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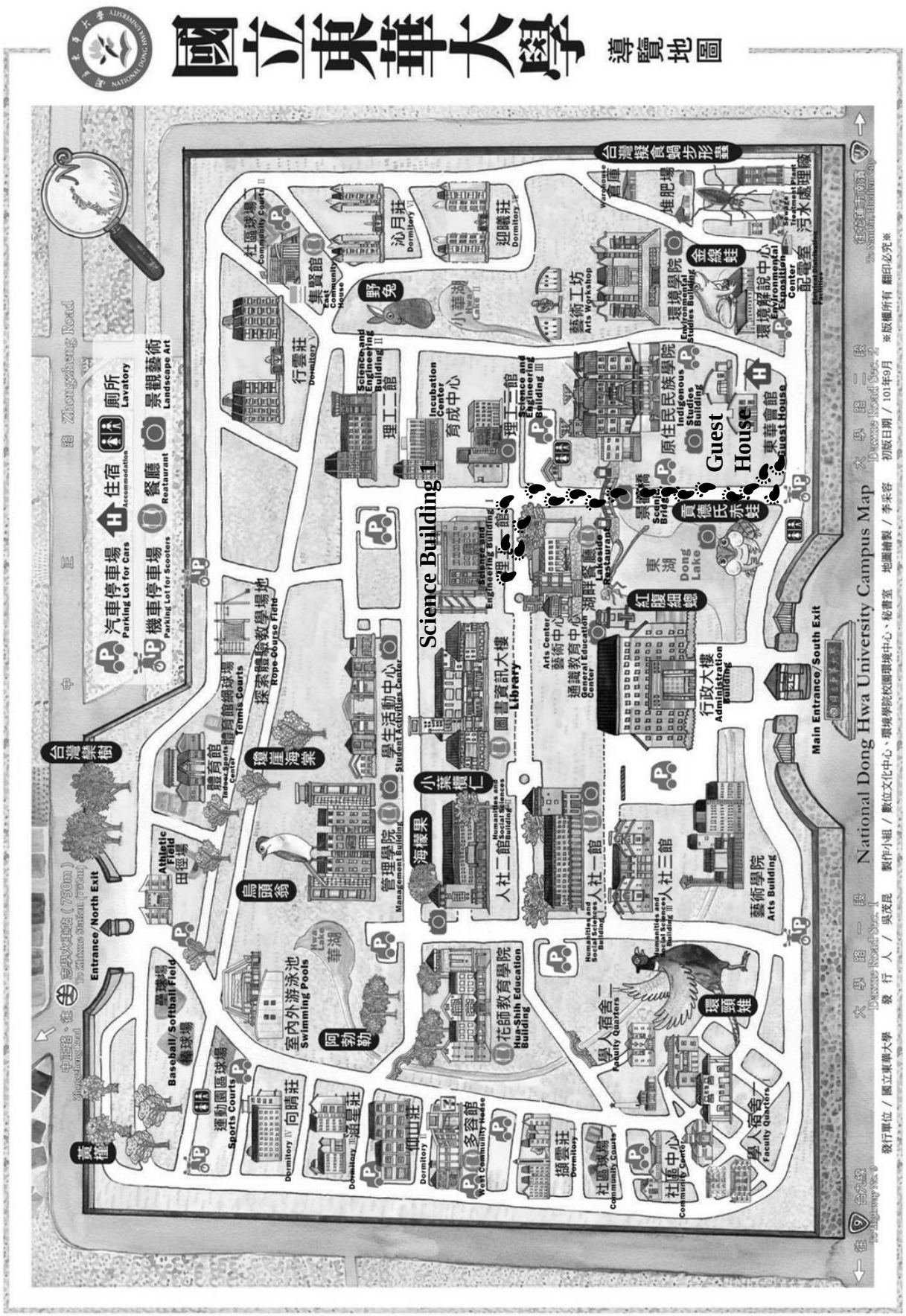
Symposium Venue map



The Second Taiwan International Symposium on Raman Spectroscopy
Program
Venue: Lecture Hall 1, Science Building 1, National Dong Hwa University, Hualien, Taiwan

June 22, Sunday

18:00–21:00		Welcome dinner

June 23, Monday

08:30–09:10		Registration
09:10–09:20		Welcome by Chia-Liang Cheng
09:20–09:30		Opening remarks by Hiro-o Hamaguchi
		Session I (09:30–12:15)
		Chair: Hiro-o Hamaguchi
09:30–10:00	Plenary 1	**Siva Umapathy**, Indian Institute of Science, India *Understanding influence of solvents on Frank-Condon active modes using ultrafast stimulated Raman loss spectroscopy*
10:00–10:30	I1	**I-Chia Chen**, National Tsing Hua University, Taiwan *Raman Spectra of Extended Metal Atom Chain Complexes*
10:30–10:45		Coffee break
		Chair: Chia-Liang Cheng
10:45–11:15	I2	**Yukio Furukawa**, Waseda University, Japan *Raman Spectroscopy of Organic Semiconductor Films Used for Electronic Devices*
11:15–11:45	I3	**Juen-Kai Wang**, National Taiwan University *and Academia Sinica*, Taiwan *High-Speed Detection Platform for Water Analysis, Food Safety and Clinical Microbiology Based on Enhanced Raman Scattering*
11:45–12:15	I4	**Yu-Ming Chang**, National Taiwan University, Taiwan *Depth-resolved micro-Raman Spectroscopy of GaN-based LED Structures*
12:20–14:00		Lunch
		Session II (14:00–18:00)
		Chair: Yu-Ming Chang
14:00–14:30	I5	**Masahiro Ando**, Waseda University, Japan *Heme-protein analysis in living single cells by Raman multivariate curve resolution approaches*
14:30–15:00	Plenary 2	**Anthony W. Parker**, STFC Rutherford Appleton Laboratory, UK *Studies on the Photoionization of α-Tocopherol and Analogue Trolox C*
15:00–15:30	I6	**Yuan-Ron Ma**, National Dong Hwa University, Taiwan *Raman scattering and Photoluminescence Studies on Various Nanostructures*

15:30–16:00		Tea break
		Chair: Yuan-Ron Ma
16:00–16:30	I7	**Tatsuyuki Yamamoto**, Shimane University, Japan *Application of Raman spectroscopy on living cells and biological tissues*
16:30–17:00	I8	**Satyen Saha**, Banaras Hindu University, India *Elucidating interactions in low melting salts and complexes by Raman Spectroscopy*
17:00–17:30	I9	**Y. Y. Yang**, Renishaw *Novel micro-Raman technology with fast imaging applications*
17:30–18:00	I10	**Ramdane Benferhat**, Horiba Scientific *Latest development in Spectroscopy for the characterization of nanostructured materials and nanodevices*
18:00–21:00		Poster session with buffet dinner

June 24, Tuesday

		Session III (08:30–12:00)
		Chair: Shinsuke Shigeto
08:30–09:00	I11	**Ashok Z. Samuel**, Gakushuin University, Japan *Unusual Acid-Base Equilibrium of an Aromatic Carboxylic Acid at the Interface*
09:00–09:30	I12	**Wen-lung Chen**, National Chiayi University, Taiwan *Probing the Structure and Function of Proteins by FT-Raman Spectroscopy*
09:30–10:00	Plenary 3	**Igor K. Lednev**, University at Albany, USA *Variety of Raman Spectroscopy for Probing Amyloid Fibrils: Deep UV, Polarized and Tip-Enhanced Raman Experiments*
10:00–10:30		Tea break
		Chair: Wen-lung Chen
10:30–11:00	I13	**Sheng Yun Wu**, National Dong Hwa University, Taiwan *Application of Raman scattering for spin-phonon coupling and magnon excitation study in nanocrystals*
11:00–11:30	I14	**Vitaly I. Korepanov**, National Chiao Tung University, Taiwan *Raman spectroscopy for nanoparticles: phonon confinement and particle size distribution in nanodiamond*
11:30–12:00	I15	**Shinsuke Shigeto**, National Chiao Tung University, Taiwan *When cells divide: Multivariate analysis-assisted Raman spectral imaging of living cells*
12:00–12:20		Closing remarks
12:20–13:20		Lunch

Symposium Oral Abstracts

Plenary 1

Understanding influence of solvents on Frank-Condon active modes using ultrafast stimulated Raman loss spectroscopy

Khokon Roy, Surajit Kayal, and S. Umapathy

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Photo-isomerization around double bond is one of the most important and the simplest form of structural distortion in a chemical reaction. On excitation to the singlet excited state, isomerization is thought to be the evolution of initially prepared Franck-Condon state towards conical intersection and followed by non-adiabatic crossing to the ground electronic state. Trans-Stilbene (tS), which undergoes ultrafast isomerization in S_1 state, used as a model compound to understand the mechanism of photo-induced isomerization reaction.

In solution phase, solvent effects could potentially influence the early time excited state dynamics after photo-excitation. Several studies have been carried out on tS to understand solvents effects and the energy flow on ultrafast time scale. Ultrafast transient Raman techniques (URLS & FSRS) have the capability to provide details of mode specific solvent induce structural changes with high temporal (~100 fs) and spectral (~10 cm^{-1}) resolution. On the other hand femtosecond transient absorption spectroscopy (TA), being an electronic absorption technique, can probe electronic state dynamics, population decay and solvent induced energy fluctuation on ultrafast time scale. TA and Raman techniques provide complementary information on electronic and vibrational nature of transient molecule in different solvents environment.

Both TA and URLS are applied to study the S_1 state of tS after 280 and 300 nms (~80 fs) photo-excitation in different solvents (acetonitrile, butyronitrile). Strong oscillatory wave-packet motion in the kinetic trace of band area of 1570 cm^{-1} band observed with-in 2 ps of the photo-excitation. Period of oscillation (220 fs) is unaffected by solvent properties but dephasing of oscillatory part shows solvent dependence (500 fs in acetonitrile, 700 fs in butyronitrile). Fourier transform of the oscillatory component gives low frequency torsional modes (90 cm^{-1}, 160 cm^{-1}, 220 cm^{-1}) which are coherently excited due to rapid energy flow from the 1570 cm^{-1} mode. The details of these results would be presented in the talk.

Acknowledgements: We would like to thank DST, DRDO and CSIR for financial support. SU would like to thank DST for the J C Bose Fellowship.

I1
Raman Spectra of Extended Metal Atom Chain Complexes

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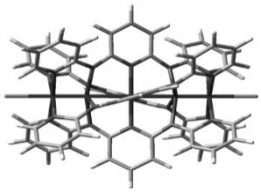

We investigate the strength of metal-metal bonds in metal-string complexes $M_3(dpa)_4X_2$, $M_5(tpda)_4X_2$ and $Cr_7(teptra)_4(NCS)_2$ (M = Co, Ni, Cr, tpda = tripyridyldiamido, teptra = tetrapyridyltriamido, X = Cl^-, NCS^-). The bands in surface-enhanced Raman scattering (SERS) and Raman spectra differ insignificantly in spectral positions, indicating no major structural variation between the solid and solution forms. For SERS measurements these complexes were bound to silver or gold nanoparticles in aqueous solution to eliminate the constraint of a crystal lattice and to maintain the complexes in thermal equilibrium; this method is convenient to identify the stable structure. In these studies, we identified both penta- and heptachromium complexes in both symmetric (*s*-) and unsymmetric (*u*-) forms. For pentachromium complexes our data agree with the results obtained from structural determination of the crystalline form. From our analysis of the vibrational normal modes, we assign the band at 280 cm^{-1} to the Cr-Cr symmetric stretching mode of the *s*-form pentachromium complex. The Raman spectra of $Cr_5(tpda)_4X_2$ are shown in Fig. 1. According to comparisons of SERS spectra obtained at either high temperatures or under oxidizing conditions, we assign 570 cm^{-1} to the stretching mode of the Cr-Cr quadruple bond in the *u*-form for the pentachromium complex and 554/571 cm^{-1} analogously for the heptachromium complex. For the second-row transition metals, we report the assignments on triruthenium metal string complexes: the infrared band at 323 cm^{-1} to the Ru_3 asymmetric stretching vibration and no Raman line to the Ru_3 symmetric stretching mode for $Ru_3(dpa)_4Cl_2$. In its first and second oxidized complexes, we observed no frequency shift in this metal-related vibrational mode. As for $Ru_3(dpa)_4(CN)_2$ complex series, we assign the infrared band at 302 cm^{-1} to the Ru_3 asymmetric stretching vibration and no Raman line to the Ru_3 symmetric stretching mode. Change in axial ligand to CN^- increasing the bonding strength in Ru-C but weakens the Ru-Ru bonding.

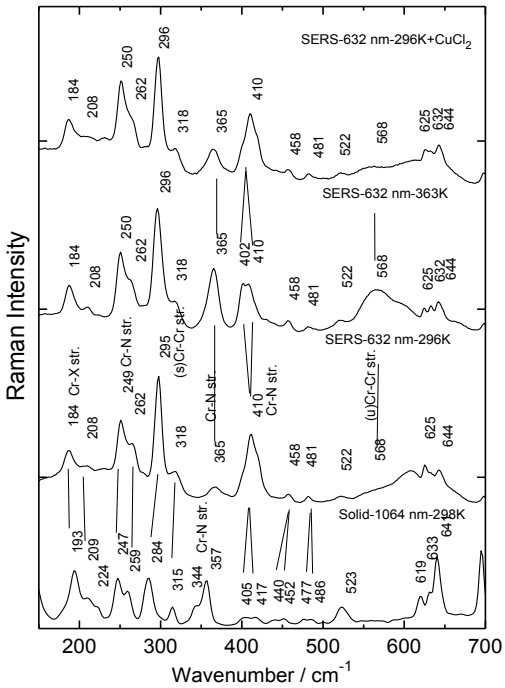

Fig.1. Structure and Raman Spectra of $Cr_5(tpda)_4X_2$

I2

Raman Spectroscopy of Organic Semiconductor Films Used for Electronic Devices

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Raman spectroscopy is a powerful tool for studying structure of organic semiconductor films used for electronic devices such as light-emitting diodes, thin-film transistors, and thin-film solar cells. Conjugated polymers are very promising as active semiconductors in the devices. Raman spectroscopy provides us with rich information about molecular and solid-state structures of polymers, charge carriers [1, 2], temperature of buried organic thin layers in the devices [3−5]. When an electron is removed from a polymer chain, a positive polaron (charge, $+e$; spin 1/2) is generated. When another electron is removed from a positive polaron, a positive bipolaron (charge, $+2e$, spin, 0) may be formed. When a positive bipolaron is unstable, two positive polarons are expected to be formed. Recently, an ionic liquid has been used as a high capacitance gate dielectric in an organic electrochemical transistor. It is called an ionic liquid transistor, which shows a large output current at a low voltage. The electrical properties of the transistors depend on carriers generated in the active layer. In this paper, we present a Raman study of carriers (positive polarons and bipolarons) generated in an ionic liquid electrochemical transistor fabricated with regioregular poly(3-hexylthiphene) (P3HT) as an active semiconductor and 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide [BMIM][TFSI] as a gate dielectric.

We observed visible and near-infrared absorption and 830-nm excited Raman spectra of P3HT films doped chemically from an $FeCl_3$ vapour and an acetonitrile solution of anhydrous $FeCl_3$. The observed Raman spectra were assigned to positive polarons and bipolarons, on the basis of electronic absorption spectra of polarons and bipolarons in a previous paper [1]. The Raman spectrum of polarons is different from that of bipolarons. Thus, we can identify positive polarons and bipolarons using Raman spectroscopy.

Raman spectra of an ionic liquid transistor were observed with excitation at 532 and 785 nm, when the gate voltage ($-V_G$) relative to the source electrode was changed from 0.0 to 2.3 V. In the range lower than 1.2 V, the observed Raman spectra were attributed to positive polarons. In the range higher than 1.7 V, the observed spectra were attributed to positive bipolarons. In the range between 1.2 and 1.7 V, the observed spectra were attributed to the coexistence of polarons and bipolarons. The relationship between the drain current and $-V_G$ was measured; the drain current exhibited a peak at the $-V_G$ of 1.8 V; the decrease in drain current at the high $-V_G$ region is a weak point for the transistor. We reported that the mobility of bipolarons is lower than that of polarons. These results show that the increase in drain current is due to the formation of positive polarons, and the decrease in drain current is associated with positive bipolarons. Positive bipolarons play a negative role in the drain current at the high $-V_G$ region. We can investigate the relationship between electrical properties and carriers in organic transistors using Raman spectroscopy.

[1] Y. Furukawa, *J. Phys. Chem*., **100**, 15644 (1996).
[2] Y. Furukawa, "Vibrational Spectroscopy of Polymers: Principles and Practice" ed. by N.J. Everall, J.M. Chalmers, and P.R. Griffiths, John, Wiley & Sons, Chichester, pp. 537 (2007).
[3] H. Tsuji, A. Oda, J. Kido, T. Sugiyama, and Y. Furukawa, *Jpn. J. Appl. Phys*., **47**, 2171 (2008).
[4] T. Sugiyama and Y. Furukawa, *Jpn. J. Appl. Phys*., **47**, 3537 (2008).
[5] R. Iwasaki, M. Hirose, and Y. Furukawa, *Jpn. J. Appl. Phys*., **52**, 05DC16 (2013).

I3

High-Speed Detection Platform for Water Analysis, Food Safety and Clinical Microbiology Based on Enhanced Raman Scattering

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[2]*Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, Taiwan, ROC*
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This presentation shows our recent development of a surface-enhanced Raman scattering (SERS) biochip that enables high-speed detection of biocides, food adulterates, and bacteria. This chip is based on two-dimensional metallic nanoparticle array embedded in nanochannels of anodic aluminum oxide (AAO) [1]. This SERS-active substrate shows highly uniform and reliable Raman enhancement. Such performance is enabled specifically by locally enhanced optical field residing at sub-10 nm gaps between adjacent nanoparticles—"hot spots"—that are confirmed with electrodynamic calculation [2] and near-field microscopy [3]. This platform can serve as the effective diagnosis method for water analysis, food inspection and clinical microbiology. Firstly, it demonstrates sensitive detection of carcinogenic malachite green in water [4]. Secondly, copper chlorophyll used as a coloring agent in fake olive oils are readily identified with this platform. Thirdly, this platform is also used to serve as early bacterial diagnosis for patients in critical condition [5,6]. The presentation then ends with outlooks of this technology.

[1]. H.-H. Wang et al., Adv. Mater. 18, 491 (2006).
[2]. B.-Y. Lin et al., Opt. Express 17, 14211 (2009).
[3]. T.-Y. Cheng et al., Phys. Chem. Chem. Phys. 15, 4275 (2013).
[4]. H.-H. Wang et al., Nanotechnology 22, 385702 (2011).
[5]. T.-T. Liu et al., PLOS ONE 4, e5470 (2009).
[6]. T.-Y. Liu et al., Nature Commun. 2, 538 (2011).

I4
Depth-resolved micro-Raman Spectroscopy of GaN-based LED Structures

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In this work, we demonstrate that depth-resolved confocal micro-Raman spectroscopy can be used to characterize the active layer of GaN-based LEDs. By taking the depth compression effect due to refraction index mismatch into account, the axial profiles of Raman peak intensities from the GaN capping layer toward the sapphire substrate can correctly match the LED structural dimension and allow the identification of unique Raman feature originated from the 0.3μm thick active layer of the studied LED. The strain variation in different sample depths can also be quantified by measuring the Raman shift of GaN A1(LO) and E2(high) phonon peaks. The capability of identifying the phonon structure of buried LED active layer and depth-resolving the strain distribution of LED structure makes this technique a potential optical and remote tool for *in operando* investigation of the electronic and structural properties of nitride-based LEDs [1].

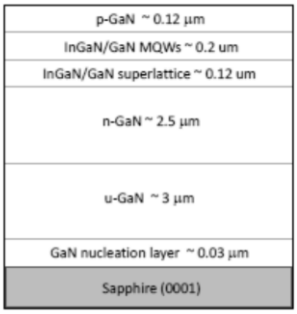

FIG. 1. Layer structure of the studied GaN-based LED sample. The total thickness of the MOCVD grown layers is ~6μm.

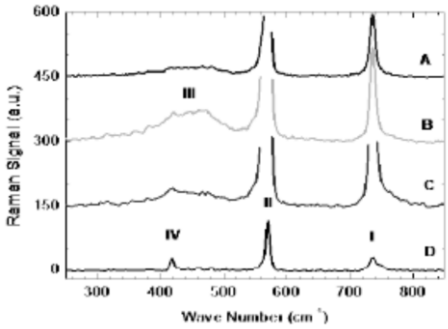

FIG. 2. Raman Spectra at different depths of the sample from the sample surface (0 μm) toward the sapphire substrate. The corresponding depths of the four Raman spectra are: (A) near sample surface at -0.5 μm, (B) MQW active layer at 0.9 μm, (C) n-GaN at 3.7 μm, and (D) inside the sapphire substrate at 14.8 μm. Note that due to the large intensities of GaN A1(LO) and E2(high) peaks, all the spectra have been truncated to fit into the plot.

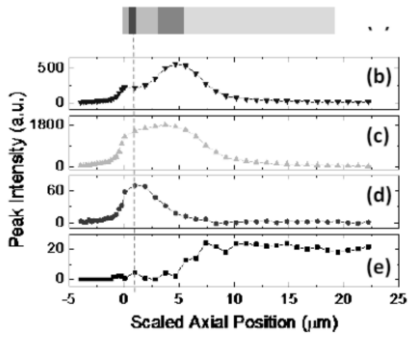

FIG. 3 : (a) Diagram of the sample layer structure, where the layers from left to right are the p-GaN capping layer, MQW/superlattice active layer, n-GaN, u-GaN, and sapphire substrate, respectively. The axial profiles are shown correspondingly to: (b) GaN A1(LO) phonon, (c) GaN E2(high) phonon, (d) MQW active layer phonon feature, and (e) sapphire A1g phonon, respectively.

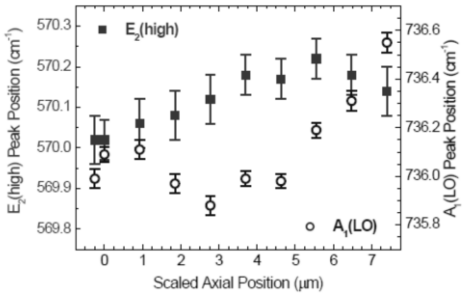

FIG. 4 : The peak frequencies of the GaN A1(LO) and E2(high) phonon modes are plotted as a function of the sample depth from the sample surface toward the sapphire substrate. The peak positions and error bars are determined by least-square curve fitting the Raman peak with single Gaussian function.

[1] W.-L. Chen, Y.-Y. Lee, C.-Y. Chang, H.-M. Huang, T.-C. Lu, and **Y.-M. Chang***, "*Depth-resolved confocal micro-Raman spectroscopy for characterizing GaN-based light emitting diode structures*", Rev. Sci. Instrum., (2013) **84**, 113108.

I5
Heme-protein analysis in living single cells by Raman multivariate curve resolution approaches

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Raman microspectroscopy is now widely used in label-free and molecular-level investigations of living cells. Although Raman spectra contain rich information on molecular structure, detailed interpretation of space-resolved spectra is often difficult because of their complexity. Raman spectra are usually interpreted as the superposition of several biochemical component spectra, as well as those of background and fluorescence. In order to decompose the complicated spectral data set into tractable component spectra, a number of chemometric methods have been developed. Especially, the multivariate curve resolution – alternating least squares (MCR-ALS) method can be a powerful tool for the molecular component distribution imaging of living cells. Under appropriate model constraints, such as non-negativity and norm regularization for spectral profiles and their concentrations, MCR-ALS technique provides physically interpretable spectral components, without a priori information on chemical components.

In this study, we have applied the MCR-ALS method to heme-protein analysis in living single cells. In biological systems, heme-proteins, metalloproteins containing iron-porphyrin complexes, play essential roles for their survival. Resonance Raman spectroscopy can detect with high sensitivity these heme-proteins in living cells. We have used a 632.8 nm excited Raman microspectroscopic system to detect myeloperoxidase and eosinophil peroxidase in white blood cells. As shown in Fig. 1, an MCR-ALS analysis with non-negativity and L1 norm constraints provides the physically sound spectra and their high contrast distribution images of the heme-proteins as well as other biomolecules, nucleic acids and proteins. We have also successfully detected cytochrome *bd* oxidase, a terminal oxidase in bacterial respiratory chains, in living *Escherichia coli.* cells (data not shown here). The change of cytochrome *bd* oxidase concentration with the change of oxygen concentration in culture media is clearly indicated by Raman microspectroscopy.

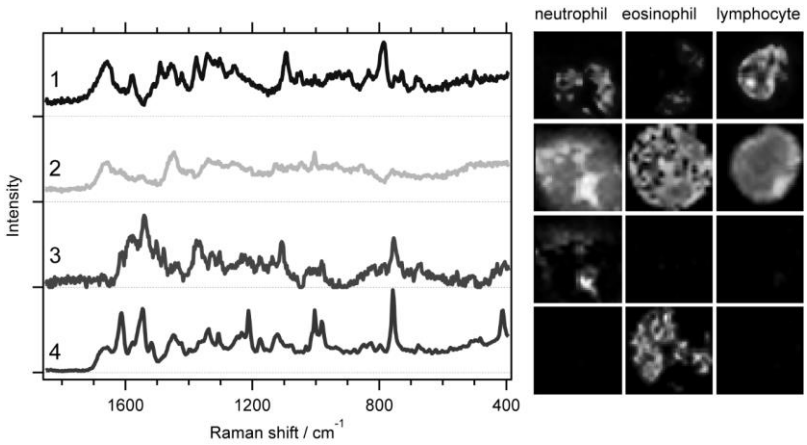

Fig.1. MCR-ALS analysis of white blood cells. Component 1 – 4 are assigned to nucleicacid, protein, myeloperoxidase and eosinophil peroxidase, respectively.

Plenary 2
Studies on the Photoionization of α-Tocopherol and Analogue Trolox C

Anthony W. Parker[1], Roger H. Bisby[2], Gregory M. Greetham[1], Philipp Kukura[3], Kathrin M. Scherer[1, 2] and Michael Towrie[1]

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Antioxidants such as vitamin E play a crucial role in biological systems, preventing oxidative stress that would otherwise result in damage to biomolecules via a series of free radical reactions. For example, in lipid, peroxidation of polyunsaturated acyl chain components of lipids leads to chemical products that cause membrane damage in cells, and may initiate atherosclerosis.

We have employed a series of ultrafast spectroscopic methods including fs-transient absorption, time-resolved infrared and femtosecond stimulated Raman spectroscopy to investigate the ultrafast chemistry of photoionization of α-tocopherol (vitamin E) and the water soluble analogue Trolox C.

These techniques have followed the formation and decay of the excited states, neutral and radical cation radicals and the hydrated electron that are produced under the various conditions examined. The study has also looked at the nature of the solvent and its role in determining whether homolytic dissociation of the phenolic –OH bond producing directly the tocopheroxyl radical, or the intermediate formation of a radical cation and the hydrated electron occurs. The results provide useful insights into the biological processes described above.

Acknowledgements

The authors thank the Central Laser Facility, Science and Technology Facilities Council (STFC) for proving access the experimental facilities. The work was jointly funded by STFC and the University of Salford.

I6
Raman scattering and Photoluminescence Studies on Various Nanostructures

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Over the past two decades, research on nanostructures has become more and more popular in various scientific fields, no matter what they are zero-dimensional (0D), one-dimensional (1D), or two-dimensional (2D). As compared with bulk materials, nanostructures obviously have much lower density and larger ratios of surface-to-volume. This is a reason why nanostructures possess fascinating and fanciful properties that can be used in practical applications. [1] Especially, metal oxide nanostructures are well known to be the most functional materials used for many applications. In this talk, photoluminescence (PL) and Raman scattering of various nanostructures will be introduced for their optical and structural properties.

Raman scattering is a powerful technique for obtaining useful information on the vibrational states of the nanostructures by investigating the confined optical phonons. In bulk crystal, the phonon eigenstate is a plane wave and the wavevector selection rule for the first-order Raman scattering requires $q \sim 0$. In contrast, the spatial correlation function of the phonon becomes finite due to its confinement in the nanostructure, and hence the $q \sim 0$ rule is relaxed. Since phonon dispersion curves are not flat, phonon confinement results in peak shift (to lower frequencies or to higher frequencies if frequency is an increasing function of wavevector) and asymmetric broadening of the Raman lines. This has been explained in a phenomenological phonon confinement.

Photoluminescence (PL) spectroscopy is a well-developed optical tool for the inspection of semiconductor materials and devices for impurities and defects. PL light emissions due to near band edage (NBE) and deep level electron transitions gap were probed by PL spectroscopy. The room-temperature PL spectra of various nanostructures showed a wide-range of emissions from ultraviolet (UV) to infrared (IR). The volatility of oxygen (O) in metal oxides leads to oxygen vacancies being the most predominant type of defect. The complicated structures give metal oxides leads the potential to yield many types of oxygen vacancies. Each oxygen vacancy offers dangling bonds and donates two electrons close to the vacancy location. The dangling bonds give rise to electronic states within the bandgap which are able to trap excited electrons, creating many trap levels within the bandgap of metal oxides leads. The great variation of in-plane and cap oxygen vacancy sites provides more trap levels, located at distinct interfacial intersections. The trap levels correspond to the ionization energies of the oxygen deficiencies and are sufficient to provide visible-light emission. Therefore, the presence of oxygen vacancies is expected to make metal oxides leads an n-type semiconductor and to play a strong role in the photoluminescence (PL) emissions.

[1] R. S. Devan, R. A. Patil, J.-H. Lin, Y.-R. Ma*, Adv. Func. Mater. 22 (2012) 3326-3370.

I7
Application of Raman spectroscopy on living cells and biological tissues

T. Yamamoto[1,2,3], Makoto Kawamukai[1,2], Yoshikazu Kinoshita[2,4], Yasuhisa Fujita[2,3,5], and Hiro-o Hamaguchi[6]

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[3]*Nanotechnology Project Center, Shimane University, Japan*
[4]*Faculty of Medicine, Shimane University, Japan*
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Introduction

Recent development in microscopic Raman spectroscopy has enabled us to study living cells and living tissues in situ. We have recently studied the effects of some physiologically active substances on the growth of fission yeast strains by Raman spectroscopy. We are working on developing a new diagnostic technique for eosinophil esophageal inflammation by Raman spectroscopy as a medical probe, as well. Recent results on these works will be introduced in our talk.

A Raman spectroscopic study on the metabolic activity of fission yeast strains

Fission yeast (*Schizosaccharomyces pombe*) is a eukaryote and a good model organism for studying human diseases or the effects of physiologically active substances on it. We can study the effects of such substances on the growth or metabolic activity of a variety of mutant strains. We have recently found out that the effects of some anti-oxidants on the growth of some strains of fission yeast by microscopic Raman spectroscopy. The effects of coenzymeQ10 (CoQ10) and its complex, included by γ-cyclodextrin, on the growth and metabolic activity of a mutant strain, *Δdps1*, which cannot produce CoQ10 by itself, was studied. CoQ10 is a coenzyme for respiration as well as a strong anti-oxidant. The *Δdps1* strain did not grow well in minimal medium due to the lack of producing CoQ10 capability. However, it grew well by an addition of the inclusion complex of CoQ10 in the minimal medium. The respiration activity of it did not recover by the addition of the complex. The addition of other anti-oxidants, glutathione, ascorbic acid, α-lipoic acid also improved the growth of the *Δdps1* strain. These results suggested that the improvement in the growth of the mutant strain by CoQ10 was brought about by the anti-oxidative activity of CoQ10, not by the recovery of the respiratory activity [1]. The relative intensity of a Raman signal observed at 1602 cm^{-1} was studied as a function of the concentration of CoQ10 using a signal at 1440 cm^{-1} as a reference. As a result, the relative intensity of the Raman signal increased with the concentration of anti-oxidants.

A Raman spectroscopic study on the eosinophil esophageal inflammation

Recently, the prevalence of eosinophilic esophagitis with dense eosinophile infiltration is increasing. The patients with this disease complain dysphagia and food impaction due to the esophageal mucosal inflammation caused by the delayed type food allergy. The characteristic point of the disease is that only esophageal mucous membrane is invaded by eosinophil. The present diagnosis of the disease is performed by the counting up the number of eosinophiles in the esophageal mucosa obtained by the endoscopic biopsy. This method inevitably forces the patients to bleed. From the view point of safety, we are trying to develop a new diagnostic technique for the eosinophil esophageal inflammation using Raman spectroscopy as a non-invasive probe. We have already found that we can distinguish eosinophile from other types of white blood cells. We are now trying to develop a new diagnostic technique to detect the existence of eosinophiles in esophageal mucous membrane by Raman spectroscopy. Our recent work on this study will also be introduced.

Reference

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I8

Elucidating interactions in low melting salts and complexes by Raman Spectroscopy

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A large number of researchers have been attracted towards research in ionic liquids (ILs) for their amazing possibilities as new materials. Many distinct properties of ILs (e.g., low melting points, high viscosities, marked amphiphilicities) are likely to originate from their unique structures and interactions. Elucidating these are of prime importance for thorough understanding and full utilization of ILs in the future. Raman spectroscopy and other spectroscopic techniques have been used for the same and will be presented. In addition, we shall also present an updates on i) insitu generation of I_2 in iodide ionic liquids, ii) decomposition of bromine-water, iii) evidence of microheterogeneity in piperidinium cation based ionic liquids.

I9
Novel micro-Raman technology with fast imaging applications

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Analysis of Raman scattered light, as a Raman spectrum, reveals key information about a sample's chemical structure. Until late 90's, Raman spectroscopy was rarely used outside a specialist research laboratory because of low sensitivity, instability and difficult-of-use of traditional double or triple grating Raman spectrometers.

In recent years, a novel confocal micro-Raman spectrometer system equipped with a combination of new technologies such as Raleigh rejection edge filters, CCD detector and research grade microscope, has been developed first by Renishaw and Leeds university of UK. Its high sensitivity (2~3 orders of magnitude higher than traditional Raman spectrometers), spatial resolution (down to < 1 um), accuracy/ repeatability and easy of use make it available for many applications in various of research and developments areas. It is particularly useful when an application requires no sample preparation, non-destructive and non-contacting analysis.

Samples as small as submicron can be analysed in seconds, non-destructively and non-intrusively, with high spectral resolution. Micro size particles, inclusions, and even single nano tube/wire could be analyzed without destroying them. Larger samples can also be imaged for their Raman or PL distributions.

An unique development of Renishaw's Global imaging technology provides the fastest way to acquire a Raman spectral image of whole sampling area at the same time without touching or moving the sample. The recent innovation of Renishaw's StreamLinePlus™ rapid Raman mapping/imaging system makes it possible to collect spatially resolved chemical information from samples as large and complex as whole pharmaceutical tablets in about 4 minutes. With StreamlineHR™ the spatial resolution of a Raman image can be as good as 250nm.

I10

Latest development in Spectroscopy for the characterization of nanostructured materials and nanodevices

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Nanoscience has attracted much attention due to the unique physical properties and potential applications such as electronic components, catalysts, sensors, biomarkers, and energy harvesters [1-5]. Among several characterization methods in nanoscience field, MicroRaman spectroscopy is widely used and well known for providing useful information on the nanostructred material's properties in a non destructive way.

Nanostructured materials' properties are of great importance for device performance. The crystallinity, composition, doping, stress and defects, which drive the electrical and optical properties of the materials are derived from MicroRaman Spectroscopy. Benefiting from the latest development in Micro Raman technology, isolated single nanowire, nanotube or nanobelt can be analysed easily.

In this presentation we will review the latest development in Raman Spectroscopy and their applications to the characterization of nanostructured materials and nanoscaled devices.

Dr Ramdane BENFERHAT got his PhD in 1987, in Solid State Physics from Paris University, Pierre et Marie Curie. His research work done at Ecole Polytechnique, the leading academic institution in France, led to several patents on Analytical Instrumentation. In 1987, he Joined HORIBA Scientific as an R&D manager and his work was awarded by the French Society of Physics in 1992, and by the French CNRS in 1993, for the successful transfer of high technology from academic to industry. Since 2007, Dr Ramdane BENFERHAT is leading the operation of HORIBA Scientific in Asia.

I11
Unusual Acid-Base Equilibrium of an Aromatic Carboxylic Acid at the Interface

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The behaviour of molecules at the interface is notably different from their behaviour in bulk water.[1] Various factors such as effective dielectric constant, electrostatic potential and even the structure of water at the interface is believed to influence their properties (e.g. acid-base equibilibrium) at the interface.[2] In the present investigation we have probed the acid-base equilibrium of an aromatic carboxylic acid gelator, *viz.* salicylic acid, at the interface of the worm-like micelle formed in the cetyltrimethylammonium bromide (CTAB) gel[3] with Raman spectroscopy.

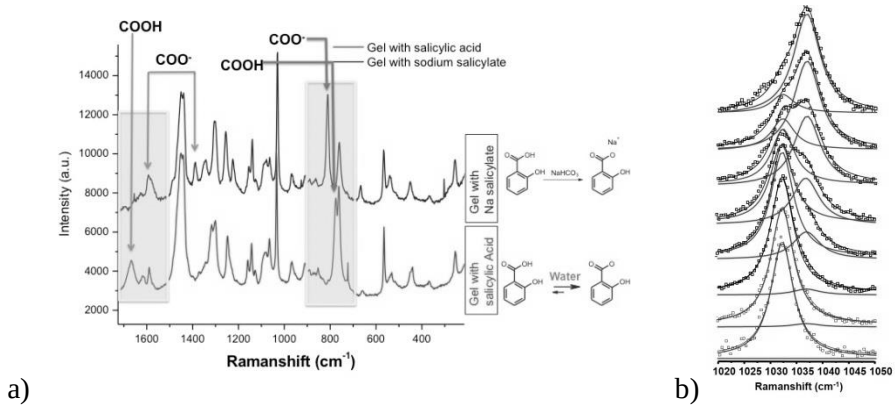

Figure 1. a) Raman spectra of CTAB gels prepared with Na-salicylate (blue) and salicylic acid (red). b) The Raman band shape variation observed due to a proton-deuterium exchange reaction in the gel at different H_2O:D_2O compositions (D_2O composition decreases from top to bottom).

We have observed that the acid and the salt of the gelator are equally capable of gelling the CTAB solution. Very similar microstructure of the gel was evident from the small angle X-ray scattering studies (Hexagonally packed cylinders). Raman spectral analysis revealed that the acid doesn't ionize into its conjugate base despite its low pKa (Figure 1a). D_2O diffusion experiments indicated that the salicylic acid exists at the hydrated domain of the interface and a proton exchange reaction occur as a consequence (Figure 1b). Further we probed the effect of changing the pH of the bulk water in the gel. Surprisingly, we found that the acid is reluctant to ionize even at pH 13 (~70%) when it is present at the interface. More interestingly, the protonation of the conjugate base (salicylate) did not occur even at a pH of 2 when present at the interface. It appears that there is a barrier for the free diffusion of ionic species into the interfacial region (H^+ and OH^-). This was substantiated by a characteristic diffusion profile observed for the diffusion of aq. $NaHCO_3$ solution into the gel. We believe that the bound-water that exist close to the micellar surface act as a barrier to the free diffusion of ions.

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I12

Probing the Structure and Function of Proteins by FT-Raman Spectroscopy

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FT-Raman spectroscopy has demonstrated several spectroscopic advantages in deciphering protein structure including simple and straightforward sample preparation, free from fluorescence interference, and rich in providing molecular information. Raman bands arising from amide I, amide III, and skeletal stretching modes of peptides and proteins are useful for characterizing backbone conformation. And valuable information could be obtained on SS/SH conversion, CH groups of aliphatic residues, and aromatic rings of amino acid residues. FT-Raman spectroscopy was employed to explore the structural change of lens proteins affected by aging and by various dietary supplements such as vitamin A, E, and Ganoderma lucidum (traditional Chinese medicine). It shows that those dietary supplements would enhance the glutathione level in eye lenses, which in turn protects protein structure from oxidative modifications.

To understand the role of disulfide bonds in protein folding/unfolding, the structure of d-crystallin and bovine serum albumin were studied by FT-Raman as the former is lack of disulfide bond while the latter has seventeen pairs of disulfide bonds. It shows that d-crystallin are more susceptible to heat and chemical-induced change than BSA. The high content of disulfide bonds in BSA serves as concrete fulcrums to stabilize protein structure. Without disruption of disulfide bonds, the secondary structure of BSA sustains only slight changes and remains in favor of a-helix. As the concrete framework of disulfide bonds collapse, the secondary structure of BSA encountered dramatic change, from a-helix into b-sheet.

Plenary 3

Variety of Raman Spectroscopy for Probing Amyloid Fibrils: Deep UV, Polarized and Tip-Enhanced Raman Experiments

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In spite of the key medical importance of amyloid fibrils, the molecular mechanism of fibrillation is not fully understood. At least in part this is because amyloid fibrils are non-crystalline and insoluble, and thus are not amenable to conventional X-ray crystallography and solution NMR, the classical tools of structural biology. We have developed and applied novel experimental approaches based on Raman spectroscopy for characterizing structure and dynamics of amyloid fibril during the last decade. These include deep ultraviolet Raman spectroscopy, polarized Raman spectroscopy of aligned fibrils and tip-enhanced Raman spectroscopy (TERS). In addition to hardware, we developed advanced statistical methods for analyzing spectroscopic data including two dimensional correlation spectroscopy (2DCoS). The application of these complimentary methods allowed for obtaining comprehensive knowledge about the mechanism of fibrillation as well as structure of fibril core and fibril surface.

I13

Application of Raman scattering for spin-phonon coupling and magnon excitation study in nanocrystals

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In this talk, we provide a review of Raman applications on Cr_2O_3 and NiO nanocrystals, in which an overview of the characteristic parameter of spin-phonon coefficient λ_{SP}, the interaction of incident light with the spin degrees of freedom, and size effects will be given. Raman scattering was utilized to study the spin-phonon and magnon configurations for these samples. The appearance of energy shift two- and four- magnon modes reflects the existence of the finite size effect. Multi-magnon excitations generated in NiO nanowalls may help to identify the Ni^{2+}—O^{2-}—Ni^{2+} superexchange mechanism associated with the short range magnetic interactions and magnon possible configurations.

I14

Raman spectroscopy for nanoparticles: phonon confinement and particle size distribution in nanodiamond

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One of the challenges of nanoparticle science and technology is to develop reliable experimental methods to characterize the particle size distribution. In principle, Raman spectroscopy could address this challenge, because the Raman spectra of nanoparticles are sensitive to the size. Still, the reliable physically consistent way of interpreting spectral pattern is yet to be developed. The first approach to describe the size effect, known as the phonon confinement model (PCM), was proposed by Richter [1] more than 30 years ago. Early works on PCM applied a one-dimensional approximation, in which the 3-dimensional phonon dispersion function is replaced by some effective 1-dimensional function, often derived from approximately averaged neutron diffraction data [2]. However, the physically meaningful way to apply the PCM would be to use 3D dispersion function instead of 1D dispersion curves.

Other important aspects not addressed in early works are to take into account the particle size distribution (as opposed to single-size approximation), and extend the integration outside the 1st Brillouin zone [3]. The working formula of such model that we use here is as follows [4]:

$$I(\omega) \cong \int \rho(\sigma) d\sigma \, \frac{\sigma^3}{N(\sigma)} \iiint_{-\infty}^{\infty} \frac{|C(q_0,q)|^2}{\left(\omega-\omega(q)\right)^2+(\Gamma_0(\sigma)/2)^2} dq_x dq_y dq_z$$

where ω is the wavenumber, q is the wave vector, $\omega(q)$ is the phonon dispersion function (3D), Γ_0 is the natural bandwidth, $\rho(\sigma)$ is the PSD and $N(\sigma)$ is the normalizing factor, and $C(0,q)$ is a Fourier coefficient.

We use the above equation to fit experimental Raman spectra of different size nanodiamonds (fig. 1). The model gives good agreement with experimental data.

In conclusion, we propose a physically consistent way of interpreting Raman spectra of nanoparticles. Since the 3D dispersion function takes into account the anisotropy of the phonon dispersion, the model built in this way has a straightforward physical meaning. We also show that, in order to provide the correct interpretation of the Raman spectra of small nanoparticles, it is critical to take into account the particle size distribution in an appropriate way.

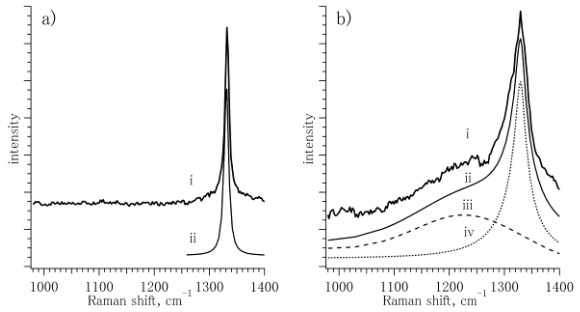

Figure 1. a) Experimental Raman spectra for HPHT-nanodiamond (av. size 68 nm) (i) and fit with formula (5) (ii). b) same for DND (av. size 3.0 nm). Lines (iii) and (iv) show the contribution from particles smaller and larger 4.6 nm into the total intensity, respectively.

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I15
When cells divide: Multivariate analysis-assisted Raman spectral imaging of living cells

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The cell cycle culminates in cytokinesis, where cells are physically split in two, and organelles and intracellular biomolecules as well as genetic information are faithfully inherited in daughter cells. *In vivo*, molecular-level investigation of this dramatic moment will not only deepen our understanding of the fundamental question of how life continues, but it will also pave the way for medical and clinical applications such as cancer diagnosis/prognosis.

Recently, my group is addressing this challenge by using a powerful spectral imaging tool that combines Raman imaging and multivariate curve resolution (MCR) analysis. In the present talk, I will first review our previous work on the cell division of fission yeast [1]. We have demonstrated time-lapse visualization of the distribution and concentration of chemical components within a single dividing yeast cell. Next I will present our latest attempt to study human colon cancer cells (HCT116). MCR-assisted Raman imaging has enabled us to obtain almost background-free intrinsic spectra of intracellular proteins and lipids and their spatial distributions with higher image contrast than the conventional univariate approach [2] could achieve. Comparison of the Raman images of M-phase and interphase colon cancer cells shows that in interphase, lipids are widespread except for the nucleus, whereas in the course of cytokinesis, they are highly localized at the cleavage furrow. Furthermore, we have observed two autofluorescence components showing considerably different distributions. These results will be discussed in relation to the biological roles that the MCR-derived components may play during cytokinesis.

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Symposium Poster Abstracts

P 1

Label-free visualization of rat eye tissue using multimodal and multiphoton spectral microscopy

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P 2

Comprehensive Raman Spectroscopic Study of 1-(4-Cyanobenzyl)-3-methylimidazolium Ionic Liquids and Their Melting Processes

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P 3

Detection of keratin molecular biomarker via Raman microspectroscopy combined with non-negative matrix factorization analysis: Discrimination of malignant vs. normal oral tissues

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P 4

Evaluation of ZnO nanoparticles for devices and medical applications by Raman spectroscopy

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P 5

Deuterium Labeled Raman Microspectroscopy of Living Yeast Cells

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P 6

The effect of solvent on Decafluorobenzophenone: a Resonance Raman study

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P 7

Toward ultrafast time-resolved chiroptical spectroscopies

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P 8

Label-free Multivariate Raman Spectral Imaging Study of Cellular Component Distributions in Colon Cancer Cells during Cell Division

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P 9

CARS spectral imaging of living algae, *Aurantiochytrium*
~Toward *in-vivo* visualization of hydrocarbon accumulation ~

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P 10

Low Frequency Raman Spectroscopic study of Intermolecular Interactions

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P 11

Study of Nanodiamond Effect on Blood Oxygen Transfer Function

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P 12

Rapid SERS Biosensor for Early Diagnosis of Influenza Virus

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P 13

In situ detection of secondary metabolites in antibiotic-producing bacteria

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P 14

Raman imaging and lipid analysis of marine diatom *Fistulifera solaris* JPCC DA0580

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P 15

Surface Enhanced Raman Scattering of Composite Au/Ag Nanoparticle Films Changed with the Ratio of AuNP to AgNP

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P 16

Investigating Lipid Metabolism *in vivo* By Mixed Stable Isotope Probing - Coupled Raman Micro-spectroscopy

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P 17

Raman Spectroscopic Study of Drug- membrane Interaction

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P 18

Resonance Raman Spectroscopy of β-Carotene in Cyclodextrin Inclusion complex

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P 19

CARS spectral imaging of iPS cells
~Toward visualization of pluripotency~

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P 1

Label-free visualization of rat eye tissue using multimodal and multiphoton spectral microscopy

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[Introduction] In diagnosis of eyes, coherent optical tomography (OCT) and confocal microscopy are widely used for *in vivo* and *in situ* evaluation of eye tissues. However, these techniques provide images based on optical information such as the refractive index differences in the tissue, and give no molecular information. Coherent anti-Stokes Raman scattering (CARS), one of the nonlinear Raman processes, enables to acquire molecular information of tissue without staining and molecular tagging. In the present study, we performed label-free imaging of rat eye tissue by using nonlinear multimodal spectral microscopy [1], which is capable of detecting multiple nonlinear optical phenomena.

[Experimental] The experimental apparatus is a nonlinear multimodal spectral microscopic system, which is developed by our group. Sample was frozen section of rat eye tissue, the thickness of which was 20 μm. The sample was fixed by formalin before the measurement.

[Results, Discussion] Figure 1 shows the result of retina. CARS, second harmonic generation (SHG), third harmonic generation (THG) and third-order sum frequency generation (TSFG) signals are shown in the NIR-visible-UV regions. Multimodal spectral images are shown in Fig. 2. In particular, at the positions of retina, SHG signals were detected around visual pigment (Fig. 2(f)). This area corresponds to the position of new visual pigment. It suggests that outer segment where visual pigment exists have non-centrosymmetric structure.

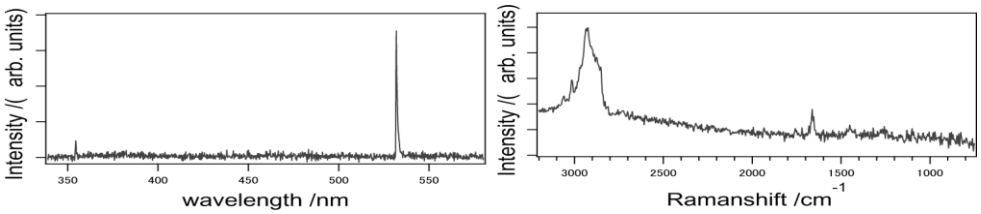

Figure1 Spectral profile of SHG, THG and TSFG (a), and imaginary part of the CARS signal (b)

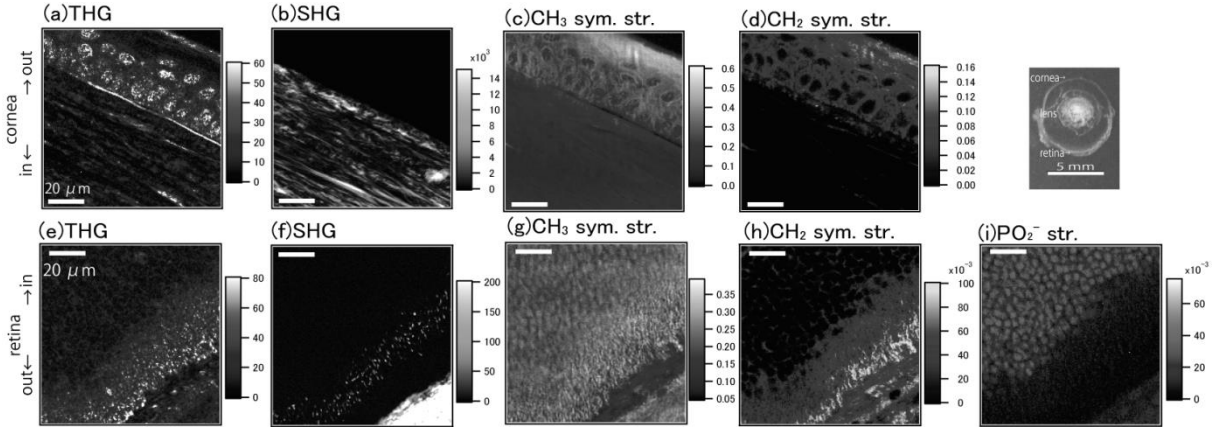

Figure2 Multimodal and multiphoton (THG, SHG and multiplex CARS) images at cornea and retina, and optical image of the frozen section of rat eye tissue

[1] Hiroki Segawa, Masanari Okuno, Hideaki Kano, Philippe Leproux, Vincent Couderc, and Hiro-o Hamaguchi, Optics Express, Vol. 20, Issue 9, pp. 9551-9557 (2012)

P 2

Comprehensive Raman Spectroscopic Study of 1-(4-Cyanobenzyl)-3-methylimidazolium Ionic Liquids and Their Melting Processes

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Nitrile-functionalized ionic liquids (ILs) are an interesting family of ILs that show better solvent and thermal properties. In this work, Raman spectroscopy is used to understand the structural aspects of nitrile-functionalized ILs. The laboratory-built Raman microspectrometer used in the present study covers a broad range of vibrational spectra from ~3000 cm^{-1} (CH stretch), to 500–2200 cm^{-1} (CN stretch and mainly cation modes), to ~10–200 cm^{-1} (intermolecular and lattice vibrations), facilitating comprehensive vibrational analysis.

We synthesized five ILs, [cbmim]X, where the cation 1-(4-cyanobenzyl)-3-methylimidazolium (abbreviated as cbmim; see Fig. 1a for chemical structure) is the anion X is Br^-, NTf_2^-, $N(CN)_2^-$, BF_4^-, and PF_6^-. According to the Raman spectral profile in the 10–1000 cm^{-1} range, the five ILs can be classified into three groups. [cbmim]PF_6 and [cbmim]BF_4 show a very similar profile and they both have a melting point higher than 100 °C. Another group, [cbmim]$N(CN)_2$ and [cbmim]Br, also shows quite a similar spectral pattern. In contrast, the Raman spectrum of [cbmim]NTf_2, which has the bulkiest anion among the five ILs studied, substantially differs from those of the other two groups. Detailed analysis of the CN stretch band at around 2230 cm^{-1} and the CH stretch bands provides qualitative structural information on the Ils. Such information is in particular useful for, [cbmim]$N(CN)_2$ and [cbmim]Pf_6, for which crystallographic data are yet to be obtained owing to the technucal difficulty in growing single xrystals of good quality. We also found that the peak position of the CN stretch band shows an anion-dependent blue shift during melting. This result seems to reflect the difference in the location of the anion with respect to the cation, as well as in the anion size.

(a)

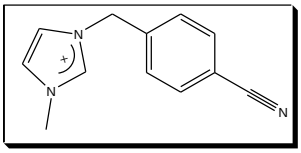
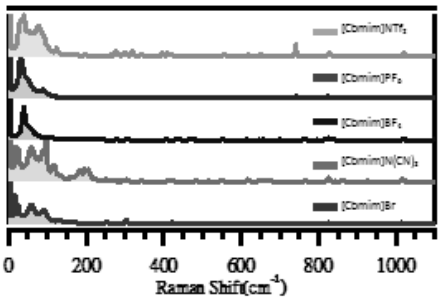
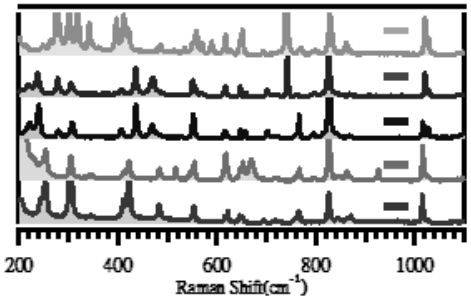


Fig.1 (a) Chemical structure of the cation cbmim.
(b) Low-frequency Raman spectra of the five Ils studied energetic molecules
(c) 10X expnsion for the Fig (b) from 200-1100

(b)



(c)



P 3

Detection of keratin molecular biomarker via Raman microspectroscopy combined with non-negative matrix factorization analysis: Discrimination of malignant vs. normal oral tissues

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Non-negative Matrix Factorization (NMF) is one of the useful analysis methods that can decompose a large spectral data into physicochemically interpretable spectra. We have already shown that Raman spectroscopy combined with NMF provides a powerful tool for studying complex biological molecular systems. [1]

In this study, we use NMF for clearer discrimination of oral squamous cell carcinoma (OSCC) and normal tissues. By providing additional molecular information, the keratin Raman spectrum, we have developed a fast and automatic method to detect keratins in OSCC tissues. First, we determine the number of components based on singular value decomposition (SVD). The SVD analysis has shown that there are five critical independent spectral components in the oral tissue spectral data set. Then with NMF analyses, for each data set of tissue samples, we capture the keratin spectral components in OSCC tissues in a straightforward manner (Fig 1 (a)) but not in normal tissues (b).

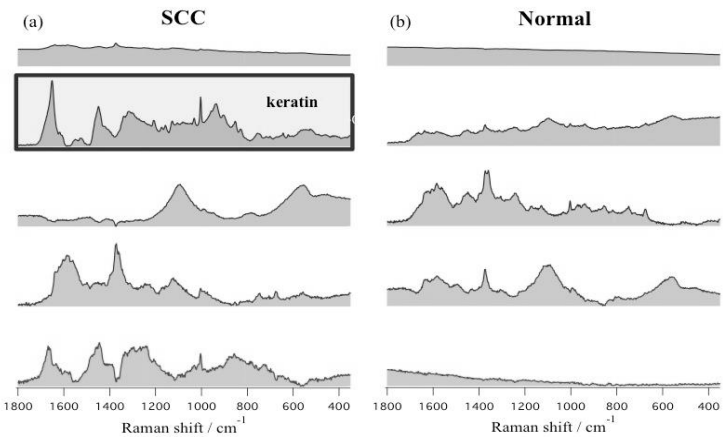

Figure 1 NMF analysis result of (a) OSCC tissue and (b) normal tissue, only (a) OSCC tissue capture keratin component.

Our study demonstrates that Raman microspectroscopy together with an NMF analysis is a powerful tool for discriminating malignant against normal oral tissues without using immunohistochemistry. In addition, this approach also provides a deeper understanding of the molecular signature which will, in the long run, prove to be very important for clinical diagnosis based on Raman spectroscopy.

1. M. Ando and H. Hamaguchi, *J. Biomed. Opt*, 2014, **19(1)**, 011016.

P 4

Evaluation of ZnO nanoparticles for devices and medical applications by Raman spectroscopy

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The development of nano-materials that are economical, simple, and practical in use is important for the applications of green and life innovations. We have been developing unique simple nanotechnologies that are safe, offer peace of mind under the concept of co-creation in nanotechnology collaboration project from material synthesis to device and clinical applications.

Zinc oxide (ZnO), well known compound as a baby powder is a low-cost and abundant material and has unique features for the use of nanomedicine. ZnO is a semi- conductor with relatively wide bandgap energy of 3.37 eV at room temperature and a large exciton binding energy of 60 meV, which results in bright room-temperature near UV emission. We have developed nitrogen doped ZnO nanoparticles and none doped ZnO nanoparticles. Nitrogen is acts as an acceptor in ZnO. Near ultraviolet light emitting diodes were demonstrated by using nitrogen doped ZnO nanoparticles [1]. None doped ZnO nanoparticles has a good fluorescent properties with strong exciton emission at room temperature and applicable for fluorescent probes for medical diagnostics. In this study, ZnO nanoparticles for these applications were evaluated by Raman spectroscopy.

We have synthesized both nitrogen doped and none doped ZnO nanoparticles by gas evaporation technique with DC arc plasma [2]. The average particle size was about 100~200 nm. Nitrogen doped particles were prepared at the chamber pressure of 75 ~150 Torr. Nitrogen atoms were incorporated via N_2 radicals formed by arc plasma at low pressure. None doped particles were prepared at higher pressure (600~760 Torr) to prevent the nitrogen incorporation. The silica-coated ZnO nanoparticles were prepared by the hydrolysis of tetraethoxysilane (TEOS) to prevent the photo quenching in the solution.

Figure 1 shows the typical Raman spectrum of ZnO nanoparticles by backscattering with the 488 nm laser. Fundamental optical modes of ZnO wurtzite structure such as A_1-LO, A_1-TO, E_1-LO, E_1-TO and E_2 (high) were observed at 540, 381, 582, 411 and 438 cm^{-1} respectively. Nitrogen related local vibrational modes were also observed from the samples prepared at low chamber pressure. The detail description of the Raman spectra for these samples will be discussed.

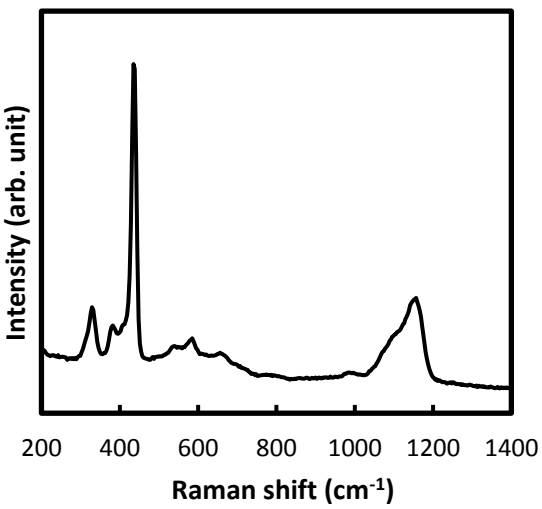

Figure 1 Raman spectrum of ZnO nanoparticles prepared at 610 Torr.

[1] Y. Fujita, K. Moriyama, Y. Hiragino, Y. Furubayashi, H. Hashimoto, T. Yoshida, Phys. Status Solidi C, 10.1002/pssc.201300645 (2014).
[2] K. Senthilkumar • O. Senthilkumar • S. Morito, T. Ohba • Y. Fujita, J. Nanopart. Res.14:1205 (2012).

P 5

Deuterium Labeled Raman Microspectroscopy of Living Yeast Cells

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Raman microspectroscopy provides a powerful means to investigate cellular metabolism in living cells. When coupled with stable isotope labeling, it becomes even more powerful owing to the additional ability to trace intracellular metabolic dynamics. Recently we have demonstrated this novel approach using ^{13}C-labeled glucose and revealed co-localization of the proteome to lipid droplets [1]. In this study, we extend our work to deuterium (^{2}H) labeling. We measured a series of Raman spectra from three distinct regions, namely, lipid droplets, the cytoplasm (protein rich), and the cell wall in living yeast cells at different incubation times in medium containing perdeuterated glucose as the primary carbon source (Figure 1). Upon ^{2}H-isotope substitution, the C-H stretch band shifts from ~2800 to 2140 cm^{-1}. Unlike other Raman bands in the congested fingerprint region (800–1800 cm^{-1}), the C-D stretch band is easily discernible because it appears in the spectroscopically silent region, thereby facilitating the tracing of the relevant metabolic process without ambiguity. In addition to the rise of the C-D stretch band, the ring breathing mode of the phenylalanine residues in proteins at 1003 cm^{-1} is found to downshift to two different wavenumbers, i.e., 961 and 975 cm^{-1}. In order to explain this isotope substitution effect, we compare the experimental results with theoretical calculation results of the phenylalanine molecule whose ring hydrogen(s) are substituted with deuterium. In the presentation, we will also discuss preliminary Raman imaging experiment on single yeast cells.

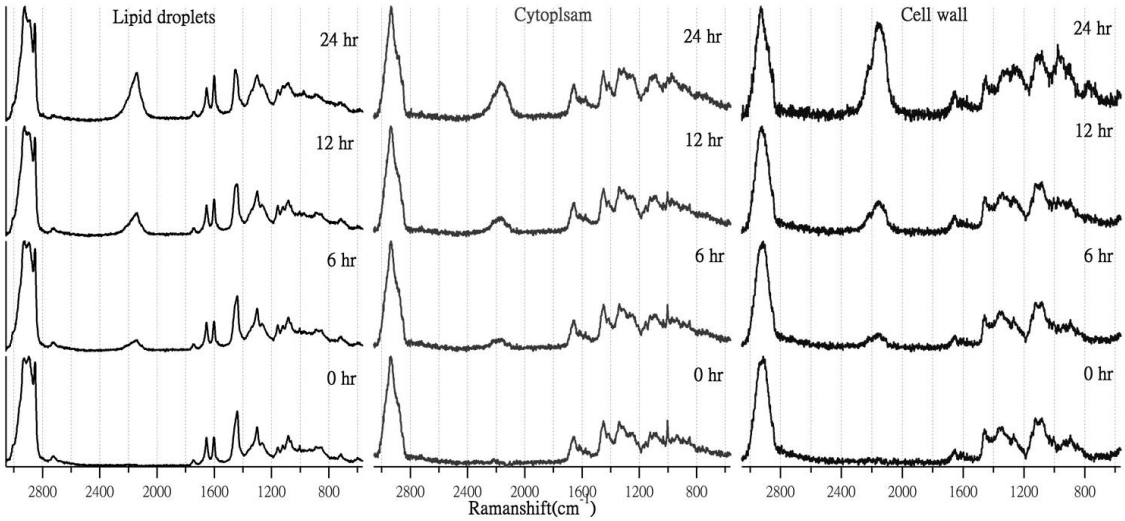

Figure 1 Averaged Raman spectra of lipid droplets, cytoplasm, and cell wall of living fission yeast cells (n = 20).

[1] H. N. Noothalapati Venkata and S. Shigeto, Stable Isotope-Labeled Raman Imaging Reveals Dynamic Proteome Localization to Lipid droplets in Single Fission Yeast Cells, *Chem. Biol.* 2012, 19, 1373-1380.

P 6

The effect of solvent on Decafluorobenzophenone: a Resonance Raman study

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The effect of solvent on the photophysical and photochemical properties of molecules in ground and excited state is an area of active research. Unlike the substituent effect, the solvent effect on the molecule has not been well understood. The solute-solvent interaction has significant influence on the structural and conformational parameters of the solute. Various spectroscopic techniques like UV-Vis, IR, and Raman spectroscopies have been exploited for understanding solvent effect. UV-Vis spectroscopy gives an idea about the change in the energy levels of the molecule, but the structural information is difficult to obtain. Though Raman spectroscopy is a better technique to understand the structural change its use is limited by the inherent weakness of the Raman signals. In this regard Resonance Raman (RR) spectroscopy has been an invaluable investigative tool. The technique is much more sensitive compared to normal Raman and modes specific to chromophore will show enhancement at resonance. So information related to chemically significant parts of the molecule can be obtained.[1]

Aromatic ketones are compounds in which the excited ^{1}n-π^* and $^1\pi$-π^* are very close to each other and the light-initiated hydrogen abstraction is the most studied photochemical reaction which involves triplet n-π^* state.[2,3] In the present work the solvent effect on ground state structure of Decafluorobenzophenone (dfbp) studied through RR spectroscopy. Carbonyl stretching frequency is sensitive to solvent polarity and it can be used as a marker to understand the solvent effect on the ground state structure of the molecule.

[1] R. J. H. Clark and T. J. Dines, Resonance Raman spectroscopy, and its application to inorganic chemistry, Angew. Chem. Int. Ed. Engl., 1986, 25, 131-158.
[2] G. Balakrishnan, S. K. Sahoo, B. K. Chowdhury and Siva Umapathy, Understanding solvent effects on structure and reactivity of organic intermediates: A Raman study, Faraday Discussions, 2010, 145, 443-466.
[3] R. Anandhi and S. Umapathy, Intermolecular hydrogen abstraction from triplet excited state of decafluorobenzophenone: a Raman investigation, J. Raman Spectrosc., 2000, 31, 331-338.

Toward ultrafast time-resolved chiroptical spectroscopies

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Time-resolved chiroptical spectroscopies, such as circular dichroism (CD) and Raman optical activity (ROA), have been expected to be powerful tools for investigating the three-dimensional structural evolution of many classes of chiral molecules during bio/chemical reactions. Although several setups for time-resolved CD (TRCD) spectroscopy have been developed so far [1], they have only limited application due mainly to the low contrast ratio of the chirality-induced signal to achiral background. Recently, in order to circumvent this difficulty, a vibrational[2] and electronic CD[3], and vibrational ROA[4] spectrometer with heterodyne detection have been developed. In these setups, linearly polarized light is used as incident light. By measuring both the phase and amplitude of the transmitted/scattered light which is polarized perpendicularly to the incident polarization, CD/ROA signals are extracted without the achiral background. However, in order to measure TRCD spectra, there remain several problems in the setup reported in ref. 2 and 3. Firstly, the probe light is not focused into the sample to avoid the polarization change by a lens. Secondly, the wavelength range is limited only from 780 nm to 820 nm, which makes it difficult to observe transient absorption signals for a variety of molecules. Herein, we report development of an improved femtosecond CD/ORD spectrometer, which covers the almost entire visible range (400 - 750 nm) and is readily applicable to TRCD measurements.

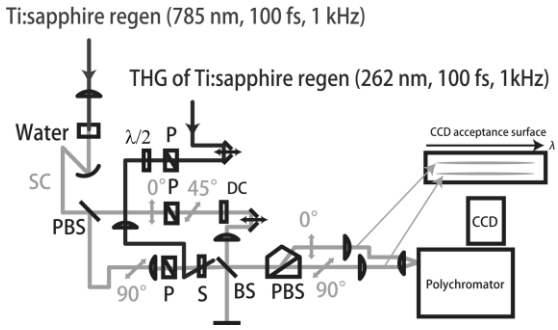

Figure 1. Our setup for femtosecond CD/ORD spectroscopy with the active heterodyne method.

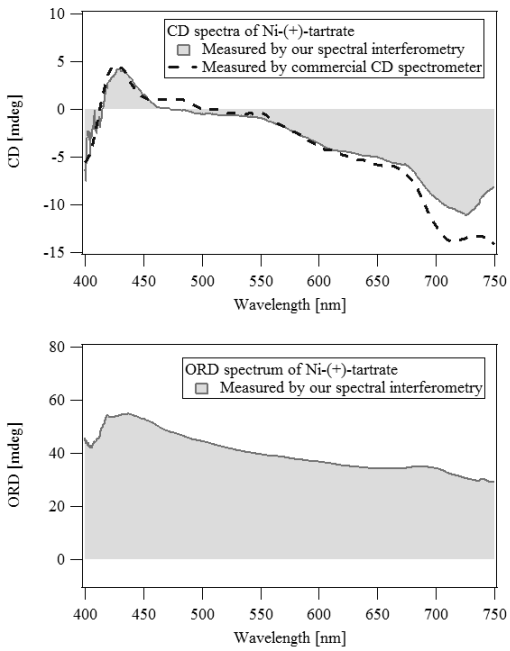

Fig. 2. CD and ORD spectra of Ni-(+)-tartrate measured by our setup (solid lines) and by a commercial CD spectrometer (broken line).

The optical setup of our spectrometer with active heterodyne detection is shown in Fig.1. A linearly polarized broadband probe pulse is introduced into the sample and the transmitted light is combined with the local oscillator (LO). By analyzing observed spectral fringes due to the interference between the transmitted light and the LO, CD and ORD spectra are extracted simultaneously. By placing a polarizer just in front of the sample, the probe light was focused into the sample without sacrifice of the polarization purity. The CD and ORD spectra of Ni-(+)-tartrate thus obtained in our setup are shown in Fig.2. The present CD spectrum is in good agreement with that measured by a conventional CD spectrometer. By combining the technique developed in this study and heterodyne-detected CARS-ROA spectroscopy[4], it is expected that time-resolved ROA measurements, which will provide more information on three-dimensional structural evolution during reactions, will also be possible in the near future.

[1] J. Meyer-Ilse, D. Akimov, and B. Dietzek, Laser Photon. Rev. 7, 495 (2013). [2] H. Rhee, *et al.*, Nature 458, 310 (2009). [3] I. Eom, S. Ahn, H. Rhee, and M. Cho, Phys. Rev. Lett 108, 103901 (2012). [4] K. Hiramatsu, M. Okuno, H. Kano, P. Leproux, V. Couderc, and H. Hamaguchi, Phys. Rev. Lett. 109, 083901 (2012).

Label-free Multivariate Raman Spectral Imaging Study of Cellular Component Distributions in Colon Cancer Cells during Cell Division

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Mitotic cell division is a crucial biological process related to the reproduction of living cells. Dysregulation of this event may lead to tumor formation. Detailed molecular-level investigation of cell division will not only deepen our understanding of the fundamental question of how life continues, but it could also open up new possibilities of diagnosis/prognosis of cancer cells. Many biological techniques have been used to study the distributions of organelles and cellular components during cell division. However, the conventional methods usually require destructive procedures or external labeling and hence are prone to alter normal cell functions or cause sample damage. Raman spectroscopy is a powerful alternative for investigating cellular components and their distributions in vivo and in a label-free manner.

In this work, we used confocal Raman microspectroscopy [1] to study colon cancer cells (HCT-116) and visualize the distributions of intracellular components such as proteins and lipids. We obtained space-resolved Raman spectra of interphase and M phase cells, and constructed Raman images by employing a multivariate data analysis method known as multivariate curve resolution (MCR) [1]. In Figure 1(a), MCR analysis of the Raman hyperspectral data has successfully yielded background-free intrinsic spectra of proteins and lipids and their spatial distributions with much higher image contrast than the conventional univariate approach can provide. As seen in Figure 1(b) and 1(c), the MCR Raman images show that the distribution of proteins looks nearly homogeneous in both types of cells, whereas lipids are localized at the cleavage furrow of dividing cells. The present results indicate that some lipid droplets or lipid-rich organelles are accumulated at the cleavage furrow during cell division, playing an important role in regulating cytokinesis [2,3]. Furthermore, we found two different autofluorescence components (denoted F1 and F2). F1 with an emission maximum at 667 nm appears at the boundary of all cells, whereas F2 (at 704 nm) is localized in the cleavage furrow of M phase cells in a very similar manner to the lipid component. This difference in distribution suggests that the two autofluorescence components arise from different chemical species in the colon cancer cell, which we are currently attempting to identify.

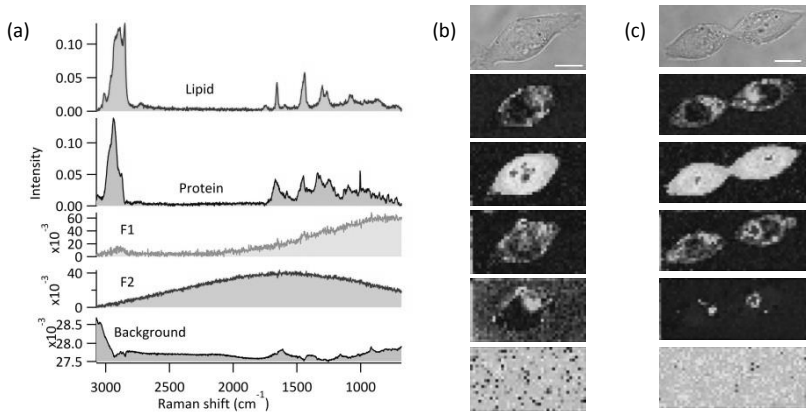

Figure 2 MCR analysis results of HCT-116 cells. MCR can provide detailed chemical information based on the derived intrinsic spectra of intracellular components (a), and construct Raman images of interphase cells (b) and M phase cells(c).

[1] C.-K. Huang, M. Ando, H. Hamaguchi, and S. Shigeto, Disentangling dynamic changes of multiple cellular components during the yeast cell cycle by in vivo multivariate Raman imaging, Anal. Chem., 2012, 84, 5661-5668
[2] K. Emoto, T. Kobayashi, A. Yamaji, H. Aizawa, I. Yahara, K. Inoue, and M. Umeda, Redistribution of phosphatidylethanolamine at the cleavage furrow of dividing cells during cytokinesis, Proc. Natl. Acad. Sci. USA., 1996, 93, 12867-72
[3] J.A. Schiel and R. Prekeris, Membrane dynamics during cytokinesis, Curr. Opin. Cell Biol., 2013, 25, 92-8

P 9

CARS spectral imaging of living algae, *Aurantiochytrium*

~Toward *in-vivo* visualization of hydrocarbon accumulation ~

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Aurantiochytrium, which is one of the algae, has attracted much attention because of its high growth rate and capability of accumulating fats. *Aurantiochytrium* is also known to produce the two main types of lipid molecules, namely squalene and triacylglycerol(TAG), in the cell. In particular, squalene has been studied intensively for practical use as energy resources alternative to fossil fuels. However, the detailed process of the squalene production is still unclear because intracellular distribution of squalene and TAG molecules are not investigated. In the present study, we have tried to identify intracellular squalene and TAG in the living cell. We used a lab-made coherent anti-Stokes Raman scattering (CARS) microscopic system, and have performed molecular vibrational imaging of *Aurantiochytrium* living cells.

Figure1 shows the Im [$\chi^{(3)}$] spectra obtained by measuring the living *Aurantiochytrium* cells. We found that the cell contains at least two types of lipid molecules. By comparing the Im [$\chi^{(3)}$] spectra measured in the cell with those of neat TAG and squalene, we identified the spectra in Figs.1(a) and (b) with those of squalene and TAG, respectively. Figure2(a) shows the CARS image obtained at the CH_2 stretch vibration. The spectra shown in Figs.1(a) and (b) are obtained at the positions of A and B in the Fig. 2(a)

In order to highlight the different distribution of squalene and TAG molecules, we have focused the Raman band of the C=C stretch vibrational mode, which is located at 1662 cm^{-1} and 1652 cm^{-1} for squalene and TAG, respectively. Figure2(b) shows a Raman-shift image of the C=C stretch vibrational mode. The distribution of TAG and qualene molecules are clearly observed.

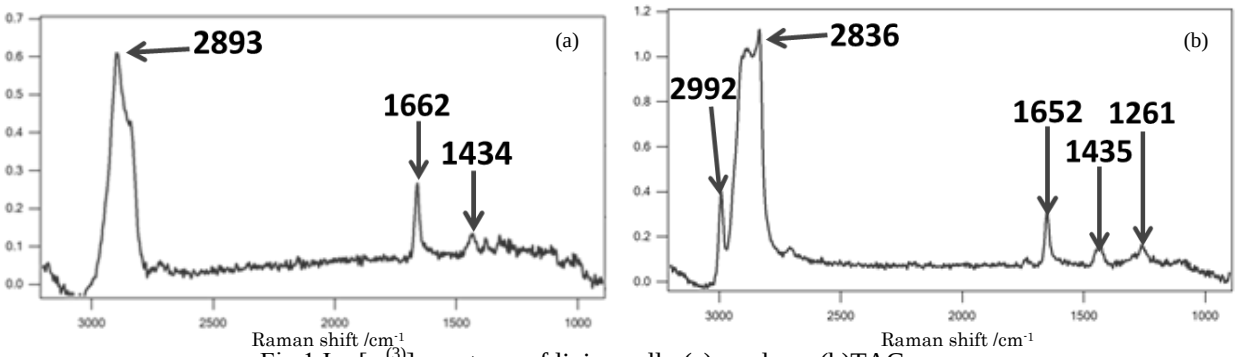

Fig.1 Im [$\chi^{(3)}$] spectrum of living cell : (a)squalene, (b)TAG

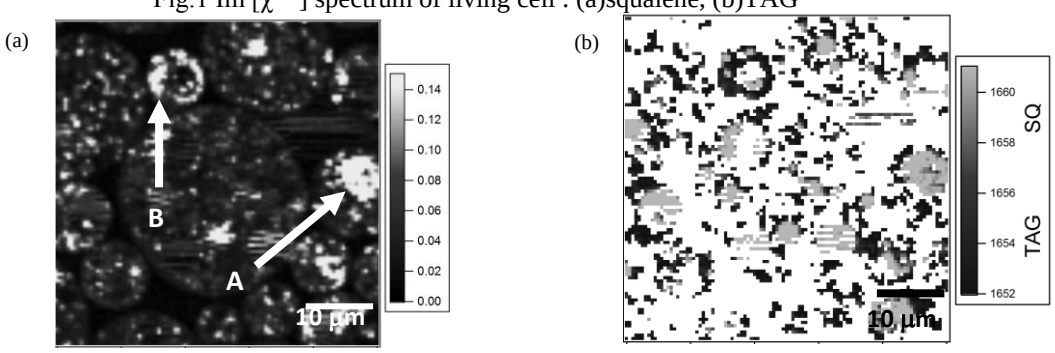

Fig.2 (a) CARS image at CH_2 stretch vibration.
(b) Raman shift image of C=C stretch vibrational mode.

P 10

Low Frequency Raman Spectroscopic study of Intermolecular Interactions

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Low frequency Raman Spectroscopy allows one to measure the chemical composition and molecular structure of many materials in the frequency range of 10-100 cm^{-1}. Effective blocking of the intense Rayleigh scattering light is essential for the measurement of low frequency Raman bands. The low frequency region of Raman spectra provides valuable information on intermolecular interactions in condensed phase materials. These intermolecular interactions comprise short as well as long range forces namely dipole-dipole, ion–dipole, dipole-induced dipole, London dispersion forces *etc*. Commercially available Raman Spectrometers equipped with holographic notch filters are not able to record signals in this low frequency region because of considerable elimination bandwidth (50 cm^{-1}). Recent developments in volume holographic grating filter technology have enabled low frequency Raman spectroscopy. Now one can explore uncharted territory that furthers the life sciences, pharmaceutical, homeland security and other fields.

We report the Low frequency Raman spectra at 785 nm using commercially available reflective volume Bragg grating[1] combined with a double monochromator and a charge coupled device (CCD) camera. These special notch filters have several advantages over thin film notch filters and multi-stage spectrometers namely significantly lower bandwidths (<10 cm^{-1}), very high transmission efficiency, high optical density and much lower cost. Many important materials exhibit strong identifiable low frequency Raman spectra as characteristics of their vibrational modes namely radial breathing modes of single- and multiwall carbon nanotubes, folded acoustic modes of multilayer superlattice structures, vibrational mode of heavy atom halides *etc*. We have used sulphur as a standard which gives discrete low frequency Raman peaks below 100 cm^{-1} which are measurable using this setup at a fraction of cost of traditional bench top instruments.

[1] C. Moser, F. Havermeyer, Ultra-narrow- band tunable laser line notch filter, 2009, 95,597-601.

P 11

Study of Nanodiamond Effect on Blood Oxygen Transfer Function

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Nanodiamond (ND) recently is tested for many biomedical applications due to its good compatibility with bio targets and non-toxicity for biological cells. Therefore, for further applications in animal models, the interaction of NDs with erythrocytes and hemoglobin (Hb, erythrocyte protein) is important to study. In this work, first we compare nanodiamonds aggregation in buffer solution (Phosphate buffered saline, PBS) or blood plasma. Then rat serum albumin (RSA) has been adsorbed on nanodiamonds surface to prevent the aggregation. ND transport by blood circulation has been studied, to understand how nanodiamonds interact with red blood cells (RBC). Raman spectroscopy was used to measure the oxygenation RBC. Raman signal of RBC depends on oxygenation degree of hemoglobin (Hb), so the spectra measurements during oxygenation or deoxygenation allow analyzing the RBC' oxygen-carrying function. To analyze direct effect of ND on Hb, the Hb was extracted from RBC and the oxygenation and deoxygenation of Hb adsorbed on ND was studied. At the low ND concentration, no any effect on the erythrocyte oxygenation and deoxygenation is observed. ND in higher concentrations increases the time of RBC oxygenation and delays deoxygenation. The same tendency is observed for Hb adsorbed on ND. Due to the complicate blood physiological conditions necessary for functioning, this subject needs more research.

P 12

Rapid SERS Biosensor for Early Diagnosis of Influenza Virus

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Influenza is an infectious disease with substantial morbidity and mortality in animal and human populations. As an RNA virus, the influenza virus is characterized by high mutational rate, necessitating frequent alterations in vaccine design for effective prevention of influenza. In addition, its segmented genome allows the reassortment to occur in the host during coinfection, resulting in antigenic shift with a potential for flu pandemics. Therefore, a rapid and accurate identification of the virus subtype particularly in the early stage of the outbreak is epidemiologically crucial in fighting against the disease. Thus far there are a limited number of direct methods to accurately and quantitatively detect the existence of influenza virus at the early phase of infection, rendering difficult the search for the source and propagation pathway of an active and acute flu epidemic and aggravating public anxiety and/or panic, as exemplified by the recent H7N9 outbreak[1]. As a result, development of specific and sensitive methods for the detection of influenza viruses has been the focus of many clinical investigations.

Surface enhanced Raman scattering (SERS) spectroscopy has been found as a promising tool for significant improvement in the detection limits of bioassays from the use of detection probes[2]. These probes are constructed from plasmonic nanostructures, such as solid gold and silver nanoparticles, taking advantage of the large enhancement in the Raman scattering signal of dyes in close proximity to such surfaces. Herein, we have developed a novel, rapid, highly sensitive biosensor protocol for diagnosis and characterization of influenza viruses in the early stage of the epidemic (as shown in Figure 1a). Using Surface-Enhanced Raman Scattering (SERS) technique and nitrocellulose membrane[3], detection limit as low as 30 ng/mL was achieved with high selectivity to different subtypes of influenza virus tested (Figure 1b). Moreover, the SERS measurement can be completed in less than 2 hours, thus making the protocol useful in early diagnosis and impediment of propagation of potential influenza pandemic.

(a) (b)

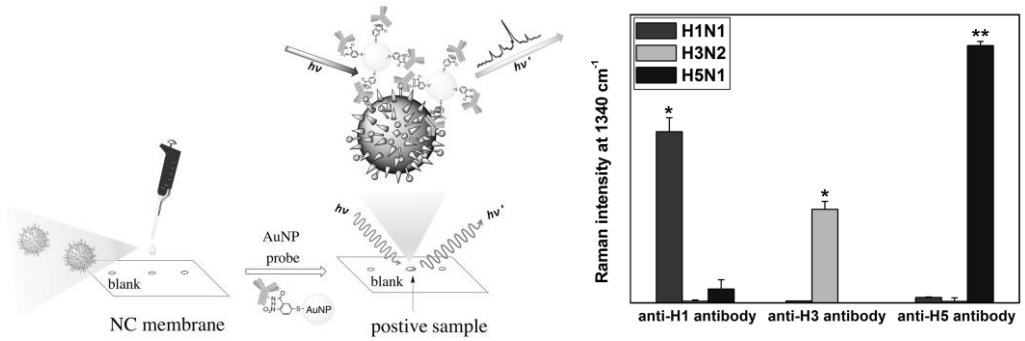

Figure 3 (a) Schematic illustration of influenza virus detection by AuNP probes and SERS. (b) Different types of influenza viruses (H1N1, H3N2, and H5N1) and PBS (blank) were taken for the selectivity tests by using a series of antibodies conjugated with DSNB and AuNPs. The $-NO_2$ symmetric stretching vibration of DSNB has a signature peak at ~1340 cm^{-1} that allows for distinct and quantitative determination of the specificity of bound influenza virus. Bar graphs show the intensities of the SERS peaks at 1340 cm^{-1} for different subtypes of influenza and confirm our bioassay with high selectivity for each subtype.

[1].Zhuang, Q.Y., S.C. Wang, M.L. Wu, et al., Epidemiological and risk analysis of the H7N9 subtype influenza outbreak in China at its early stage. Chinese Sci Bull, 2013. **58**(26): pp. 3183-3187
[2].Driskell, J.D., K.M. Kwarta, R.J. Lipert, et al., Low-level detection of viral pathogens by a surface-enhanced Raman scattering based immunoassay. Anal Chem, 2005. **77**(19): pp. 6147-54
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P 13

In situ detection of secondary metabolites in antibiotic-producing bacteria

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The secondary metabolites which usually have the useful functions including antibiotics have been discovered from the microorganisms. The conventional methods to identify or quantify the antibiotics depend on the analysis of whole microbes by time-consuming and laborious solvent extraction-based methods such as GC-MS. Therefore, because of the demand for the cost-effective production and new antibiotics producers, a non-destructive technique for compositional analysis is required. Here, we report the first application of Raman microspectroscopy for *in situ* detection of microbially derived antibiotics in living miroorganisms [1].

Raman measurements were conducted using a laboratory-built confocal Raman microspectrometer equipped with a 532-nm laser, an inverted microscope and a CCD detector. The lateral and depth resolutions of Raman imagine system were 0.3 and 2.6 µm respectively.

In this study, *Streptomyces nodosus* known as the producers of an antifungal antibiotic Amphotericin B (AmB) were used as a model for antibiotic-producing Actinomycetes. AmB is an elongated cyclic molecule consisting of hydrophobic polyene and hydrophilic polyhydroxyl domains. The Raman spectrum of standard AmB showed the specific two bands at 1559 cm^{-1} corresponding to C=C stretch and 1157 cm^{-1} corresponding to C-C stretch in the conjugated chain of AmB. The mycelia of *S. nodosus* cultivated in AmB production medium showed the Raman bands at 1556 cm^{-1} and 1154 cm^{-1} in addition to the bands from the biomolecules such as cytochromes and proteins. We considered these two bands obtained from the mycelia under AmB-inducing condition could be assigned to AmB. In comparison with the spectra of standard AmB, the spectra of the mycelia showed the Raman peak shifts to lower wavenumber by 3 cm^{-1}. This result was speculated to suggest that AmB was accumulated in the molecular aggregate states in living cells. To investigate the time-course change of AmB production, the center and the edge area of mycelia were measured on day 2 to day 5. As a result, the band intensity of AmB increased due to time course. These changes were more significant in the center area than in the edge area, which suggested AmB was more accumulated in the center area. Moreover, the Raman images of mycelia and single hypha revealed the distribution of cell components. While proteins and cytochromes were distributed within the entire cells, AmB was distributed heterogeneously with no correlation between the localization of AmB and other biomolecules. These results showed that the AmB production is enhanced locally in the mycelia and the single hyphae.

In summary, Raman microspectroscopy enabled us to detect AmB from a small amount of *S. nodosus* non-destructively and to reveal the locally distribution of AmB in living cells. These results suggest that this technique has the potential to be applied to the real-time monitoring of the microorganisms producing antibiotics or to the screening of novel antibiotics candidates.

[1] R. Miyaoka, M. Hosokawa, M. Ando, T. Mori, H. Hamaguchi and H. Takeyama. *In situ* detection of antibiotics Amphotericin B produced in *Streptomyces nodosus* using Raman microspectroscopy. Marine drugs. 2014. 12(5). 2827-2839.

Raman imaging and lipid analysis of marine diatom *Fistulifera solaris* JPCC DA0580

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[2]*ASMeW, Waseda University*
[3]*National Chiao Tung University*

Microalgae are considered as a promissing source for biofuels production. In our laboratory, marine diatom *Fistulifera solaris* JPCC DA0580 was selected from a marine microalgal culture collection as a high lipid-producing. This strain shows high growth rate and can accumulate triglyceride (TG) up to 60% of the dried mass under nutrient limitation. The imaging of proteins, carotenoids and lipids in *F. solaris* JPCC DA0580 living cells using Raman spectroscopy and the microcavity array-based single-cell patterning technique was presented last year. In addition, the $I_{1,650}/I_{1,440}$ ratio, which is a marker of degree of lipid unsaturation, was demonstrated to decrease in the oleosome during lipid accumulation phase. The finding is beneficial for the biodiesel quality. Therefore, in this study, the further measurement of detailed in vivo fatty acids compositions in the oleosome of *F. solaris* JPCC DA0580 was achieved by fitting analysis of Raman spectrum.

The compositional ratio of fatty acids in algal lipid was calculated from the coefficients of the linear combination of the least-square fitting according to the spectra of pure fatty acids. The fitted spectrum was processed by using the least-square method in the region containing the Raman markers associated with lipid saturation or unsaturation (1048–1112 cm^{-1}, 1215–1508 cm^{-1}, and 1640–1722 cm^{-1}). First, we evaluated the accuracy of the fitting analysis in model fatty acid mixture samples. The fatty acid compositions in the living cell and extracted lipids that were determined by Raman fitting was further compared with that determined by regular GC-MS analysis (Table 1) . The result showed that the fatty acid composition obtained by Raman fitting was in accordance with the regular GC-MS analysis.

This method can provide a quantitative and real-time information of compositions, chain lengths, and degree of unsaturation of lipids in living cells for improving the cultivating parameters or for determining the harvest timing during large-scale cultivations for microalgal biodiesel production. Therefore, this technique is a potential tool for in vivo lipidomics for understanding the dynamics of lipid metabolisms in various organisms.

Table 1 Fatty acid compositions analyzed by GC-MS and Raman fitting analysis.
After 72 h of cultivation under nutrient-depleted condition, *F. solaris* JPCC DA0580 cells were analyzed by Raman microspectroscopy. Extracted lipids were obtained from the same culture and analyzed accordingly.

		Fatty acid composition (%)		
Condition	Method	C16:0	C16:1	C20:5n3
Extracted lipid	GC-MS	45.6 (±0.3)	49.6 (±0.3)	4.8 (±0.1)
Extracted lipid	Raman fitting	46.0 (±2.3)	49.4 (±2.8)	4.6 (±0.6)
In vivo (oleosome)	Raman fitting	41.2 (±0.7)	55.5 (±0.7)	3.3 (±0.4)

[1] Huawen Wu, Joanne V. Volponi, Ann E. Oliver. Et al.,PNAS,2011,108, 3809–3814

Surface Enhanced Raman Scattering of Composite Au/Ag Nanoparticle Films Changed with the Ratio of AuNP to AgNP

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Surface Enhanced Raman Scattering (SERS) has high potential in the development of biological or chemical sensing. Assembled nanostructures consisting of noble metal nanoparticles are often applied to SERS spectroscopies because of strong surface plasmon resonance (SPR). Our previous study [1], had shown that a novel centrifuge assembly method could be used to fabricate gold nanoparticle (AuNP) films with good coverage, morphology, and well-control thickness. We also found the assembled AuNP films have strong interparticle coupling effect on their SPR, which red shift with the decreases of interparticle spacing. In this report, we fabricated composite AuNP and AgNP films with different volume ratios of AuNP to AgNP. We found the intensity of Raman spectrum of R6G (Rhodamine 6G) on composite Au/Ag film could even be stronger than pure AgNP films when AuNP/Ag/NP ratio is between 3% and 20%. The intensity was increased with the ratio in the range of 3% to 10%, and some of all composited films are larger than pure AuNPs. These results indicate that disperse composite film AuNP in surrounding AgNPs could contribute a strong SERS.

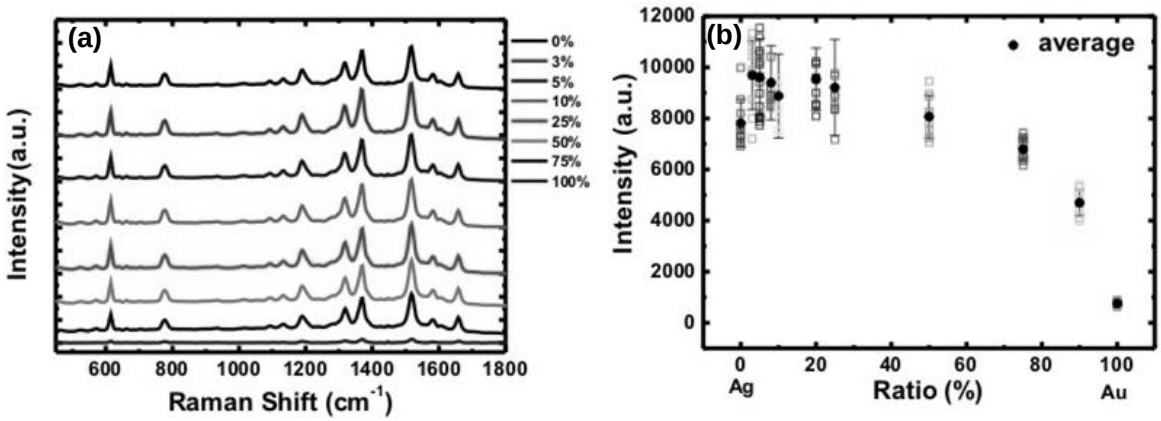

Figure 1. (a) Raman spectrum of R6G molecule with different ratio AuNP/AgNP composite films. (b) The average and deviation of 1517cm^{-1} Raman peak intensity; the light source use 5μw 633 nm laser.

[1] I-C. Ni, S.-C. Yang, C.-W. Jiang, C.-S. Luo, W. Kuo, K.-J. Lin, S.-D. Tzeng: J. Phys. Chem. C **116** (2012) 8095.

P 16

Investigating Lipid Metabolism *in vivo* By Mixed Stable Isotope Probing - Coupled Raman Micro-spectroscopy

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Lipids, which help to store energy in a compact form, have variety of roles in biological systems and their metabolism is central to life. Hence it is of both interest and fundamental importance to understand lipid metabolic pathways *in vivo*. Traditionally, cellular biochemistry is studied by chromatographic and mass spectrometric based methods which are not applicable to *in vivo* studies. Here we show that combination of stable isotope probing (SIP), Raman microspectroscopy and multivariate curve resolution can serve as a valuable approach in metabolomics research. Raman microscopy when combined with stable heavy isotopes (show identical chemical behaviour with naturally occurring counterparts), which have been employed to study cellular metabolism, confers *in vivo* capability. To demonstrate, we studied ergosterol biosynthesis in single living fission yeast cells grown in mixtures of normal (^{12}C) and ^{13}C-glucose as the sole carbon source. A marker band for ergosterol, which corresponds to in-phase C=C stretching of the 5,7-diene moiety (Fig 1), appears at 1602 cm^{-1} in ^{12}C medium but shifts to 1542 cm^{-1} in ^{13}C medium. A gradual shift via partially substituted intermediates (at 1586 and 1555 cm^{-1}) was observed by changing $^{12}C/^{13}C$ ratio in the medium (Fig 2). By carefully looking into the biosynthetic pathways and by comparing the observed peak positions with calculation results on isotope-substituted ergosterol, it is possible to understand how ^{13}C is incorporated in the conjugated C=C moiety of the molecule. The multivariate spectral data analysis revealed intrinsic spectra and their relative abundances of all isotopomers. Our findings show that SIP-Raman provides new, complementary and otherwise unobtainable pieces of molecular information at the single cell level.

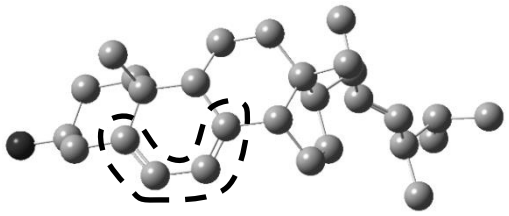

Fig 1: Optimized structure of ergosterol by DFT calculations. The carbon atoms highlighted with dashed line form the 5, 7-diene structure whose vibration is seen at 1602 cm^{-1} experimentally.

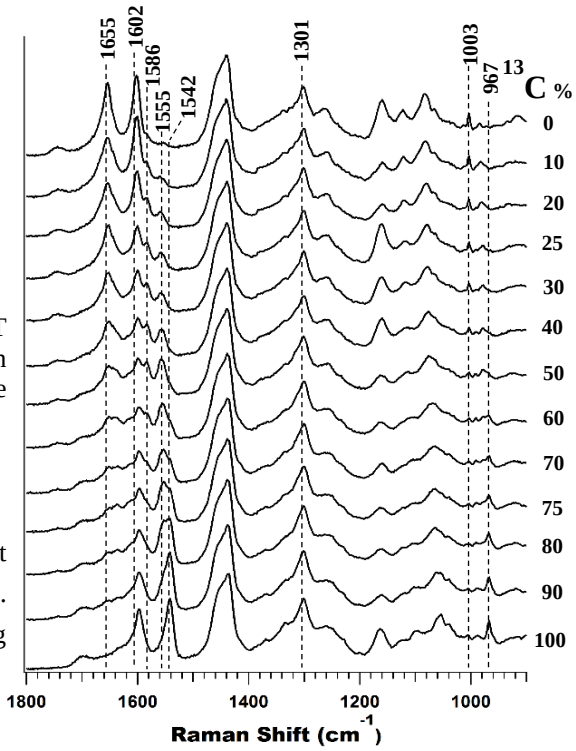

Fig 2: Lipid rich Raman spectra of fission yeast in different carbon isotope (^{12}C & ^{13}C) mixtures. ^{13}C composition is given in percentage indicating the rest to be ^{12}C.

P 17

Raman Spectroscopic Study of Drug- membrane Interaction

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The study of drug-membrane interaction is important to improve the efficiency of the drug, hence their biological performance. The unilamellar lipid vesicles (liposomes) have become an extremely useful model system for cell membrane. These have been used to study the drug-membrane interactions and membrane permeabilization. These vesicles have also been used as potential drug delivery vehicles. The lipid, 1,2- dipamitoyl-sn-glycero-3-phosphocholine (DPPC) has been used for the preparation of vesicles and chlorpromazine as a drug to study the drug-membrane interaction. We have used the electroformation method and also the hydration method for the preparation of vesicles. The Confocal Raman Micro-spectroscopy was used to study the drug membrane interaction. The shift and change in the peaks of the spectra of the lipid vesicles due to the interaction with the drug provides information about the membrane disorderness.

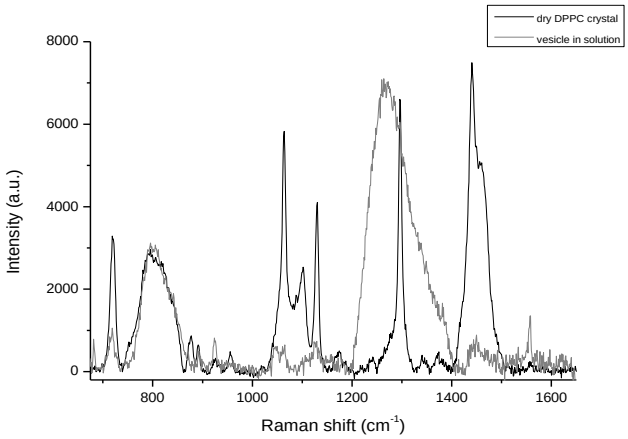

Figure 1. Raman spectra of DPPC vesicle in solution and DPPC crystal.

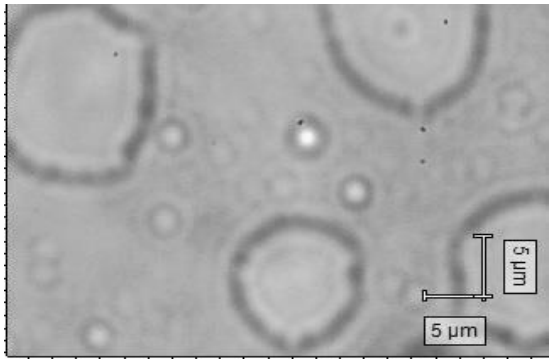

Figure 2. Image of the vesicles formed by hydration method.

References

1. Peter Walde, Katia Consentino, Helen Engel, and Pasquale Stano, Gaint Vesicles: Prepration and Applications, Chembiochem, 2010, 11, 848-865.
2. Christoph Herold, Grzegorz Chwastek, Petra Schwille, and Eugene P. Petrov, Efficient Electroformation of Supergiant Unilamellar Vesicles Containing Cationic Lipids on ITO-Coated Electrodes, Langmuir, 2012, 28, 5518-5521.

Resonance Raman Spectroscopy of β-Carotene in Cyclodextrin Inclusion complex

S-T, Wu[1], A. Chiou[1,2], Tatsuyuki Yamamoto[3] and H. Hamaguchi[4]

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[2]*Biophotonics& Molecular Imaging Research Center, National Yang Ming University, Taiwan*
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Carotenoids are common in many colorful fruits or vegetables and play important roles in the human body. Because carotenoids are antioxidants, they can reduce the risk of Cardiovascular disease (CVD), Age-Related Macular Degeneration (AMD) and so on. Here we report resonance Raman spectra of a typical carotenoid, β-carotene, in cyclodextrin inclusion complex in aqueous suspension, as a model of a carotenoid in biological environments. In principle, the peak position of a Raman band is not expected to shift by changing the excitation wavelength. However, we have found that the peak position of the *v*1band of β-carotene in cyclodextrin inclusion complex changes with excitation wavelength.

As shown in Figure 1, the peak position of the *v*1band changes significantly from 1521.5 cm^{-1}at 488.0 nm to1516.1cm^{-1} at 514.5 nm, and further to1514.7cm^{-1}at 531.9nm.The sample of β -carotene inclusion complex is heterogeneous in suspension, and particles move randomly in water (Figure 1, inset image). We use Raman mapping to check the peak positions with different particles in suspension. It is found that different particles give different peak positions. The peak shifts of the*v*1bandthus found suggest that the β-carotene molecule can take a number of different structures in inclusion complexes. This finding provides an important basis for understanding structural variety of carotenoid existing in biological environments as in the human body.

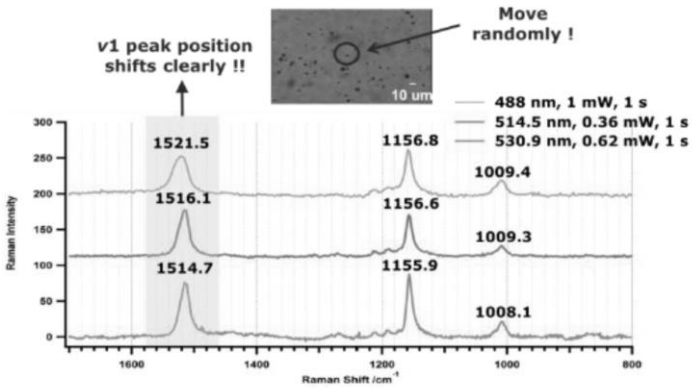

Figure 1 Resonance Raman Spectra of β-carotene in cyclodextrin inclusion complexes.

P 19

CARS spectral imaging of iPS cells

~Toward visualization of pluripotency~

Hiroaki Yoneyama[1], Hiroki SEGAWA[2], Nishimura Ken[3], Fukuda Aya[3], Hisatake Koji[3], Hideaki Kano[1]

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Induced pluripotent stem cells (iPS cells) have potential to differentiate wide variety of tissues and organs. Recently, many studies have been carried out in order to apply iPS cells to tissue engineering and regenerative medicine. However, it is difficult to find pluripotency of iPS cells without staining or molecular tagging. In the present study, we have performed molecular vibrational imaging of iPS cells using nonlinear Raman spectroscopy in order to find the spectroscopic signature of pluripotency.

We used homebuilt multiplex coherent anti-Stokes Raman scattering (CARS) microspectroscopy system. We measured two kinds of cell colonies; the one is ordinary iPS cells, and the other is non-pluripotent cells, which is prepared by reducing the amount of Klf4. Klf4 is one of the transcription factors, which is in general introduced into somatic cells with Oct3/4, Sox2, and c-Myc in order to induce reprograming. Genetically, these two cells can be distinguished by expression of Nanog, which is one of the markers of pluripotency. In the present study, Nanog is labeled with Gleen Fluorescent Protein (GFP), in order to confirm pluripotency.

The results are shown in Figure 1 and 2. Figure 1 shows $\mathrm{Im}[\chi^{(3)}]$ spectrum of iPS cells, in particular at the position of nucleolus. The spectrum is $1.5\mu m^2$-averaged around arrowed region in Fig.2. We find several Raman bands mainly due to protein and nucleic acid. Figure2 shows multiplex CARS images reconstructed from each Raman bands. A single iPS colony is clearly visualized with unique vibrational contrast.

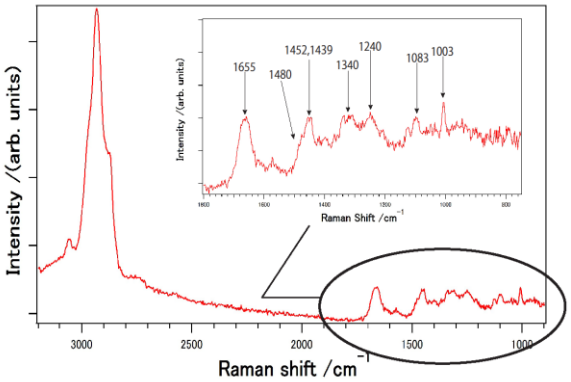

Figure 1. $\mathrm{Im}[\chi^{(3)}]$ spectra of iPS cells indicated by the arrow in Fig. 2

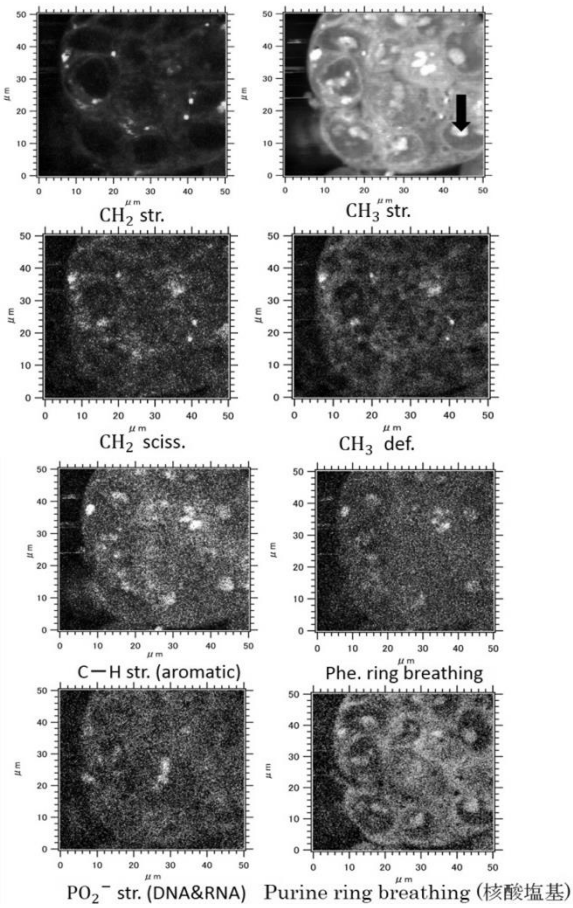

Figure 2. CARS images of iPS cells

Taiwan Association of Ramam Spectroscopy Summer Camp 2014, Hualian

This summer camp is suitable for the new coming graduate students and undergraduate students. The purposes of summer camp led to create a warming interaction environment amongst teachers and students. Symposium attended speakers will provide lectures for students concerning basic, development and applications of Raman spectroscopy. That might be helpful for students to find out the innovative idea, inspiration and interest on spectroscopy and related fields.

Speakers

Hiro-o Hamaguchi
National Chiao Tung University, Taiwan

Chia Liang Cheng
National Dong Hua University, Taiwan

Igor K. Lednev
State University of New York, Albany, USA

Anthony W. Parker
STFC Rutherford Appleton Lab, UK

Siva Umapathy
Indian Institute of Science, India

Information

Date: June 24th-25th, 2014
Location: Stuart Villa, Hualian
Fee: NTD 500/ person

Contact

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We also have Symposium of Taiwan Association of Ramam Spectroscopy in NDHU at 6/22-6/24, welcome to join us!

TARS 2014, Hualien TISRS 2014, Hualien IR Laboratory NDHU, Hualian, Taiwan 國立東華大學



Taiwan association of Raman Spectroscopy Summer Camp

Date: June 24-25, 2014
Venue: Stuart Villa, Yanliao, Hualien, Taiwan
Agenda:

June 24 , Tuesday

13:30–13:45		Assemble for leaving NDHU
13:45–14:15		Travel from NDHU to Stuart Villa
14:15–14:45		Take off luggage
14:45–15:00		Opening remark
		Session-I (15:00–17:45)
		Chair: Pei-Hua Chung
15:00–15:45	L1	**Hiro-o Hamaguchi**, National Chiao Tung University, Taiwan *Raman Spectroscopy: From the very beginning to the state-of-the-art*
15:45–16:15		Tea break
		Chair: Chang-You Song
16:15–17:00	L2	**Chia-Liang Cheng**, National Dong Hwa University, Taiwan *Raman Spectroscopy in Nanobiotechnology*
		Chair: Jem-Wei Yu
17:00–17:45	L3	**Igor K. Lednev**, University at Albany, USA *Variety of Raman Spectroscopy for Probing Amyloid Fibrils: Deep UV, Polarized and Tip-Enhanced Raman Experiments*
18:00–21:00		Buffet Dinner

June 25, Wednesday

08:00–09:00		Breakfast & Key return
		Session-II (09:00–10:40)
		Chair: Ankit Raj
09:00–09:45	L4	**Siva Umapathy**, *Indian Institute of Science, India* *Time resolved stimulated Raman Spectroscopy*
09:45–09:55		Break
		Chair: Liang-Cheng Kuo
09:55–10:40	L5	**Anthony W. Parker**, STFC Rutherford Appleton Laboratory, UK *Raman Spectroscopy of Bone and the Invention of Spatially Offset Raman Spectroscopy*
10:40–11:00		Closing remark
11:00–11:30		Assemble & Get in Bus (Collect lunch box on the bus)
11:30–13:00		Back to Hualien Train Station/ NDHU

L1
Raman Spectroscopy: From the very beginning to the state-of-the-art

Hiro-o Hamaguchi

Department of Applied Chemistry and Institute of Molecular Science
National Chiao Tung University, Taiwan

The history of Raman spectroscopy began with the discovery of the Raman effect by C. V. Raman and K. S. Krishnan reported in a paper entitled "A new type of Secondary Radiation" (C. V. Raman and K. S. Krishnan, *Nature* **121**, 501 (1928)). They focused the sun light into liquid samples in a glass cuvette using two lenses with 230 cm and 5 cm focal lengths. By looking at the sample liquid from the direction perpendicular to the sun light, they saw a light path (Figure 1 (a)). If two filters, green-yellow and blue-violet filters, were inserted in the incident light pathway (Figure 1 (b)), the light path disappeared, because the incident sun light was totally absorbed by the two filters. However, if the blue-violet filter was moved to the scattered light pathway (Figure 1 (c)), they again saw the light path. This result indicates that the shorter-wavelength light passing through the green-yellow filter is converted to some longer-wavelength scattered light to transmit the blue-violet filter. This "new type of secondary radiation" was identified as what we now call Raman scattering.

In the experiment by Raman and Krishnan, the light source was the sun, the spectrum analyzer was a set of filters and the detector was the naked eyes. Now, we use a laser, a spectrometer and a CCD detector, instead. The efficiency of Raman measurements increased tremendously: the measurement time decreased from hours to milliseconds, by one-millionth. The state-of-the-art technology produces a hand-held Raman spectrometer that is suitable for field use, not just the laboratory use. No one actually knows the real potential of Raman spectroscopy. So it is a very good idea to learn more about it.

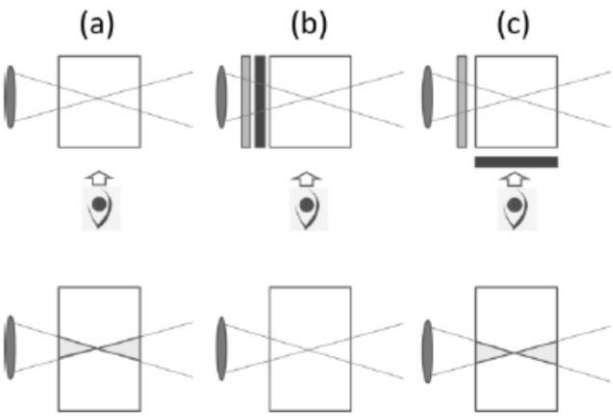

Figure 1. Schematic diagram of the experiments by Raman and Krishna.

L2
Raman Spectroscopy in Nanobiotechnology

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The use of Raman spectroscopy has become increasingly important in many research fields ever since the Raman scattering process was demonstrated by CV Raman more than eighty six years ago. Although Raman spectroscopy found its position initially in molecular spectroscopic studies, with the advancement on instrumentation both in spectral and spatial resolution as well as detection sensibility, it is now used in many cross-disciplinary researches which provided new insights in how we look at things, and endless possibilities.

Raman Spectroscopy provides a, in many cases, noninvasive method to investigate biological samples. Spectral identification and Raman mapping allows observing the interaction of bio molecules. Combining with nanotechnology-based instrumentation, Raman spectroscopy now plays important roles in a new field termed nanobiotechnology.

In this lecture, we will give examples on how Raman spectroscopy can be applied, along with other technology, in this interdisciplinary field of research. In our group, we have devoted our efforts on using nanodiamond (ND) to build a smart nano-bio-probe for bio/medical applications. The goal is to utilize ND's superb surface properties as a platform for drug loading for efficient anticancer drug delivery while ND's Raman and fluorescence properties are used for bio imaging. Several successful cases have been demonstrated in both cellular and animal models.

L3

Variety of Raman Spectroscopy for Probing Amyloid Fibrils: Deep UV, Polarized and Tip-Enhanced Raman Experiments

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In spite of the key medical importance of amyloid fibrils, the molecular mechanism of fibrillation is not fully understood. At least in part this is because amyloid fibrils are non-crystalline and insoluble, and thus are not amenable to conventional X-ray crystallography and solution NMR, the classical tools of structural biology. We have developed and applied novel experimental approaches based on Raman spectroscopy for characterizing structure and dynamics of amyloid fibril during the last decade. These include deep ultraviolet Raman spectroscopy, polarized Raman spectroscopy of aligned fibrils and tip-enhanced Raman spectroscopy (TERS). In addition to hardware, we developed advanced statistical methods for analyzing spectroscopic data including two dimensional correlation spectroscopy (2DCoS). The application of these complimentary methods allowed for obtaining comprehensive knowledge about the mechanism of fibrillation as well as structure of fibril core and fibril surface.

L4
TIME RESOLVED STIMULATED RAMAN SPCTROSCOPY

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Understanding a chemical reaction requires detail knowledge of the molecular vibrational motions on a multidimensional potential energy surface. Typical time scale range for a molecular vibration is from 10 fs to 1 ps. In order to observe a chemical reaction in real time, high time (as fast as a vibration) and spectral resolution (vibrational line width) are a necessity. In addition, one needs to monitor the dynamics of many vibrational modes simultaneously to get a better understanding of the reaction mechanism. Ultrafast time resolved stimulated Raman spectroscopy has both time (~ 100 fs) and spectral resolution (~20 cm^{-1}) to observe dynamics of many vibrational modes in real time. This technique can also provide very useful information on the rate of energy flow which can be intra (IVR) and intermolecular (vibrational cooling) and details on the coupling of solute vibrations to the surrounding solvents. Based on this principle, femtosecond stimulated Raman spectroscopy (FSRS) technique has been applied to probe the dynamics of cis-trans isomerization, charge transfer dynamics, excited state proton transfer dynamics *etc*. [1], [2]. Ultrafast Raman loss spectroscopy (URLS) is a similar nonlinear Raman technique [3], [4]. A typical stimulated Raman experiment employs a narrowband Raman pump laser beam (pulse width of ~1 ps) and a broadband probe laser beam (pulse width ~ 100 fs), and a photo excitation pump beam (~ 100 fs). In such a case the temporal resolution is fully determined by the actinic pump-probe cross-correlation, while the spectral resolution is only set by the Raman pump pulse spectral width. In FSRS, the probe is a continuum Stokes field with respect to the narrowband Raman pump pulse, whereas in URLS the probe is chosen to cover the entire Stokes and anti-Stokes regions with respect to the Raman pump wavelength and the scattered light is only observed on the anti-Stokes side. We have studied the photo-isomerization of trans-Stilbene in different solvents after 280 nm (~ 100 fs) phot-excitaion (figure 1). The main marker band is C_{et}=C_{et} stretch (1560 cm^{-1}). Analysis of URLS spectra of this Franck-Condon active mode within 2 ps after photo-excitation shows oscillatory behavior (figure 1(b)). The origin of this oscillation is due to the coupling of Franck-Condon active mode with the low frequency modes.

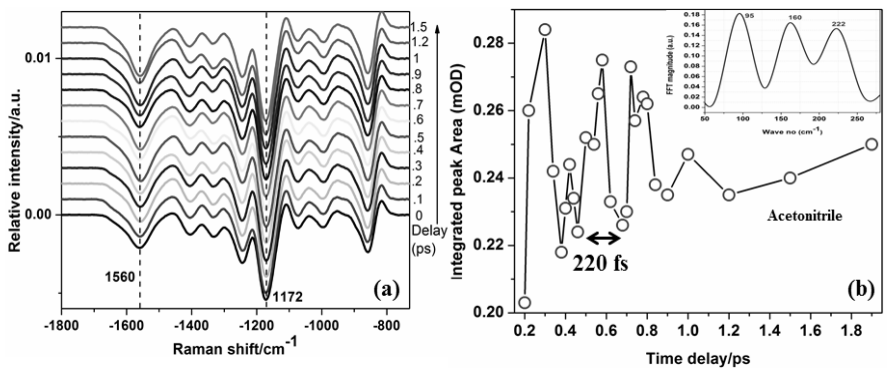

Fig. 1 Few URLS spectra of trans-Stilbene in the S_1 state are shown (a). Integrated intensity of 1560 is shown with respect to time delay. Inset is FFT of the observed oscillation. Main band observed in FFT plot are 90, 160, 222 cm^{-1}

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L5
Raman Spectroscopy of Bone and the Invention of Spatially Offset Raman Spectroscopy

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Bone is a complex material engineered by nature to fulfil numerous functions including supporting and protecting (particularly during movement) the various organs of the body and the place where red and white blood cells are produced and minerals are stored. Bone is a type of dense connective tissue comprising mainly of a matrix inorganic mineral salts and collagen (organic components of proteins).

Raman spectroscopy is able to measure a chemical "finger- print," that provides details of molecular functional groups associated with the mineralized components of bone tissue including phosphate, carbonate as well as the collagen components from bands of the proteins such as amide I and III (indicators of secondary protein conformation), proline, hydroxy- proline, and lipids Raman spectroscopy therefore has several advantages over X-ray methods that are used conventionally which only measures the mineral component in bone and is an ionising form of radiation. These advantages make the development of Raman spectroscopy to diagnose the state of health of bone tissue particularly attractive. However, to do so and produce a clinical useable device it was essential to overcome limitations of Raman that is traditionally used as surface technique for solids. The idea was to move away from the usual detection methods employed in collecting Raman photons directly from the point of illumination and collect the scattered Raman light slightly away from the irradiated zone. This new method is **S**patially **O**ffset **R**aman **S**pectroscopy (SORS).

The tutorial will describe Raman spectroscopy of bone, the SORS technique as well as the numerous steps leading to a working device able to make *in-vivo* measurements on patients.

Acknowledgements

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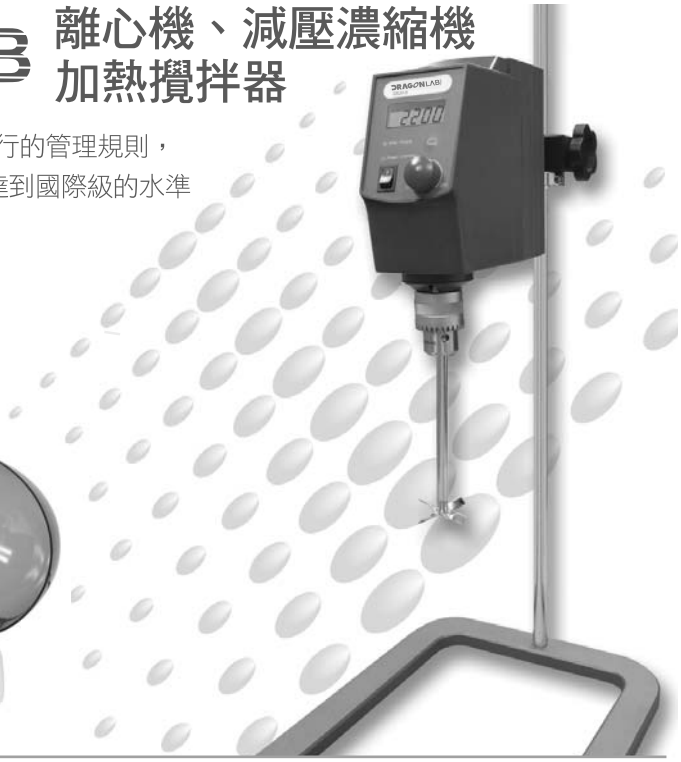

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