Early Identification of Microcolonies via Forward Scattering Technology

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Overview

A label-free and nondestructive optical elastic light scattering method has been developed for food-borne bacteria detection and identification. The developed system (also called BARDOT) has achieved 91-100% accuracy in detection and identification of bacterial cultures at species and strain levels for different genera such as Listeria, Staphylococcus, Salmonella, Vibrio, and Escherichia [1-3]. However, the current investigation requires 24-36 hours of incubation for colonies to grow into the size of 1.2~1.5 mm. As a work shift (8 hours) is typically done by both industry and government agencies, which produce micro-colonies of around 100-200 µm for most of the most common fastidious bacteria, we proposed to extend the current research and continuous analysis of scatter signatures of micro-colonies with the goal of reducing the overall bacterial detection time. In order to achieve this, we developed the two objectives as follows:

1. To understand the correlation between the forward scattering patterns and the morphological features of bacterial colonies
2. To extend the application to microcolony detection

Methods and Approach

A bacterial colony is a biological spatial light modulator which modulates the phase and amplitude of the incident wavefront as it propagates through it [4]. A Gaussian shape with two key parameters (central height $H_0$ and 1/e diameter $D_0$) is adopted to evaluate the amplitude and phase modulation to the incoming wavefront. The scattering pattern of the far-field is correlated to the modulated wavefront through a scalar diffraction theory. One of the two key parameters of a Gaussian is varied incrementally, while the other is kept constant to numerically calculate the scattering patterns and then to compare them among significant trends.

Maximum Diffraction Angle & Number of Diffraction Rings

The local maxima is the result of the interference of two or more wavevectors pointing at the same direction. Therefore, the maximum diffraction angle is governed by the direction of the wavevector which is normal to the emerging wavefront. This implies that the magnitude of the slope of the wavefront is the key factor in determining the maximum diffraction angle. The diffraction rings are dependent upon the center thickness and not dependent on the colony diameter. This is because the optical path difference (OPD) increases as the $H_0$ increases and so does the number of diffraction rings.

Theoretical Background

A diffraction based model for forward light scattering (FLS) from bacterial colonies

Scattering Pattern Comparison from μ-BARDOT system

Experimental Results

Morphological measurements of microcolonies from CDM

Profile measurement was performed on three species: E. coli DH5α, Listeria monocyogenes[4344] and Salmonella Montevideo. To quantitatively address the aspect ratio difference for the three species, 1-D cross-sections of the representative height profiles were compared in Fig 5(a). The profile of (red line) showed approximately 200 µm diameter. Experiments were conducted to measure and compare morphological differences among E. coli, Listeria and Salmonella. While $E$. coli was indicated to be more of a ‘flat’ colony shape for this diameter range.

Some correlations between colony morphology and its forward scattering pattern

$H_0$ increased $D_0$ constant, the maximum diffraction angle and the total number of rings increased.

$H_0$ constant $D_0$ increased, the maximum diffraction angle decreased and the total number of rings remained constant.

Conclusions and Future Work

The main purpose of the research is to understand the basic biophysics behind this FLS phenomenon and to explore the possibility of extending this technique for microcolony detection. When laser is directed through a colony of multiple phase structures and morphological information is encoded on the passing optical wavefront which results in unique forward scattering patterns on the imaging plane. In order to quantify the amplitude and phase modulation, a Gaussian stack-layer model was proposed based on the profile measurement data. Correlation between colony morphology and its forward scattering pattern was established through computational simulation. Experiments were conducted to measure the colony morphology with focus on microcolonies to identify their phenotypic differences. Light scattering experiments were performed on an μ-BARDOT system and the results were compared with the simulation results.

In the future, we are going to do the quantitative phase measurement using Transport of intensity Equation (TIE) [6, 7] to solve the parabolic wave equation. The basic idea is to reconstruct a phase map based on the light intensity and its longitudinal derivative measurements along the propagation direction. Fig 8 shows the mechanism how to retrieve quantitative phase using one focused and two defocused (+/-4z from the focal plane) microscopic images.

References