

1 Rapid detection and identification of bacteria directly
2 from whole blood with light scattering spectroscopy
3 based biosensor

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Abstract

Bacterial infections are one of the major causes of death worldwide. The identification of a bacterial species that is the source of an infection generally takes a long time, and often exceeds the treatment window for seriously ill patients. Many of these deaths are preventable if the bacterial species can be identified quickly. Here we present an optical spectroscopic method for rapid detection and identification of bacteria directly from whole blood using a light scattering spectroscopy technique. This technique was originally developed to detect pre-cancerous changes in epithelial tissues, characterize changes in tissue on the cellular scale, and characterize biological structures comparable to or smaller than a single wavelength. We demonstrate here that not only can an inexpensive light scattering spectroscopy-based biosensor rapidly detect and identify four bacteria species in the blood, responsible for the majority of death causing infections, but that species-level identification can potentially be made based on approximately one thousand bacterial cells per milliliter of blood. Observing entire colonies or performing susceptibility testing is therefore not required.

Keywords: Light scattering spectroscopy; Bacteria; Sepsis; Rapid detection; Whole blood

1. Introduction

Bacterial infections, which can cause sepsis and septic shock, are one of the major causes of death worldwide (Holmes et al., 2016). More than three million antibiotic-resistant bacterial infections occur in the U.S. each year and more than 50,000 people die as a result (Kadri, 2020). Sepsis occurs in 1-2% of all hospitalizations in the U.S, but accounts for 17% of in-hospital deaths. Presently, the identification of a bacterial species that is the source of an infection takes anywhere from several days to a week. This long identification time exceeds the treatment window for seriously ill patients and a broad-spectrum antibiotic treatment is initiated based on other information from the patient assessment. However, the choice of antimicrobial agent is a guess, which is often incorrect, resulting in the death of the patient in approximately 40% of severe sepsis cases (Paolucci et al., 2010), with every hour delay in the correct antimicrobial prescription reducing the survival rate by 7.6% (Kumar et al., 2006). Many of these deaths are preventable if the bacterial species can be identified quickly, allowing a more specific antibiotic treatment to be initiated immediately. Additionally, the fast identification of bacterial species in non-emergency scenarios will reduce the likelihood of emerging bacterial strains with antibiotic resistance, and improve patient response to treatment. Given the high financial costs associated with treating new antimicrobial-resistant strains of bacteria, reducing their likelihood can significantly reduce healthcare costs.

Current clinical methods depend on culture for bacterial identification (e.g. blood culture, urine culture, sputum culture). To perform a culture, a

sample of body fluid is added to bacterial broth media. This sample is maintained at body temperature and monitored over a period of up to 5 days. Bacteria that are easy to culture can be identified within 1 or 2 days, while bacteria that are more difficult to culture may take up to 5 days to culture, or may not give a positive result at all. After a bacteria is cultured, it is plated and tested for antibiotic susceptibility in order to identify the species. This is another time-consuming step and recent efforts have attempted to replace this step with more advanced techniques (Schubert et al., 2011). Other methods have shown bacterial identification through PCR, even at earlier stages in the process (Obara et al., 2011). However, a bacteria-specific primer is required for PCR to be successfully used and this is not available for a large number of bacterial species.

Several attempts have been made to decrease the time it takes to identify the bacterial species in blood or other biofluid samples (Liu et al., 2011; Nguyen et al., 2019) by performing bacteria detection without culture directly from whole blood. The techniques employ various flavors of nucleic acid amplification, such as Iridica Plex ID (Abbott Molecular, Des Plaines, IL), SeptiFast (Roche Diagnostics, Risch-Rotkreuz, Switzerland) (Sinha et al., 2018), or MinION nanopore sequencing (Oxford Nanopore Technologies, Oxford, United Kingdom) (Ashton et al., 2015; Bradley et al., 2015) or amplification-free technologies, such as a droplet digital detection technology IC 3D (Velox Biosystems, Irvine, CA) (Kang et al., 2014). However, the majority of these techniques still suffer from common problems of inability to detect non-targeted pathogens and a long (at least several hours)

turnaround time, which so far, has limited their ability to make a large impact on patient treatment decisions.

Optical techniques have also been used for bacteria characterization. For example, time-dependent fluorescence and Raman spectroscopy were employed by Layne, Bigio and colleagues (Layne et al., 1985). Sharaha and colleagues (Sharaha et al., 2017) demonstrated Fourier-transform infrared spectroscopy (FTIR) of the *Escherichia coli* bacterial strain. The feasibility of using light scattering to detect and differentiate bacteria has also been tested. By employing laser beam scattering, Wyatt demonstrated different angular dependence of scattering for three bacterial strains, namely, *Streptococcus lactis*, *Serratia marcescens* and *Neisseria catarrhalis*, with concentrations as low as $5 \cdot 10^6$ CFU/mL (Wyatt, 1969). To describe concentration of viable bacteria in a sample we use here and throughout the paper a standard microbiology unit of CFU/mL, where CFU stands for a colony-forming unit (Goldman and Green, 2008), independently of whether the colony were grown, as in the above cited paper, or the measurements were performed without culture directly from biofluid. Light scattering has been employed for characterization of individual strains of bacteria of different sizes and shapes at rather high 10^7 – 10^8 CFU/mL concentrations (Katz et al., 2003). Angle-resolved light-scattering in combination with the flow cytometry-based approach were used to differentiate two strains of *Escherichia coli* bacteria (Konokhova et al., 2013), while other light scattering patterns-based approaches have also been tested (Romanov and Yurkin, 2021). Angular imaging patterns of a forward-scattering laser beam were also employed for detection of bacterial pathogens in food (Banada et al., 2009). Finally, an

optical scatter imaging (OSI) technique was developed to probe local particle size *in situ*, and was used to detect the *Escherichia coli* bacterial strain by Boustany and colleagues (Zheng et al., 2009).

In this paper we present a novel optical spectroscopic method for rapid detection and identification of bacteria directly from whole blood, using light scattering spectroscopy (LSS) based techniques. Originally, LSS or elastic scattering spectroscopy (ESS) was developed to characterize changes in tissue on the cellular scale and to detect pre-cancerous changes in epithelial tissues (Perelman et al., 1998; Backman et al., 2000; Drezek et al., 2003; Sokolov et al., 2004; Lovat et al. 2006; Qiu et al., 2010; Zhang et al., 2017). Later, LSS (Fang et al., 2003; Melo et al., 2014) and LSS-based microscopy techniques (Liu et al., 2005; Itzkan et al., 2007; Huang et al., 2009) were employed to characterize biological structures comparable to or smaller than a single wavelength, such as subcellular organelles and exosomes. We demonstrate here that not only can LSS detect and identify four bacteria species in the blood, responsible for the majority of death causing infections, but that species-level identification can potentially be made based on approximately one thousand bacterial cells per milliliter of blood, without the need for observing entire colonies or performing susceptibility testing.

2. Materials and methods

2.1. Light scattering spectroscopy system for bacteria detection

Light scattering spectroscopy is based on the detection of a light scattering spectrum that depends on the fundamental characteristics of the particles,

such as their size and refractive index. LSS typically utilizes the convenient approximation of assuming that the particles can be adequately modeled as spheres of unknown size and refractive index (Perelman et al., 1998). The exact solution of light scattering by spheres can be solved and is known as Mie theory, with the results depending on the diameter of the sphere, the relative refractive index, the wavelength of light, the polarization, observation angle, and distance to the particle (Mie, 1908).

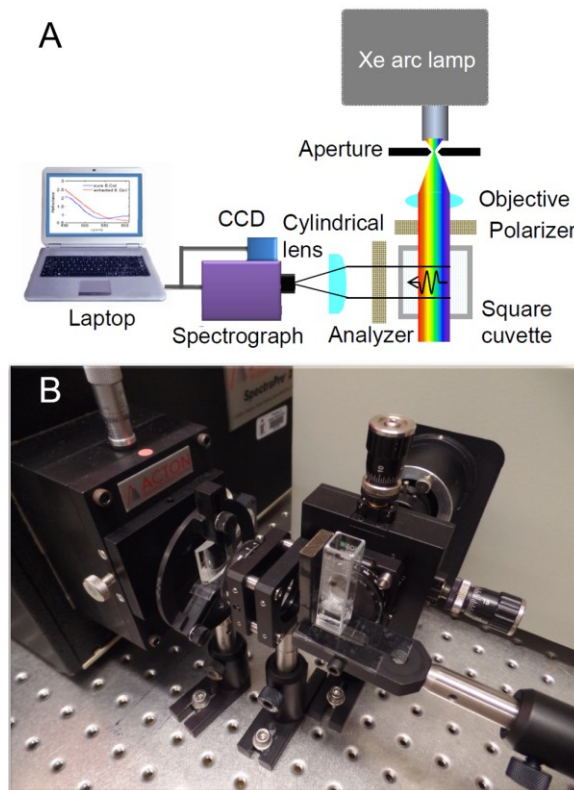


Fig. 1. Schematic (A) and photograph (B) of the LSS system for bacteria detection and identification.

Light scattering spectra of bacteria were collected using the experimental system shown in Fig. 1. A 75 W xenon-arc lamp (Newport) was used as a source of white light. A 10X objective was employed to illuminate a sample placed in a 1 cm path length square glass cuvette (Hellma 101-OS).

The sample consisted of a liquid suspension of either individual strains of bacteria, mixtures of various strains of bacteria, or samples of bacteria in human blood. As the polarization of the linearly polarized light is mostly retained in the 90-degree scattering when the polarization is perpendicular to the scattering plane for weak arbitrarily shaped scatterers, including bacteria (Koch and Ehrenfeld, 1968), as well as scatterers with the reflectance symmetry with respect to the scattering plane (Chýlek, 1977) that are not necessarily described by weak scattering, such as spherical scatterers with high relative refractive indices, two polarizing filters with their polarization direction perpendicular to the scattering plane (denoted by polarizer and analyzer in Fig. 1A) were employed. By polarizing the incoming light and detecting the component of the scattered light which is perpendicularly polarized relative to the scattering plane, one can reduce the contribution of multiple scattering and thus increase the bacteria-specific contribution of a single scattering. The scattered light was collected by a cylindrical lens and delivered onto the entrance slit of the imaging spectrograph (Princeton Instruments SP-2300i) coupled to a high-efficiency CCD with thermoelectric cooling (Andor Technology iXon DV885 EMCCD), providing light detection in the 450–700 nm wavelength range. The detector was controlled by a computer, onto which the data was transferred, stored, and processed.

In order to calibrate the LSS system and establish the ability of LSS to detect and differentiate submicrometer particles, we first performed experiments with polystyrene microspheres with four different diameters: 82 ± 6 nm, 380 ± 15 nm, 510 ± 11 nm, and 745 ± 10 nm suspended in water, with diameters and standard deviations provided by the manufacturer

(Polysciences, Inc). We used 82 nm microspheres to calculate the ratio of the Mie theory-based spectrum for the 90-degree scattering and the experimental spectrum. By multiplying that ratio on the spectra of the 380 nm, 510 nm, and 745 nm microspheres, we obtained the calibrated spectra with the characteristics of the experimental system taken into account.

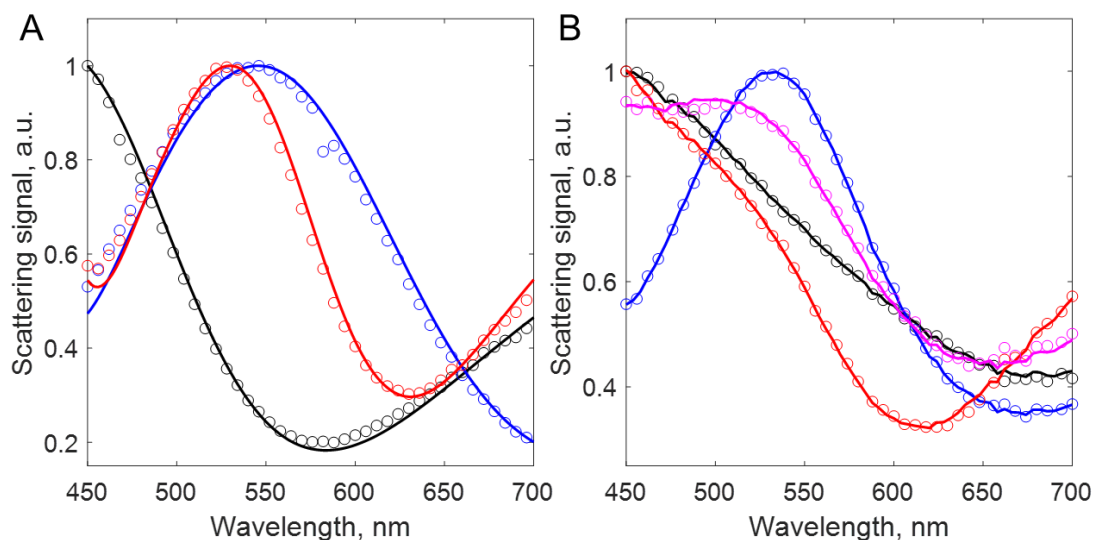


Fig. 2. Light scattering spectroscopic measurements using bacteria-size microsphere scatterers. (A) Comparison of LSS experimental data (circles) and theory (lines) for 380 nm (black), 510 nm (blue), and 745 nm (red) microspheres. The reconstructed sizes are 390 nm, 500 nm and 735 nm, respectively. (B) Comparison of LSS experimental data and theory for 1:1 mixture of 380 nm and 510 nm (black), 510 nm and 745 nm (blue) and 380 nm and 745 nm (red), and 1:1:1 mixture of 380 nm, 510 nm, and 745 nm (purple) microspheres.

The spectra predicted by Mie theory were fitted to the calibrated experimental data (see Fig. 2A) using least-squares minimization (Fang et al., 2003). The reconstructed mean sizes and standard deviations of the microspheres were 390 ± 14 nm, 500 ± 10 nm, and 735 ± 8 nm, and appeared to be in excellent agreement with the manufacturer provided specifications for all sizes of microspheres used in the experiments (the

manufacturer does not provide the functional form of the size distributions for either size of microspheres). Based on these experiments, the accuracy of the LSS experimental system and algorithm for the spherical scatterers is estimated to be 10 nm.

We also developed an approach capable of reconstructing concentrations of individual scatterers present in the interrogation volume of the LSS system. The approach does not require Mie theory based modeling of the scatterers' spectra, but can just employ a prior compiled "LSS library" of spectra of individual scatterers, such as various types of bacteria strains, to obtain their concentrations. In this case, no assumption about the bacteria shapes or refractive indices is needed.

The experimentally measured LSS spectrum can be presented as a weighted sum of the spectra of the scatterers present in the interrogation volume

$$S(\lambda) = \sum_{i=1}^N c_i S_i(\lambda) + \varepsilon(\lambda) \quad (1)$$

where N is the number of scatterers, c_i and $S_i(\lambda)$ are the concentrations and spectra of individual components present in the LSS library, respectively, and $\varepsilon(\lambda)$ is the error which includes the experimental noise of the system, as well as the contributions of unknown scatterers that may exist in the sample. In that case, the concentrations of the components could be found by employing the conventional linear least square minimization approach:

$$\sum_{\lambda} \left(S(\lambda) - \sum_{i=1}^N c_i S_i(\lambda) \right)^2 \Rightarrow \min \quad (2)$$

To test the performance of this approach, we measured three binary and one ternary mixtures of polystyrene microspheres in water. The reconstructed spectra of the mixtures are presented in Fig. 2B and the related concentrations are given in Table 1.

Table 1. Reconstruction of the concentrations from mixtures of 380 nm, 510 nm, and 745 nm microspheres using LSS spectra. Here column A provides actual relative concentrations of the microspheres and column R provides the reconstructed concentrations. Sample 1 - 380 nm and 510 nm, sample 2 - 510 nm and 745 nm, and sample 3 - 380 nm and 745 nm mixtures (50% : 50%), and sample 4 - 380 nm, 510 nm, and 745 nm (33.3% : 33.3% : 33.3%).

Samples	380 nm		510 nm		745 nm	
	A	R	A	R	A	R
1	50%	46.3%	50%	53.7%	0	0
2	50%	48.4%	0	0	50%	51.6%
3	0	0	50%	45.9%	50%	54.1%
4	33.3%	30.4%	33.3%	37.4%	33.3%	32.2%

As one can easily see, the approach can reconstruct the relative concentrations of the binary and ternary mixtures of microspheres with a better than 95% accuracy.

2.2. Preparation of bacteria samples in water and whole blood

Four typical gram-positive and gram-negative bacteria strains, *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10798), *Klebsiella pneumoniae* (ATCC 13883) and *Staphylococcus aureus* (ATCC 25923), were purchased from American Type Culture Collection (ATCC) and cultured according to the ATCC guidelines. Two strains, *P. aeruginosa* and *S. aureus*, were streaked onto trypticase soy agar plates, *E. coli* was streaked onto

Luria-Bertani (LB) agar plates and *K. pneumoniae* was streaked onto Difco™ Nutrient Agar (BD 213000, BD, Franklin Lakes, NJ) plates. After overnight incubation at 37°C, a single colony of each strain was cultivated in 10 mL of the respective broth at 37°C with shaking at 200 rpm. Here *P. aeruginosa* and *S. aureus* were grown in trypticase soy broth; *E. coli* in LB broth and *K. pneumoniae* in Difco™ Nutrient broth (BD 234000, BD, Franklin Lakes, NJ). After 14 to 16 hours of incubation, suspension of each bacteria was centrifuged at 2000 g for 10 minutes at room temperature. The pellets of bacteria were resuspended with 10 mL of phosphate-buffered saline (PBS) and centrifuged (2000 g, 10 min, room temperature). This washing procedure was repeated twice to remove any culture broth residue. After the final wash, bacteria were suspended in PBS.

The optical density (OD) of suspensions was then measured with a BIO-RAD SmartSpec Plus spectrophotometer (Bio-Rad, Hercules, CA, USA) at 600 nm and correlated with bacteria number densities in suspensions (Chance and Mawhinney, 2000; DePamphilis and Adler, 1971). The same amount (10 mL) of each culture medium without inoculating bacteria was incubated for 14 to 16 hours, centrifuged, washed twice and resuspended with PBS, serving as blanks for OD measurements or negative controls for LSS measurements. The concentration of resuspended bacteria was determined using spectrophotometry. This cell concentration was reconfirmed by spiral plating (AUTOPLATE 4000, Exotech Inc., FL, USA) the suspension onto the respective agar plates to obtain total viable counts.

To perform LSS measurements, each bacteria sample was resuspended in Milli-Q water to obtain 10^7 CFU/mL, 10^5 CFU/mL, 10^4

CFU/mL and 10^3 CFU/mL concentrations, which were confirmed by spectrophotometry. The LSS spectra for each 400 μ L bacteria sample placed in a 1 cm path length square glass cuvette were collected at room temperature using an integration time of 10 s (0.1 s with 100 repeats). For each concentration, three samples were prepared, and three measurements were performed on each sample.

Bacterial samples in whole blood were prepared by mixing 50 μ L of bacteria suspensions in PBS at concentrations of 10^7 CFU/mL with whole blood obtained from healthy adult volunteers. The resulting concentration of bacteria in whole blood was 10^3 CFU/mL. The protocol was reviewed by the BIDMC Institutional Review Board, and the requisite approvals were obtained.

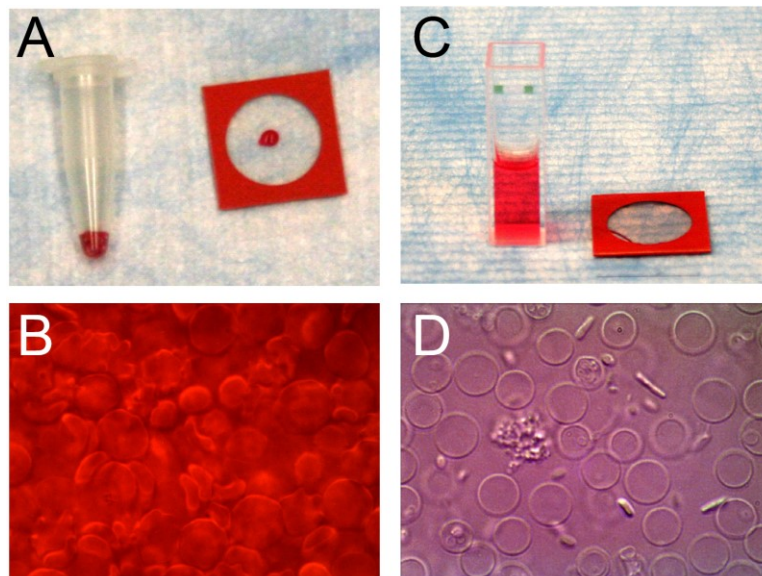


Fig. 3. Sample of *E. coli* bacteria in whole blood, obtained for realistic bacterial concentration in blood during mild sepsis (A), and the DIC microscopy image of the sample (B). The same sample after adding Milli-Q water to lyse the cells (C), and the DIC microscopy image of the sample (D). *E. coli* bacteria can be clearly observed, along with the erythrocyte ghosts.

To minimize contribution of red blood cells in the samples, the cells were lysed by hypotonic shock using cold water. Bacteria samples in whole blood (200 μ L) were mixed with ice-cold Milli-Q water (600 μ L). When red blood cells lyse, the hemoglobin spreads throughout the sample, making it look pinkish. However, the medium inside and outside of the cell becomes the same, making the scattering cross section of the cell ghost to be approximately 0.1% of the cross section of the intact red blood cell. As scattering is significantly reduced, the solution becomes significantly more clear. The mixtures were lightly agitated for 2 min or until the solution became fully clear which indicated that red blood cells lysis was complete (Fig. 3D) (Janse et al., 2006). Prior to the LSS measurements, the samples were kept still for 3 min at room temperature, allowing the cell debris to settle.

3. Results and discussions

3.1. Detection and identification of bacteria in water suspensions

We performed LSS experiments with water suspensions of four bacterial species: *E. coli*, *K. pneumonia*, *P. aeruginosa*, and *S. aureus*. These four species account for nearly 80% of the total sepsis cases caused by specific bacteria (Ani et al., 2015). To establish that each bacteria strain has a unique, easily identifiable LSS spectrum, initial measurements were performed at the relatively high concentration of 10^7 CFU/mL. The microphotographs of the bacteria presented in Fig. 4A show that it is not possible to differentiate *E. coli* from *P. aeruginosa*, or *S. aureus* from *K.*

pneumonia just using their microscopic appearance. However, LSS spectra of the bacteria strains presented in Fig. 4B are rather unique, clearly differentiating each type of bacteria.

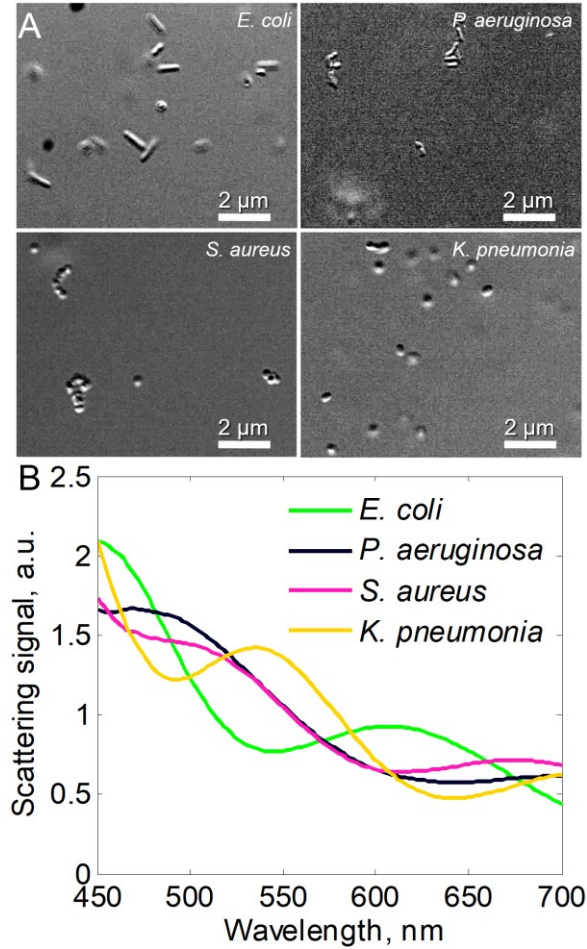


Fig. 4. Microphotographs (A) and LSS spectra (B) of four bacterial strains: *E. coli*, *P. aeruginosa*, *S. aureus*, and *K. pneumonia* suspended in water at 10^7 CFU/mL concentration.

We should note here that the experimental LSS spectra of bacteria presented in Fig. 4B are linear combinations of three components: (1) LSS spectra of bacteria in the sample, $S_b(\lambda)$, averaged over all possible orientations of the bacteria, (2) LSS spectra of small (under 100 nm) particles always present in the suspension, which has a typical $1/\lambda^4$ Rayleigh

wavelength behavior, and (3) experimental noise. The Rayleigh contribution should be removed from the spectrum with the removal procedure described previously (Fang et al., 2003).

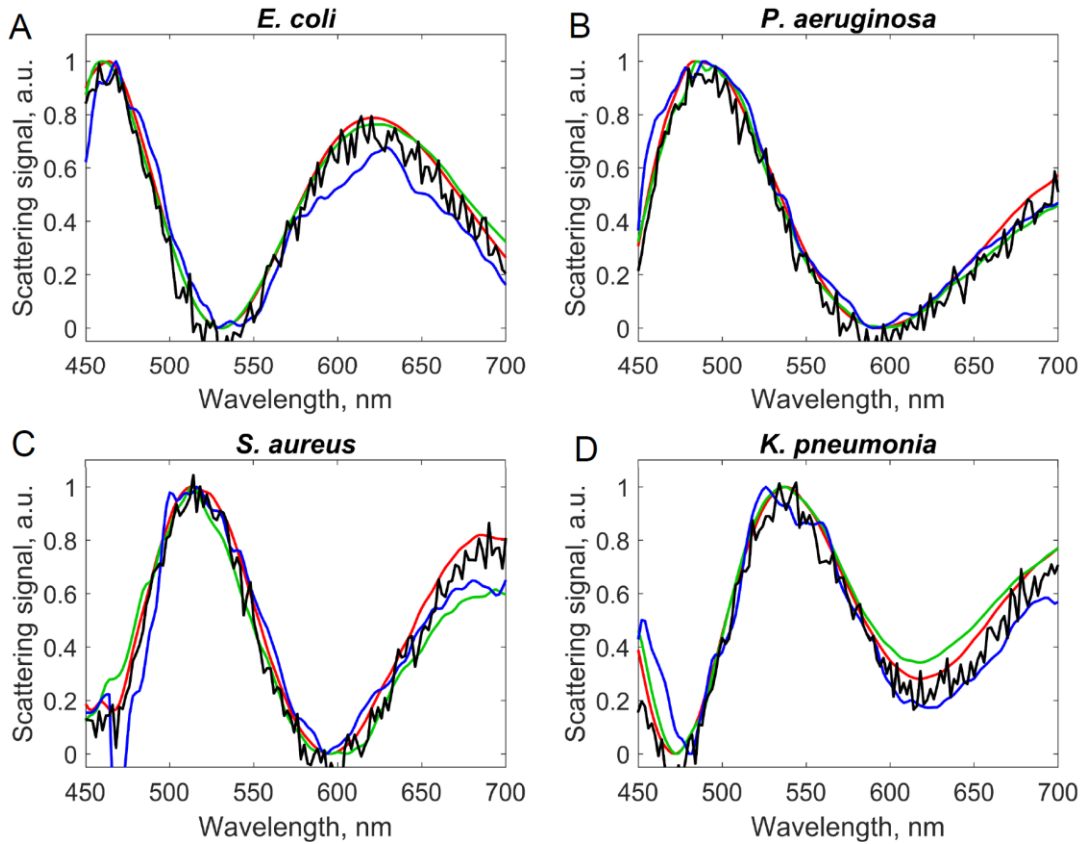


Fig. 5. Rayleigh contribution corrected LSS spectra of four bacterial strains for (A) *E. coli*, (B) *P. aeruginosa*, (C) *S. aureus*, and (D) *K. pneumonia*, at concentrations of 10^7 CFU/mL (red), 10^5 CFU/mL (green), and 10^4 CFU/mL (blue), and 10^3 CFU/mL (black).

The resulting Rayleigh contribution corrected LSS spectra for concentrations of bacterial strains of 10^7 , 10^5 , 10^4 and 10^3 CFU/mL are presented in Fig. 5 and clearly demonstrate that each bacteria type has a unique and reproducible $S_b(\lambda)$. Moreover, even at concentrations of 10^3 CFU/mL, which is the characteristic of mild sepsis (Cheng et al., 2013), the

bacteria spectra are still easily recognizable despite the presence of a certain amount of noise in the spectra.

These experimental Rayleigh contribution corrected LSS spectra can serve as markers for bacteria identification. No assumption about the bacteria shapes or refractive indices is required, while the independence of the spectral shape on the bacteria concentration is demonstrated in Fig. 5.

3.2. Rapid detection and identification of bacteria in whole blood

In order to test the feasibility of the LSS technique to detect and identify bacteria relevant to sepsis treatment clinical conditions, we performed measurements in the whole blood samples (see Methods). We prepared a total of 16 samples, with samples 1 through 4 having *E. coli* in whole blood, 5 through 8 having *S. aureus*, 9 through 12 having *P. aeruginosa*, and 13 through 16 had *K. pneumonia*. Red blood cells are present in a whole blood sample and are significantly larger than bacteria. Given that they have a different scattering spectrum, the overall amount of light scattering from red blood cells could be a problem. Therefore, as explained in the Methods section, red blood cells were lysed by hypotonic shock using cold water. After the cell lysis, the samples were kept still for 3 min at room temperature, allowing the cell debris to settle. Typical raw LSS data for *E. coli* bacteria extracted from a whole blood sample is presented in Fig. 6A, and the LSS spectrum after Rayleigh correction can be seen in Fig. 6B.

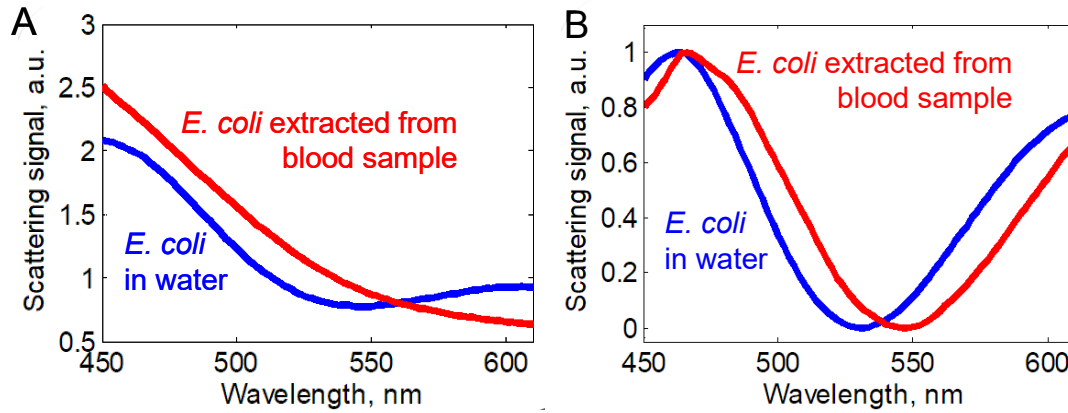


Fig. 6. Raw LSS data for *E. coli* bacteria extracted from whole blood sample (A), and LSS spectrum after Rayleigh correction (B), with spectra in water provided as a reference.

Since the majority of clinical sepsis cases contain dangerous amounts of just one bacteria type within the patient's blood (Ani et al., 2015), and given that this bacteria should be rapidly identified, only one type of bacteria was present in each of the testing samples. Using the spectral unmixing approach described in Sec. 2.1, we reconstructed relative concentrations of bacteria in each of the samples (see Table 2). The first column for each bacteria strain shows the actual percentages of bacteria in four samples, and the second column provides the average percentages reconstructed from the LSS measurements. For example, for samples 1 through 4, we obtained an average percentage of *E. coli* to be more than 95%, with three outliers giving less than 5% in total. The overall accuracy of the LSS method in identification of bacteria in blood samples appears to be approximately 97%, which is quite encouraging.

Table 2. Identification of bacteria in whole blood samples with LSS. Here column A provides actual percentages of the bacteria and column R – reconstructed percentages.

Samples	<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>K. pneumonia</i>	
	A	R	A	R	A	R	A	R
1 – 4	100%	95.5%	0	2.8%	0	1.5%	0	0.2%
5 – 8	0	4.4%	100%	90.1%	0	0.1%	0	5.5%
9 – 12	0	0	0	0	100%	100%	0	0
13 – 16	0	0.2%	0	0	0	0	100%	99.8%

Although the integration time for LSS measurements is ten seconds, the overall procedure time is approximately three minutes when two preparation steps of red blood cell lysis and cell debris settlement are taken into account. Based on our results, we believe that the LSS method is capable of rapid detection and identification of bacteria strains in whole blood samples at bacteria concentrations characteristic of the mild sepsis.

4. Conclusions

In this article, we describe a new optical spectroscopic method for rapid detection and identification of bacteria directly from whole blood, without the need to perform bacteria culture. Several typical gram-negative (*E. coli*, *K. pneumonia*, *P. aeruginosa*) and gram-positive (*S. aureus*) strains of bacteria, known to cause sepsis, were identified with LSS. Each of these strains requires different antibiotic treatment, with *E. coli* normally treated with fluoroquinolones or azithromycin, and recently with rifaximin, *K. pneumonia* with aminoglycosides and cephalosporins, *P. aeruginosa* with carbapenems, polymyxins, and recently tigecycline, and *S. aureus* with penicillin. The fact that each of these bacteria requires quite specific antibiotics for treatment emphasizes the importance of their rapid identification.

396 The method detects physical and biochemical properties of bacteria,
397 related to their sizes, shapes and refractive indices, utilizing principles of LSS.
398 The inexpensive LSS bacteria detection and identification technique does not
399 require complex sample preparation. The only preprocessing step consists of
400 adding distilled cold water to a whole blood sample to lyse the red blood cells,
401 steering it and waiting for approximately three minutes for the cell debris to
402 settle. Cold water induces blood cells swelling followed by cell rupture due to
403 hypotonic shock, while bacteria are kept intact because they have a rigid
404 exoskeleton composed of peptidoglycan, which protects them from osmotic
405 pressure (Silhavy et al., 2010).

406 We should note here that although there are a number of commercially
407 available lysing products or reagents that can lyse red blood cells more
408 efficiently, we have chosen hypotonic shock using ice-cold water, which is
409 one of the simplest and most commonly used methods to lyse red blood cells.
410 This choice was made because chemical disruption of the red blood cell
411 membrane with detergents or alkali materials employed in chemical lysis can
412 alter the properties of the bacterial sample (Han et al., 2019). In addition,
413 some red blood cell lysis buffers are specially formulated to be used for DNA
414 or RNA isolation from blood, which is not suitable for the detection of bacteria
415 in our study.

416 In the LSS based detection, unlike PCR, free DNA from lysed cells does
417 not interfere with the LSS measurement because the signal is dominated by
418 the largest scatterer in the suspension. Furthermore, species-level
419 identification can be made based on a small number of bacterial cells without
420 the need for susceptibility testing involving bacterial colonies' growth.

Sample preparation with LSS system takes only a few minutes, while spectroscopic measurements are performed in several seconds, which makes the approach ideal for rapid detection and identification of bacteria directly from whole blood in hospital or ambulatory settings or even in the field.

It is important to emphasize that 80% of all sepsis infections in hospitals are due to the 4 bacteria species presented here. By adding 7 additional bacteria species (*Streptococcus pneumoniae*, *Listeria monocytogenes*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Proteus mirabilis*), this number can be brought to approximately 95% (Ani et al., 2015). While the questions of the spectral uniqueness and reproducibility for those additional bacteria species require additional consideration, in principle, by increasing the “LSS bacteria library” to 11 bacteria species, one should be able to rapidly identify the sepsis causing bacteria in the majority of clinical cases.

As the method differentiates bacteria based on their size, shape and refractive index, it is also important to note that individual strains of the same bacterial species have remarkably reproducible shapes and narrow distributions of sizes (Männik et al., 2012; Amir, 2014). For example, the strain of *S. aureus* bacteria used in this work is the methicillin-sensitive (MSSA) strain, which accounts for 95.7% of all *S. aureus* caused sepsis (Ani et al., 2015). This bacteria has a spherical shape and is approximately 100 nm smaller than the other strain of *S. aureus* which is methicillin-resistant (MRSA) (Raju et al., 2007; Onyango et al., 2013). This difference in size is significantly larger than the accuracy of the LSS measurements. Moreover, according to the standard of care, each of the bacterial species is treated

with just several very specific antibiotics. For example, independently of the strain, *S. aureus* is treated with penicillin, and thus the identification of bacterial species with the LSS system would immediately result in a very significant reduction in the choice of antibiotics used for treatment.

Finally, the presented method is optimized for identifying bacteria from blood for rapid sepsis diagnostics. It is capable of achieving bacteria detection in concentrations as low as 10^3 CFU/mL in blood, in approximately three minutes, giving the physician immediate information. Therefore, due to the simplicity and feasibility for manipulation, this method could potentially be adapted as part of a rapid clinical laboratory routine.

Declaration of competing interest

The authors declare that they have no conflict of interest.

CRedit authorship contribution statement

Le Qiu: Methodology, Investigation, Resources, Project administration, Writing - original draft. Lei Zhang: Investigation, Writing - original draft. Gary L. Horowitz: Methodology, Writing - review & editing. Vladimir Turzhitsky: Methodology, Investigation, Writing - review & editing. Mark F. Coughlan: Writing - review & editing. Maria Glyavina: Writing - review & editing. Umar Khan: Visualization. Yuri N. Zakharov: Investigation, Resources. Edward Vitkin: Data curation, Software, Formal analysis. Irving Itzkan: Methodology, Writing - review & editing. Lev T. Perelman: Conceptualization, Methodology, Project administration, Writing - original draft, Funding acquisition, Supervision.

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