Engineering Applications of Artificial Intelligence 67 (2018) 100–110

Contents lists available at ScienceDirect

Engineering Applications of Artificial Intelligence

journal homepage: www.elsevier.com/locate/engappai

Automated fluorescence microscopy image analysis of *Pseudomonas aeruginosa* bacteria in alive and dead stadium

Marcin Woźniak [a,*], Dawid Połap [a], Leon Kośmider [b], Tomasz Cłapa [c]

[a] Institute of Mathematics, Silesian University of Technology, Kaszubska 23, 44-100 Gliwice, Poland
[b] School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Department of General and Analytical Chemistry, Jagiellońska 4, 41-200 Sosnowiec, Poland
[c] Department of General and Environmental Microbiology, Poznań University of Life Sciences, Szydłowska 50, 60-656 Poznań, Poland

ARTICLE INFO

MSC:
92C55
68U10
68T10
68T45

Keywords:
Fluorescence microscopy
Bacteria detection
Automated image analysis

ABSTRACT

Fluorescent microscopy techniques take advantage of observing even single cells in live and dead stadium, and make it possible to selectively recognize specific components of biomolecular structures. This methodology is based on Green Fluorescent Protein (GFP) recognition of biochemical activities of individual microbial cells visible in screening. Unfortunately, recognition perception of human professional for fluorescent signals can be affected by various environmental factors what can lead to false interpretation of the results. Therefore intelligent computer method for fluorescent signal counting can be a great assistance at work.

In this article we present experimental research results on the development of new automated fluorescence microscopy image analysis, implemented for *Pseudomonas aeruginosa* bacteria. Proposed method is composed of two stages of image processing. In the first, we enhance the image and extract only important bacteria shapes into simplified image. In the second, this simplification is used for detection of rod shape and spherical shape bacteria. At the end of processing statistical analysis is performed to evaluate number of bacteria in dead and alive stadium.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Research on novel image processing methods result in many applications to various aspects of technology and science. One of them is automated processing of images in search of particular features that can be extracted for classification and recognition purposes. Most often these are used for automated assistance in medicine and biotechnology.

Images can be processed for content retrieval, where devoted metric learning helps on extracting features i.e. useful for automated lung diseases classification presented by Ramos et al. (2016). Similarly to assistance on diseases detection it is also important to work on prevention. To successfully prevent potential infections and diseases it is necessary to early detect microorganisms and bacteria that cause human disease. *Pseudomonas aeruginosa* is increasingly prevalent human pathogen. It is measuring from 0.5 μm to 3.0 μm where its main habitat is mainly soil and water as well as surface of plants. This bacterium is able to colonize various environments utilizing compounds, and therefore posing constant threat to humans. *P. aeruginosa* mainly cause infection in people with weakened immune system i.e. suffering from Cystic Fibrosis (CF) and hospitals patients, where it is one of the most important and dangerous microorganisms causing nosocomial infections. Patients with CF or immunocompromised patients suffering from burns or undergoing cytotoxic chemotherapy are especially exposed to *P. aeruginosa*. Therefore this pathogen is often identified as main reason for high rates of mortality as reported by Van Delden and Iglewski (1998), Garred et al. (1999) and Hentzer et al. (2003). According to the research of Cirz et al. (2006) treatment of *P. aeruginosa* infections is very difficult because of the high resistance to antibiotics and ability to interfere with the host immune system as shown by Akiko et al. (2008). Despite progress in medicine, CF and other *P. aeruginosa* infections are still problematic since knowledge about pathogenicity of this bacterium remains incomplete. Therefore prevention from infections with *P. aeruginosa* is necessary, i.e. by early detection of this bacterium and therefore extermination form the environment.

* Corresponding author.
E-mail addresses: Marcin.Wozniak@polsl.pl (M. Woźniak), Dawid.Polap@polsl.pl (D. Połap), Leon.Kosmider@gmail.com (L. Kośmider), Tom.Clapa@gmail.com (T. Cłapa).

https://doi.org/10.1016/j.engappai.2017.09.003
Received 11 December 2016; Received in revised form 26 April 2017; Accepted 4 September 2017
0952-1976/© 2017 Elsevier Ltd. All rights reserved.

1.1. Related works

It is possible to fight against medically important microorganisms, however in advance we need to identify them. Classic detection methods are based on microflora cultivation what involves selective media, however it is time consuming, laborious and prone to statistical and methodological errors as discussed by Moter and Gobel (2000). Therefore fluorescent microscopy techniques take advantage in recent years. This methodology allows to observe single cells in live or dead stadium. Moreover we are able to selectively recognize specific components of biomolecular structures what is very important for proper comprehensive analysis of images. Starkuviene et al. (2004) and Bottari et al. (2006) presented research results on application of fluorescent microscopy screening to identify microorganisms in complex microbial communities. Similarly Gitai (2009) and Borkowski et al. (2015) presented studies on cytotoxic bacterial cells by application of fluorescence screening techniques. Method presented in Starkuviene et al. (2004) is based on Green Fluorescent Protein (GFP), that can be implemented in vary studies. However, to investigate location of GF-protein it is necessary to use fluorescent microscopy. Identification and localization of green signal can be hard to do, especially in cases when the signal is obtained as a large number of fluorescent cells. Thus, computer programs and algorithms, able to identified that signal without mistakes are necessary. Similar problems with cell identification we can observe after studying work (Bottari et al., 2006). FISH (Fluorescent In Situ Hybridization) method can be a powerful tool for identification of microorganisms and specific genes even in multispecies communities. Unfortunately, without 'good eye' of researcher for fluorescent signals identification, errors could be made, thus lead to false interpretation of the results. Multiple staining techniques applied to fluorescent microscopy can help on information retrieval to identify biochemical activities of individual microbial cells. Unfortunately this kind of assay is limited to balance dye properties, instrumental capabilities and software design according to Brehm-Stecher and Johnson (2004). Constant development in fluorescent probe techniques make it possible to implement intelligent software able to assist on imaging acquisition, improving resolution, and quantitative analysis for efficient documentation of bacteria detection. In this case computer method for fluorescent signal counting would be a great simplification of time-consuming calculations.

In this article we discuss developed method to analyze fluorescent microscopy images of *P. aeruginosa*. Motivation for our research comes from the fact that even human professionals recognition may be affected by various environmental factors what can lead to false interpretation of the results. In the research we have developed a methodology for fluorescent images processing that enables counting of microorganisms in constant time without influence from environmental factors. This methodology contributes to the development of automated decision support in medicine, where in particular this type of image processing can be useful in laboratories working on microbiology and biotechnology, where it is necessary to evaluate medicine or treatments by fast and reliable estimation of the population of bacteria.

The methodology presented in the following sections is discussed for sample preparation for fluorescence screening. Applied intelligent processing of the image detects *P. aeruginosa* in alive and dead stadiums. Parallel to this, proposed method is performing statistical analysis of detected bacteria to calculate and classify depicted bacterial shapes into two main classes: alive stadium and dead stadium. Number of bacteria classified as alive can indicate how dangerous for humans examined sample and therefore source environment can be. Number of bacteria in dead stadium can indicate that applied chemical treatment is efficient and source environment becomes barren what prevents growth of bacteria, see Fig. 1.

2. Proposed method

Classic approach to fluorescence microscopy screening is based on five independent steps: sample preparation, image acquisition, data handling, analysis and interpretation discussed by Pepperkok and Ellenberg (2006). In these steps information contained in the image is processed to evaluate number of bacteria in alive and dead stadium. This evaluation is very important to judge the sample and therefore environment that it was taken at. Unfortunately, analysis and interpretation are mainly not automated and therefore cause problems to medical staff at clinics and microbiology institutes.

We propose novel approach to the process of automated evaluation of fluorescence microscopy images, in which input image $\mathcal{I}$ is processed to extract separately bacteria in dead and alive stadium. This extraction is crucial for the following statistical analysis to calculate the number of dead and alive bacteria, which gives explicit answer about the results of medical or chemical treatment applied to sanitize a source place of examined sample. The method is developed to assist in automated estimation of fluorescent microscopy images for the same microscopic field but at different filters for alive bacteria stadium and for dead bacteria stadium. This is non trivial operation, since to perform this calculation we must count only shapes of *P. aeruginosa* bacteria. Moreover various bacteria may be combined with each other so we must skip the large aggregate structures for which it is not possible to judge what is the participation of dead to alive.

The process starts when a medical sample at the source place is taken. It is placed in fluorescence microscope, where two pictures are taken: $(\mathcal{I}_{alive})$ in blue tint for alive stadium and $(\mathcal{I}_{dead})$ in red tint for dead stadium. The problem is that many bacteria are visible in both pictures due to morphology of dying. Therefore automated approach is necessary to assist in efficient evaluation. Proposed method is using both input images of each sample, which are processed in the following stages. In the processing we use pixels $\mathbf{x_i} \in \mathcal{I}$ and their spatial coordinates $(x_{i,1}, x_{i,2})$ values in row and column.

2.1. Stage I — preprocessing

First stage is applied to both images $(\mathcal{I}_{alive})$ and $(\mathcal{I}_{dead})$ for which we apply conversion to gray scale. Next we improve the image contrast and reduce pixels presenting noise that come from the source image or appear after transformations. After, images $(\mathcal{I}_{alive})'$ and $(\mathcal{I}_{dead})'$ are processed to convert them into simplified images $(\mathcal{I}_{alive})''$ and $(\mathcal{I}_{dead})''$ showing only white shapes of bacteria over black background. Finally heuristic method is applied to verify extraction results, which are forwarded as images $(\mathcal{I}_{alive})'''$ and $(\mathcal{I}_{dead})'''$ to detection stage.

2.1.1. Conversion and enhancement

Conversion between input images and final simplified black and white version is necessary for automated classification, since it is hard to compare pictures in different colors. We need to unify pictures $(\mathcal{I}_{alive})$ and $(\mathcal{I}_{dead})$ to a similar scale, in which we simultaneously sharpen the shapes of bacteria visible in both images through the use of contrast modeling. At the same time, because of applied processing, we are able to endure a part of the picture noise parallel to enhance shape edge of the visible bacteria, which is then suitable for processing by the proposed statistical detector.

Filtering to gray scale is done by application of recommendation ITU-R Rec. BT.709-5 by ITU Radiocommunication Sector from Geneva Switzerland, which sometimes is simply called Lumia standard. Transformation to gray scale utilize simple process for $R(\mathbf{x_i})$ (red), $G(\mathbf{x_i})$ (green) and $B(\mathbf{x_i})$ (blue) values of pixels. We replace original red, green, and blue values with the new gray value according to

$$Gray = R(\mathbf{x_i}) \cdot 0.2126 + G(\mathbf{x_i} \cdot 0.7152 + B(\mathbf{x_i}) \cdot 0.0722). \quad (1)$$

Constant values in Eq. (1) are presented after calibration of the method for most precise detection. In the following numerical experiments we

دریافت فوری ← متن کامل مقاله

ISIArticles
مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات