Development of automatic detection and identification instrument for bacterial colonies with light scattering

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ABSTRACT

A novel microbiological instrument that automatically locates the bacterial colony and captures the forward-scattering signature is presented. The new instrument consists of a two laser source, CCD image sensors and line scanner. The line scanner and it's related optics utilizes transmission characteristics of Petri-dish plate and captures the transmitted intensity to provide the number of colonies and 2-dimensional coordinate information of the bacterial colonies. Image processing algorithm is applied to separate the background image from the bacterial colonies and 2-dimensional center of each colony is calculated. Then the Petri-dish plate is automatically aligned to respective centroid with trajectory optimization method such as Cross Entropy (CE) algorithm. Second monochromatic image sensor captures the forward-scattering signatures and performs a fine adjustment to align the bacterial colonies to the incident light via minimizing the 2-dimensional balancing of the quadrant signal. The final scattering signature is stored and analyzed to provide rapid identification and classification of bacterial sample.

INTRODUCTION

Bacterial contamination in food and other source is monitored through standard laboratory practice of counting the number of colonies (colony forming units, CFU) and there are various different ways to improve the colony counting efficiency through computerized image processing. Previous research has applied distance transform, multiple/adaptive threshold, predefined intensity model, and modified Hough transform to enhance the image processing schemes. However, additional step of identifying and classifying the types of bacterial colony is required to deliver the valuable information of pathogenicity. Identification and classification of microbiological samples are performed using a serological method, morphological method, and proteomics/genomics. Among these, morphological method applies an interrogating agent to extract the internal information from the sample such that either individual cell characteristics or colony morphology characteristics are used to rapidly and accurately identify the microorganism. Recent study on from Guo and other colleagues showed the possibility of using transmission and reflection type of bacterial colony scattering and suggested the feasibility of using transmission type (forward light scattering) as an rapid, non-invasive and agent-free method of bacterial colony detection and using Zernike moment invariants for classification[1]. In addition, Bae[2] *et al* proposed the biophysical model to predict the forward scattering along with newly designed forward-scatterometer which is named as **B**acterial **R**apid **D**etection using **O**ptical scattering **T**echnology (BARDOT). Here we suggest a combining these two main instruments such that widely-used colony counting instrument could be extended to identify the species of bacterial colony under investigation without the need of expensive equipment such as PCR. The proposed instrument could be manufactured as a portable-laboratory system which could be directly used in the field of outbreak area.

MATERIALS AND METHODS

Hardware of BARDOT system

As shown in Fig. 1, automated BARDOT system consists of following three major components: colony counter/locator part, forward-scattering part, and 2D motorized stage part. The colony locator and counter part is composed of line scan laser (Lasiris 501L-635-5mW), line scan optics using two cylindrical lens (f=198 mm) and, line scanner (Hamamatsu 512 pixel, 25 μm pitch, and 2.5 mm length). The line scan laser is a 635 nm laser with 5mW mm circular beam.

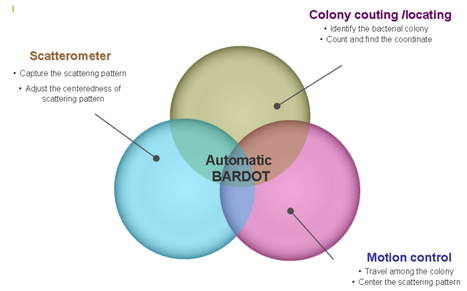

Figure 1. Components of BARDOT system

The line scan optics produces laser line of 4" x 0.5 mm. The line scanner has 512 resolution with 2.5 mm length. The laser line scan is adopted to produce a 2D transmission map of bacterial colony and agar plate which is later processed to find the number of bacterial colony and the 2D location of the center of colonies. After the line scanner provides the information about the sample plate, the sample is translated to a forward scatterometer position. The forward-scattering part is composed of a laser diode module of 635 nm wavelength (Lasiris 501L-635-1mW) and a monochromatic CCD image sensor (Silicon Imaging SI1280 FM-CL) with 1280 × 1024 resolution with 6.7 × 6.7 μm unit pixel size. Motion control part consists of three axis stepping motor (Velmex NEMA 17) with the controller and the pulse and encoder (US digital E2-400-197 IHT) signal is communicated through NI PCI-6602.

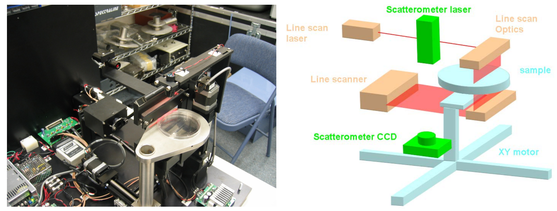

Figure 2. Picture and schematic diagram of BARDOT

Software of BARDOT System

The software part also consists of three parts. Automatic BARDOT system acquire the sample information and align the sample to the incident laser until the last scattering signature of the colony is captured. For the colony locating/counting part, a Visual C++ program is written to locate the center of the bacterial colony. Based on the transmission data from the line scanning, a threshold 2D map data is constructed. Based on this data, we apply a region-growing segmentation[3] algorithm to count the number of colonies on the 2D map data. Simultaneously, the outer border of the segmented cluster is acquired such that the degree of circularity is computed to filter the colony with background noises. In addition to that, the degree of circularity information is applied to filter the colonies that are multiply touching each other since these kind of colony produces distorted scattering pattern not generated from their individual morphological differences.

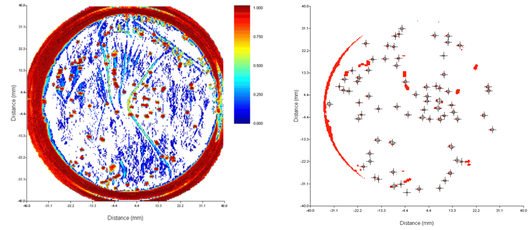

Figure 3. Transmission data of bacterial colony with the center location

After the colony is counted and located, there is an issue of how efficiently traveling through all of the identified colonies. The 2-dimentional loop of the Visual C++ code shows a horizontal and vertical loop. Therefore the growing region algorithm will assign a colony count value whenever it finds a cluster starting from the bottom left corner. Therefore natural numbering sequence will follow the scanning loop and the total traveling trajectory will include much of redundant movement which is not optimized for fast and efficient traveling. This is the so-called Traveling Salesman Problem (TSP) which is finding the route that minimizes the total travel length and visiting the each location only once. Here we applied the TSP algorithm with Cross Entropy (CE) method[4] as shown in Fig. 4. Once the centroid of each cluster is computed, we create a cost matrix which has the travel length from one node to the adjacent node and compute the trajectory that minimize the entropy of the all possible traveling route.

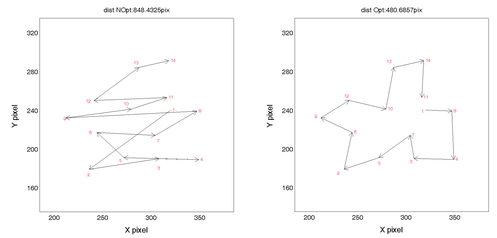

Figure 4. Comparison of non-optimized and optimized traveling with cross entropy (CE) algorithm

After the traveling trajectory has been determined the petri-dish plate is moved to the computed center location. Although mechanical and geometrical center is computed accurately, the scattering center which creates a concentric scattering pattern might be different.

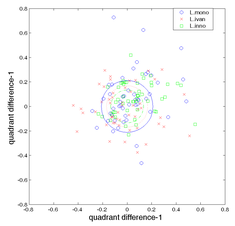

Figure 5. Quantitative assessment of centeredness of scattering pattern from human operation for three *Listeria* species

Quantitative value of centeredness is computed through quadrant balancing. The result of human operated scattering image shows a 1σ error of 0.13-0.23 which is equivalent to 0.13 mm – 0.2 mm from the scattering center as shown in Fig. 5. The displacement of the motor showed a nonlinear relationship since the characteristics of light scattering of Fig.6. The dash-dot line case shows when the offset of computed centroid of bacterial colony and true center is less than 100 μm.

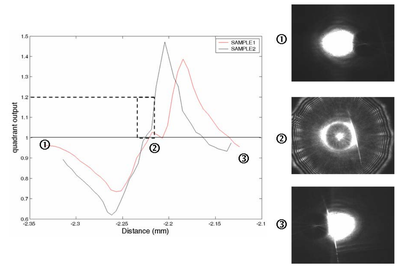

Figure 6. Relationship of quadrant balancing signal and scanning across the *L. monocytogenes* colony

CONCLUSIONS

The hardware and software component of BARDOT system has been constructed. The hardware consists of colony locater/counter, forward-scatterometer, and motion controllers. The software designed to located and captured the centered scattering pattern is segmentation with region growing algorithm, trajectory optimization algorithm with cross entropy (CE), and centering algorithm with balancing the quadrant signal

REFERENCES

1. B. Bayraktar, P. P. Banada, E. D. Hirleman, A. K. Bhunia, J. P. Robinson, and B. Rajwa, J. Biomed. Opt. 11, 034006 (2006)
2. E. Bae, P. P. Banada, K. Huff, A. K. Bhunia, J. P. Robinson, and E. D. Hirleman, Appl. Opt., 46, 3639-3648 (2007)
3. D. Phillips, *Image processing in C*, 1st Ed. (R&D Publication, Inc, 1994).
4. R.Y. Rubinstein, A Unified Approach to Combinatorial Optimization, Monte-Carlo Simulation, and Machine Learning, 1st Ed. (Springer-Verlag, 2004).

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