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研究課題名（和文）Molecular interaction analysis of lipid bilayer nanodomains by scanning probe nanospectroscopy in fluid environments

研究課題名（英文）Molecular interaction analysis of lipid bilayer nanodomains by scanning probe nanospectroscopy in fluid environments

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研究成果の概要（和文）：本研究では、632.8 nmおよび785 nmの励起波長を使用可能なナノ分光システムを構築する事ができた。また、本システムは環境影響を受けやすい試料に対応するための環境制御システム中に収納されている。システムの設計には、数値シミュレーション（FDTDおよび Mathematica）を用いて、光学系とプローブの最適な実験パラメータ（開口数，レーザー照射角度，プローブの傾き）を算出した。金プローブを作製するために、3電極の電気化学エッチングシステムを作製し、この金プローブを作製するための方法論を確立した。

研究成果の学術的意義や社会的意義
It is important to develop nanospectroscopy systems that have both high spatial resolution and chemical sensitivity in order to study phenomena occurring at the nanometer scale. The development of a nanospectroscopy system in this project would help study both biological and non-biological samples.

研究成果の概要（英文）：In this work, we were able to construct a nanospectroscopy system that can operate at 632.8 nm and 785 nm wavelengths and is housed in an environment control system to accommodate environment sensitive samples. The system was designed through the use of numerical simulations (FDTD and Mathematica) to obtain the optimal experimental parameters of the optics and probe (numerical aperture, laser illumination angle, tip tilt). A three-electrode electrochemical etching system was made to fabricate Au tips and a methodology was established to make these Au tips.

研究分野：Engineering

キーワード：Raman spectroscopy　surface science

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１．研究開始当初の背景

(1) The cell membrane and its structure can influence cellular activity. One particular structure of interest in the cell membrane is the lipid bilayer or lipid rafts. Lipid bilayers can form nanodomains and are hypothesized to be involved in cell processes such as cell death and host-pathogen interactions. The role of lipid bilayer nanodomains (LBN) in cells, however, is still unclear.

(2) In order to understand the role of LBN in the cell membrane, their direct observation is necessary especially in various physiological conditions. To image these nanometer scale structures and simultaneously characterize their physiochemical properties, a scanning probe microscope integrated with a spectrometer is essential. Through this nanospectroscopy system, tip-enhanced Raman spectroscopy (TERS) and tip-enhanced terahertz Raman spectroscopy (TE-THzRS) can be utilized to detect both high frequency Raman (fingerprint region, 200 cm^-1 to 2000 cm^-1) and low frequency Raman (terahertz Raman, 10 cm^-1 to 200 cm^-1) for a full chemical picture of the sample. The sample can be placed in a microfluidic device in order to vary the physiological conditions such as temperature, pH levels and proteins.

２．研究の目的

The purpose of this research is to develop a nanospectroscopy system that can image and characterize *in situ* the formation of LBN and its behavior in a dynamic liquid environment (e.g. varying temperature, pH levels and proteins). All this is possible through the subnanometer spatial resolution and high chemical sensitivity provided by the proposed nanospectroscopy system.

３．研究の方法

In order to conduct the proposed research, the nanospectroscopy system was initially designed based on the existing scanning probe microscope system. The existing system was originally designed for TERS using a 632.8 nm laser, while the proposed system must also be capable to do both TERS and TE-THzRS using a 785 nm laser. A change in the optics was essential. Numerical simulations were also needed to determine the appropriate TERS/TE-THzRS probe for the experiments.

４．研究成果

(1) Through numerical simulations, the electric field profiles on a gold (Au) substrate for both normal and oblique incident illuminations were both visualized and compared. It was found through finite-difference time-domain (FDTD) simulations that for oblique illumination, the field profile of electric field in the z-direction was more intense as compared to normal incidence (Figure 1). The illumination wavelength used was 785 nm while the numerical aperture (NA) used for the simulations was 0.6, which is the NA of the objective lens in the existing system.

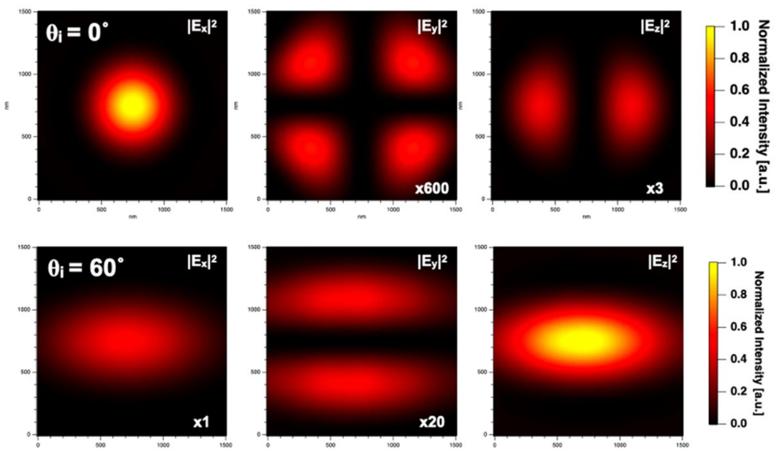

Figure 1. Calculated electric field distribution on an Au substrate for normal (θ_i = 0°) and oblique incidence (θ_i = 60°).

The use of a parabolic mirror to illuminate the tip-sample junction was also explored. Mathematica calculations were also conducted to visualize the electric field

distribution on the Au substrate (Figure 2). The illumination wavelength used was 785 nm while the NA of the parabolic mirror was 1.515.
(2) The electric field enhancement at the tip apex was also simulated using FDTD at

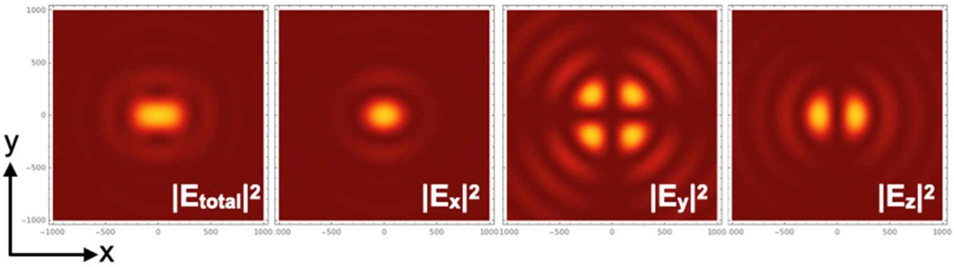

Figure 2. Calculated electric field distribution when using a parabolic mirror (NA = 1.515).

various angles of illumination. It was found that there is an optimal angle to enhance the electric field at both the z and x direction, although the electric field at the x direction is one order of magnitude weaker than the electric field in the z direction (Figure 3). If the electric field in the x direction can be optimized further, it would be possible to obtain considerable enhancement in both the x and z directions. Such findings would be useful to excite samples that have Raman modes in the in-plane and out-of-plane directions.

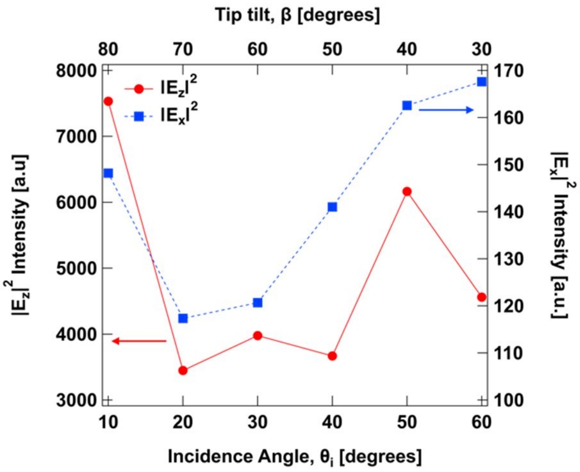

Figure 3. Comparison of electric field intensities in the x (Ex) and z (Ez) directions at the tip-sample gap (d = 1 nm) for different angles of incidence and tip tilt.

(3) Tips were successfully mounted on a home-made scanning probe head and the head along with the sample stage plus the detection optics were mounted in an environment control chamber.

(4) A three-electrode system (based on the work of Yang, et. al *J. Phys. Chem C* 2018, 122, 16950-16955) was constructed (Figure 4a) and sharp tips with a diameter of 50 nm were fabricated (Figure 4b). A methodology to make these Au tips was established and can be used for Au wires of varying diameters (e.g. 100 microns, 250 microns, and 300 microns).

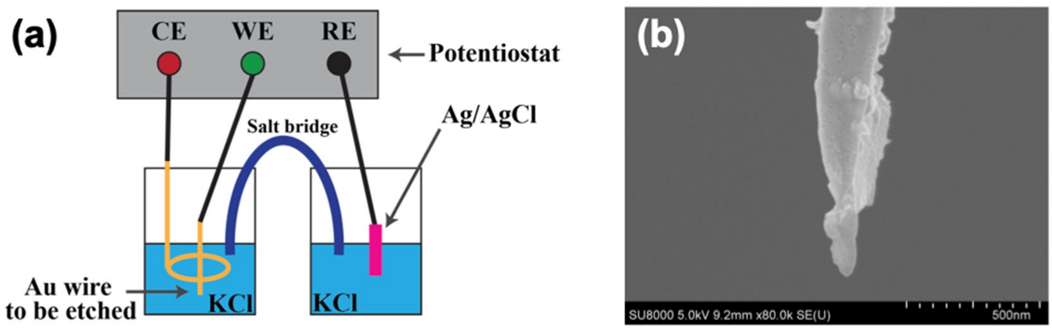

Figure 4. (a) schematic of 3-electrode electrochemical system used to etch the Au tips and (b) example of etched tip with a diameter of 50 nm.

(5) The developed nanospectroscopy system can utilize both 632.8 nm and 785 nm laser wavelengths although not simultaneously. Such versatility is important to accommodate a variety of samples and also tips whose plasmon resonance varies depending on the

material used for the tip. The system is housed in a metal environment control chamber whose main purpose is to stabilize both the temperature and the humidity in the experimental space and to block out stray light, mechanical and vibrational noise.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計2件（うち招待講演　1件／うち国際学会　2件）

１．発表者名 Maria Balois*, Norihiko Hayazawa,Satoshi Yasuda, Katsuyoshi Ikeda, Bo Yang, Emiko Kazuma, Yasuyuki Yokota, Yousoo Kim, and Takuo Tanaka
２．発表標題 Imaging of Sub-nanometer Strain Variations in Monolayer Defect-Free Graphene using Tip-Enhanced Raman Spectroscopy
３．学会等名 第81回応用物理学会秋季学術講演会 JSAP-OSA Joint Symposia（国際学会）
４．発表年 2020年

１．発表者名 Maria Vanessa Balois*, Norihiko Hayazawa, and Takuo Tanaka
２．発表標題 Development of near-field scanning optical microscope systems for nanomaterial characterization
３．学会等名 40th Philippine-American Academy of Science & Engineering (PAASE) & 2020 Annual PAASE Meeting & Symposium (40th APAMS)（招待講演）（国際学会）
４．発表年 2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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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