# 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*, *Staphulococcus*, *Salmonella*, *Vibrio*, and *Escherichi* [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 desired by both industry and government agencies, which produces micro-colonies of around 100~200 μm for most interested species. Therefore, we proposed to extend the current research and continue 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:

❑To understand the correlation between the forward scattering patterns and the morphological features of bacterial colonies

❑To extend the application to microcolony detection

- To experimentally characterize morphology of microcolonies
- To predict scattering patterns from morphology measurements
- To compare the predicted scattering patterns with experimental results to validate our understanding

In addition, we concluded the incubator design concept with a final design proposal to facilitate the complete automation of this technology in detecting bacterial colonies in the hundred micro range.

## Methods and Approach

A bacterial colony is a biological spatial light modulator which modulates the phase and amplitude of the incoming wavefront as it propagates through it [4]. A Gaussian shape with two key parameters (central height $H_0$ and 1/e3 diameter $D$) is adopted to evaluate the amplitude and phase modulation to the incoming wavefront. The scattering pattern on the far field is correlated to the modulated wavefront through a scalar diffraction theory. One of the two morphology parameters is varied incrementally, while the other is kept constant to numerically calculate the scattering patterns and then to compare among them for significant trends.

With the understanding of how the morphology characteristics of colonies affect the features of the forward scattering patterns, we designed experiment to measure and compare morphological differences among different bacterial species. A laser confocal displacement meter (CDM) was used to measure the colony morphology, phase contrast images were taken for the measured colonies for reference. A μ-BARDOT system was setup for acquiring the forward scattering patterns from microcolonies. The experimental work was compared with the theoretical prediction.

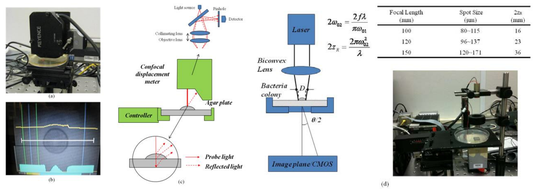
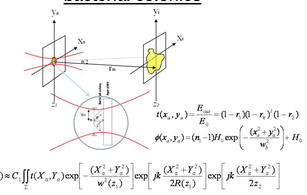



Fig 1. Experimental Apparatus: a) laser confocal displacement meter (CDM); b) Video output from CDM; c) Schematic diagram of CDM; d) μ-BARDOT system for capturing the scattering pattern

## Theoretical Background

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



$$t(x_a, y_a) = \frac{E_{out}}{E_{in}} = (1 - r_1)(1 - r_a)^2(1 - r_2)$$

$$\phi(x_a, y_a) = (n_1 - 1)H_0 \exp\left(-\frac{(x_a^2 + y_a^2)}{w_0^2}\right) + H_0$$

$$E_z(X_s, Y_s) \approx C_z \iint_{\Sigma} t(X_a, Y_a) \exp\left[-\frac{(X_a^2 + Y_a^2)}{w^2(z_1)}\right] \exp\left[jk\frac{(X_a^2 + Y_a^2)}{2R(z_1)}\right] \exp\left[jk\frac{(X_s^2 + Y_s^2)}{2z_2}\right]$$

$$\times \exp[jk\phi(X_a, Y_a)] \exp[-i2\pi(f_X X_a + f_Y Y_a)] dX_a dY_a$$

Fig 2. Schematic diagram to illustrate the scalar diffraction model with a Gaussian shape to describe colony morphology and a stacked layer model to approximate the phase and amplitude modulation [5]

### Some correlations between colony morphology and its forward scattering pattern

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


Fig 3. Radial plot of the forward scattered light intensity distribution on the image plane calculated with Gaussian shape assumption and scalar diffraction theory [5]

### Colony Morphology vs. Ring Count

$H_0$ was the major factor contributing to the total number of diffraction rings observed at the imaging plane irrespective of the $D$ value. The total number of peaks which remained relatively constant across the diameter but increased as the $H_0$ values increased.

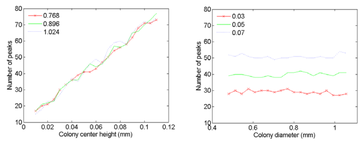



Fig 4. The number of ring counts vs. colony height for three different diameters (left); The number of ring counts vs. colony diameter for three different heights (right). [5]

### 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 $H_0$ is increased, and so does the number of diffraction rings.

## Experimental Results

### Morphological measurements of microcolonies from CDM

Profile measurement was performed on three species: *E.coli* DH5α, *Listeria* monocytogenesF4244 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 with 9.3 μm peak value which gave approximately 20:1 aspect ratio while the rest two bacteria showed approximately 10:1 ratio. Fig 6(b) displays the aspect ratio distribution for ten bacterial colonies for each genus. The result clearly indicated a trend of 10:1 aspect ratio for *Salmonella* and *Listeria*, while *E.coli* was indicated to be more of a 'flat' colony shape for this diameter range.

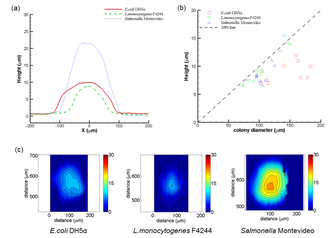



Fig 5. Profile comparison for three different bacteria:
(a) 1-D height profiles across the peak locations in microcolonies;
(b) the aspect (diameter/height) ratio distribution with a 10:1 ratio line;
(c) 2-D contour plots corresponding to the 1-D profile plots in (a).

### Scattering Pattern Comparison from μ-BARDOT system

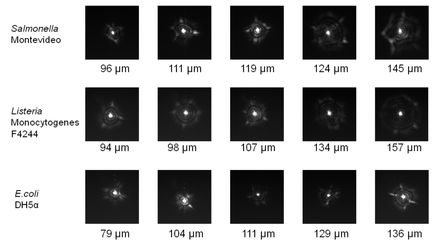



Fig 6. A series of forward scattering patterns for three genera of microcolonies of (Top) *Salmonella* Montevideo; (Middle) *L.moncytogenes* F4244 and (Bottom) *E.coli* DH5α.

### Experimental Results and Theoretical Prediction Comparison

$$N_{rings} \cong \frac{\Delta\Phi}{2\pi} \qquad \theta/2_{max} \cong \frac{1}{k}\left(\frac{d\Delta\Phi}{dr}\right)_{max}$$

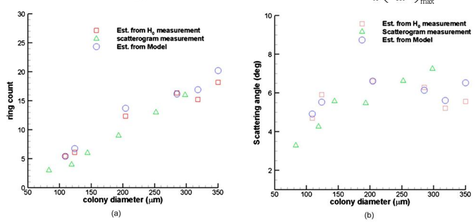



Fig 7. Comparison among the experiment, the simulation results and the estimation equations. (a) shows the comparison of the number of rings versus the colony diameter up to 350 μm. (b) displays the similar comparison for maximum diffraction angle. [5]

## 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, its complicated phase structure 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 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 (+/-Δz from the focal plane) microscopic images.

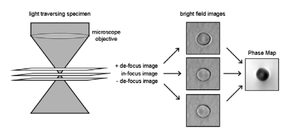



Fig 8. Quantitative phase measurement using TIE

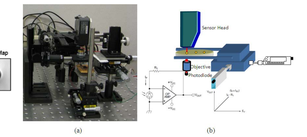



Fig 9. Absorption measurement experimental setup

Secondly, phase and profile information gives the real part of a complex refractive index, and the imaginary part which is called the extinction coefficient represents the amount of absorption loss when the electromagnetic wave passes through the object. Fig 9. shows the ongoing platform for transmittance measurement. As the laser light from the sensor head scans through the surface of the colony to obtain its profile data point by point, we have a micro-objective lens located at the bottom to collect all the transmitted light and focus it onto a photodiode, which induces a current amplified by an op-amp to provide a linear relationship between the light intensity and the voltage reading. The current system development is still ongoing.

## References

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