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科研費 KAKENHI

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Development of artificial intelligence assisted Raman microscopy for reliable and automated examination of urine sediment

研究課題名（英文）Development of artificial intelligence assisted Raman microscopy for reliable and automated examination of urine sediment

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：尿は 3,000 以上の多様な代謝物を含む体液です。尿の正確な組成を決定することや，その収集と保管は困難であり，費用がかかります。本研究課題では，いくつかの製剤を調査し，尿分析の開発における人工尿の適合性をAI を活用したラマン分光法を用いて検討しました。ラマンスペクトルは，液体または空気乾燥条件下でポータブルラマン分光計とラマン顕微分光計の両方を使用して測定されました。いくつかの実験パラメータを最適化することにより，標準的なヒト尿とよく一致する人工尿スペクトルを実現しました。この AU 製剤をラマン分光法ベースの尿分析の開発中にヒトの尿の代わりに使用して成功させることができると信じています。

研究成果の学術的意義や社会的意義
Human urine flows through kidneys, ureters, bladder, urethra etc. and it accurately reflects change in all the organs. Separate screening tests are done for each disease. Proposed urinalysis by Raman microscopy helps to screen multiple conditions in one step, reduce cost/time and contribute to SDG3

研究成果の概要（英文）：Urine is a rich body fluid with over 3,000 metabolites. Determining exact composition of urine, its collection and storage are difficult and expensive. During the development of new modalities of urinalysis, therefore, artificial urine has many advantages as it is both practical and fast to obtain over human urine for research and educational purposes. In this work, we investigated several formulations and examined suitability of artificial urine for the development of AI assisted Raman spectroscopy based urinalysis. Raman spectra were measured using both portable Raman spectrometer and a Raman micro-spectrometer under liquid or air-dried conditions. By optimizing several experimental parameters, we realized artificial urine spectra that matched well with standard human urine. We believe this formulation of AU can be used to replace human urine during development of Raman spectroscopy based urinalysis successfully.

研究分野：Bioanalytical Chemistry

キーワード：Raman Spectroscopy　Single cell analysis　Disease diagnostics　Molecular imaging　Artificial Intelligence　Machine learning　Explainable AI

１．研究開始当初の背景 (Background)

Global burden of kidney and urinary tract diseases are on the raise and significantly affects elderly people. It is of great concern in ageing societies such as Japan. In fact, United Nations Sustainable Development Goals 3 (SDG-3) targets good health and well-being for people globally. One of the proposed means to achieving it is to develop early warning systems. So, development of multipurpose screening tools for renal and urinary tract illnesses is need of the hour. In this context, urine is an attractive target. Depending on pathological condition, urine and urine sediments may contain epithelial cells, blood cells, casts, salts/crystals and microorganisms which can be used for screening. Wide range of tests including dipsticks have been developed to detect biochemical changes in urine but are unreliable because contamination is a widespread problem. So microscopic examination of urine is necessary for proper screening / diagnosis. Current efforts rely only on morphological changes (shape/size) which fail to identify many urinary components. On the other hand, Raman spectroscopy relies on molecular information and has recently demonstrated excellent classification of various biomaterials including cells and microorganisms. Therefore we proposed to develop AI assisted Raman Spectroscopy based Urinalysis. However, urine is a rich body fluid with over 3,000 metabolites. Many aspects of our lives such as age, gender, nature and time of food intake, function of various organs including kidneys etc. are reflected in its composition and investigating urine has its own challenges. Determining exact composition of urine, its collection and storage are difficult and expensive. Therefore, during the development of new modalities of urinalysis, artificial urine (AU) has many advantages as it is both practical and fast to obtain over human urine for research and educational purposes. In this work, we prepare various formulations of AU and examined its suitability for the development of AI assisted Raman spectroscopy based urinalysis.

２．研究の目的 (Purpose)

Human urinary system includes many organs - kidneys, ureters, bladder, urethra etc. (Figure 1). Presently, screening depends on target disease and separate screening tests are done for each disease which is expensive and time-consuming. Since urine flows through all these organs, it accurately reflects change in any/all of them. Therefore efficient investigation of urine and urine sediment by microscopy can help to screen multiple conditions simultaneously in just one step, reduce cost/time and help to contribute to SDG-3.

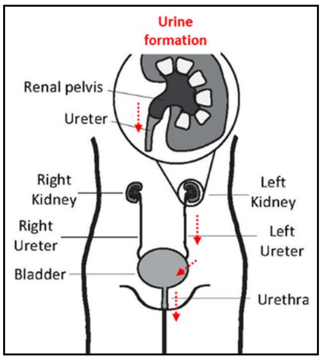

Figure 1. Human urinary system and flow of urine

３．研究の方法 (Methods)

We investigated several AU formulations reported in the literature. AU was prepared according to formulation shown in Table 1 modified from Sarigul et al., Scientific Reports, 9:20159 (2019) and measured using both portable Raman spectrometer (pRS) and a Raman micro-spectrometer (RM) under liquid/air-dried conditions with the following measurement parameters.

Table 1. AU formulation

Chemical	Molarity(mM)	Quantity(g/100ml)
Na_2SO_4	11.965	0.1700
$C_5H_4N_4O_3$	1.487	0.0250
$Na_3C_6H_5O_7 \cdot 2H_2O$	2.450	0.0720
$C_4H_7N_3O$	7.791	0.0881
CH_4N_2O	249.750	1.5000
KCl	30.953	0.2308
NaCl	30.053	0.1756
$CaCl_2$	1.663	0.0185
NH_4Cl	23.667	0.1266
$K_2C_2O_4 \cdot H_2O$	0.19	0.0035
$MgSO_4 \cdot 7H_2O$	4.389	0.1082
$NaH_2PO_4 \cdot 2H_2O$	18.667	0.2912
$Na_2HPO_4 \cdot 2H_2O$	4.667	0.0831

Raman Microspectrometer: Nanofinder: objective - 100x, excitation laser wavelength - 532 nm, laser power - 2mW

Portable Raman Spectrpmeter: EZRaman-I: excitation laser wavelength - 785 nm, laser power - 46mW

４．研究成果 (Results)

We compared the Raman spectra between AU and human urine and could see that most of the peaks corresponded to each other, especially those for urea and creatinine. The peaks at 527, 1006, and 1160 cm−1 indicate the presence of urea and the peaks at 681, 846, and 908 cm−1 creatinine, with attribution assigned to the symmetric stretching peak C─N for the peak at 1006 cm−1 and C─NH2 and C═O stretching and vibrations of the aromatic ring for the peak at 681 cm−1, confirming the peaks found in other studies of urine. We could successfully obtain spectra of urine by both pRS and RM. Due to higher throughput, urine Raman spectra from RM has high signal/noise (S/N) compared to pRS. We observed that liquid AU has higher S/N than air-dried AU on pRS. Spectra of urine in 1s has low S/N and exposure over 10s gives reliable data under measured conditions. Increase of laser power results in higher S/N as expected. Effect of volume is negligible. Therefore, we believe this formulation of AU can be used to replace human urine during development phases.

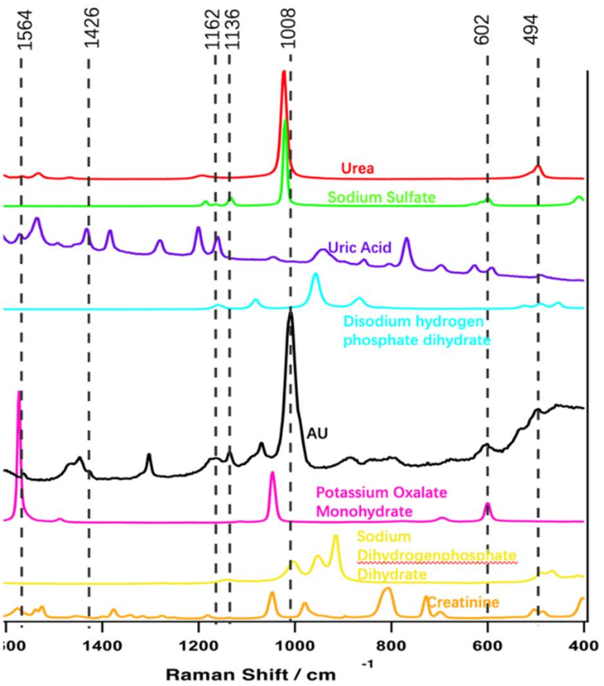

Figure 1. Raman Spectra of AU compared with various components measured using Raman micro-spectrometer.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　4件／うちオープンアクセス　4件）

１．著者名 Panneerselvam Rajapandiyan、Sadat Hasan、Hohn Eva-Maria、Das Anish、Noothalapati Hemanth、Belder Detlev	４．巻 22
２．論文標題 Microfluidics and surface-enhanced Raman spectroscopy, a win?win combination?	５．発行年 2022年
３．雑誌名 Lab on a Chip	６．最初と最後の頁 665～682
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/d1lc01097b	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Noothalapati Hemanth、Iwasaki Keita、Yamamoto Tatsuyuki	４．巻 258
２．論文標題 Non-invasive diagnosis of colorectal cancer by Raman spectroscopy: Recent developments in liquid biopsy and endoscopy approaches	５．発行年 2021年
３．雑誌名 Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy	６．最初と最後の頁 119818～119818
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.saa.2021.119818	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 －

１．著者名 Mojidra Rahul、Hole Arti、Iwasaki Keita、Noothalapati Hemanth、Yamamoto Tatsuyuki、C Murali Krishna、Govekar Rukmini	４．巻 10
２．論文標題 DNA Fingerprint Analysis of Raman Spectra Captures Global Genomic Alterations in Imatinib-Resistant Chronic Myeloid Leukemia: A Potential Single Assay for Screening Imatinib Resistance	５．発行年 2021年
３．雑誌名 Cells	６．最初と最後の頁 2506～2506
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/cells10102506	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

１．著者名 Anjikar Ajinkya、Jadhav Priyanka、Hole Arti、Paneerselvam Rajapandian、Ingle Arvind、Yamamoto Tatsuyuki、Noothalapati Hemanth、C. Murali Krishna	４．巻 2
２．論文標題 Removal of hemolysis interference in serum Raman spectroscopy by multivariate curve resolution analysis for accurate classification of oral cancers	５．発行年 2023年
３．雑誌名 Sensors & Diagnostics	６．最初と最後の頁 390～397
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D2SD00137C	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

１．著者名 Zakaria Riki、Andriana Bibin. B.、Watanabe Takumu、Maryani Anisa、Paramitha Pradjna N.、Kuntana Yasmi P.、Kusaka Yukako、Noothalapati Hemanth、Iwasaki Keita、Oshima Naoki、Hashimoto Kosuke、Matsuyoshi Hiroko、Ishihara Shunji、Yamamoto Tatsuyuki、Sato Hidetoshi	４．巻 285
２．論文標題 Development of in-situ Raman diagnosis technique of eosinophil esophagitis	５．発行年 2023年
３．雑誌名 Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy	６．最初と最後の頁 121804～121804
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.saa.2022.121804	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Yoshikiyo Keisuke、Takahashi Miho、Narumiya Yu、Honda Mikito、Iwasaki Keita、Ishigaki Mika、Nagato Edward G.、Noothalapati Hemanth、Shimizu Hidehisa、Murota Kaeko、Yamamoto Tatsuyuki	４．巻 3
２．論文標題 Co-ingestion with γ-cyclodextrin improves bioavailability of α-linolenic acid in Perilla frutescens seed oil	５．発行年 2023年
３．雑誌名 Food Hydrocolloids for Health	６．最初と最後の頁 100116～100116
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.fhfh.2023.100116	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 －

〔学会発表〕　計11件（うち招待講演　4件／うち国際学会　7件）

１．発表者名
Hemanth Noothalapati and Tatsuyuki Yamamoto
２．発表標題
Label-free visualization of fungal cell and spore wall architecture by confocal Raman microscopy
３．学会等名
American Chemical Society Spring 2021（国際学会）
４．発表年
2021年

１．発表者名
Hemanth Noothalapati, Keita Iwasaki, Asuka Araki, Tatsuyuki Yamamoto, Riruke Maruyama
２．発表標題
MCR-ALS assisted identification of spectral markers for objective discrimination of breast cancer from mammary epithelial cells by Raman microspectroscopy
３．学会等名
11th International Conference on Advanced Vibrational Spectroscopy（国際学会）
４．発表年
2021年

1．発表者名 Hemanth Noothalapati and Tatsuyuki Yamamoto
2．発表標題 Raman Microspectrocopy: From label-free single cell imaging to disease diagnosis
3．学会等名 Indo-Japan Sakura Science Program（招待講演）
4．発表年 2021年

1．発表者名 Hemanth Noothalapati, Keita Iwasaki, Ajinkya Anjikar, Asuka Araki, Riruke Maruyama, Tatsuyuki Yamamoto
2．発表標題 Machine learning assisted Raman microspectroscopy for objective discrimination of breast cancer cells from normal mammary epithelial cells
3．学会等名 Saratov Fall Meeting SFM'21 & 9th International Symposium on Optics and Biophotonics（国際学会）
4．発表年 2021年

1．発表者名 Hemanth Noothalapati, Keita Iwasaki, Ajinkya Anjikar, Asuka Araki, Riruke Maruyama, Tatsuyuki Yamamoto
2．発表標題 Accurate classification of breast cancer cells from normal mammary epithelial cells by machine learning assisted Raman microspectroscopy
3．学会等名 12th International Symposium of Indian Scientist Association in Japan（国際学会）
4．発表年 2021年

1．発表者名 Hemanth Noothalapati, Rahul Mojidra, Arti Hole, Keita Iwasaki, Tatsuyuki Yamamoto, Murali Krishna C and Rukmini Govekar
2．発表標題 Objective discrimination of drug resistant cells in chronic myeloid leukemia by Raman spectroscopy and multivariate curve resolution analysis
3．学会等名 第 19 回医用分光学研究会
4．発表年 2021年

1．発表者名
Hemanth Noothalapati, Ajinkya Anjikar and Tatsuyuki Yamamoto

2．発表標題
Artificial intelligence assisted Raman spectroscopy for reliable classification of cancer cells

3．学会等名
International Conference on Nanoscience and Photonics for Medical Applications（招待講演）（国際学会）

4．発表年
2022年

1．発表者名
Hemanth Noothalapati, Imrul Hossain and Tatsuyuki Yamamoto

2．発表標題
Visualizing cell wall dynamics during yeast sporulation process by Raman microspectroscopy and MCR-ALS technique

3．学会等名
27th International Conference on Raman Spectroscopy（国際学会）

4．発表年
2022年

1．発表者名
Hemanth Noothalapati

2．発表標題
Explainable AI assisted identification of molecular signatures in Raman spectroscopy for reliable and automated classification of cancer cells

3．学会等名
American Chemical Society Spring 2023（国際学会）

4．発表年
2022年

1．発表者名
Hemanth Noothalapati

2．発表標題
Label-free analysis of single cells by Raman microspectroscopy

3．学会等名
4th Meeting on Biometrics of redox, oxidative stress and phytochemicals（招待講演）

4．発表年
2022年

1．発表者名 Hemanth Noothalapati
2．発表標題 Raman microspectroscopy: From single cell imaging to diagnostics
3．学会等名 第28回中国四国支部分析化学若手セミナー（招待講演）
4．発表年 2022年

〔図書〕　計0件

〔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			
インド	ACTREC, Tata Memorial Center, Mumbai	SRMAP, Amravati, India		