様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科研費 KAKENHI

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研究課題名（和文）Novel label-free tool for infections diagnosis based on Nano-Electro Optical Tweezers

研究課題名（英文）Novel label-free tool for infections diagnosis based on Nano-Electro Optical Tweezers

研究代表者
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研究成果の概要（和文）：急性感染症の原因を区別することは、21 世紀初頭に人類が直面している大きな臨床課題です。私のプロジェクトでは、材料科学、ナノフォトニクス、バイオメディカルの連携に基づく新しいアプローチを採用し、捕捉された単一の細菌の電気光学特性と表現型を、短期間で高感度に特定しました。ナノフォトニクス ツールを使用して、自然環境に浮遊する細菌の検出、成長、生存、および繁殖のメカニズムを研究し、その行動を明らかにしました。これらのプロセスを単一の微生物レベルで理解することで、微生物の生態、抗生物質耐性の発達、および細胞生物学の基本原理についてより深い洞察を得ることができます。

研究成果の学術的意義や社会的意義
Rapid and reliable identification of pathogenic bacteria is crucial across various fields such as healthcare, food safety, and environmental sciences. My research demonstrates a nanophotonic platform capable of providing valuable and repeatable bacterial information in liquid environments.

研究成果の概要（英文）：The distinction between causes of acute infections is a major clinical challenge which is faced by humanity at the dawn of the 21st century. My project identified with high sensitivity the electro-optical properties and phenotypes of a single trapped bacterium by employing a novel approach based on the engagement of material science, nanophotonics and biomedicine in a short timescale. By using nanophotonic tools, I studied bacteria's detection, growth, survival, and reproduction mechanisms suspended in their natural environment to shed light on their behavior. By understanding these processes at the single-micro-organism level, we can gain deeper insights into microbial ecology, antibiotic resistance development, and the fundamental principles of cellular biology.

研究分野：Nanophotonics and Biophotonics

キーワード：Nanophotonics　Raman spectroscopy　Single-bacterium level　Bacteria detection

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様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Several diseases are caused by bacteria and other micro-organisms. Therefore, the rapid and accurate identification of these micro-organisms is essential for effective treatment and the prevention of further infections. Currently, diagnostic methods for bacterial infections rely on culture-based approaches such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). While these culturing methods produce highly accurate results, they are time-consuming, require complex sample pretreatment, and necessitate expertise from operators. Consequently, there is a strong need for alternative methods that offer more convenient, rapid, and sensitive features for bacterial detection and identification. Raman spectroscopy has proven to be a useful optical tool for identifying bacterial species. However, high concentrations of bacteria are required to detect several weak vibration bands. This limitation reduces the effectiveness of Raman spectroscopy in cases where bacterial concentrations are low, making it less practical for rapid and sensitive detection of bacterial infections. Furthermore, Raman spectroscopy alone may not provide sufficient information regarding antibiotic susceptibility, which is crucial for effective treatment. Determining the susceptibility of bacteria to specific antibiotics typically requires additional methods to assess the growth and viability of bacteria in the presence of antimicrobial agents. This highlights the need for integrated approaches that combine the strengths of Raman spectroscopy with other diagnostic techniques to achieve both accurate identification and rapid assessment of antibiotic susceptibility.

In addition, metamaterials that operate at THz frequencies have micron-size apertures that can serve as ideal platforms for fungal and bacterial detection because these micro-organisms have sizes comparable to the size of micro-aperture. Moreover, combining these plasmonic structures with techniques that determine antibiotic susceptibility, such as electric current-based methods, would allow for comprehensive and rapid valuations. Likewise, Fano-resonant asymmetric metamaterials have been used for biosensing and nanoparticle trapping. Due to the strong interference between super-radiant and subradiant plasmonic modes, Fano-like resonance structures are highly sensitive to changes in the local refractive index. Consequently, such sensitive properties make Fano-like structures particularly attractive as biomedical and chemical sensing platforms compared with traditional sensing schemes like culture-based methods. Furthermore, integrating metamaterial structures with methods for assessing antibiotic susceptibility, such as electric current-based techniques, can enhance the diagnostic capabilities. This integrated approach not only allows for precise identification of bacterial species but also enables the rapid determination of their antibiotic susceptibility. This could significantly improve the speed and accuracy of diagnosing bacterial infections and identifying the most effective treatments, addressing a critical need in clinical microbiology.

２．研究の目的

Bacterial mutations involve changes in their genetic material, which can result from mechanisms such as point mutations, gene duplication, and other genetic recombination events. Functionalization requires that these genetic changes provide some advantage or new capability to the organism, making this process particularly challenging. Moreover, a single technique is often insufficient for a comprehensive evaluation of antibiotic susceptibility due to the diversity of pathogenic bacteria and the wide range of antibiotics required testing them. To address these limitations, a multiparameter approach using multiple methods in parallel is necessary for a more thorough and accurate assessment of antibiotic susceptibility. Hence, the project proposed a novel diagnostic platform that can offer direct and accurate evaluation of detection of bacteria at the single-bacterium level within a short time.

This platform integrates metallic metamaterial with optical tweezers Raman spectroscopy and an electric current to achieve this goal. Plasmonic metamaterial enable precise manipulation and observation of individual bacteria, while the application of an

electric current provides additional insights into the bacterial response to antimicrobial agents. By combining these technologies, the project provided an alternative approach to overcome the limitations of existing methods and resulted in a robust solution for the direct assessment of bacterial susceptibility to antimicrobial drugs.

３．研究の方法

As a first step, I determined the theoretical absorption spectral peak of the proposed metamaterial structure using Comsol Multiphysics software. The absorption peak spectrum (Fig.1(a)) with a resonance at 6030 nm is used as an indicator of the theoretical resonance position of the metamaterial structure. I designed the metamaterial to operate at the THz regime because biological samples possess mid-infrared vibrational fingerprints that can be used for their identification. Then, using a focused ion beam I fabricated an array of 17x17 microholes on a metallic substrate with a slit size comparable to the volume of the bacterium (Fig. 1(b)). By introducing an array of microholes, many trapping sites can be activated at the same time leading to simultaneously analysis of several bacteria locally in well-defined positions. Figure 1(c) shows the electromagnetic field enhancement of a single unit.

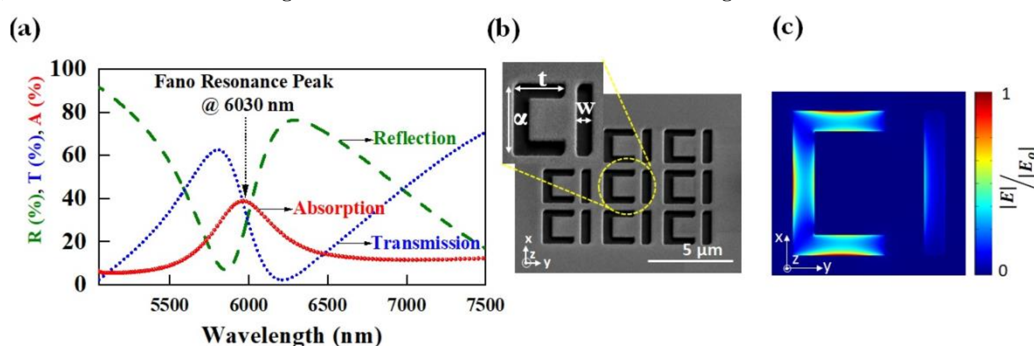

Figure 1. (a) Theoretical reflection, transmission, and absorption spectra for the metamaterial platform, noting the resonance peak at 6030 nm close to amide protein vibrations. (b) Scanning electron microscope (SEM) image, viewed at 52° from the surface normal of the structure. The geometrical dimensions of each metamolecule unit (enlarge image) are vertical slit α=2.8 ± 0.3 μm, horizontal slit t = 1.7 ± 0.2 μm, slit width w = 0.41 ± 0.02 μm, and periodicity p = 3.6 ± 0.2 μm. (c) The electromagnetic field enhancement of a single metamolecule at a simulated resonance of 6030 nm for the y-direction. The plasmonic hotspots are located at the edges of the C-type nano-aperture.

Afterwards, I prepared solutions using Gram-type positive or Gram-type negative bacteria to study the growth rate mechanism. For example, as gram-negative bacteria I chose Escherichia coli BL21 cells, without any plasmid construct for antibiotic resistance. E. coli is a Gram-negative bacterium and interacts strongly with gold nanostructures via lipopolysaccharide carboxylate groups. The primary culture was set up in Luria-Bertani (LB) broth medium and cultured overnight in a shaking incubator. The following day, the overnight culture was subcultured in LB broth without any antibiotics and grown to mid-exponential phase (Fig.2(a)). The E. coli cultures were diluted 1/1000 to a final concentration of 10^4 CFU/ml. Note that during the stationary phase (Fig.2(b)), a bacterial concentration of 10^8 CFU/mL was used for the experimental process.

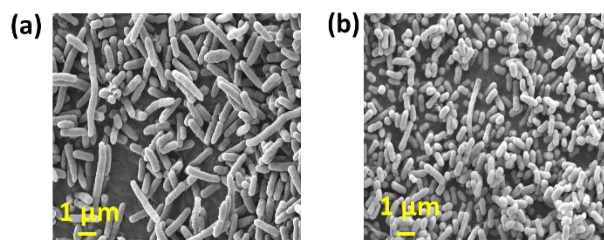

Figure 2. Scanning electron microscope images showing the morphology of E.coli strains grown to the exponential phase in LB broth cultures shaking in an incubator shaker. (a) Mid-exponential phase and (b) stationary phase of E. Coli.

The experimental set up consist of an optical tweezers Raman spectroscopy which a ND:YAG laser beam at 532 nm with a maximum output power of 17 mW focused using a high numerical aperture (NA = 1.25) oil immersion objective lens onto the sample. The metamaterial structure was sealed with a glass cover slip and an adhesive microscope spacer of 10 μm form a microwell. Six μl of bacterial solution were pipetted into the well and the device was mounted on top of a piezoelectric translation stage.

４．研究成果

Raman signatures of E. coli were recorded at several locations on the metamaterial under off-resonance laser excitation at 530 nm, where the photodamage effect is minimized.
When using metallic structures with resonances matched to the excitation laser, both the laser and the scattering light are absorbed by the structures themselves. The absorbed photon energy leads to excitation of the electrons in the metal and the subsequent nonradiative decay of excited electrons converts the energy to heat. The thermal disturbance caused by plasmonic heating may affect the photochemical degradation of the bacterial cells showing low surface enhanced scattering spectrum reproducibility. However, in this project I overcame this obstacle because the resonance of the metamaterial device is red-shifted from the laser excitation, thereby minimizing the laser-induced heating that can contribute to photodamage of biological entities.

As the sizes of the E.coli are comparable to the micro-gaps of the metamaterials, its local immobilisation leads to an increase in the Raman sensitivity. In Figure 3(a), the measured Raman spectrum of Escherichia coli on a 50 nm gold film and a glass substrate shows that the spectra do not display the same peaks as those obtained with the metamaterial under the same interrogation settings (Fig.3 (b)). I assumed that these spectral peaks are attributable to enhancement from the Fano-resonant mode supported by the metamaterial.

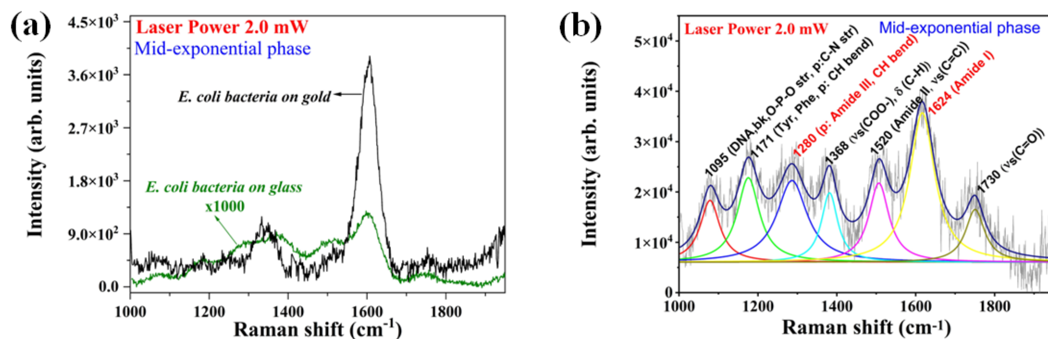

Figure 3. (a) Raman signal of E. coli bacteria in the mid-exponential phase on gold (black line) and microscope glass (green line × 1000) substrates. The Raman signal from the glass substrate is rescaled by a factor of 1000 for ease of viewing. (b) Raman spectrum of E. coli bacteria in the mid-exponential phase on the metamaterial using 2.0 mW incident laser power.

Since bacteria are living organisms and do not necessarily respond or grow identically over several days, even if the growth conditions are kept the same, I noted that the bacterial concentration of 10^4 CFU/mL used, might be the optimum to provide the most reproducible spectra. I also observed that the time-dependent signal related to bacterial amide peaks increased during the bacteria’s mid-exponential phase while it decreased during the stationary phase (Fig.5). Specifically, the intensity of peptide (amino acids and proteins) peaks progressively increased as the bacteria moved from the exponential phase to the mid-exponential phase. In this case, the peptidoglycan layer of the bacterium wall is around 12 nm and as the electromagnetic field penetration depth is 100 nm, the intensity of the Raman peaks of the subcellular components increases. However, the thickness of the peptidoglycan layer of the bacterium wall in

the stationary phase is larger compared to that during the mid-exponential phase and this may lead to a decrease in the Raman peak intensities.

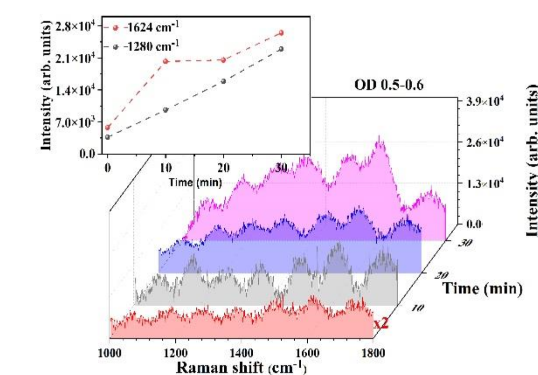

Figure 5. Raman spectra of single-trapped bacteria in the mid-exponential phase on a metamaterial device over time. Inset shows a linear relationship between the amide peak intensity magnitude and time.

The last part of this project was the combination of electric- and near-field which allow me to asses a wide range of different bacteria and monitoring of their response to a variety of antibiotics ideally down to timescales of several minutes. Specifically, by employing optical tweezers Raman spectroscopy I distinguished with high precision whether the bacterium was Gram-type positive or Gram-type negative in a heterogeneous medium and I studied their growth rate mechanism. Simultaneously, by employing the electric current in response to the abiotic challenge I investigated its survival and its reproduction mechanics in a short timescale.

The results of the project have been presented at several national and international conferences, showing the innovative approach and significant findings to the scientific community. Additionally, the project has led to multiple publications in peer-reviewed journals, highlighting the robustness and impact of the research. These publications have further validated the platform's potential to revolutionize bacterial detection and antibiotic susceptibility testing, positioning it as a critical tool in the fight against antibiotic resistance.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　5件）

１．著者名	４．巻
Domna G. Kotsifaki, Sile Nic Chormaic	In press
２．論文標題	５．発行年
The role of temperature-induced effects generated by plasmonic nanostructures on particle delivery and manipulation: a review	2022年
３．雑誌名	６．最初と最後の頁
Nanophotonics	1-20
掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1515/nanoph-2022-0014	有
オープンアクセス	国際共著
オープンアクセスとしている（また、その予定である）	該当する

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Nanotechnology	25507
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10.1088/1361-6528/abbca9	有
オープンアクセス	国際共著
オープンアクセスとしている（また、その予定である）	該当する

１．著者名	４．巻
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３．雑誌名	６．最初と最後の頁
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掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1063/5.0032846	有
オープンアクセス	国際共著
オープンアクセスとしている（また、その予定である）	該当する

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掲載論文のDOI（デジタルオブジェクト識別子）	査読の有無
10.1016/j.scitotenv.2020.143927	有
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2．論文標題 Asymmetric split-ring plasmonic nanostructures for the optical sensing of Escherichia coli	5．発行年 2023年
3．雑誌名 Biomedical Optics Express	6．最初と最後の頁 4875
掲載論文のDOI（デジタルオブジェクト識別子） 10.1364/BOE.497820	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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1．発表者名 Domna G. Kotsifaki
2．発表標題 Manipulation, trapping, and biosensing using metamaterial devices
3．学会等名 Optica Biophotonics Congress: Optics in the Life（国際学会）
4．発表年 2022年～2023年

1．発表者名 Domna G. Kotsifaki
2．発表標題 Plasmonic Fano-resonant metamaterial for nanoparticle trapping and biosensing (invited talk):
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4．発表年 2022年

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3．学会等名 Optics& Photonics Japan 2022, Utsunomiya, Japan, 14 November 2022.（国際学会）
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2021年

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2．発表標題
Analysis of small plastics in coastal surface water samples of Okinawa using optical tweezers-Raman spectroscopy
3．学会等名
OMC2021, Online, Japan, 21 April 2021（国際学会）
4．発表年
2021年

〔図書〕　計1件

1．著者名 Domna G. Kotsifaki, Viet Giang Truong, Sile Nic Chormaic	4．発行年 2022年
2．出版社 Springer Lect. Notes Nanoscale Sci.	5．総ページ数 347
3．書名 Plasmon-Enhanced Light-Matter Interactions - Plasmon-enhanced optical forces and tweezers	

〔産業財産権〕

〔その他〕

－

6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関