural interior consisting of very loose, broad cells. These features can also be observed in the line scan for a given row in both figures as shown in Figure 1(e) and (f). In addition, its border, while generally circular, is not sharp. The changes in colony morphology are due to changes in the shape, size, and cell-cell adhesivity of cells that are spontaneously differentiating to an unspecified fate or are no longer pluripotent.

Based on visual characteristics of stem cell colony health, each colony can be rated according to at least four quality categories. These visual quality criteria are: 1. Textural homogeneity, 2. Textural tightness or fine grain, 3. Border sharpness, and 4. Border circularity.

Though the first two criteria are independent factors, together they define the textural characteristics of an undifferentiated stem cell colony. Homogeneity refers to the uniformity of the textural qualities across the colony. Alone, this criterion does not discriminate among various types of uniform texture. Thus, the second criterion of textural tightness dictates the specific type of texture that constitutes an undifferentiated colony; namely, an undifferentiated colony consists of small cells, densely packed together to reveal a tight texture. Jointly, these first two criteria state that a good stem cell colony exhibits a homogeneous, tight texture throughout.

Proposed Methodology: Based on these observations of the texture we present a computer vision based algorithm with high day-to-day consistency to automatically classify images of stem cells. Employing a non-parametric support vector...
machine classifier with wavelet subband energy features and parametric Kullback-Leibler (KL) distance approach with wavelet coefficient modeling, we perform segmentations of stem cell colonies into areas of differing quality. We restrict emphasis here to texture analysis. The complete study is described in [4]. The Approach is briefly described in the next section, while Section 3 presents early results, including method validation based on another application, individual egg cell analysis. Section 4 concludes the article.

2. APPROACH

2.1. Overview

Based on the above observations, the proposed methodology [4] consists of a multi-step approach that relies on features obtained from image texture and border crispness analysis, combined with prior information. Referring to Figure 2, Step 1, Pre-Processing, sets up the images in the right format. Step 2, Texture Analysis, consists of two different parts. In the first part, Step 2a, we produce qualitative graphical aids derived from pixel-level textural segmentation of stem cell colonies into regions of varying quality. We characterize colony texture using the subband energies of a wavelet decomposition and employ both a non-parametric support vector machine (SVM) as the classifier that produces the segmentation and a statistical wavelet coefficients based classifier using the Kullback-Leibler distance (KLD) adapted to the task of textural image segmentation. This technique was originally proposed by Vetterli and Do for content-based image retrieval [5]. From these segmentations, we can provide stem cell researchers with a rich set of descriptive graphical representations of their colonies. In Step 2b, we consider the colony level quantitative classification of full colonies into four pre-determined quality categories. In a hierarchical approach, we derive colony-wise textural features from Step 2a’s pixel-level graphical outputs. In Step 3, Border Crispness Analysis, we derive a new set of colony-wise border quality features, specifically border sharpness and circularity. In step 5, the features extracted from Steps 2 and 3 are combined together with prior information obtained in step 4 as inputs to a multiclass support vector machine (SVM) Classifier Decision System. In the remainder of this section, we describe the texture analysis.

2.2. Pixel-Level Features

Wavelet Subband Energies. In both the parametric and non-parametric SVM approach, we employ energy measures of the wavelet subband coefficients as the texture feature set. Specifically, we perform a 2-level wavelet pyramid decomposition using the Daubechies 4 wavelet on an $M \times M$ pixel window around the given image pixel in consideration.

For each of the resulting detail coefficient sets $cD[b]$, $b = 1, 2, \ldots, B$, we calculate $MD_b$ the mean deviation [6] and $E_b$ the variance, where $b$ is the index of the subband.

Generalized Gaussian Density of Wavelet Coefficients. As in Vetterli and Do [5], we adopt for the parametric approach, after verification, a generalized Gaussian distribution for the coefficients of the wavelet decomposition (GGD) at each subband. Specifically,

$$p(x; \alpha, \beta) = \frac{\beta}{2\alpha \Gamma(1/\beta)} e^{-((|x|/\alpha)^eta}$$  

(1)
where $x$ is a vector of wavelet coefficients, $\alpha$ is a width parameter proportional to the standard deviation, and $\beta$ is a thickness shape parameter. A third parameter, the location parameter or mean, is assumed zero in (1). Each colony class would have a representative density function (1), and for each colony pair, we derive the KL distance, as discussed below.

2.3. Colony Level Features

After segmenting each colony interior by textural quality, we derive various colony-level features from these segmentations. For both the SVM and KLD pixel level we calculate the mean and variance across each pixel of both the binary pixel classifications function $f(x_i)$ and the confidence values function $h(x_i)$. In addition to these eight colonywise features we introduce two additional outlier-based features to augment the variance features above in characterizing homogeneity. If the local mean value for a given pixel’s window differs by more than two standard deviations from the confidence mean across the entire colony, we declare the pixel to be an outlier. We define the SVM texture outlier and KLD texture outlier features to be the total number of outlier pixels across the colony using the SVM and KLD classifiers, respectively. More precisely it is the number points where the local mean deviates from the colonywise mean by more than twice the colonywise variance.

2.4. Classification Algorithms

**Non Parametric – Support Vector Machine (SVM).** Although several non-parametric classification methods exist such as neural networks, $k$-nearest neighbor and logistic regression, we choose to employ Vapnik’s support vector machine classifier [7]. One particularly attractive feature of SVM is that it offers a compact representation of the classes to be differentiated. Another attractive feature is the multitude of Kernels one can use for feature generation [8]. For this work, best results were obtained using the polynomial Kernel function.

The results of the SVM are the two functions $h(x)$ and $f(x)$, the signed Euclidean distance from the new input point $x$ to the optimal hyperplane, and $f(x)$ returns the binary $+1$ or $-1$ classification of $x$ respectively.

To extend the standard binary SVM multiclass case we apply the multiway classification function [9],

$$H(x) = \arg\max_{j \in \{1, \ldots, k\}} h_j(x)$$

(2)

to classify a colony $x$ into one of the $k$ classes. Intuitively, (2) dictates that we classify the colony $x$ to the class which is the most confident of $x$ belonging to it rather than not. For $k = 2$, this multiclass approach collapses to the standard binary SVM classifier.

**Parametric – Kullback-Leibler Distance.** We adapt a methodology proposed by Vetterli and Do for the task of content-based image retrieval [5]. Vetterli and Do show that a new point $x$ should be classified to the class whose characteristic wavelet coefficient distribution is closest to the distribution of the new point as measured by the Kullback-Leibler distance [5]. Similarly the KLD also produces a classification function $f(x)$ and a confidence measure $h(x)$. For the joint density functions corresponding to the wavelet coefficients of two different colonies. The KL distance is defined as

$$D_{KL}(1, 2) = \int p_1(x) \ln \left( \frac{p_2(x)}{p_1(x)} \right) dx.$$  

(3)

The distance yields a simple expression for the GG densities of (1) [4, 5].

**Training.** The SVM and KLD classifiers described above must both be trained and tested from among our limited supply of stem cell colony images. For each classification run, we randomly split the available colonies into disjoint training and test sets so that we do not test on colonies used for training. Additionally, we perform each run ten times using a random training/test partition of the available colonies each time. We then take the average classification rate over the ten runs as our performance measure.

3. RESULTS

**Stem Cell Colonies.** Table 1 shows results for 4 different classes of colonies, ranging from uniformly undifferentiated (class 1) to uniformly differentiated (class 4), with two intermediate classes of diverse cell population. We are especially successful in separating category 4 from the other categories, achieving no worse than 88.0% and up to 96.7% performance across all three comparisons. Figure 3 is another illustration of the preliminary success of the algorithm. The Figure shows a representative colony from both class 1 (A) and class 3 (B). Frames (C) and (D) were identified by the expert as ambiguous, having aspects of more than one class. The expert was particularly interested in how the algorithm fares against these two samples, which are often wrongly attributed to class 2. The algorithm clearly classified frame C as class 1 and frame D as class 3. This result was confirmed by the expert.

**Validation on Egg Cell Images.** We have a sequence 58 image frames of an Rhesus egg. The healthy egg, shown in Figure 4 (A), is destroyed starting at frame 30 (B), and by frame 55 (C), it is dead. The texture of all the image frames were decomposed, and the wavelet coefficients densities parameters were computed. The KL distance between the densities of each frame and those of the first (blue curve, bottom), and last (red curve) frames are computed. For instance, for the blue curve, the first 29 frames are healthy and the KL distance with the first frame is small. As soon as the egg is damaged, the KL distance vis a vis the first, and all healthy frames, increases. The blue and red curves show the wavelet texture analysis, and the KL distance based on the generalized Gaussian density model, as a consistent efficient discriminator between a healthy and dying egg.
Table 1. Sample Classification Results.

<table>
<thead>
<tr>
<th>Categories</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs.</td>
<td>81.4%</td>
<td>90.5%</td>
<td>96.7%</td>
</tr>
<tr>
<td>2 vs.</td>
<td>86.2%</td>
<td>88.0%</td>
<td></td>
</tr>
<tr>
<td>3 vs.</td>
<td></td>
<td>93.5%</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Four colonies. Colonies C and D both contain attributes from class 2, and humans often classify them as such, though they do not belong to that class. The algorithm correctly classified colonies C and D as belonging to class 1 and 3, respectively.

4. CONCLUSION

We have introduced a non-invasive non-destructive hierarchical methodology that provides a classification tool to aid in the stem cell quality control process. The process is automatic and can be executed at higher temporal and spatial resolutions, with more consistency and reliability than the human eye. Current research aims at expanding our training set, and at further developing data driven classification and prediction techniques and evaluating their statistical performance. Finally, the ideas and methodologies presented are applicable to a wide range of both medical and non-medical applications.

5. REFERENCES


