Biophysics for Biomedical and Environmental Sciences

Editor: Monica Florescu

Transilvania University of Brasov Press
2016

EDITURA UNIVERSITĂŢII *TRANSILVANIA* DIN BRAŞOV
Adresa: 500091 Braşov,
B-dul Iuliu Maniu 41A
Tel:0268 – 476050
Fax: 0268 476051
E-mail: editura@unitbv.ro

Editură acreditată de CNCSIS
Adresa nr.1615 din 29 mai 2002

Descrierea CIP a Bibliotecii Naţionale a României
Biophysics for biomedical and environmental sciences / ed.:
Monica Florescu. - Braşov : Editura Universităţii "Transilvania", 2016
Conţine bibliografie
ISBN 978-606-19-0768-7

I. Florescu, Monica (ed.)

577.3

CONTENTS

FINDING NEW TOOLS FOR OLD ISSUES – NOVEL STRATEGIES FOR BACTERIAL ULTRASENSITIVE DETECTION

N. E. DINA[1]

Abstract: *Currently, microbial culture-based and molecular assays (immunological or nucleic acid technologies) are among the most commonly used methodologies in detection and identification of microbial pathogens. This work reviews the development in the field of the surface-enhanced Raman scattering (SERS)-based approaches for pathogen detection, including whole-cell fingerprinting, label-free strategies and handheld diagnostic systems, which hold great promise as rapid, reliable and affordable alternatives for infection screening and treatment.*

Key words: *SERS, microbial pathogens, ultrasensitive detection, high throughput screening, bacterial infection, optofluidic system.*

1. Introduction

Pathogen detection is the critical means in issues related to public health and food safety; and only the rapid, sensitive and efficient tools can enable the end users to make accurate assessments on the risks of infections (humans and animals) or contaminations (foods and other commodities) caused by microorganisms. One of the major concerns is the increasing rate of mortality due to **sepsis** from 27% to 41% (only in the German hospitals) in 6 years [1]. Neonatal sepsis, for instance, is present as a cause-specific mortality and morbidity indicator in World's Health Statistics Report for 2015 with significant contribution [2].

The conventional methods in clinical bacteraemia diagnostic take between 1-2 days and involve plating, growth, colony counting and examination inside hospital setting. The current limitations of these standard protocols for sample analysis and infections' diagnosis are constantly challenged by trauma situations, on-site medical interventions, rapid analysis in outdoor conditions (caused by military actions, related to water and environmental research), food safety and biodefense. Thus, fast alternatives, reliable solutions for the healthcare providers are to identify. The stringency is given by the current drive to decrease time of diagnosis and to prevent infection-related morbidity and mortality. An innovative detection and molecular diagnosis tool is now embodied in the combination of Raman spectroscopy with nanotechnology [3-7].

Generally, only bulk samples or concentrated solutions may be investigated by

[1]Department of Molecular and Biomolecular Physics, National Institute of R&D of Isotopic and Molecular Technologies, Cluj-Napoca

using Raman spectroscopy owing to sensitivity limitations. However, a particularity, the nanoscience phenomena of **surface-enhanced Raman spectroscopy (SERS)** has already addressed such concerns, by allowing limits of detection down to single molecules [8] or single cells [9, 10]. In addition, by using ultrasensitive Raman spectroscopy, the detection benefits from high specificity due to molecular fingerprinting, whilst cumbersome sample preparation and invasive probing/treatment are avoided. These unique characteristics of SERS-based approaches offer great potentials in applications such as pathogen detection, infection diagnosis and **high throughput screening (HTS)** [11]. The main asset remains the rapid, within minutes [12-15], and reliable spectral response of bacteria, so crucial in sepsis awareness and prevention.

Spectral pathology, an emerging molecular diagnose approach, is exploring biochemical fingerprinting via characteristic bond vibrations of the cellular components, changes in Raman/SERS profiles related to disease onset and progression [16]. As intrinsic molecular information is probed by the inelastic scattering of light, no external label is required and only minute sample volumes are necessary, making Raman scattering spectroscopy a very appealing tool.

While remarkable progress has been made in nanotechnology in the last decades, by developing various nanostructured metallic surfaces used for nanoparticles [17, 18], nanorods [19], dendrites [10], etc.; challenges or drawbacks in real-life applications such as pathogen's detection and biomolecular screening remain to be overcome [20]. SERS-based approaches are still in their experimental stages and require further research and validation before being considered suitable for routine analysis in **point-of-care (POC)** tests or for production on a commercial scale [21]. Thus, in this short review, the development in optical biosensors by employing multiple-labelling or label-free models and their practical relevance in pathogen's detection is updated.

2. Microfluidic approaches in SERS sensing

SERS-based biosensors become more and more popular as a result of the intensive drive towards using vibrational spectroscopy techniques for clinical purposes in diagnostics. Infections are still a world wide spread cause of hospitalization and contribute in a worrying rate to decease through severe sepsis [1, 2].

Microfluidic devices enable microorganisms' detection at single-cell level by using low-cost materials like PMMA (Polymethylmethacrylate) [22] or PDMS (Polydimethylsiloxane) [23] and benefit from increased throughput capability. These materials are transparent at optical frequencies (240 nM – 1100 nM), facilitating the observation of contents in micro-channels visually or through a microscope. Moreover, the molding technique allows mass-producing of microfluidic chips from a mold. By sealing the tubings and injecting the fluid in the detection window, the pathogens are not released in the ambient atmosphere

and contamination is avoided. It enables cell culture by controlling the amount of gas through PDMS or dead-end channels filling [24]. In the food industry inert metals are commonly used, especially stainless steel and aluminium. Stainless steels contain alloys such as chromium to increase resistance to corrosion (rusting). With progress in the processing technologies and the integration of nonconventional (i.e. not silicon-based) materials, a new field named **microelectromechanical systems (MEMS)** emerged [23]. These miniaturized systems overcome many demerits compared to bulkier analytical instruments: they support precise control of fluids flowing under laminar regime, minimize the analysis volumes required of reagents and samples, favour short reaction times, enable multiplexed analysis, require less power to operate, are portable, and involve low production costs. Today, structures can be fabricated with sub-micrometre precision, flows of liquids can be precisely controlled using integrated or external pumps and valves, and quantitative detection of various analytes can be assessed with high sensitivity using optical-based techniques.

Micro-particle separation plays an important role in several microfluidic approaches assuring the detection in complex matrices such as full blood, food-related media, etc. [25]. Density gradient centrifugation and tangential flow filtration or cell sorting systems are the commonly used approach to isolate the target cells from such heterogeneous samples. Several strategies combined with microfluidic devices are sedimentation, filtration, cell deviation, cell trapping, etc.

Sensing for pathogens is provided either by "ON" and "OFF" signals from typical reactions of nucleic acids, enzymes, antibodies or aptamers (indirect detection) or whole-organism fingerprint and a whole array of additional information like cell-drug interaction or biochemical screening and quantification (direct detection). In each case the specific values for purity and cell health must meet requirements for further analysis and assessments. Furthermore, the huge and expensive equipment used in biomolecular detection fields, conventional antibody-antigen reaction-based such as enzyme-linked immunosorbent assay (ELISA), involve diffusion-dominated kinetics that are too slow and difficult to carry target molecules to the detection surface, thus resulting in low sensitivity (~100 nM). This aspect in addition to long hybridization time (4–6 h), high costs due to elaborate enzyme tagging and sophisticated optical instruments that are not portable, limit their practical relevance.

The combination of **optical tweezers (OT)** with fully-integrated microfluidic systems enables the design and concept of new devices for biological analysis with enhanced sensitivity and ready-to-use also by non-specialized personnel [26]. Miniaturized OT-microfluidic models solve common issues by space-saving analysis platforms, reduced maintenance work for critical alignment procedures, and enhanced versatility through the integration of **optical traps.** Optical trapping is achieved by tightly focusing a laser through a high numerical aperture (NA) objective. **Optical micro-tweezers (μOT)** based on fibre bundles can trap a single cell and excite its Raman response [22, 27]. Important to notice that in this

particle-size range, μOT provides a trapping efficiency comparable to that of OT based on high-NA microscope objective, as the tests performed on red blood cells and tumour cells (colon cancer cells) diluted in phosphate buffered saline solution unveiled. The Raman signature recorded for a cell optically trapped into the microfluidic channel was mainly dominated by the lipids and protein specific bands, but with a high noise signal contribution [26].

Laser Trapping Raman Systems (LTRS) consist of a laser source for trapping and potentially a second laser source for Raman excitation; a microscope to focus the trapping laser beam, image the trapped particle, and collect the Raman signal; a spectrometer and a detector to record the Raman spectra. In order to minimize the elastic scattering from the trapping and exciting laser while maximizing the Raman signal, a notch filter is usually required. Since laser tweezers trap particles near the focal point of the objective lens, the particles are necessarily aligned in the high intensity part of the beam, enabling efficient Raman excitation. As a result, most LTRS systems use the trapping laser to also act as the Raman excitation light source, although a second laser can provide additional flexibility [25]. Raman spectra of trapped yeast cells revealed distinct spectra depending on whether the yeast cells are alive or dead [25, 28]. Soon afterwards, it was applied to obtain SERS from single optically trapped bacterial spores [29].

LTRS in microfluidics involves the ability to continuously trap, characterize, and release individual particles/spores/cells for on-line Raman-based particle identification or for a series of successively arriving particles with different properties.

In addition to optical trapping, microfluidic systems have also been combined with electrostatic trapping [30, 31]. Moreover, combining microfluidics with SERS enables much faster Raman measurements, and makes possible to identify cells and characterize cellular chemical dynamics in flow, without needing to trap the particles [25]. Microfluidic microbial culture devices were recently designed in order to facilitate the cell seeding process by utilizing capillary action and the rapid determination of minimum inhibitory concentration (MIC) of antibiotics was assessed especially for bacteria with unknown resistance mechanism [24].

A promising approach including an **optofluidic system** and rapid on-chip SERS detection and identification of bacteria was very recently developed [32]. The novel biochip for rapid separation and concentration of bacteria for further label-free and highly sensitive on-chip detection/analysis was successfully tested on *S. aureus, E. coli,* and *P. aeruginosa*. By combining the biased AC electroosmosis (ACEO) and dielectrophoresis (DEP) with a 3D electric field design, a separation mechanism and selective concentration of bacteria from a heterogeneous sample within 5 minutes was achieved [32]. Those two working principles combined resulted in the high-purity selective concentration of target nanocolloids/bacteria into the center electrode and a powerful microfluidic platform for rapid on-chip SERS detection of pathogen from blood samples. In the same study is presented a cell-based biochip using rapid and label-free approach represented by AC electric

field induced electro-rotation (eROT) and developed to evaluate the drug susceptibility of cancer cells. In this case, DEP single-cell trapping is used for subsequent eROT detection and drug-cell interaction is successfully monitored.

Figure 1 sketches the main steps for bacterial detection by using integrated microfluidic devices, SERS sensing and chemometric techniques.

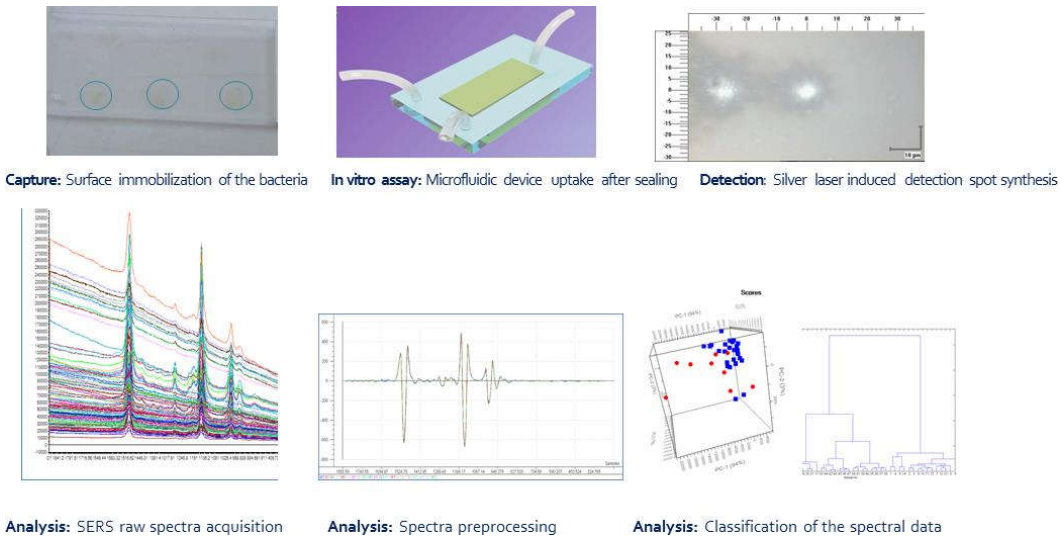

Fig. 1. *Schematic illustration of representative components and steps required in high throughput Raman/SERS-based techniques applied to date for integrated biosensors for bacterial detection: capture - detection - analysis phases of the sensing process.*

Schröder et al. developed a spectroscopy-based rapid test that reveals the resistance of *Enterococci* towards vancomycin within 3.5 hours (including the pre-cultivation step) [6]. By means of integrated DEP, bacteria were directly captured from dilute suspensions on a micrometer-sized region of a chip. Then Raman spectroscopic analysis of the trapped bacteria over a time span of two hours in absence and presence of antibiotics revealed characteristic differences in the molecular response of sensitive as well as resistant *E. faecalis* and *E. faecium*. The Raman spectra are projected into a statistical model and confirm whether the pathogens are sensitive (positive vancomycin effect score) or resistant (negative vancomycin effect score) towards vancomycin. Thus, antibiotic-bacteria interactions can be rapidly detected and explained with Raman-based systems, an emerging clinical tool for the challenging nosocomial pathogens' management.

Spectroscopic methods provide a high potential to become general applicable for bacteria, independently of their taxonomic profiles and show practical advantages compared to most gene- or antibody-based arrays that require special knowledge of the geno- or phenotypes of the resistant bacteria.

Spectroscopy in synergy with DEP [6], 3D hybrid AC electrokinetic strategy for concentration [12, 32], lateral flow immunochromatography [33], and

chemometrics is already applied with various clinical scopes. Among vibrational spectroscopies, SERS is confirmed as a label-free reliable tool that is able to identify and analyze cell/bacteria/virus/protein/molecule without DNA hybridization and antibody-antigen reaction.

3. Handheld SERS-based diagnostic systems

The key features of a HTS technique are being simple, fast, cheap and reliable. SERS is a promising candidate considering the trivial sample preparation required the rapid analysis, its high sensitivity and molecular specificity. Therefore, SERS meets the basic needs of sensing and diagnostics, non-traditional and mobile imaging and it's a matter of time to answer the question whether it can supersede current, conventional techniques. Portable, miniaturized Raman instruments are already tested *in vivo* for clinical use, for instance in real-time tumoral margins assessment and resection [34]. The advent of single-cell sensing and the ability to realize real-time, *in vivo* analysis turn Raman-based ''**cellular medicine**'' more and more feasible.

Most recent label-free SERS-based sensing attempts show clinically relevant results [19, 35]. Kotanen et al. identified five types of bacteria pooled in human serum by evaluating the SERS profiles from preloaded and recovered bacteria with Principle Component Analysis (PCA) and Partial Least Squares Differential Analysis (PLSDA) models. The protocol involves lysis filtration and recovery of bacteria followed by irradiation with a 785 nm laser **handheld Raman spectrometer** on a silver nanorod substrate [19].

Spectral acquisition times have been reduced to maximum several minutes for hundreds of spectra as a result of technological progress. Unfortunately, the laborious data analysis performed for evaluation, validation and calibration of the chemometric models necessary for classification and comparison of the bacterial spectral fingerprints is still consuming valuable time and computational resources.

Considering the label-free aspect, SERS-based detection will represent a more valuable tool when reducing the post-processing steps of data analysis; since the extraction of such a large volume of spectral information still requires complex multivariate analysis. Moreover, the common analysis of the Raman/SERS spectral profiles is related to the changes in intensity of individual bands that can only provide semi-quantitative information. Still, subtle changes in biochemical composition are noticeable when careful analysis of the absolute intensity and of each contribution is carried out. By employing support vector machines [35] and complex data processing and dedicated software, the results are remarkable. Leave-one-batch-out cross validation, where the multi-level model (PCA-PLS-LDA or PLS-LDA-LDA) is tested with data of an independent biological replicate, yields >85% sensitivity and even higher specificity with respect to the prediction based on spectral minor changes [6, 19, 35].

This review has emphasized some of the key developments in SERS assays for bacterial detection, molecular diagnose and point-of-care analysis. A comparison

between Raman and SERS-based approaches is presented in Figure 2 by highlighting their assets and particular detection approaches.

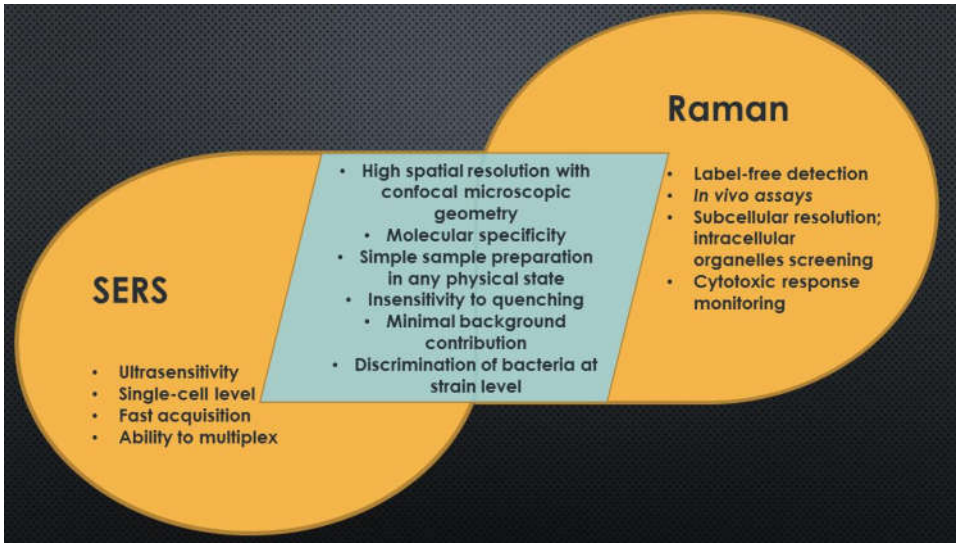

Fig. 2. *Advantages and common attributes of Raman and SERS detection methods.*

SERS nanotags are highly multiplexable based on excitation from a single laser wavelength [36-38], an important consideration for portable diagnostic instrumentation. In general, SERS nanotags are of great benefit to the point-of-care market, since they can be leveraged in a variety of assay systems to create highly sensitive, multiplexed detection of numerous biological targets.

A new subject for the spectroscopic-based detection approaches might become **quorum sensing**. Bacteria have a quorum sensing mechanism used to coordinate certain behaviours such as biofilm formation, virulence, and antibiotic resistance, based on the local density of the bacterial population. Quorum sensing can occur within a single bacterial species as well as between diverse species, and can serve as a simple indicator of population density or the diffusion rate of the cell's immediate environment. Various molecules can be used as signals: common classes of signalling molecules are oligopeptides in Gram-positive bacteria, N-acyl homoserine lactones (AHL) in Gram-negative ones.

SERS is between the few methods of detection with ultra-sensitivity, able to sense even o single molecule of biological interest. By thoroughly comprehending this communication principle between cells, SERS might provide valuable input in bacterial biofilm control strategies [39]. In contrast to bactericidal approaches, SERS would exert less selective pressure which in turn would reduce the likelihood of resistance development. This might be the paradigm shift acknowledged by SERS for the old clinical issues related to bacteraemia and sepsis.

Conclusions

The intensive effort that has been exerted to develop feasible SERS-based biosensors for bacterial detection will definitely enable future strategies for replacing conventional culture-based clinical assays for sepsis prevention. New tools of ultrasensitive detection and diagnose are needed since more and more bacteria evolve to the stage of negating a wide spectrum of antibiotics, even the latest generation ones. The promising miniaturized, handheld spectrometers will in time become more of an omnipresent icon of future point-of-care analysis and will turn traditional clinical analysis in lab setting obsolete.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS –UEFISCDI, project number PN-II-RU TE-2014-4-0862.

References

1. Fleischmann, C.; Thomas–Rueddel, D.O.; et al. Hospital Incidence and Mortality Rates of Sepsis: An Analysis of Hospital Episode (DRG) Statistics in Germany From 2007 to 2013. *Dtsch. Arztebl. Int.* **2016**, 113, 159-166.
2. WHO, *World Health Statistics 2015*, **2016**, 16-17.
3. Eberhardt, K.; Stiebing, C.; et al. Advantages and limitations of Raman spectroscopy for molecular diagnostics: an update. *Expert Rev. Mol. Diagn.* **2015**, 15, 773-787.
4. Pahlow, S.; Meisel, S.; et al. Isolation and identification of bacteria by means of Raman spectroscopy. *Adv. Drug Del. Rev.* **2015**, 89, 105-120.
5. Kumar, V.; Kampe, B.; et al. Classification and identification of pigmented cocci bacteria relevant to the soil environment via Raman spectroscopy. *Environ. Sci. Pollut. Res.* **2015**, 22, 19317-19325.
6. Schröder, U.-C.; Beleites, C.; et al. Detection of vancomycin resistances in *enterococci* within 3 ½ hours. *Sci. Rep.* **2015**, 5, 1-7.
7. Hanf, S.; Bögözi, T.; et al. Fast and Highly Sensitive Fiber-Enhanced Raman Spectroscopic Monitoring of Molecular H_2 and CH_4 for Point-of-Care Diagnosis of Malabsorption Disorders in Exhaled Human Breath. *Anal. Chem.* **2015**, 87, 982-988.
8. Kneipp, K.; Wang, Y.; et al. Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS). *Phys. Rev. Lett.* **1997**, 78, 1667-1670.
9. Kneipp, K.; Haka, A.S.; et al. Surface-Enhanced Raman Spectroscopy in Single Living Cells Using Gold Nanoparticles. *Appl. Spectrosc.* **2002**, 56, 150-154.

10. Wang, P.; Pang, S.; et al. Label-free mapping of single bacterial cells using surface-enhanced Raman spectroscopy. *Analyst* **2016**, 141, 1356-1362.

11. Jamieson, L.E., Byrne, H.J. Vibrational spectroscopy as a tool for studying drug-cell interaction: Could high throughput vibrational spectroscopic screening improve drug development? *Vib. Spectrosc.* **2016,** *in press*.

12. Cheng, I.F.; Chang, H.-C.; et al. Rapid (<5min) Identification of Pathogen in Human Blood by Electrokinetic Concentration and Surface-Enhanced Raman Spectroscopy. *Sci. Rep.* **2013**, 3, 1-8.

13. Muhamadali, H.; Subaihi, A.; et al. Rapid, accurate, and comparative differentiation of clinically and industrially relevant microorganisms via multiple vibrational spectroscopic fingerprinting. *Analyst* **2016**, 141, 5127-5136.

14. Liu, H.; Lu, L.; et al. Rapid detection and differentiation of *Listeria monocytogenes* and *Listeria* species in deli meats by a new multiplex PCR method. *Food Control* **2015**, 52, 78-84.

15. Fan, C.; Hu, Z.; et al. Rapid detection of food- and waterborne bacteria using surface-enhanced Raman spectroscopy coupled with silver nanosubstrates. *Appl. Microbiol. Biotechnol.* **2011**, 92, 1053-1061.

16. Byrne, H.J.; Baranska, M.; et al. Spectropathology for the next generation: Quo vadis? *Analyst* **2015**, 140, 2066-2073.

17. Mircescu, N.E.; Zhou, H.; et al. Towards a receptor-free immobilization and SERS detection of urinary tract infections causative pathogens. *Anal. Bioanal. Chem.* **2014**, 406, 3051-3058.

18. Zhou, H.; Yang, D.; et al. SERS detection of bacteria in water by *in situ* coating with Ag nanoparticles. *Anal. Chem.* **2014**, 86, 1525-1533.

19. Kotanen, C.N.; Martinez, L.; et al. Surface enhanced Raman scattering spectroscopy for detection and identification of microbial pathogens isolated from human serum. *Sens. Biosensing Res.* **2016**, 8, 20-26.

20. Teng, J.; Yuan, F.; et al. Aptamer-Based Technologies in Foodborne Pathogen Detection. *Front. Microbiol.* **2016**, 7, 1-11.

21. Troy, D.J.; Ojha, K.S.; et al. Sustainable and consumer-friendly emerging technologies for application within the meat industry: An overview. *Meat Science* **2016**, 120, 2-9.

22. Bragheri, F.; Minzioni, P.; et al. Design and optimization of a reflection-based fiber-optic tweezers. *Opt. Express* **2008**, 16, 17647-17653.

23. Temiz, Y.; Lovchik, R.D.; et al. Lab-on-a-chip devices: How to close and plug the lab? *Microelectron. Eng.* **2015**, 132, 156-175.

24. Takagi, R.; Fukuda, J.; et al. A microfluidic microbial culture device for rapid determination of the minimum inhibitory concentration of antibiotics. *Analyst* **2013**, 138, 1000-1003.

25. Redding, B.; Schwab, M.; et al. Raman Spectroscopy of Optically Trapped Single Biological Micro-Particles. *Sensors* **2015**, 15, 19021-19046.

26. Liberale, C.; Cojoc, G.; et al. Integrated microfluidic device for single-cell trapping and spectroscopy. *Sci. Rep.* **2013**, 3, 1-7.

27. Liberale, C.; Minzioni, P.; et al. Miniaturized all-fibre probe for three-dimensional optical trapping and manipulation. *Nat. Photon.* **2007**, 1, 723-727.
28. Xie, C.; Dinno, M.A.; et al. Near-infrared Raman spectroscopy of single optically trapped biological cells. *Opt. Lett.* **2002**, 27, 249-251.
29. Alexander, T.A.; Pellegrino, P.M.; et al. Near-Infrared Surface-Enhanced-Raman-Scattering-Mediated Detection of Single Optically Trapped Bacterial Spores. *Appl. Spectrosc.* **2003**, 57, 1340-1345.
30. Lesser-Rojas, L.; Ebbinghaus, P.; et al. Low-Copy Number Protein Detection by Electrode Nanogap-Enabled Dielectrophoretic Trapping for Surface-Enhanced Raman Spectroscopy and Electronic Measurements. *Nano Lett.* **2014**, 14, 2242-2250.
31. Perozziello, G.; Catalano, R.; et al. A microfluidic device integrating plasmonic nanodevices for Raman spectroscopy analysis on trapped single living cells. *Microelectron. Eng.* **2013**, 111, 314-319.
32. Cheng, I.-F.; Chen, Tzu-Ying; et al. Minute-Level Speed Identification and Assessment of Bacteria/Cells Using Electrokinetic Assistance. *International Journal of Engineering and Technology Innovation (IJETI)* **2016**, 6, 225-231.
33. Stambach, N.; Carr, S.; et al. Rapid Detection of Listeria by Bacteriophage Amplification and SERS-Lateral Flow Immunochromatography. *Viruses* **2015**, 7, 6631-6641.
34. *Raman4Clinics, https://www.raman4clinics.eu*.
35. Silge, A.; Schumacher, W.; et al. Identification of water-conditioned Pseudomonas aeruginosa by Raman microspectroscopy on a single cell level. *Syst. Appl. Microbiol.* **2014**, 37, 360-367.
36. Samanta, A.; Das, R.K.; et al. Multiplexing SERS nanotags for the imaging of differentiated mouse embryonic stem cells (mESC) and detection of teratoma in vivo. *Am. J. Nucl. Med. Mol. Imaging* **2014**, 4, 114-124.
37. Bedics, M.A.; Kearns, H.; et al. Extreme red shifted SERS nanotags. *Chem. Sci.* **2015**, 6, 2302-2306.
38. Dinish, U.S.; Balasundaram, G.; et al. Actively Targeted In Vivo Multiplex Detection of Intrinsic Cancer Biomarkers Using Biocompatible SERS Nanotags. *Sci. Rep.* **2014**, 4, 1-7.
39. Coughlan, L.M.; Cotter, P.D.; et al. New Weapons to Fight Old Enemies: Novel Strategies for the (Bio)control of Bacterial Biofilms in the Food Industry. *Front. Microbiol.* **2016**, 7, 1-21.