

# Towards a Single Parameter Sensing for Bacteria Sorting through Optical Fiber Trapping and Back-Scattered Signal Analysis

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**Abstract:** We investigated if a recently proposed method can differentiate yogurt bacteria trapped by a polymeric lensed fiber tip, through back-scattered signal analysis. Results suggest that it can be a valuable contribution for foodborne analysis/bacteria identification. © 2018 The Author(s)

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## 1. Introduction

Pathogens and other “good” bacteria are virtually everywhere: in food, soil, water and even in the human digestive flora [1]. The presence of some pathogens in food or water is a serious concern, considering that a single microorganism can lead to unwarranted deaths and illness [1]. The advent of Biotechnology has greatly contributed to the development of novel food testing assays [1, 2]. However, there is an increasing need for more specific, faster and more sensitive methods, given the rising number of ready-to-eat meals commercially available [1]. The current methods for bacteria sorting and differentiation are based on conventional techniques, which rely on the isolation and proliferation of the selected microorganism(s); or bioluminescence processes; Flow Cytometry techniques; immunological methods; Polymerase Chain Reaction (PCR) or Spectroscopic techniques [1]. However, the majority of these methods are complex, time consuming and expensive. A rapid method able to identify and isolate a single microorganism can be of utmost importance for foodborne pathogens detection and water quality testing [1]. Taking these aspects into consideration and the possibility of miniaturization of devices, optical fiber tools have been considered valuable alternatives to the above methods [2].

Considering the low-cost and rapid method to simultaneously trap and differentiate synthetic and biological particles recently proposed in [3], we decided to investigate whether it is able to distinguish smaller specimens as different types of bacteria using the same type of polymeric lensed optical fiber tools. Through this method, a single particle can be simultaneously isolated through optical trapping and identified uniquely using a short-term portion of the back-scattered signal [4]. Using the same type of polymeric lens on the top of optical fibers, we tried to trap and differentiate two types of yogurt bacteria (*Lactobacillus Acidophilus* and *Streptococcus Thermophilus*) using a single signal-derived attribute created using Multivariate Data Analysis. The method proposed here showed a similar performance to the method proposed in [4]. Thus, it can be a great contribution to the development of a novel low-cost and rapid method for bacteria isolation and identification.

## 2. Methods

### 2.1. Polymeric Lenses Fabrication Method

The polymeric lenses used for trapping were fabricated on top of cleaved optical fibers (Thorlabs SM 980-5.8-125, Thorlabs, New Jersey, USA) through a self-guided photopolymerization method developed by Soppera et al. [3, 5]. This process is mainly based on the assemble of cross-linked polymeric structures through monomers linking - in this case of pentaerythriol triacrylate (PETIA) - triggered by light of a specific wavelength [5, 6]. A solution more commercially known as Irgacure 819, which is sensitive to wavelength values between 375 and 450 nm, was used as photo-initiator in this process [6]. Considering these specifications, a violet 405 nm laser (LuxX cw, 60 mW, Omicron) was then used to trigger the polymeric cross-linking chain reaction [6]. The fabrication process can be summarized by the following steps. At first, the cleaved end of an optical fiber is positioned vertically in a moving stage, while the violet laser is aligned to be injected in the optical fiber distal end, to excite its fundamental mode (note that the selected fiber is multimode at the polymerization wavelength value). Then, the optical fiber extremity is slowly dipped into the solution with the initiator and the monomer substances at a proportion of 0.2%, respectively. After slowly removing the immersed tip from the solution, a polymer drop is formed in its extremity, which is then irradiated through the core during 60 seconds at a power of, at least, 20  $\mu$ W at 405 nm, and consequently cured [3, 6].

## 2.2. Optical Trapping and Sensing Setup

The optical manipulation setup built to manipulate and acquire the back-scattering signal was based on a home-made inverted microscope composed by a 20x objective connected to an image acquisition system (with a CMOS camera, EO-2018C from Edmund Optics), a 4-axis motorized micromanipulator ( $x, y, z$  and angular) and the signal acquisition module. In order to acquire the back-scattered signal, one of the two entries of a 50/50 980 nm fiber coupler with a 1x2 topology was spliced to a pigtailed 980 nm laser (500 mW, Lumics, ref. LU0980M500) and the other to the back-scattered signal acquisition module. The opposite single entry of the optical coupler was spliced to the optical fiber tip fabricated through the process described above and inserted into a metallic capillary controlled by the motorized micromanipulator. This capillary was tilted at  $50^\circ$ , since previous experiments showed that trapping phenomena is only possible for fiber tip inclination angles  $>30^\circ$  [6]. This bilateral configuration allowed both laser light guidance to the optical fiber tip through the optical fiber and the acquisition of the back-scattered signal through a photodetector - PDA 36A-EC, Thorlabs - connected to a data acquisition board (DAQ from National Instruments, Texas, USA). This DAQ transmitted the acquired signal to the laptop at a sampling rate of 5kHz. A drop of a solution containing a commercial natural yogurt sample composed by both types of bacteria (rod-shaped *Lactobacillus Acidophilus* and spherical *Streptococcus Thermophilus*) diluted in Phosphate-buffered Saline (PBS) was placed over a glass coverslip placed over the inverted microscope setup. The spherical lensed fiber tip was then dropped into this solution for bacteria manipulation and simultaneous back-scattered signal acquisition. The output laser power was set to  $\approx 50$  mW during the experiment [6]. Four bacteria from the *Lactobacillus* and five from the *Streptococcus* type were trapped using the fabricated polymeric lens.

## 2.3. Bacteria Trapping and Sensing Using Polymeric Lenses

### 2.3.1. Back-Scattered Signal Acquisition and Processing Steps

After the polymeric tip be immersed in the solution of PBS with diluted natural yogurt, 80 seconds of back-scattered signal were acquired at a sampling rate of 5 kHz, when each one of the analyzed bacteria (four *Lactobacillus* and five *Streptococcus*) was trapped in front of the tip. *Lactobacillus* had a rod-like shape and were, in average,  $0.9 \pm 0.4 \mu\text{m}$  wide,  $14 \pm 7 \mu\text{m}$  long and had  $0.7 \pm 0.3 \mu\text{m}$  in height. *Streptococcus* had a symmetric circular geometry and, in average,  $1.8 \pm 0.7 \mu\text{m}$  of diameter. Short-term segments of signal were also acquired for the case of no bacteria in front of the tip for four different places, representing the class “No particle/bacteria trapped”. “No particle” signal acquisitions were performed by moving the polymeric tip into an empty area, where, despite the laser being turned on, no particle was trapped. Acquired signal portions were divided according to the following classes: “No particle trapped”; “*Lactobacillus* bacteria trapped” and “*Streptococcus* bacteria trapped”. After acquisition, the following signal processing steps were performed using a custom built MATLAB 2015a<sup>®</sup> script [3]. At first, in order to remove low frequency noise such as the 50 Hz component of the electrical grid, the signal was filtered using a second-order 500 Hz Butterworth high-pass filter [3]. Then, each entire 80 seconds signal acquisition was split into epochs of 2 seconds for each class. The  $z$ -score of each 2 s portion was computed in order to remove noisy signal epochs (signal portions which corresponding  $z$ -scored magnitude exceeded 10 were discarded). The final dataset was then composed by 135, 144 and 185 samples (2 s signal portions) for “No particle”, “*Lactobacillus*” and “*Streptococcus*” classes, respectively. Processed signal portions are depicted in fig. 1.

Considering that a set of 43 back-scattered derived parameters was already successfully used to differentiate simple biologic and synthetic particles using polymeric lenses on the top of optical fibers in [3], we extracted these same characteristics (table 1) from each signal portion and investigated their potential to distinguish bacteria. However, the inclusion of so many attributes would be difficult to design a reading system for bacteria identification. Thus, the Linear Discriminant Analysis (LDA) was applied to generate a single parameter, given its adequacy to the previous differentiation task described in [3]. The LDA consists in determining a subspace of lower dimension, in which the

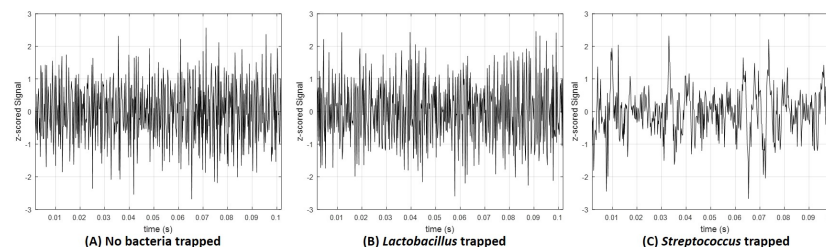


Fig. 1. Sketches of processed back-scattered signal portions acquired when (A) no particle; (B) a *Lactobacillus* or (C) a *Streptococcus* bacteria was trapped using the fabricated polymeric lens.

Table 1. Table summarizing the back-scattered signal parameters set evaluated (based on [3]).

Type	Group	Number	Feature/Parameter
Time Domain	Time Domain Statistics	1	Mean (M)
		2	Standard Deviation (SD)
		3	Skewness (Skew)
		4	Kurtosis (Kurt)
		5	Interquartile Range (IQR)
		6	Entropy (E)
	Time Domain Histogram	7	$H_{Nakagami}$
Frequency Domain	Discrete Cosine Transform (DCT)	8 ... 27	1st ... 20th Coefficient ( $E_{DCT}[1] \dots E_{DCT}[20]$ )
		28	Number of coefficients that capture 98% of the original signal ( $N_{DCT}$ )
		29	Total spectrum Area Under Curve (AUC) ( $AUC_{DCT}$ )
		30	Maximum peak amplitude ( $Peak_{DCT}$ )
	31	Total spectral power ( $P_{DCT}$ )	
	Wavelet Packet Decomposition	32 ... 37	Haar Relative Power 1st ... 6th level ( $E_{Haar}^1 \dots E_{Haar}^6$ )
		38 ... 43	Db10 Relative Power 1st ... 6th level ( $E_{Db10}^1 \dots E_{Db10}^6$ )

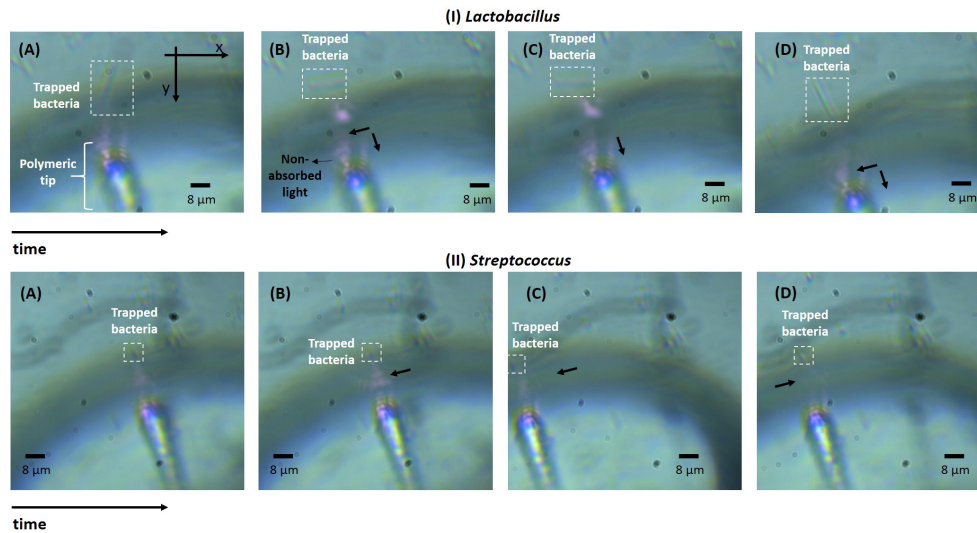


Fig. 2. Video frames showing (I) *Lactobacillus* yogurt and (II) *Streptococcus* yogurt bacteria trapping in PBS using the polymeric lensed fiber tip. (I, II)(A-D) Bacteria are moved due to displacements of the optical fiber tip along both transverse and longitudinal directions by optical trapping.

data points of the original problem are “separable”. Using this method, it is possible to build a single parameter that results from a linear combination of the original features [3].

Statistical analysis was performed to study the sensing ability of each of the 43 features separately and the final LDA-derived one, using the Statistics Toolbox from MATLAB R2015a<sup>®</sup>. Non parametric statistical tests were applied, due to the fact that some of the features analyzed failed to be normally distributed (Shapiro-Wilk Normality Test). The Kruskal-Wallis test was applied to each feature to evaluate its differentiation power in a 3-classes comparison manner. A post-hoc pairwise analysis was also conducted for each one of the variables after the Kruskal-Wallis for 3 conditions. The Mann-Whitney test (2 conditions) was then performed to evaluate the differentiation ability of each feature in a pairwise manner (“No particle” vs. “*Lactobacillus*”; “No particle” vs. “*Streptococcus*” and “*Lactobacillus*” vs. “*Streptococcus*”).

### 3. Results and Discussion

In fig. 2 are depicted two video sequences showing the effective optical trapping of both types of yogurt bacteria *Lactobacillus* and *Streptococcus* using the lensed fiber tips. It is possible to observe that *Lactobacillus* can be moved along both the directions (transverse and longitudinal) by optical trapping. Due to its asymmetrical shape, *Lactobacillus* was displaced according to different movement patterns depending on the bacteria surface location where the resultant of trapping forces was exerted on. Thus, it was possible to trap both types of bacteria and acquire the back-scattered signal, ensuring that the radiation detected was derived exactly from each trapped bacteria.

Relatively to the statistical analysis performed considering each feature separately, only 7 of the 43 features analyzed were able to significantly differentiate the three classes at a statistical significance level of 0.05. Six were wavelet-derived parameters ( $E_{Haar}^8$ ,  $E_{Haar}^9$ ,  $E_{Haar}^{10}$ ,  $E_{Db10}^8$ ,  $E_{Db10}^9$  and  $E_{Db10}^{10}$ ) and only one was based on time-frequency

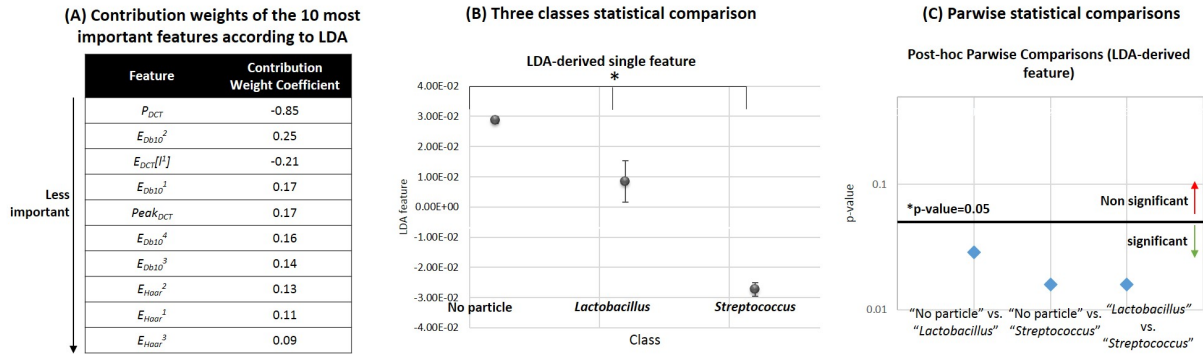


Fig. 3. LDA single feature for bacteria sorting results. (A) Table showing the 10 most important features and corresponding weight contributions to the final LDA feature. (B) LDA values for each bacteria class (\*p-value, Kruskal-Wallis test  $< 0.05$ ). Error bars correspond to standard errors. (C) p-value results obtained in the post-hoc comparisons for each pair of classes (\*p-value  $< 0.05$ ). X axes values are in logarithmic scale.

signal analysis ( $\mu_{Nakagami}$ ). The wavelet-derived features also revealed to be highly relevant attributes in [3]. However, none was successful in differentiating “No particle” from “*Lactobacillus*” class in this study, regarding the pairwise comparisons. Thus, similarly to the method reported in [3], the LDA technique was needed to gather all the relevant contribution of the original 43 features and remove the noisy information. The resultant single feature was then able to simultaneously separate classes in a three-classes and in a pairwise manner (fig. 3.(B) and 3.(C)). By observing the contribution weights of each original feature, we can also conclude that frequency-derived attributes are more relevant than the time-derived ones, as already verified in [3]. In fact, the  $P_{DCT}$  attribute was again the most important parameter to the differentiation task. Note that, in this experiment, the input laser signal was not modulated in frequency, on contrary to the previous study [3], which enhances even more the importance of the information provided from the frequency component of the signal. Thus, differences in particles shape, optical characteristics and in the nature of their content are reflected in the frequency component of the light that they scatter. Since the geometry of *Lactobacillus* and *Streptococcus* bacteria are completely different and the particles differentiated in the previous study ([3]) were all spherical, we can conclude that, in fact,  $P_{DCT}$  is a robust feature relative to scatterers size, shape, refractive index, complexity degree, etc. The LDA turned the complex problem proposed here into a simple one, through which is possible to sort different kind of yogurt bacteria using a single signal parameter and no complex and time-consuming imaging methods. Additionally, the results obtained also showed that the proposed lenses are able to trap specimens as small as  $1 \mu\text{m}$  of diameter or width, like bacteria.

#### 4. Conclusions

In conclusion, we present a novel low-cost and rapid sensing method to differentiate yogurt bacteria through a single parameter based on the back-scattered radiation emitted by an isolated bacteria, when trapped using one of the proposed polymeric lenses. This method can be a valuable contribution for bacteria sorting methods and individualization of hazardous specimens for foodborne analysis.

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