Integrated multi-channel optical system for bacteria characterization and its potential use for monitoring of environmental bacteria

IGOR BUZALEWICZ,1,* AGNIESZKA SUCHWALKO,2 PAWEŁ TRZCINSKI,3 LIDIA SAS-PASZT,3 BEATA SUMOROK,3 KATARZYNA KOWAL,4 RYSZARD KOZERA,5,6 ALINA WIELICZKO,7 AND HALINA PODBIELSKA1

1Bio-Optics Group, Department of Biomedical Engineering, Faculty of Fundamental Problems of Technology, Wroclaw University of Science and Technology, 27 Wybrzeże S. Wyspiańskiego Street, Wroclaw, Poland
2QUANTUP, 30/9 Litewska Street, Wroclaw, Poland
3Rhizosphere Laboratory, Agrotechnical Department, Research Institute of Horticulture, 1/3 Konstytucji 3 Maja Street, Skierniewice, Poland
4Independent Researcher, Leeds, UK
5Faculty of Applied Informatics and Mathematics, Warsaw University of Life Sciences SGGW, 159 Nowoursynowska Street, Warsaw, Poland
6School of Computer Science and Software Engineering, University of Western Australia, 35 Stirling Highway, WA 6009 Crawley, Perth, Australia
7Department of Epizootiology and Veterinary Administration with Clinic of Infectious Diseases, Wroclaw University of Environmental and Life Science, 45 Grunwaldzki Square, Wroclaw, Poland

*igor.buzalewicz@pwr.edu.pl

Abstract: The potential use of a novel multichannel optical system towards fast and non-destructive bacteria identification and its application for environmental bacteria characterisation on the strain level is presented. It is the first attempt to use the proposed optical method to study various bacteria species (Gram-negative, Gram-positive) commonly present in the environment. The novel configuration of the optical system enables multichannel examination of bacterial colonies and provides additional functionality such as registration of two-dimensional (2D) distribution of monochromatic transmission coefficient of examined colonies, what can be used as a novel optical signature for bacteria characterization. Performed statistical analysis indicates that it is possible to identify representatives of environmental soil bacteria on the species level with the 98.51% accuracy and in case of two strains of Rahnella aquatilis bacteria on the strain level with the 98.8% accuracy. The proposed method is an alternative to the currently used preliminary bacteria examination in environment safety control with the advantage of being fast, reliable, non-destructive and requiring minimal sample preparation.

© 2019 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

1. Introduction

Bacteria identification is an essential foundation of the microbiological medical and veterinary diagnosis, but it is also important in such areas, as food safety control, monitoring environmental contamination and in agriculture. Therefore, many national and international efforts are made towards the development of novel detection and identification techniques, primarily leading to the reduction of costs and analysis time. Many methods based on different detection principles are commonly available for identification of environmental bacteria. The most widely used techniques include biochemical, molecular and mass spectrometric methods [1–7]. The predominant used molecular methods involve amplification of the gene and the analysis based on the polymerase chain reaction [7–9]. The identification process is preceded by isolating the bacterial DNA, the restriction enzyme digestion, electrophoresis separation
and hybridisation, which is connected to radioactive labelling. Its fundamental disadvantages are the genetic plasticity and variability of targeted organisms, the need to prepare high-quality samples containing only the genetic material to be tested, without any impurities and the destructive character of the examination. Alternative methods use fluorescence in situ hybridization (FISH) technique, which is cytogenetic method utilizing fluorescence probes [10], biochemical techniques, such as BIOLOG, or novel culturing methods [11] or mass spectroscopy using MALDI – TOF (Matrix - Assisted Laser Desorption - Ionization - Time Of Flight) [3,4,12] that provide new insights of the microbiological diversity in the environment.

It should be pointed out that all described above techniques have destructive character, which means that it is not possible to verify the same sample using different methods. Moreover, they require a number of chemical reagents, fluorescence and/or immunological markers etc. The cost and the complexity of these methods prevent their widespread use, limiting them to the professional laboratories, only. Therefore, the demand for alternative methods is rapidly growing.

Novel methods based on light scattering and diffraction on bacterial colonies grown on solid nutrient media have considerable advantages since they are non-destructive and do not require any advanced sample preparation [13–17]. These techniques provide an attractive alternative to conventional procedures such as molecular, biochemical, immunological and immune-enzymatic ones, enabling the non-contact and non-destructive measurements, with no need for sample preparation or labelling and considerable simplification of the instrumentation. Bacteria identification systems based on forward-light scattering or diffraction approaches, which are being developed are: BARDOT (Bacterial Rapid Detection using Optical Scattering Technology) at Purdue University in the United States [14,17,18] and BISLD (Bacteria Identification System by Light Diffraction) at Wroclaw University of Science and Technology in Poland [13,19–22]. Both techniques are using the statistical methods and classification models to quantitatively determine the bacteria species and strains based on the registered optical signatures of bacterial colonies. However, the assumed methodology: kind of features extracted from these signatures, features ranking/selection, determination of the most predictive features, as well as classification process, are entirely different, what is analysed in details in [20].

BISLD is a non-contact method carrying out a non-destructive measurement, and there is no need for advanced and time-consuming sample preparation. Bacterial colonies generate diffraction patterns exhibiting unique species- and strain-dependent features, which can be used in bacteria identification supported by image processing and statistical methods. Moreover, identification is reliable, fast, not expensive and what is extremely important, it does not require any knowledge in advance about the sample, as it is in the case of the immunological tests. Through automatization of the bacteria sample preparation, this method can be used for microbiological examination by the non-professional staff without the microbiological experience, what additionally increases the prevalence of use for example by agricultural centres without microbiological laboratories. As it was already mentioned, proposed optical examination of biological samples has non-contact and non-destructive character, and therefore in case of results that need further confirmation, the sample can be verified by other methods, thus offering a significant advantage in comparison to biochemical and molecular methods.

The primary goal of the presented work was examining the possibility of identification of the environmental bacteria, using the light diffraction phenomena. In this study, the BISLD technique with a novel system integrated, reconfigured, with the multichannel optical arrangement was tested to differentiate bacteria present in soil and plants roots on a strain level. The multichannel optical systems are commonly used for characterization of various kinds of samples; therefore the application of this concept for bacterial colonies examination is an obvious consequence. Such approaches were used for multichannel registration of the
multispectral forward scattering patterns of bacterial colonies for three different wavelengths [23]. Presented here optical system enables the multi-channel examination of bacterial colonies' optical signatures (diffraction patterns and 2D transmission coefficients map), automatic registration of optical patterns and accurate bacteria identification, what significantly improves examination performance and increases the potential of use in the fully automated microbiological laboratories. The system composes of an optical set-up, which records bacteria diffraction patterns combined with advanced statistical algorithms used for the analysis of these diffraction patterns. Here, we demonstrate a multivariate statistical analysis of the Fresnel patterns' features as an efficient way for characterising bacteria genus or species and building the classification features vectors. There are no reports on such initial classification features ranking before building the bacteria classification model. Moreover, the multichannel configuration of the present system enables also detection of the 2D monochromatic light transmission coefficient of bacterial colonies, which provides additional information explaining the spatial structure of colonies diffraction patterns. In [24] the concept was presented of analysis of light transmission properties of bacterial colonies by point by point measurements of their optical density, however in proposed novel approach it is possible to obtain the morphology and spatial distribution of the transmission properties by single shot microscopic imaging, without the time-consuming scanning and the use of the high-resolution translation stages.

In the present study eight various bacteria species commonly existing in the environment, particularly in soil, were examined. Among them, there were representative environmental samples - plant growth promoting rhizobacteria (PGPR): Rahnella aquatilis (x31E and x21N) and Bacillus subtilis (Sp27). It is commonly known that the relationship of soil microorganisms with plants is very close and intimate and plants growth is directly dependent on the presence of the beneficial bacteria living in the soil [25]. Unfortunately, all the knowledge about beneficial soil microbial organisms cannot be practically used without identification of the microbes in the soil. Construction of an effective identification tool for quick recognition of beneficial bacteria and other groups of microorganisms enables their characterisation and use them for practical applications. To the best of our knowledge, this is a first attempt to use novel light diffraction method for soil bacteria identification, which can create perspectives of their application in commercial agriculture practice and environmental contamination control centres. The results obtained as a part of herein studies have shown, that this newly developed technique can be suitable for practical implementation in environmental monitoring of bacteria presence with the differentiation on the level of the bacteria strains.

2. Methods and materials

2.1 Methodology of bacteria identification system by light diffraction (BISLD)

There are many methods for bacteria identification used in microbiological laboratories. The BISLD system proposed in this paper is inexpensive, fast and reliable. The presented method is based on a modified optical sensing system, described in [13,20–22,26–28]. The influence of various growth conditions of bacteria and other factors affecting the diffraction patterns of bacteria colonies were already thoroughly examined [13,22,29]. Bacteria identification based on the statistical analysis of Fresnel diffraction patterns of bacterial colonies has been developed, as well [19–22,27]. The functional diagram of the proposed method is illustrated in the Fig. 1. It presents all the necessary phases of the identification technique divided into successive steps.
2.2 Configuration of the new, multichannel optical biosensor

BISLD system, developed in our Group at the Wroclaw University of Science and Technology, is based on scalar diffraction theory foundations [13]. The fundamental concept of the BISLD system is based on the already verified assumption that in case of bacterial colonies growing on the solid nutrient medium, the variation of the optical properties (refractive indices and transmission coefficients) and morphology properties (profile, size and colony shape) depending on the spatial orientation and metabolism of the bacteria cells in the colony, are responsible for generating Fresnel diffraction patterns that are unique for each bacteria genus and species [13,22,29,30]. Therefore, it is possible to examine the colonies at different stages of their evolution or after different incubation times. Already reported results have shown, that it is possible to identify bacteria genus and species basing on their Fresnel diffraction patterns supported by advanced statistical analysis with very low identification error of 1.30-1.43% [22]. The set-up was reconfigured and, and the main optical elements were integrated into cage-system (see Fig. 2). Moreover, the additional measurements channels were incorporated.

Fig. 2. The BISLD system configuration: (1) - the laser diode module (635 nm, 1 mW, collimated, Thorlabs), (2) - amplitude filters wheel (OD: 0-2, Thorlabs), (3) - beam expander BE (1.5X, Thorlabs), (4) - iris diaphragm (diameter: 0-2.5 cm, Thorlabs) with automatically controlled diameter, (5) - transforming lens L (f = 12 cm, Thorlabs), (6) - pellicle beam splitter (T/R = 50:50, Thorlabs), (7) - holder with the sample of bacterial colonies in Petri Dish integrated with an automatic X-Y-Z translation stage, (8) – beam splitter (T/R = 50/50, Thorlabs), (9) - CCD camera (EO-1312, Edmund Optics) with imaging objective (f = 3.5 cm, Edmund Optics) for diffraction patterns recording, (10) high-resolution CCD camera (DCU223M, Thorlabs) with imaging objective (f = 12 mm, Edmund Optics), (11) high-sensitivity CMOS camera (DC1240M, Thorlabs) with imaging microscope objective (Nikon, 4x, WD = 30 mm) and (12) - computer unit.
First, an additional channel for registering the image of all bacterial colonies on Petri dish was included to enable the automatic localisation of bacterial colonies grown on the medium. This system module combines the cage-mounted pellicle beam splitter (6), which enables recording of bacterial colonies on Petri dish in reflection mode, the additional camera (10) with imaging objective (f = 12 mm, Edmund Optics) and the ring illuminator for uniform illumination of the Petri dish. This modified optical system enables computer-controlled automatic localisation and positioning of the bacterial colony respectively to the light beam, automatic adjusting the diameter of the beam by controlling the iris-diaphragm diameter and registration of Fresnel diffraction patterns of bacterial colonies located on analysed Petri dish.

Moreover, the second additional measurement channel for registration of the magnified images of individual bacterial colonies was incorporated for determination of two-dimensional (2D) monochromatic transmission coefficient, providing the additional information characterising the examined bacterial colonies and their optical properties. This module illumination system combing described above elements (1)-(6) ensures the uniform illumination of the individual bacteria colony (7). The light transformed on the bacterial colony is directed by the beam splitter (8) to CMOS camera (11) with an imaging microscope objective. The automatization and the additional registration channels significantly increase the functionality and facilitate the measurements in the proposed optical system.

2.3 Bacterial samples preparation

In this study eight bacteria species (Gram-negative, Gram-positive), were examined: *Citrobacter freundii* (PCM 531), *Escherichia coli* (PCM 0119), *Proteus mirabilis* (PCM 547), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (PCM 2267), *Staphylococcus intermedius* (PCM 2405), *Bacillus subtilis* (Sp27) and *Rahnella aquatilis* (x31E and x21N). The six species were obtained from the Polish Collection of Microorganisms, registered with the World Federation of Culture Collections (WFCC No. 106). The environmental cultures of *Rahnella aquatilis* (strains: x31E and x21N) and *Bacillus subtilis* (Sp27) were obtained from the Rhizosphere Laboratory of the Microbiological Department at the Research Institute of Horticulture (Poland). The bacterial samples were prepared in the laboratory of the Department of Epizootiology and Veterinary Administration with Clinic of Infectious Diseases of the Wroclaw University of Environmental and Life Science. The procedure of sample preparation and incubation standardisation was already reported [13,22]. Bacteria suspensions were first incubated for 18 hours at a temperature of 37°C. Bacteria suspensions in respective $10^{-5}$ and $10^{-6}$ dilutions were seeded on the surface of the solid nutrient medium in Petri dishes with Columbia agar (Oxoid), to obtain 12-20 colonies per plate, and were again incubated at 26°C for the next 18 hours. The same nutrient medium was used for all bacterial species/strains to omit the initial differentiation and a priori information about their species/strains introduced by their metabolic properties. Fixing the parameters of the incubation process (nutrient medium, temperature and time of incubation etc.) and defined diameter of the colony, guarantees the proper comparison of the bacteria colonies diffraction patterns.

In a practical situation, the examined samples are a mixture of various bacteria species/strains cells. To investigate the possibility of bacteria identification using the proposed method, a sample containing the mixture of four bacteria species: *Bacillus subtilis*, *Citrobacter freundii*, *Escherichia coli*, *Proteus mirabilis*, was prepared. From the $10^{-7}$ dilutions of each bacteria species suspension, the appropriate volumes: 200 µl (*Bacillus subtilis*), 200 µl (*Citrobacter freundii*), 300 µl (*Escherichia coli*) and 300 µl (*Proteus mirabilis*) were mixed. The percentage presence of bacteria species in this mixture was as follows: 20% (*Bacillus subtilis*), 20% (*Citrobacter freundii*), 30% (*Escherichia coli*) and 30% (*Proteus mirabilis*). Next, the 50 µl of this mixture were seeded on the surface of the solid nutrient medium and incubated for 18 hours at 37°C.
2.4 Statistical analysis of the Fresnel diffraction patterns of bacterial colonies

The colonies of different bacteria species/strains exhibit unique morphological and optical properties, which are also influencing the spatial distribution of the diffracted light intensity in the form of the Fresnel diffraction patterns. After the recording of the diffraction pattern, edges and centres of pattern, are determined. Image processing and feature extraction stages are sometimes thought as necessary part of the analysis, but of a little significance. However, the determination of proper numerical features mapping the natural morphology and texture of the patterns under study in the regions that differentiate mostly various species/strains of bacteria is critical for building effective predictive models. Every model will perform classification much better, if it is built upon very distinct features in comparison with a case of building it upon some randomly chosen essential features. That is why, apart from selecting prediction model that gives the smallest error, it is so important to extract and select numerical features that reflect changes in diffraction patterns caused by the variety of bacteria species/strains. The workflow of the statistical analysis of bacterial colonies diffraction patterns is presented in Fig. 3.

![Diagram of statistical analysis workflow](image)

**Fig. 3.** The workflow of the statistical analysis of diffraction patterns.

2.4.1 Feature extraction and selection

The visual inspection of diffraction patterns reveals the fact that they vary mostly in the number of naturally occurring, ring-shaped intensity maxima, their width, separation and texture. Therefore, the sectional analysis of the diffraction patterns was proposed to map the naturally occurred diffracted light spatial intensity variations, what makes the method unique among other using light diffraction as they all treat patterns as a whole. Each examined pattern is therefore partitioned into 10 disjoined rings of equal thickness what allows to describe the differences in the thicknesses of the naturally occurring regions utilising numerical features. Additionally, the linear normalisation algorithm is applied to provide comparable light intensity conditions. Performed image processing operations were already described with all details in [19–21]. Feature extraction and selection stage is one of the steps that most evolved since the method was developed. For each ring, numerical features denoting morphological and textural properties based on the central statistical moments, are
calculated. Based on the visual inspection, features chosen as possible predictors are: mean and standard deviation denoting brightness and roughness of the regions of interest, respectively, as well as skewness referring to the measure of the symmetry of the shape of the pixel intensities distribution within each ring, kurtosis being a measure of the flatness of a distribution of the pixel intensities within each ring, smoothness, uniformity and entropy. To indicate features that best differentiate the diffraction patterns between bacteria species/strains a two-step algorithm was designed and applied in the feature selection step. The first step is defining a ranking of the most predictive feature groups (features within the whole pattern, e.g. mean in all rings) performed by the use of QDA (Quadratic Discriminant Analysis). The second step is ranking the features from the best predictive groups (e.g. in which ring mean value of the pixels’ intensity is differentiating bacteria in the best way) performed utilising Fisher divergence Signal-to-Noise Ratio (SNR) and analysis of variance ANOVA. Features are chosen for the prediction models according to the second step ranking.

2.4.2 The classification and classification performance assessment

After the extraction and selection of the most predictive features of the Fresnel patterns of bacterial colonies, the classification and classification performance assessment, are performed. This task consists of building classification models QDA (Quadratic Discriminant Analysis) and SVM (Support Vector Machine) that allow the identification of unknown bacterial colonies diffraction patterns and assessment of the performed analysis with the use of cross-validation (CV) with stratified sampling to ensure homogenous distribution of the samples belonging to various classes (bacteria species/strains) along with multi-class case sensitivity and specificity measures. Image processing was performed by using a dedicated macro, written in the ImageJ software (http://rsb.info.nih.gov/ij/) with human interaction [31]. Further statistical analysis was accomplished with the use of the R Project for Statistical Computing [32]. This work aims to examine the potential of the proposed method for the identification of the environmental bacterial samples on the species- and strain level. Therefore, firstly, to show, how the method deals with the separation and classification of the environmental samples: Bacillus subtilis and Rahnella aquatilis, we used the same workflow of data set, but the set was divided into four classes: Rahnella aquatilis x21N strain, Rahnella aquatilis x31E strain, Bacillus subtilis (Sp27), and the rest of the data marked as Other bacteria. On the other hand, to demonstrate the possibility to differentiate bacteria on the strain level, we used the same workflow of data set, but contrary to the previous case, the set was divided into three classes: Rahnella aquatilis x21N strain, Rahnella aquatilis x31E strain, and the rest of the data marked as Other bacteria.

2.5 Analysis of the 2D transmission coefficient of the bacterial colonies

To explain the correlation between the diffraction patterns of bacterial colonies and their optical transmission properties, the discrete 2D transmission coefficient distributions inside the region occupied by colony based on the microscopic transmission images under monochromatic illumination (635 nm), were determined. These optical signatures are describing the species-/strains-dependent optical transmission properties of bacterial colonies respectively to the transmission properties of the used nutrient medium. The methodology and physical foundations of the 2D transmission coefficient evaluation were described in details in [33]. The 2D transmission coefficient is expressed by \( T(i, j) \) as the discrete transmission coefficient for the particular pixel \((i, j)\) of the recorded transmission image of single colony and describes the relative transmission coefficient of the bacterial colony, normalized to the transmission properties of the nutrient medium, on which the colonies are grown. The \( T(i, j) \in [0;1] \) values are dimensionless units. The value 0 refers to the total attenuation of the light passed through the nutrient medium by an opaque object, whereas value 1 to the total transmission of this light by a transparent object. The 2D transmission coefficient distribution corresponds to the spatial geometry of the bacterial colony and light absorption properties of
the bacterial cells and extracellular material forming the dense areas of the colony. It can be used for explanation of the specific light transformation on bacterial colonies, as well as for bacterial colony characterisation. It should also be mentioned, that the 2D transmission coefficient determines the relative light transmission of the bacterial colony respectively to the nutrient medium transmission, what eliminates the possible changes of its values and spatial distribution caused by the thickness of the nutrient medium and its thickness.

The validation of the proposed approach was performed by using the neutral density filters with known transmission coefficient: 10% (NE501B, Thorlabs) 32% (NEB505B, Thorlabs), 40% (NEB504B, Thorlabs), 79% (NE501B, Thorlabs) for 633 nm as test objects. The filters were placed on the surface of a nutrient medium, which was uniformly illuminated with the light at 633 nm (FWHM = 10nm). The relative transmission was determined according to the presented methodology. The standard deviations of the evaluated transmission coefficient respectively to catalogue value were found to be between 0.05 and 9%.

3. Results

3.1 Fresnel diffraction patterns of bacterial colonies

Eight bacterial species were examined, including two strains of one of them. Therefore, in the classification process, 9 different classes were formed: 7 bacteria species and 2 strains of *Rahnella aquatilis*. Altogether 50 Fresnel diffraction patterns with a unique spatial distribution of the diffracted light intensity were generated for each class of bacteria species/strains, giving a total number of 450 registered patterns. The examples of the diffraction patterns for each class are shown in Fig. 4.

![Representative diffraction patterns of bacterial colonies examined in the study](image)

Moreover, the registered diffraction patterns exhibit a highly repeatable spatial distribution of the diffracted light intensity (see Fig. 5), what confirms the species-/strain-dependence of bacterial colonies Fresnel patterns for standardised incubation conditions. In a day-to-day examination of the environmental samples, they contain a mixture of cells of different bacteria species. Therefore, to check the possibility to distinguish bacteria based on the Fresnel patterns of the multi-species colonies on the same plate, an examination of the sample containing four bacteria species, was carried out. The examples of registered Fresnel patterns of spatially separated colonies are shown in Fig. 6.
The sample contained 19 bacterial colonies; however only 17 colonies were spatially separated and were suitable for diffraction patterns recording. Analysis of the obtained Fresnel patterns enabled to identify the species of different colonies in the sample, as well as the fraction (in %) of bacteria species presented in the sample. This experiment shows that the BISLD technique can be used for examination of multi-species samples of spatially separated colonies and effective identification.

3.2 The 2D transmission coefficient of bacterial colonies

In presented here new optical system the additional imaging channel was incorporated to provide the additional optical signature of bacterial colonies in the form of spatial distribution of 2D light transmission coefficient. These optical signatures are determined by image processing analysis of the microscopic transmission images of bacterial colonies and are describing the transmission of the colony relative to the nutrient medium transmission. The exemplary 2D distributions of the transmission coefficient images of various bacteria colonies
are presented in Fig. 7. Obtained results have shown that colonies of different bacteria species/strains exhibit unique light transmission properties. The analysis of these new optical signatures of bacterial colony allows explaining the specific spatial distribution of Fresnel diffraction patterns of bacterial colonies.

The recorded Fresnel patterns of Bacillus subtilis have a form of a central round intensity maximum with irregular boundaries, additionally modulated by the speckle's patterns (Fig. 4). The spatial distribution of the transmission coefficient for this bacteria was very heterogeneous, and its values were changing in a wide range from 0.1 to 0.8 (see Fig. 7(a)). This effect was associated with the local fluctuations of the colony thickness dependent on the variation in the composition of the bacteria cells and accumulation of extracellular material across the colony. The heterogeneous profile of the bacterial colony and effects on the edges of different areas led to a specific spatial distribution of the Fresnel patterns intensity and a presence of the speckle effect typical for strongly scattering objects with highly heterogeneous morphological and optical properties.

![Fig. 7. The representative monochromatic 2D transmission coefficient distribution of: (A) Bacillus subtilis, (B) Rahnella aquatilis (x31E), (C) Rahnella aquatilis (x21N), (D) Citrobacter freundii, (E) Escherichia coli, (F) Proteus mirabilis, (G) Pseudomonas aeruginosa, (H) Staphylococcus aureus, (I) Staphylococcus intermedius colonies obtained under monochromatic illumination (color scale represents values of transmission coefficient).](image)

The diffraction patterns of the two strains of Rahnella aquatilis shown in Fig. 4 have few dissimilarities. The pattern of the x31E strain exhibits a heterogeneous variation of light transmission across the colony. The pattern of the x21N strain has a visible dark centre with bright edges, then a zone of fluctuating intensity and bright edge. In the case of both colonies, bright spikes around the outer zones of the colonies, can be observed. These dissimilarities between the diffraction patterns of bacteria from the same species but two different strains indicate that the optical methods can be applied to differentiate the bacteria on the strain level. The 2D transmission coefficient values were lower in case of x21N strain. The differences in the optical features between the colonies cause that light diffraction dominates on the peripheral area of the x21N colony, where the spokes-like irregular edges of the light
transmission zones were recorded, while in case of the x31E strain colony the light transmission through the central region was significantly higher and the side-lobes maxima have lower intensities. As shown in Figs. 7(b) and 7(c), the 2D transmission coefficient values varied from 0.4 to 0.6 for x31E and from 0.2 to 0.6 for x21. In both cases, the lowest values of the transmission coefficient were found in the centre of the pattern and then increased towards the edges of the colony. This can be associated with the accumulation of the material in the centre of the colony, which also results in the highest thickness within the central area. Also, the x31E strain had distinct zones/rings of the coefficient, whereas x21N had three zones, where the intermediate zone was a transition between the two zones with specific spokes-like features and heterogeneous spatial distribution of the 2D transmission coefficient surrounding the central region of the colonies.

Analysing results obtained for *Citrobacter freundii* colonies, the minimum intensity of the diffracted light in the centre of the Fresnel diffraction patterns was surrounded by the annular-shaped maximum with irregular spatial distribution and numerous radially arranged sidelobe’s intensity maxima. The spatial distribution of diffraction patterns intensity was associated with the specific transmission properties of the colony (see Fig. 7(d)). The light transmission was the lowest in the central region of the colony and the 2D transmission coefficient values are varying between 0.25 to 0.35. This zone was surrounded by the peripheral zone with higher transmission coefficient values ranging from 0.45 to 0.78. However, the boundaries of the regions with different light transmission properties had irregular shape and several local 2D transmission coefficient extrema resulting in a heterogeneous spatial distribution of the coefficient inside the region occupied by the colony. The presence of the central zone with limited light transmission was responsible for the presence of the central minimum of the diffracted light intensity in Fresnel patterns. Moreover, the irregular shape of the zones with different transmission properties was leading to the irregular spatial distribution of the intensity maxima, as well as the side-lobes maxima.

The Fresnel patterns of *Escherichia coli* colony were circular with a central maximum and radially arranged side-lobes maxima and more regular than patterns of the *Citrobacter freundii*. The 2D transmission coefficient maps were found to be more regular than those recorded for other bacteria (see Fig. 7(e)). The homogeneity of the spatial distribution of the transmission coefficient results in a regular shape of the intensity maxima of the diffracted light and spatial arrangement. The diameter of the colony and the thickness of the zones with different transmission properties were higher than in the case of the *Citrobacter freundii*, therefore according to the similarity theorem of the Fourier transform [34], it can be linked to the inversely proportional changes of the diameter and lateral size of the Fresnel diffraction patterns.

The diffraction patterns of the *Proteus mirabilis* colonies had one circular high-intensity maximum in the central region and round-shaped, concentric lower intensity maxima at the peripheral region of Fresnel pattern. In the 2D transmission coefficient map (see Fig. 7(f)), the two zones of different light transmission were distinguished: one with a regular shape in the central region of the colony, where the transmission coefficient values were within the range of 0.15 to 0.3, and the second one, annular shaped in the peripheral region of the colony, where the values of 2D transmission coefficient were varying from 0.3 to 0.6. The spatial distribution of the transmission coefficient values is causing the knife-edges diffraction effect leading to the dark spot in the centre of Fresnel pattern surrounded by round-shaped maxima with decreasing light intensity.

High fluctuations of the transmission coefficient values similar to the *B. subtilis* colonies were observed for the *Pseudomonas aeruginosa* colonies (see Fig. 7(g)). The values of 2D transmission coefficient were above 0.5 in all regions of the colony, and higher than in the case of the *B. subtilis*. It indicates higher light transmission through colony comparing to the other tested bacteria. Although it is difficult to distinguish the zones with uniform light transmission, it is possible to observe the corrugated shaped region of the transmission
coefficient’s maxima. The analysed coefficient values range from 0.3 to 0.9. The heterogeneous distribution of the 2D transmission coefficient associated with the local distribution of bacterial cells and extracellular materials lead to the heterogeneous spatial distribution of diffracted light intensity with irregular-shaped maxima (see Fig. 4). This heterogeneity of 2D transmission maps and the diffraction patterns can be caused by the high motility of the rod-shaped *Pseudomonas aeruginosa* cells.

In the performed test, two different species of *Staphylococcus aureus* and *Staphylococcus intermedius* with spheroid-shaped bacterial cells, were examined. Obtained results showed that colonies of these symmetrical bacteria cells exhibit significantly higher structural homogeneity than colonies formed by the rod-shaped bacteria. The homogeneous morphology of the colony leads to a homogeneous spatial distribution of both: 2D transmission coefficient and Fresnel diffraction patterns. In case of the *S. aureus* the 2D transmission coefficient’s map (see Fig. 7(h)) had a form of the discs and limited light transmission properties, the coefficient’s values were varying from 0.28 to 0.35 in the central region of the colony, and from 0.40 to 0.48 in the peripheral region, respectively. Contrary, in the case of *S. intermedius* species (see Fig. 7(i)), colonies showed weaker light transmission and the coefficient’s values were within 0.19 to 0.28 range in the central and 0.35 to 0.60 range in the peripheral region of the colony. The highest light transmission of *S. intermedius* colony was observed near to the colony edges, what can suggest that the thickness of the colony in this region decreased. The diversity of the light transmission through colonies of both species resulted in noticeable differences in the spatial distribution of the Fresnel pattern. In case of the *S. aureus*, a low-intensity spot in the centre of diffraction patterns was surrounded by the concentric round maxima. The pattern had a similar shape as an opaque disc [13]; however, despite limited transparency of bacterial colonies, the values of the diffracted light intensity in the central region of the *Fresnel* patterns are higher than in the case of the opaque disc.

On the other hand, in case of *S. intermedius* colonies due to low values of the transmission coefficient in the central region, a dark spot surrounded by the annularly arranged speckles was observed in the centre of the registered *Fresnel* patterns. It was correlated with limited transparency in the central zone of the colony and slightly higher transmission near the zone edges. In the peripheral region of the *Fresnel* pattern, concentric round maximum, with lower intensity than in *S. aureus* diffraction patterns, was observed. The form of the speckle patterns is caused by strong absorption in the central region of the colony and heterogeneous transmission properties near the colony edges.

The 2D transmission coefficient’s distributions are correlated with the bacterial colony morphology and internal structure, bacterial cells and extracellular material concentration, as well as their optical absorption properties, what constitutes an additional optical signature suitable for bacteria characterisation.

3.3 Features extraction and selection

In partitioning zones of each diffraction patterns, the quantitative features describing the changes of the spatial distribution of diffracted light intensity, were determined. For the selection of the suitable predictors for the classification models, an advanced multi-step numerical analysis of the collected diffraction patterns was carried out. In the first step, the diffraction pattern images were used to attain information about following morphological and textural features from 8 groups, based on central statistical moments: mean, standard deviation, skewness, flatness, smoothness, uniformity, entropy, radius. The extracted features were analysed using QDA [22,35,36] to identify and rank groups of features with the lowest model error for bacteria identification. The predominant features were: mean, entropy, radius and standard deviation in the given order. The groups of features were analysed utilising Fisher divergence Signal-to-Noise Ratio (SNR) and analysis of variance ANOVA. The
obtained classifications from this second step ranking of the most relevant group of features are shown in Fig. 8.

![Graphs](image)

Fig. 8. ANOVA (a) and SNR (b) based features discriminative power ranking, features are labelled -name.x, where a name is bacteria strain, and x is the ring number starting from the pattern centre.

### 3.4 The classification and classification performance assessment

Suitability of the method for identification of environmental bacteria was verified through the analysis of the registered Fresnel diffraction patterns divided into following four classes: *Rahnella aquatilis* x21N strain, *Rahnella aquatilis* x31E strain, *Bacillus subtilis* (Sp27) and the rest marked as Other bacteria. Specific features were extracted from the patterns and ranked using ANOVA and SNR statistical protocol. The classification performance assessment was performed by cross-validation (CV) with stratified sampling. The best predictors were used in QDA and SVM predictive model building. The results of the analysis depicted in Table 1 indicate that bacteria can be assigned to the right class with an error only 1.59%.
Table 1. Identification results of soil bacteria using two QDA and SVM classifiers and ANOVA and SNR selection methods were used for ranking predictive features. A number of best predictors (features) used in the model building, identification error, multi-class sensitivity and specificity, are depicted. Most relevant results are marked in bold.

| number of features | error [%] | sensitivity | specificity |
|-------------------|-----------|-------------|-------------|
| QDA ANOVA         | 21        | 1.97        | 0.9790      | 0.9804      |
| SNR               |           | 16          | 2.25        | 0.9432      | 0.9822      |
| SVM ANOVA         | 26        | 2.01        | 0.8980      |             |
| SNR               | 24        | 1.59        | 0.9329      | 0.9923      |

To demonstrate the suitability of the proposed method and the optical system for bacteria differentiation on the strain level, further statistical analysis was performed. The data set was divided into three groups: two strains of *Rahnella aquatilis* and all the rest of the bacteria under study, marked as Other bacteria. Similarly to previously obtained rankings (ANOVA and SNR), predictive models were built (QDA and SVM) along with their assessment. The results from Table 2 prove the possibility of distinguishing *Rahnella aquatilis* strains by the presented method with high specificity.

Table 2. Identification results for *Rahnella aquatilis* strains. Two feature selection methods (ANOVA and SNR) were used for the features predictive properties ranking. A number of best predictors (features) used for the model building, identification error, multi-class sensitivity and specificity, are depicted. Most significant results are marked in bold.

| number of features | error [%] | sensitivity | specificity |
|-------------------|-----------|-------------|-------------|
| QDA ANOVA         | 10        | 1.57        | 1.0000      | 0.9665      |
| SNR               | 18        | 1.80        | 0.9868      | 0.9645      |
| SVM ANOVA         | 15        | 0.21        | 1.0000      | 0.9930      |
| SNR               | 23        | 0.42        | 0.9880      | 0.9954      |

Performed examinations of environmental samples: *Rahnella aquatilis* (x31E and x21N) and *Bacillus subtilis* colonies have shown that their Fresnel patterns exhibit unique features, which can be used to discriminate these colonies of soil and roots bacteria from the other examined bacteria colonies. Moreover, it was demonstrated that the differences between diffraction patterns of closely related bacteria colonies: two *Rahnella aquatilis* strains are associated with differences of their optical properties as transmission/absorption of bacterial colonies.

4. Discussion

4.1 The multichannel optical system configuration for bacteria identification

Obtained results have shown that proposed technique enables distinguishing colonies of different microorganisms grown on a nutrient medium on the same plate through analysis of their diffraction patterns. The new multichannel optical system configuration enables automatic registration of series of bacterial colonies diffraction patterns and 2D transmission coefficients’ maps for bacterial colonies characterisation. The proposed methodology of determination of bacterial colonies' transmission properties by single shot microscopic imaging, without the need for scanning measurements as in other approaches [24] can significantly limit the time and improve the examination of the colony optical properties. These modifications of the optical system significantly facilitate the measurements and offer a more complex characterisation of the species-/strains-dependent optical properties of bacterial colonies.
4.2 The 2D transmission coefficient's maps of bacterial colonies as novel optical signatures for their characterisation

Light transmission properties of physical objects are significantly affecting the illuminating monochromatic beam and leading to the diffraction of light. This phenomenon was used in our study to differentiate bacterial strains. A detailed analysis of correlation of obtained bacterial colonies diffraction patterns (see Fig. 4) and their 2D transmission coefficients (see Fig. 7) helps to build up a knowledge and to understand the nature of the light diffraction on these colonies. Although, the analysis transmission properties of the bacterial colonies by means of point by point scanning of local value of their optical density was already proposed in [24], the methodology of the proposed approach for determination of 2D transmission coefficients of the bacterial colonies by single shot microscopic imaging was not analyzed nor investigated in the literature before. The new approach allows to eliminate the time-consuming scanning procedures, need of additional translation stages' use and provide additional morphological and optical properties, which can be used in bacteria differentiation or identification. The diversity of colony profiles in various bacteria characterised by the local changes of the colony thickness is associated with the species and strain-dependent accumulation of the bacteria cells and extracellular material in the region of an area occupied by the colony and their specific absorption coefficients, which affect the light transmission properties of bacterial colonies. The light diffraction effects take place at the boundaries and edges of zones within the colony with different transmission properties. Physical features of these zones: size, shape and transmission coefficient values lead to the unique spatial distribution of the diffraction pattern’s intensity. The presence of the different transmission zones and their features inside the region occupied by colony are associated with the concentration of the bacterial cells and extracellular material exhibiting the specific absorption coefficient, as well as their spatial distribution, which is affecting the spatial geometry of the colony and in consequence the pathlength of the light transmitted through the colony. It should be mentioned that chemical composition of the nutrient medium can significantly affect also the composition of the extracellular material produced by bacterial cells, what may lead to the change of the absorption properties of the colony. Moreover, the kind of nutrient medium is also affecting the spatial accumulation of the extracellular material and cells, what is manifested by the changes of the colony morphology. In consequence, it will also cause the change of the spatial distribution of the 2D transmission coefficient’s maps of the bacterial colonies. It means that this kind of optical signature will be sensitive to the chemical composition of the nutrient medium, but also the species-/strains-dependent bacteria metabolism. Therefore, the 2D transmission coefficient's maps of bacterial colonies grown on nutrient media can be additional optical signature, which can be used for bacteria characterisation. The statistical analysis of the pattern with a comparison to the built model enables to capture these differences between the strains and species and differentiate the colonies from each other. Obtained results have shown a direct correlation between the light transmission properties of different bacteria species/strains colonies and the spatial distribution of their Fresnel diffraction patterns.

Moreover, performed in [23] examination of the multispectral forward scattering patterns of bacterial colonies for three different wavelengths of the illuminating beam have shown that the differences between the multispectral patterns can be used for bacteria differentiation. The bacteria discrimination potential of these optical signatures is based on the different optical properties of bacterial colonies for different wavelengths. Therefore, it would be valuable to determine the multispectral 2D transmission coefficients' maps of bacterial colonies for different bacteria species and characterise their light transmission properties for different wavelengths or spectral bands, because they can explain these changes between the multispectral patterns of the same colonies, and can provide additional features for bacteria differentiation.
4.3 Diffraction signatures of bacterial colonies

Visual inspection of obtained results (see Fig. 4) reveals that diffraction patterns exhibit the diversity of their spatial structures in all regions of the occupied observation plane, what confirms that proposed approach of partitioning diffraction patterns for extraction of classification features is more appropriate for bacteria identification than the global analysis of diffraction patterns. Differences can be observed in patterns size, brightness, texture and morphology (in each case the diffraction patterns were recorded at the same distance from the bacterial colonies). Obtained results indicate that although all analyzed diffraction patterns exhibit characteristic symmetry associated with round shape of bacterial colonies, the intensity spatial distributions of the diffracted light also exhibit unique properties: specific mesh structure of intensity modulation, presence of different number of the round-shaped intensity maxima or radial intensity maxima, as well as the presence of the round spot or discs. These differences give the fundamental advantage of the proposed method, which is based on the already verified assumption that in the case of bacteria colonies growing on the solid nutrient medium, the variation of the optical and morphological properties, as well as bacterial cells’ metabolic properties, are responsible for generation of the unique Fresnel diffraction patterns for each bacteria species and strains. Moreover, the performed analysis of the correlation between the 2D transmission coefficient's map of a bacterial colony and their diffraction patterns have shown that such significant factor influencing this patterns as their chemical composition and spatial geometry can be characterised by analysis of the spatial distribution of the light transmission coefficient. Therefore, one of the essential features of the proposed method is the similarity of diffraction patterns registered for chosen bacterial colonies of the same class (e.g. single strain), but a diversity of the spatial structures between the classes (e.g. two strains of the same species) (see Fig. 5).

The experiments carried out on the sample containing 4 bacteria species (see Fig. 6) showed that it is possible to differentiate a single colony through the analysis of the diffraction pattern. Based on the obtained results it was possible to estimate the percentage of bacteria species present in the sample: \textit{B. subtilis} (~5%), \textit{Citrobacter freundii} (~21%), \textit{E.coli} (~31%), \textit{Proteus mirabilis} (~31%), which are related to the volumes of each bacteria species suspensions in the mixture. Only in the case of the \textit{Bacillus subtilis} the deviation of the percentage presence in the mixture occurs. Therefore, the proposed method is accurate for the colonies, which are spatially separated. In the case of the two overlapping colonies (red-dashed line on Fig. 6), the direct bacteria identification is impossible, because the diffraction pattern of this complex colony is a superposition of individual colonies Fresnel patterns. The overlapping of the colonies disturbs the proper identification, however, by choosing proper dilution of bacteria suspension may help to solve this problem. Visual observation of the diffraction patterns enables differentiation of the bacteria species/strains present in the tested sample, however to obtain the quantitative information about the species/strain classification, the qualitative examination should be supported with proposed statistical analysis.

4.4 Features extraction and selection

The proposed algorithm, in contrary to those used by other research groups [14,23], splits the diffraction patterns into disjoined regions and thus allows extraction of features that reflect the naturally occurring differentiation in the distribution of texture and morphology of the diffraction patterns. The data set consists of 80 local features for each of 10 rings in each of 450 diffraction patterns. The BARDOT method used 120 features (in the form of global Zernike moment invariants) describing each forward-scattering pattern as inputs for the analysis procedure. The order of Zernike moment-based features was high enough to include not only low-frequency shape information but also high-frequency features details of the light-scatter patterns [37]. Moreover, contrary to the BARDOT and other recently proposed methods [15,16], in our approach, the selection or ranking of the most bacteria differentiating features is performed to build the most appropriate classification model based on the most
discriminative features [20]. Both performed rankings (see Fig. 8) have shown that most significant features are calculated from partitioning zones situated close to the centre of the diffraction patterns (as they are located at the top of both rankings), while those at the edges are of less importance. Furthermore, both rankings suggest that entropy is the most significant predictive feature. Entropy is referred to Shannon entropy that quantifies expected value of information carried by each of the pattern rings. As the rankings exhibit significant differences, both of them were used for further identification purposes. These results indicate that the optical properties and internal structure of the central region of the colony, which are revealed in the central region of the diffraction patterns, are the most critical factors for bacteria differentiation. Moreover, it confirms that the proposed approach for the diffraction patterns partitioning for extraction the classification features is more appropriate for bacteria identification than the global analysis of diffraction patterns [14,15,17,23].

4.5 The classification and classification performance assessment

The analysed soil bacteria were distinguished best by SVM model based on the SNR features ranking. The best model (SVM with SNR ranking) used 24 features and gave error as small as 1.59% with the sensitivity 0.9329 and specificity 0.9923. The worse of built models was QDA with SNR ranking, and it gave an error of 2.25%, but this model uses only 16 features what means that it is much simpler than the best one. The results were consistent with the expectations since the SVM is a more complex algorithm than QDA and deals better with multidimensional data. SVM also built more complex models (based on more features). Sensitivity and specificity values close to 1 suggest an excellent selection of predictive models for the task. Obtained results proved that the presented method could be used successfully for environmental bacteria identification on the species level.

Moreover, the proposed method can identify bacteria also on the strain level. The performed examination of the diffraction patterns of two strains of *Rahnella aquatilis* bacteria has shown that classification models gave lower errors with fewer features and better sensitivity in almost all tested cases, except for the QDA with SNR that used two more features. The lowest error is just 0.2% (SVM model with ANOVA based ranking), while the worst is only 1.8% (as for all bacteria identification QDA model with SNR based ranking), what proves that the presented method is suitable for differentiation of the bacterial strains, not only species. It was shown, that the proposed method based on registration of the unique diffraction signatures of bacterial colonies grown on solid nutrient media has perspectives to be used as a useful tool for better exploration and precise identification of environmental bacteria samples.

5. Conclusions

The newly developed bacteria identification system based on Fresnel diffraction patterns of bacterial colonies can find a potential use for monitoring of the environmental bacteria presence. The proposed method is based on registration of the unique diffraction signatures of bacterial colonies grown on solid nutrient media. Proposed algorithm in contrary to those used by other research groups in similar methods splits the diffraction patterns into disjoined regions and thus allows extraction of features that reflect the naturally occurring differentiation in the distribution of texture and morphology of the diffraction patterns. In consequence, our method gives information about total bacteria amount with the distinction of individual bacteria species/strains. It enables to examine the bacteria mixture seeded on a nutrient medium. Our technique allows to distinguish colonies of different bacteria, located on the same plate based on their diffraction patterns.

The novel multichannel optical systems enable the automatization of the bacterial colonies localisation and positioning, recording of their diffraction patterns and their 2D transmission coefficients. These additional monochromatic 2D transmission coefficient map of bacterial colonies are exhibiting similar species-/ strains-dependent features as the diffraction patterns.
Therefore, they can be used for explaining the physical foundation of Fresnel patterns generation by bacterial colonies, as well as the alternative optical signatures which can be used for characterisation of the morphological and optical properties of bacterial colonies, what was demonstrated.

Presented study is the first attempt to use the proposed optical method supported by statistical and image processing algorithms for identification of the soil bacteria species/strains including also the rhizospheric bacteria indicated as PGPR bacteria (B. subtilis, Rahnella aquatilis). Therefore, the proposed, quick method developed for identification of soil and PGPR bacteria can contribute to improved understanding of their biodiversity, interactions (signalling, growth promotion actions, disease suppression etc.) that occur between plants and bacteria. Exploration of physiological diversity of rhizospheric bacteria capable of reducing the disease incidence and promoting plant growth is essential for practical applications of microbial inocula and bioproducts in crop production technologies. The newly developed tool for identification of soil bacteria will be helpful for improving the selection, characterisation, and management of biological control especially will enable their practical applications.

The further works will be focused on registration of additional optical signatures of bacterial colonies for multi-parametric characterisation of bacteria species and strains.

Funding
The Wroclaw University of Science and Technology (No. 0401/0008/17 and No. 0401/0008/18).

Acknowledgments
The Bioavlee Ltd. is acknowledged for fruitful discussions on the topic and technical support.

Disclosures
The authors declare that there are no conflicts of interest related to this article.

References
1. S. W. Wessel, H. C. van der Mei, D. Morando, A. M. Slomp, B. van de Belt-Gritter, A. Maitra, and H. J. Busscher, “Quantification and qualification of bacteria trapped in chewed gum,” PLoS One 10(1), e0117191 (2015).
2. J. Kok, L. C. Thomas, T. Olma, S. C. A. Chen, and J. R. Iredell, “Identification of bacteria in blood culture broths using matrix-assisted laser desorption-ionization Sepsityper™ and time of flight mass spectrometry,” PLoS One 6(5), e23285 (2011).
3. D. Ziegler, A. Mariotti, V. Pfugler, M. Saad, G. Vogel, M. Tonolla, and X. Perret, “In situ identification of plant-invasive bacteria with MALDI-TOF mass spectrometry,” PLoS One 7(5), e37189 (2012).
4. S. Sauer, A. Freiwald, T. Maier, M. Kube, R. Reinhardt, M. Kostrzewa, and K. Geider, “Classification and identification of bacteria by mass spectrometry and computational analysis,” PLoS One 3(7), e2843 (2008).
5. D. I. Cattoni, J.-B. Fiche, A. Valeri, T. Mognin, and M. Noellmann, “Super-resolution imaging of bacteria in a microfluidics device,” PLoS One 8(10), e76268 (2013).
6. L. R. Dartnell, T. A. Roberts, G. Moore, J. M. Ward, and J.-P. Muller, “Fluorescence characterization of clinically-important bacteria,” PLoS One 8(9), e75270 (2013).
7. R. I. Amann, W. Ludwig, and K. H. Schleifer, “Phylogenetic identification and in situ detection of individual microbial cells without cultivation,” Microbiol. Rev. 59(1), 143–169 (1995).
8. P. Marschner, D. Crowley, and R. Lieberci, “Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize,” Mycorrhiza 11(6), 297–302 (2001).
9. P. Marschner, C.-H. Yang, R. Lieberci, and D. Crowley, “Soil and plant specific effects on bacterial community composition in the rhizosphere,” Soil Biol. Biochem. 33(11), 1437–1445 (2001).
10. C. H. Jaeger 3rd, S. E. Lindow, W. Miller, E. Clark, and M. K. Firestone, “Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan,” Appl. Environ. Microbiol. 65(6), 2685–2690 (1999).
11. P. H. Janssen, P. S. Yates, B. E. Grinton, P. M. Taylor, and M. Sait, “Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia,” Appl. Environ. Microbiol. 68(5), 2391–2396 (2002).
12. A. Croxatto, G. Prod’hom, and G. Greub, “Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology,” FEMS Microbiol. Rev. 36(2), 380–407 (2012).
13. I. Buzalewicz, A. Wieliczko, and H. Podbielska, “Influence of various growth conditions on Fresnel diffraction patterns of bacteria colonies examined in the optical system with converging spherical wave illumination,” Opt. Express 19(22), 21768–21785 (2011).

14. P. P. Banada, K. Huff, E. Bae, B. Rajwa, A. Aroonnual, B. Bayraktar, A. Adil, J. P. Robinson, E. D. Hirleman, and A. K. Bhunia, “Label-free detection of multiple bacterial pathogens using light-scattering sensor,” Biosens. Bioelectron. 24(6), 1685–1692 (2009).

15. P. R. Marcoux, M. Dupoy, A. Cuer, J. L. Kodja, A. Lefebvre, F. Licari, R. Louvet, A. Narassiguin, and F. Mallard, “Optical forward-scattering for identification of bacteria within microcolonies,” Appl. Microbiol. Biotechnol. 98(5), 2243–2254 (2014).

16. U. Minoni, A. Signoroni, and G. Nassini, “On the application of optical forward-scattering to bacterial identification in an automated clinical analysis perspective,” Biosens. Bioelectron. 68, 536–543 (2015).

17. Y. Tang, H. Kim, A. K. Singh, A. Aroonnual, E. Bae, B. Rajwa, P. M. Fratamico, and A. K. Bhunia, “Light scattering sensor for direct identification of colonies of Escherichia coli serogroups O26, O45, O103, O111, O121, O145 and O157,” PLoS One 9(8), e105272 (2014).

18. E. Bae, D. Ying, D. Kramer, V. Patsekin, B. Rajwa, C. Holdman, J. Sturgis, V. J. Davison, and J. P. Robinson, “Portable bacterial identification system based on elastic light scatter patterns,” J. Biol. Eng. 6(1), 12 (2012).

19. A. Suchwalko, I. Buzalewicz, and H. Podbielska, “Identification of bacteria species by using morphological and textural properties of bacterial colonies diffraction patterns,” in Proceedings of SPIE, F. Remondino, M. R. Shortis, J. Beyerer, and F. Puente León, eds. (2013), p. 87911M.

20. A. Suchwalko, I. Buzalewicz, and H. Podbielska, “Statistical identification of bacteria species,” in Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education, A. Méndez-Vilas, ed. (Formatex Research Center, 2013), pp. 711–721.

21. A. Suchwalko, I. Buzalewicz, A. Wieliczko, and H. Podbielska, “Bacteria species identification by the statistical analysis of bacterial colonies Fresnel patterns,” Opt. Express 21(9), 11322–11337 (2013).

22. A. Suchwalko, I. Buzalewicz, and H. Podbielska, “Bacteria identification in an optical system with optimized diffraction pattern registration condition supported by enhanced statistical analysis,” Opt. Express 22(21), 26312–26327 (2014).

23. H. Kim, B. Rajwa, A. K. Bhunia, J. P. Robinson, and E. Bae, “Development of a multispectral light-scatter sensor for bacterial colonies,” J. Biophotonics 10(5), 634–644 (2017).

24. H. Kim, N. Bai, A. K. Bhunia, G. B. King, E. D. Hirleman, and E. Bae, “Development of an integrated optical analyzer for characterization of growth dynamics of bacterial colonies,” J. Biophotonics 6(11-12), 929–937 (2013).

25. J. Vessey, “Plant growth promoting rhizobacteria as biofertilizers,” Plant Soil 255(2), 571–586 (2003).

26. I. Buzalewicz and H. Podbielska, “Optical sensing of bacteria by means of light diffraction,” OSA Tech. Dig., 6–7 (2010).

27. A. Suchwalko, I. Buzalewicz, and H. Podbielska, “Computer-based classification of bacteria species by analysis of their colonies Fresnel diffraction patterns,” Proc. SPIE 8212, 82120R13 (2012).

28. H. Podbielska, I. Buzalewicz, A. Suchwalko, and A. Wieliczko, “Bacteria classification by means of the statistical analysis of fresnel diffraction patterns of bacteria colonies,” in Biomedical Optics, BIOMED 2012 (2012), p. BSu5A.5.

29. I. Buzalewicz, K. Łżewski, M. Kujawińska, and H. Podbielska, “Degeneration of Fraunhofer diffraction on bacterial colonies due to their light focusing properties examined in the digital holographic microscope system,” Opt. Express 21(22), 26493–26505 (2013).

30. I. Buzalewicz and H. Podbielska, “Current trends of innovations in microbiological diagnosis by light diffraction,” in Advances in Intelligent Systems and Computing (Springer, Cham, 2017), 526, pp. 267–275.

31. M. D. Abràmoff, P. J. Magalhães, and S. J. Ram, “Image processing with ImageJ,” Biophoton. Int. 11(7), 36–42 (2004).

32. R. R Development Core Team, “R: A Language and Environment for Statistical Computing,” R Found. Stat. Comput. 1(2.11.1), 409 (2011).

33. I. Buzalewicz, M. Kujawińska, W. Krauze, and H. Podbielska, “Novel perspectives on the characterization of species-dependent optical signatures of bacterial colonies by digital holography,” PLoS One 11(3), e0150449 (2016).

34. J. W. Goodman, Introduction to Fourier Optics, McGraw-Hill Physical and Quantum Electronics Series (Roberts & Company, 2005).

35. I. Guyon, S. Gunn, M. Nikravesh, and L. Zadeh, Feature Extraction, Foundations and Applications, Studies in Fuzziness and Soft Computing (Springer, 2006), 207(11).

36. C. J. Huberty, Applied Discriminant Analysis (1994).