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Automated Gram-staining Characterization of Digital Bacterial Cell Images

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***Abstract*-- The Gram-staining technique is used as a tool for the differentiation of Gram-positive and Gram-negative bacteria, as a first step to determine the identity of a particular bacterial sample. In this paper, an automated image analysis has been developed for the monitoring of the Gram-staining characteristics of bacteria. The experimental results are compared with manual results obtained by microbiological expert. The results demonstrate the efficiency of the proposed method.**

***Keywords**: Gram-staining, color image analysis, thresholding, histogram entropy*

1.0 INTRODUCTION

The most important differential stain used in bacteriology is Gram stain, which was discovered by Danish Bacteriologist Hans Christian Gram[1]. It is the only procedure based on staining technique, which divides bacteria into two large groups, namely, Gram-positive and Gram-negative. The procedure of staining is diagrammatically represented in the Fig.1.

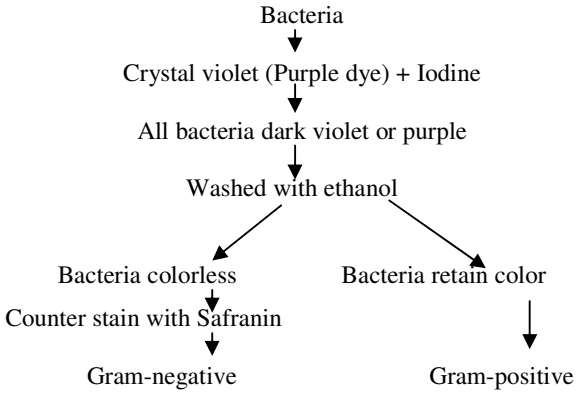


Fig. 1 Procedure of Gram-staining

Gram's method is the most important and fundamental orthodox method for bacterial identification. **Gram staining** (or **Gram's method**) is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram variable and Gram in determinant groups as well.

Gram staining is a common procedure in the traditional bacteriological laboratory. The technique is used as a tool for the differentiation of Gram-positive and Gram-negative bacteria, as a first step to determine the identity of a particular bacterial sample [6]. Generally, the amount of Gram-positive or Gram-negative bacteria, as stained by the classical technique, is estimated by visual inspection and manual counting under a simple optical microscope. This procedure can be facilitated by automated image analysis. Automated image analysis of activated sludge has been proposed previously for filaments bacteria abundance on monochrome image (da Motta et al. 2001). For Gram color images, Saida et al.[4] have proposed a photometric characterization (by Gram stain index) for bacterial population in various aquatic environments, where the bacteria were deposited on a membrane. Denis Pandolfi and Marie-Noelle Pons [3] have also proposed Gram-staining characterization of activated sludge filamentous bacteria by automated color analysis in HSI color space. The objective of the present study is to develop a method of automated color image analysis for Gram characterization of bacteria is in YCbCr color space. The Gram-positive bacteria would appear in blue color and Gram-negative bacterial species would appear red. The experimental results show effective improvement due to change in color space.

II. MATERIAL AND METHODS

A smear of bacteria was deposited on a glass slide and thoroughly air-dried. It was stained for 1 min in Crystal Violet solution, 1 min in iodine solution, washed for 10s in ethanol and finally, counterstained with safranin for 1 min. The glass slide was examined under oil immersion at 100x – 250x magnification with direct illumination in a Dialux 20 microscope equipped with a 3 CCD Sony color camera and connected to a PC. We have considered 100 color images for present study using the above setup.

III. PROPOSED METHOD

The purpose of the automated image analysis of digital bacterial cell images is to identify the type of bacteria, whether it is Gram-positive or Gram-negative based on their color features.

The YCbCr color space is used [5]. This color space is widely used for digital video. In this format, luminance information is stored as single component (Y), and

chrominance information is stored as two color-difference components (Cb and Cr). The Cb represents the difference between the blue component and a reference vale. The Cr represents the difference between the red component and a reference value. These features are defined for video processing purposes and so are not meaningful concerning human experience.

The following equations transform RGB in [0,1] to YCbCr in [0,255].

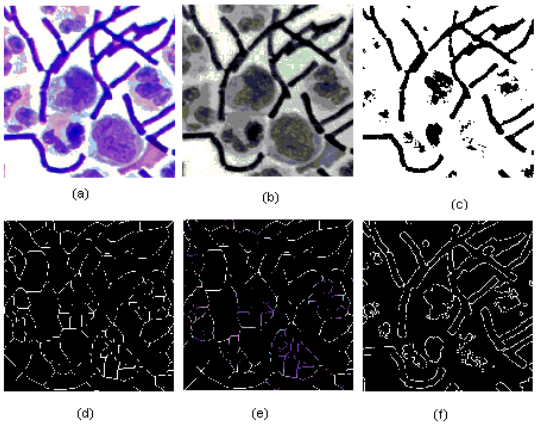
Y =16+65.481 R+128.553 G+24.966 B
Cb=128-37.797 R-74.203 G+11.000 B
Cr=128+112.000 R-93.786 G-18.214 B

The proposed algorithm for the Gram-staining characterization based on color image analysis in YCbCr color space is given below:

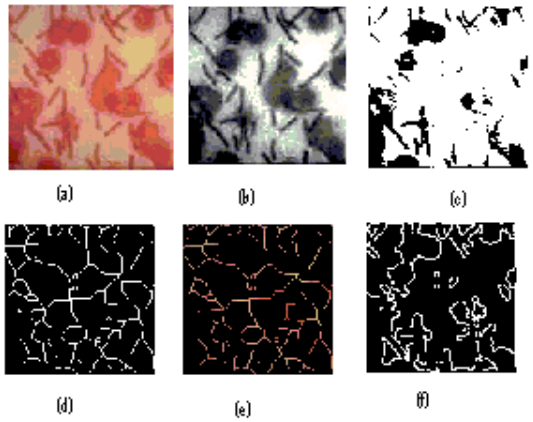
Algorithm: Gram-staining characterization
Input : Digital Gram-stained bacterial cell images.
Output : Gram-stain characterization (Gram-positive or Gram-negative)
Step 1. Input cell image (color stained)
Step 2: Convert the RGB image into YCbCr
Step 3: Enhance Luminance (Y) component by histogram equalization
Step 4: Perform global thresholding on Y based on histogram entropy yielding binary image (i.e. 0 for background and 1 for objects (bacteria + debris)
Step 5: Apply morphological operations, namely, erosion, reconstruction and dilution to remove debris
Step 6: Skeletonize the image of step 5 and superimpose skeleton image on original image.
Step 7: Classify non-background pixels as Red pixels or Blue pixels using the criteria: a pixel in YCbCr image is a Red pixel of its R-value > B value; otherwise, it is a Blue pixel.
Step 8: Obtain the border pixels from the image of step 5.
Step 9: The bacteria corresponding to a fragment in image of step 7, is Gram-negative if (i) number of Red pixels > number of Blue pixels, and (ii) more than 60% of border pixels are Red pixels; otherwise, the bacteria is Gram-positive.

IV. EXPERIMENTAL RESULTS AND DISCUSSIONS

For the purpose of experimentation, 100 color bacterial Gram-stained digital images of both Gram-positive and Gram-negative are considered. The implementation is done on a Pentium P-IV 1.0 GHz machine using MATLAB 7.0. The input color bacterial image (Fig. 2(a)) is transformed from RGB into YCbCr color space and luminance (Y) primitive is enhanced by histogram equalization in order to increase the image contrast (Fig. 2(b)). Automated thresholding based on histogram entropy is applied on the contrast image and the result is a binary image with two grey levels i.e. 0 for the background and 1 for the region of the interest i.e. objects (Fig.2(c)). Then the skeletonized image has been obtained by performing morphological operations (Fig.2(d)). All the skeleton fragments shorter than 5 pixels and containing a branch end are eliminated from the skeletonized image using morphological operation. Then the skeletonized image is superimposed on original image and the skeleton overlapping pixels are extracted (Fig.2(e)). These pixels are considered to be blue (B) when its level on the blue primitive of image is higher than its level on the red (R) primitive. Inversely, a pixel is considered to be red when R>B. Next, we obtain the border pixels from thresholding binary image (Fig.2(f)). Finally, we classify the bacteria corresponding to a fragment in skeletonized image using the criteria, namely: the bacteria is Gram-negative if (i) number of Red pixels are greater than (>) number of Blue pixels, and (ii) more than 60% of border pixels are Red pixels; otherwise, the bacteria is Gram-positive. The experimentation is also done by a change of color space to, namely, Lab color space [7].



Gram-positive bacteria



Gram-negative bacteria

Fig. 2(a) Original color image(RGB), (b) contrast image (luminance plane)of (a), (c) binary image of (b), (d) skeletonized image of (c), (e) superimposed image of (d) on (a), (f) border image of (c), for Gram-positive and Gram-negative bacterial cells.

In order to validate the method, the results of the automated Gram analysis are compared with those obtained by manual assessment by microbiological expert. The experimental results are in good agreement with the visual inspection by the microbiological expert. The comparison of the identification performance of proposed and manual method is given in Table 1. It is observed that the Gram-stain characterization results are better in YCbCr color space than that in RGB or Lab color space in comparison with manual method.

TABLE 1: COMPARISON OF IDENTIFICATION PERFORMANCE OF PROPOSED METHOD, DENNISH ET AL. METHOD AND MANUAL METHOD.

Color model	Total No. of images	Gram-positive	Gram-negative	Misidentification
Proposed (YCbCr)	100	58	34	8
Proposed (Lab)	100	60	30	10
Dennish Pondolf[3] (RGB)	100	56	30	14
Manual	100	58	42	--

5. CONCLUSION

An automated image analysis procedure has been developed to identify the characteristics of Gram-stain bacterial image. The experimental results are compared with the visual inspection done by a microbiologist and are found to be in good agreement with manual results. The comparison of the identification performance of the proposed method along with other fundamental structural and chemical attributes of bacterial cell walls will be considered in our future work.

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