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Automatic hemolysis identification on aligned dual-lighting images of cultured blood agar plates

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ABSTRACT

Background and Objective: The recent introduction of Full Laboratory Automation systems in clinical microbiology opens to the availability of streams of high definition images representing bacteria culturing plates. This creates new opportunities to support diagnostic decisions through image analysis and interpretation solutions, with an expected high impact on the efficiency of the laboratory workflow and related quality implications. Starting from images acquired under different illumination settings (top-light and back-light), the objective of this work is to design and evaluate a method for the detection and classification of diagnostically relevant hemolysis effects associated with specific bacteria growing on blood agar plates. The presence of hemolysis is an important factor to assess the virulence of pathogens, and is a fundamental sign of the presence of certain types of bacteria.

Methods: We introduce a two-stage approach. Firstly, the implementation of a highly accurate alignment of same-plate image scans, acquired using top-light and back-light illumination, enables the joint spatially coherent exploitation of the available data. Secondly, from each segmented portion of the image containing at least one bacterial colony, specifically designed image features are extracted to feed a SVM classification system, allowing detection and discrimination among different types of hemolysis.

Results: The fine alignment solution aligns more than 98.1% images with a residual error of less than 0.13 mm. The hemolysis classification block achieves a 88.3% precision with a recall of 98.6%.

Conclusions: The results collected from different clinical scenarios (urinary infections and throat swab screening) together with accurate error analysis demonstrate the suitability of our system for robust hemolysis detection and classification, which remains feasible even in challenging conditions (low contrast or illumination changes).

1. Introduction

Besides its leading role in the development of modern microbiology, bacteria culturing on agar plates remains a gold standard procedure for bacteria identification in the workflow of Clinical Microbiology Laboratories (CML) all around the world. By looking at the bacteria culture, the skilled microbiologist obtains a first (so called presumptive) interpretation of possible ongoing infections affecting the patients' heath. This is a fundamental step in the diagnostic process to initiate a prompt and appropriate antibiotic therapy and to guide the selection of specific analytic and diagnostic phases (e.g. antibiogram, MALDI-TOF mass spectrometry, PCR for pathogen DNA or RNA amplification) that allows the confirmation or definition of a more effective treatment. Moreover, there is diagnostically relevant information about pathogenic identification and virulence that is immediately available from bacteria culturing which is impossible or difficult to see from other procedures. One of the most important examples from this is hemolysis associated with bacteria colonies cultured on blood agar plates.

Hemolysis is a peculiar process caused by hemolysin, a group of proteins produced by certain microorganisms, causing the lysis (i.e. the dissolution) of the red blood cell membrane in the growth substrate. The ability to identify the occurrence of this process is very important in order to classify known types of pathogens and, in some type of analysis, such as throat swab screening, is a very effective way of distinguishing positive samples. Moreover, the presence of hemolysis is also a factor of virulence for some bacteria (see for instance E. Coli [1]). Traditionally, microbiologists seek the presence of hemolysis by looking at back-lit plates.

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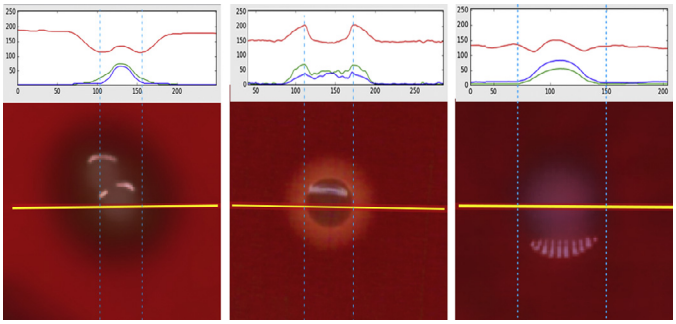

Fig. 1. Color distribution (RGB channels) along the colony axis (yellow line) in the cases of Alpha (Left), Beta (Middle) and Gamma (Right) hemolysis. The images are taken from back-lit plates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Hemolysis can manifest itself in three different ways [2] (also depicted in Fig. 1):

- Alpha (α) hemolysis: partial lysis of red blood cell membrane that visually produces a green or brown discoloration in the medium.
- Beta (β) hemolysis: associated with a complete lysis of red blood cells, it produces a lightened (yellowish) and near transparent halo around the colony.
- Gamma (γ) hemolysis: despite the apparently contradictory term, this indicates the lack of hemolysis. There should be no reaction in the surrounding medium so that the area near the margin of the colony looks uniform and red (the color of the blood agar substrate).

The assessment of the presence of hemolysis has a significant impact in terms of the speed of presumptive identification of dangerous pathogens but does not involve trivial visual tasks for the microbiologist. The difficulties faced are due to the fact that timely recognition may not be easy because of the mild effects of hemolysis, especially in its early stages. In fact, hemolysis produced by bacteria takes time to form, and so the sooner even the initial stages can be recognized, the faster the diagnostic process will be, because it allows the identification on plates incubated for a shorter time.

The need to find automated solutions for the quantitative assessment of bacteria growth (especially for the tedious but diagnostically relevant counting of the number of Colony Forming Units CFU on a plate) is documented in surprisingly far-dated trials [3,4]. Despite the merit and intuitions of these pioneering works, the complexity and variety of pre-analytical procedures in CMLs have meant that sample preparation and plate reading remained manual and exclusively carried out by skilled technicians and microbiologists until recently. Visual CMLs diagnostics using computer vision/intelligence solutions is now strongly motivated by the ongoing rapid diffusion of Full Laboratory Automation (FLA) systems [5] which are expected to change radically the working patterns of modern CMLs. FLA systems are able to process automatically hundreds to thousands of samples per day in standardized conditions and produce huge streams of digital images documenting the bacterial growth from the seeded and incubated plates. This new digital revolution is establishing needs and new outlooks of advanced image analysis and understanding tools able to improve the speed of diagnostics and reliability of the widespread clinical procedures involving (digitized) bacteria culture plates. As a reference FLA system for the creation of our experimental database (see Section 2), we consider the WASPlab^{TM}(Copan, Italy), which is able to record high definition plate images at different incubation times and in different lighting conditions, including combinations of front-light and back-light, to produce naturally appearing images for specialist readings on diagnostic workstations. The hemolysis halo can vary greatly in terms of both color and dimension: the width of hemolysis that we want to isolate goes from 0.25 mm to 2.5 mm and over. High spatial resolution images such as those generated by the line scan system mounted on WASPlab enable the observation of phenomena which are barely visible to the naked eye and our objective is to identify hemolysis as early as possible.

There are two main technical achievements of our work: we designed a keypoint based solution to achieve the robust, fast and accurate alignment of images acquired under widely different lighting conditions and displaying critical aspects that make this task particularly challenging. Based on this alignment we developed a joint dual-image classification of bacterial colony segments for the detection and classification of even the subtle effects produced by hemolysis. This is a task that is often problematic even for the skilled specialist and for which we obtained fully satisfactory results in different clinical scenarios (a near perfect recall on unseen images from clinical routine at the cost of an acceptable number of false positives).

An overall scheme of the proposed system is presented in Fig. 2. This is divided in two consecutive phases. The first issue is related to the fact that the information relevant to the precise detection of hemolytic effects is distributed over images taken with different illumination settings, so these images must first be finely spatially co-registered. This is mainly due to the fact that colony borders are clearly visible in front-light images (while they are blurred in back-light ones), whereas the hemolytic halo is only visible in back-light images. The alignment cannot be taken for granted in practice and, in our case, only a coarse alignment of the two kinds of image is in fact initially provided. We exploit an automatically extracted feature-point based alignment procedure (see Section 3) that, notwithstanding the high morphological and color heterogeneity of the considered images, actually succeeds, in the large majority of cases, in achieving the desired alignment result. Once the images are co-registered, we adopt a machine learning approach based on specifically designed feature evaluation and SVM classification (see Section 4) to detect and distinguish between the different types (Alpha, Beta or Gamma) of hemolysis on both single colony and whole plate setups. The experimental tests, documented in Section 6, were conducted on different clinically relevant scenarios and demonstrate the workability and high performance of our solution. Further considerations and a preliminary evaluation of the clinical impact of the proposed method are given in Section 7.

2. Dataset creation

Since the assessment of the effect of hemolysis requires the presence of red blood cells, here we only consider bacteria cultures on blood agar plates. Blood agar is a generic medium (i.e. it allows the growth of very different types of bacteria) and is one of the most commonly used in microbiology analysis. It is cheaper than other more selective media but, on the other hand, it allows a greater variety of morphology and concentration (load) in the produced growth. To meet the requirements of high reliability and robustness of the diagnostic process, a database of hemolytic and non-hemolytic colonies must be created that actually represents this variety, including many hemolysis examples of different shape, intensity and dimension. Failure to recognize hemolytic segments (i.e. false negatives) must not occur. With this in mind, we collected a dataset of 235 plates, produced by the inoculation on REMEL 5% sheep blood agar media of urine samples collected during routine lab screening tests. Images have been digitized by means of WASPLab automation system which acquires, by linear scanning, 16-mega-pixel color images. These high spatial resolution images (0.0265 mm/pixel) are produced by moving the plate