ζ-Bidens®: A novel computer program for studying bacterial colony features

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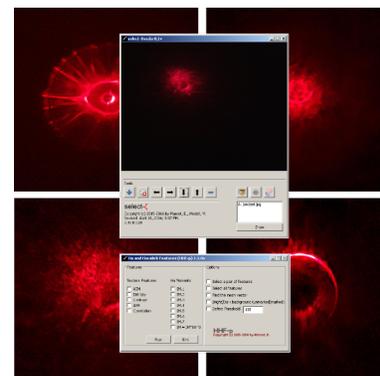2. Fisheries Research Center, Food Safety Department, 5ta. Avenida y 246, Playa, Havana, Cuba

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Abstract

Optical forward-scattering systems supported by image analysis methods are increasingly being used for rapid identification of bacterial colonies (*Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio cholera*, etc.). The conventional detection and identification of bacterial colonies comprises a variety of methodologies based on biochemical, serological or DNA/RNA characterization. Such methods involve laborious and time-consuming procedures in order to achieve confirmatory results. In this article we present ζ-Bidens, a novel software for studying bacterial colony features. The software ζ-Bidens was programmed using C++, Perl and wxBasic programming languages. A graphical user interface (GUI), an image processing tool and functions to compute bacterial colony features were programmed. We obtained versatile software that provides key tools for studying bacterial colony images as: texture analysis, invariant moment and color (CIELab) calculation, etc., simplifying operations previously carried out by MATLAB ® applications. The new software can be of particular interest in fields of microbiology, both for bacterial colonies identification and the study of their growth, changes in color and textural features. Additionally ζ-Bidens offers to the users a versatile environment to study bacterial colonies images. ζ-Bidens is freely available from:

⊠ http://ds-bidens.sourceforge.net/

Keywords: bacterial colony; free software; visual patterns; image analysis

1 INTRODUCTION

$\mathscr{I}$n recent years have been increasing interests in developing computer programs for studying biological images. Advances in imaging devices have been remarkable, and high-quality images can now be easily acquired. However, less progress has been made developing software for the specific requirement of these image analyses. Multipurpose software packages are expensive and must be customized according to the user´s purpose, re-

quiring considerable expertise and time [1]. Most commercially available software packages are proprietary, meaning that the underlying methods of analysis are hidden from the researcher [2].

Colonies display bacterial capacities for multicellular coordination which can be useful in nature where bacteria predominantly grow as films, chains, mats and colonies. Multicellularity regulates many aspects of bacterial physiology, including DNA rearrangement systems. In some bacterial species, colony development involves swarming (active migration of cell groups). Swarm colony development displays precise geometrical controls and periodic phenomena [3].

The conventional detection methods based on biochemical, serological or DNA/RNA characterization involves additional steps of sample enrichment in a liquid culture medium, plating on selective agar media to obtain single colonies, and use of special reagents for DNA isolation and PCR. The detection process could take about $3 - 5$ days to get confirmatory results and are labor intensive. PCR-based methods though faster is highly dependent on efficient DNA isolation and also is limited by its accuracy in detecting live bacterial cells, leading to false positive identification. Rapid identification of *Listeria monocytogenes* and other Listeria species (with 91-100 % accuracy), *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholera* is possible using optical forward-scattering system (scatterometer) and image analysis software for features extraction (scalar values), calculated from images of the scattering patterns (scatter images) [4,5] .

Study of bacterial colony features (BCF) can be a powerful tool to develop new methods for bacterial colonies rapid identification, but it is not easy for a researcher because it involves several complex procedures, such as image processing and features calculation. In this article we present ζ-Bidens, a novel computer program for studying BCF. ζ-Bidens is characterized by the following features: **(1)** The software is easily operated with the aid of a graphical user interface (GUI); **(2)** No special computer devices for image processing are required; **(3)** A large number of samples (say 10,000) can be processed; and **(4)** The calculated features (scalar values) can be easily exported for analysis by other software for further studies. ζ-Bidens is free and easy-to-use software.

2 FEATURES AND FUNCTIONALITY

§ Image Analysis

A tool named Select-ζ (Fig. 1, b) allows selecting bacterial colony images (BCI). First, a user takes images of samples (colonies on agar plates or scatter images (Fig.1, e)) using a digital imaging device, and imports them as full-color bitmap files into a computer memory device. Select-ζ uses this image files as input files and a user processes them by the following procedure. The user must define bacterial colony radium using *the radium button* and clicking on bacterial colony center. Next, it is possible to select bacterial colonies clicking on the center of them. The user could undo/save bacterial colony selection utilizing *undo and save buttons*. The bacterial colonies selected are saved at ζ-*Bidens Virtual Memory* (BVM), waiting for features calculations (scalar values).
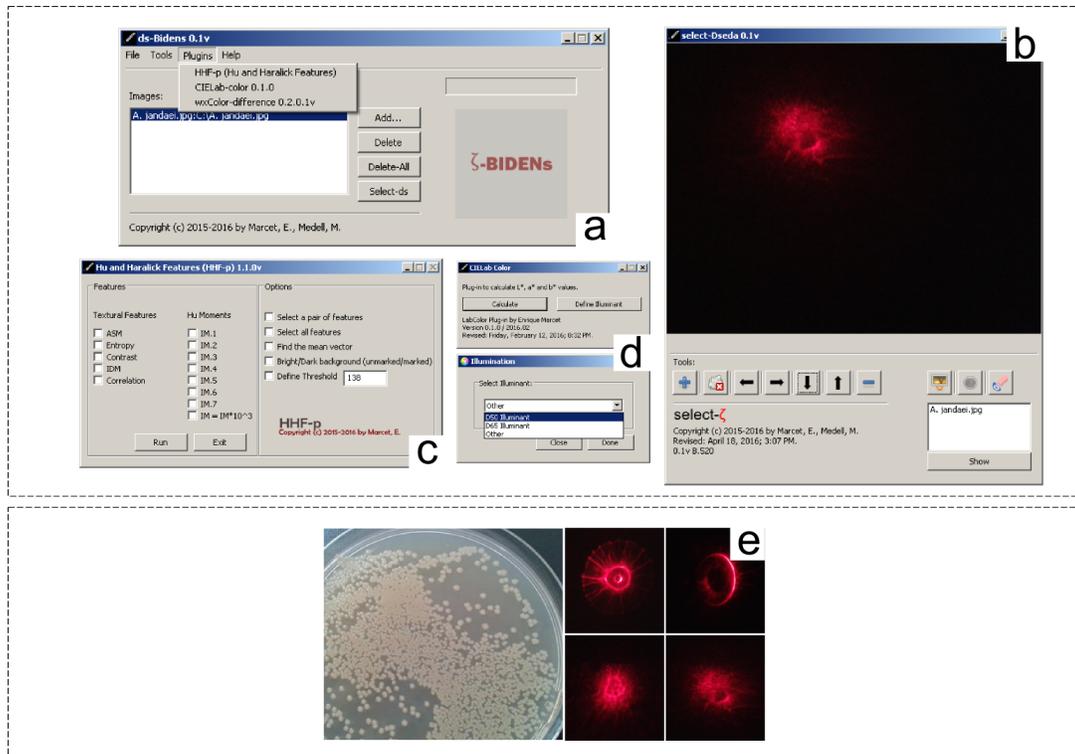
Figure 1. ζ-Bidens principal tools: (a) the main ζ-Bidens menu comprises plug-ins, tools, help and file options. Using *Add* button the user can import full-color images for subsequent image processing; (b) Select-ζ application is a tool for selecting bacterial colonies on imported images; (c) HHF-p: plug-in for features calculation (Haralick Textural Features and Hu Invariant Moments); (d) CIELab-color plug-in allows the calculation of CIELab color of selected images; (e) *Aeromonas spp* scatter images (right) and *Aeromonas spp* colonies on agar plates (left).

§ Features Extraction

ζ-Bidens possess several plug-ins for features extraction such as HHF-p, CIELab-color and wxColor-difference as shown in Figure 1. HHF-p (Fig.1, c) allows the calculation of Hu Invariant Moments [6] and Haralick Textural Features [7]. Through HHF-p the user can make **.csv** files with feature values. CIELab-color plug-in (Fig.1, d) allows the calculation of BCI color, while wxColor-difference uses CIEDE2000 method [8] to obtain color-difference between two bacterial colony images.

§ Plug-in Manager and Repository

Plug-in Manager Application (PMA) allows adding/deleting plug-ins. The user can add new plug-ins by the following procedure: a user must download new plug-ins from software website and to add them using PMA. Computer programmers could develop new plug-ins for ζ-Bidens using wxBasic language (http://wxbasic.sourceforge.net/) and for improving existent plug-ins. Following the software user's guide, computer programmers can convert C++/Perl plug-ins in order to use them in ζ-Bidens.

§ Example of use

Pathogenic bacterial contamination in products not only put the public at risk, but also is costly to companies because of routine product testing, product recalls and liabilities. For example, *Listeria monocytogenes* is one of the major foodborne pathogens with the highest mortality rate (20-28%) among the food pathogens.

Traditional methods for detection and identification of bacterial contaminants, such as *Listeria monocytogenes*, typically require 3-7 days and use biochemical or antibody or nucleic acid based tests [9]. ζ-Bidens helps to study *Listeria monocytogenes* bacterial colony scatter images obtained using a scatterometer system [9] by improving options, simplifying the process and therefore shortening the time of the assay, etc.

§ Implementation and Availability

ζ-Bidens is implemented in C++, wxBasic and Perl. The entire package is freely available for downloading from: ✉ http://ds-bidens.sourceforge.net/ and includes: **1)** the ζ-Bidens binary executable, **2)** all source codes with a Makefile, **3)** an actualized plug-ins repository, **4)** a user manual, **5)** a language package (English and Spanish), **6)** an example data and examples of running the different options offered by the software. The software was designed and optimized for Windows operating systems, however, can also be adjusted for Linux and Mac OS (changing wxBasic and Perl Virtual Machines).

3   Conclusion

We have developed ζ-Bidens, an extensive software that facilitates bacterial colony images studies. We are continually developing the software and upcoming versions in the near future will offer additional plug-in. Additional plug-ins can be developed on demand according to the new required characteristics of the users.

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