ALSCTIM: Advances in Live Single-Cell Thermal Imaging and Manipulation

November 10-12, 2014
OIST Seaside House

[Program at a glance]

Monday, November 10

09:30-10:00	Opening by Organizers
10:00-11:00	Keilmann "Basics and history of s-SNOM and nano-FTIR"
11:00-12:00	Suzuki "Thermal activation and temperature measurement of single living cells"
12:00-13:30	Lunch@ Chura Hall
13:30-14:30	Braun "Biomolecule Thermophoresis"
14:30-15:30	Okabe "Intracellular temperature imaging using polymeric thermometer and quantitative microscopy"
15:30-16:00	Coffee Break
16:00-17:00	Poster Panel Disucussions in lobby (Ground floor)
17:00-18:00	Dong "Single-molecule Raman mapping with sub-nm spatial resolution"
18:00-18:30	Free time
18:30-20:30	Working Dinner@ chura Hall (Poster Panel Disucussions in lobby)

Tuesday, November 11

09:30-10:30	Kroy "Hot Brownian Motion"
10:30-10:50	Coffee Break
10:50-11:50	Ono "Micro thermal sensor for heat detection of a biological cell"
12:00-13:30	Lunch@ Chura Hall
13:30-15:00	Campus tour
15:00-16:00	Hillenbrand "nano-FTIR spectroscopy of individual protein complexes"
16:00-17:00	Lohmüller "Optothermal Manipulation of Cells with Plasmonic Nanoparticles"
17:00-17:30	Coffee Break
17:30-18:30	Business round table discussion: Keilmann, Braun, Bursgens, Kawase (tentative)
18:30-20:30	Working Dinner@ Chura Hall (Poster Panel Disucussions in lobby)

Wednesday, November 12

09:30-10:30	Ogura "Lecture title: Vibrational Spectroscopic Investigation of Molecular Mechanisms of Aerobic Respiration"
10:30-10:50	Coffee Break
10:50-11:50	Kajihara "Near-field microscopy of thermally excited waves"
12:00-13:30	Lunch@ Chura Hall
13:30-14:30	Bürsgens "Localized optothermal heating of nanoparticles for DNA melting"
14:30-15:30	Kawase "Novel Techniques for THz-wave measurement"
15:30-16:00	Coffee Break
16:00-17:00	Hamaguchi "Molecular fingerprinting and mapping of living cells by Raman microspectroscopy"
17:00-18:30	[Keynote] Keilmann "Infrared nanoscopy of biological and soft matter"
18:30-20:30	Banquet@ Chura Hall (Poster Panel Disucussions in lobby)

Dieter Braun
Professor, LMU Munich

Biomolecule Thermophoresis

Dieter Braun is an associate professor in the Department of Physics at the Ludwig Maximilians University of Munich. He received a Diploma and PhD in Physics, working with Peter Fromherz at the MPI for Biochemistry. After working as DFG Scholar at Rockefeller University with Albert Libchaber, he led his own Emmy Noether group on biomolecule thermophoresis and thermal trapping in Munich, leading to the award winning Startup company NanoTemper (50 employees) founded by two of his first PhD students. The company uses thermophoresis to quantify biomolecule binding in complex liquids. He has received the Schloessmann Award in Optical Methods in Modern Biology and recently the Klung-Wilhelmy Weberbank prize, Germany's highest paying biannual award for physicists younger than 40.

Federico Bürsgens
Managing Director, GNA Biosolutions GmbH, Germany

Localized optothermal heating of nanoparticles for DNA melting

Federico is one of the founders of GNA Biosolutions GmbH, a spin-off company from Ludwigs-Maximilians-University Munich/Germany. He holds the post of Managing Director with the primary responsibilities for GNA's finances as well as technology-wise for the optoelectronic and software development. Federico started his entrepreneurial career in high-school and thus gained many years of business planning experience.
Federico began his undergraduate studies of physics at the University of Münster/Germany, and then enrolled at the Universitiy of Würzburg/Germany and the University of Texas at Austin/US, where he earned his Master's degree in physics. During his entire studies Federico was a fellow of the German National Academic Foundation (Studienstiftung). Afterwards, Federico moved to Munich/Germany where he worked in the Center for Nanoscience and obtained his PhD in physics from the University of Munich in 2008. He is author and co-author of 6 international patent families on optical heating of nanoparticles for DNA detection.

Zhenchao Dong
Professor, University of Science and Technology of China

Single-molecule Raman mapping with sub-nm spatial resolution

Dr. Zhenchao Dong is currently a full professor at Hefei National Laboratory for Physical Sciences at the Microscale (HFNL), University of Science and Technology of China (USTC). His research interest is in the field of single-molecule optoelectronics and nanoplasmonics, particularly on scanning tunneling microscopy (STM) based single-molecule electroluminescence and single-molecule Raman scattering. The aim of his research is to understand the underlying physics that governs the optoelectronic behavior of single molecules at the nanoscale, particularly on the light generation and energy transfer in a plasmonic nano-environment. Dong is currently the PI of several research projects supported by the MoST, NSFC, and CAS. He has published more than 120 papers indexed by SCI (H-factor=27), including 8 first-author or corresponding-author papers in Nature, Nature Photonics, Phys. Rev. Lett., J. Am. Chem. Soc., and Angew. Chem. He has presented many invited talks in prestigious international conferences, including APS, ACS, IVC, ICN+T, NFO, etc. He is the recipient of "National Outstanding Scientific and Technological Workers Award" from CAST in 2010 and a couple of other awards and honors in 2013, including "the CAIA Outstanding Award", "USTC President Award for Outstanding Research Achievements", and "Top 10 Advances in Science and Technology of China".

Hiro-o Hamaguchi
Professor, National Chiao Tung University

Molecular fingerprinting and mapping of living cells by Raman microspectroscopy

Hiro-o Hamaguchi received his D. Sc. Degree in physical chemistry from the University of Tokyo in 1975. From 1997 till 2012, he was a Professor of Chemistry at the University of Tokyo. He is now a Chair Professor at National Chiao Tung University, Taiwan. His recent research efforts are directed toward complicated molecular systems including solutions and liquids, ionic liquids, and also living cells and human organs, with the use of time- and space-resolved Raman spectroscopy. He is the author of 270 scientific papers. He received Meggers Award (2005), the Spectroscopical Society of Japan Award (2006), Chemical Society of Japan Award (2009) and TRVS Award (2009) and Medal with Purple Ribbon (2012). His hobby is associating with nature, currently, in particular, collecting Taiwanese stag beetles.

Reiner Hillenbrand

Ikerbasque Research Professor and Group Leader, CIC nanoGUNE

nano-FTIR spectroscopy of individual protein complexes

Rainer Hillenbrand is Ikerbasque Research Professor and Nanooptics Group Leader at the nanoscience research center CIC nanoGUNE in San Sebastian (Basque Country, Spain), and a Joint Professor at the University of the Basque Country. He is co-founder of the company Neaspec GmbH (Martinsried, Germany), which develops and manufactures near-field optical microscopes. From 1998 to 2007 he worked at the Max-Planck-Institut for Biochemistry (Martinsried, Germany), where he led the Nano-Photonics Research Group from 2003 to 2007. He obtained his PhD degree in physics from the Technical University of Munich in 2001. Hillenbrand's research activities include the development of optical near-field nanoscopy and infrared nanospectroscopy, and its applications in nanophotonics, graphene plasmonics, materials sciences and biology.

Yusuke Kajihara

Associate Professor, The University of Tokyo

Near-field microscopy of thermally excited waves

Yusuke Kajihara received the B.E., M.E., and Ph.D. degrees in precision engineering in 2001, 2003, and 2007, respectively, from the University of Tokyo. He spent 4 and half years as a Japan Science and Technology (JST) postdoctoral researcher at the University of Tokyo (the group of Prof. Susumu Komiyama). Since 2012, he has been a faculty member at Institute of Industrial Science, the University of Tokyo. He was a visiting researcher at Max-Planck Institute in 2011 (the group of Dr. Fritz Keilmann). His research interests include terahertz microscopy and nanoscale manufacturing.

Kodo Kawase

Professor, Department of Electrical Engineering, Nagoya University

(Visiting principal researcher in RIKEN Sendai)

Novel Techniques for THz-wave measurement

Kodo Kawase received B.S. degree in electronic engineering from Kyoto University in 1989, and Ph. D degree in electronic engineering from Tohoku University in 1996. He became an Initiative Researcher at RIKEN in 2001. He became a Professor of Graduate School of Engineering, Nagoya University in 2005. He received the 1997 Young Scientist Award from the JSAP, the 1998 Excellent Presentation Award, the 2000 and 2006 Prize of Laser Engineering from the Laser Society of Japan, the 2002 Marubun research and encouragement award and 2006 Marubun Special Research Award by the Marubun Research Promotion Foundation (MRPF), the 2005 Young Scientists' Prize by the Commendation for Science and Technology by the Minister of Education, Culture, Science and Technology (MEXT). He has been conducting research activities in several directions within the THz field. He developed several types of widely tunalbe THz sources using nonlinear optical effects, and suggested a whole range of real-life applications.

Flitz Keilmam

Senior Researcher, Ludwig-Maximilians-Universität, München, Germany

(i) Infrared nanoscopy of biological and soft matter

(ii) Basics and history of s-SNOM and nano-FTIR

Fritz Keilmann (born 1942) studied meteorology and physics in München and received a Dr. rer. nat. for research on infrared plasma diagnostics. As postdoc of Ali Javan at MIT he developed antenna-based harmonic mixing and high-power THz lasers. He has been a staff researcher of the Max-Planck Society from 1973 to 2012: Initally at the MPI für Festkörperforschung Stuttgart, he pioneered far-infrared nonlinear optics and spectroscopy of solids, and investigated phonon physics, microwave biological effects, carrier dynamics of semiconductors, far-infrared ellipsometry of superconductors, and cyclotron pumping of quantum-Hall edge states.

In 1995 he relocated to the MPI für Biochemie Martinsried where he pioneered infrared scattering near-field microscopy, and also coherent Fourier-transform infrared spectroscopy using frequency-comb beams. From 2007 - 2012 he was with the MPI für Quantenoptik Garching, in the DFG Cluster "Munich-Centre for Advanced Photonics", developing broad-band infrared spectroscopic near-field microscopy. He has been a guest researcher at

UC Santa Barbara and UC San Diego. He has been awarded the Kenneth J. Button prize 2009.

He is presently a Scientific Advisor to Neaspec GmbH, producer of near-field infrared microscopes, and a Senior Researcher at LMU München investigating soft matter and biological nanocomposites.

Klaus Kroy
Professor, Universität Leipzig

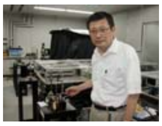
Hot Brownian Motion

Klaus Kroy is a theoretical physicist working in the field of soft and biological matter or `soft mesoscopics'. Some of his interests are aeolian sand transport, non-equilibrium dynamics of hot nanoparticles, proteins and stiff polymers, and the inelastic mechanics of the cytoskeleton. He received his PhD from the Technical University of Munich in 1998. Since 2004 he is a professor at the University of Leipzig, Germany. He has also previously worked at the ESPCI Paris (France), the University of Edinburgh (UK), and the Hahn-Meitner Institute Berlin (Germany).

Theobald Lohmüller
Group leader, LMU Munich

Optothermal Manipulation of Cells with Plasmonic Nanoparticles

Theo Lohmüller studied Chemistry and Biology at the University of Erlangen-Nürnberg and the University of Würzburg, Germany. In 2004 he joined the group of Prof. Joachim P. Spatz at the Heidelberg University and the Max-Planck Institute for Intelligent Systems in Stuttgart (formerly Metals Research). In 2008 he was awarded his doctoral degree in Chemistry for his work on "Nanostructured Functional Materials". After working as a Research Scientist for BASF at the Global Research Center in Singapore he joined the group of Prof. Jay T. Groves as a DFG PostDoc Fellow at UC Berkeley and the Lawrence Berkeley National Laboratory, Materials Sciences Division in 2009. In April 2011 he returned to Germany to join the chair of Prof. Jochen Feldmann at the Physics Department at LMU Munich as a group leader. In 2011 he became an extraordinary member of the Center of Nanoscience (CeNS) at the LMU Munich and since 2014 he is a principal investigator within the Nanosystems Initiative Munich (NIM).



Takashi Ogura
Professor, Picobiology Institute, Graduate School of Life Science, University of Hyogo

Vibrational Spectroscopic Investigation of Molecular Mechanisms of Aerobic Respiration

[Education history]
1981 Graduated from College of Arts and Natural Sciences, The University of Tokyo

1983 Graduated from Graduate School of Medical Science, Osaka University

[Work history]
1986 JSPS fellow
1986 Assistant Professor, Institute for Molecular Science, Okazaki National Research Institutes
1998 Associate Professor, Graduate School of Arts and Sciences, The University of Tokyo
2003 Professor, Graduate School of Science, Himeji Institute of Technology
2004 – Present Professor, Graduate School of Life Science, University of Hyogo

[Other history]
1987 Obtained D. Sci. degree from The University of Tokyo
2011 Chairman, Organizing Committee, The 49th
2011 – Present Director, Picobiology Institute, Graduate School of Life Science, University of Hyogo
2013 – Present Visiting Scientist, RIKEN

Takahito Ono
Professor, Tohoku University

Micro thermal sensor for heat detection of a biological cell

He is currently a Professor at Department of Mechanical Systems and Design in Tohoku University. He received the D.E. degree in mechatronics and precision engineering from Tohoku University in 1996. During 1996–2001, he has been a Research Associate in the Department of Mechatronics and Precision Engineering, Tohoku University. During 2001-2009, he has been an Associate Professor. From 2009, He is a Professor at Department of Mechanical Systems and Design in Tohoku University.
Professor Ono's expertise is in the area of nanoelectromechanical systems (NEMSs), silicon based nanofabrication,

ultrasensitive sensing based on resonating devices, scanning probe technologies, nanoprobe sensing for nanoscale science and engineering, and hetero-integration of LSI-MEMS. Currently, he is project reader of "Microsystem Integration Research Initiative in Tohoku University, which is the project to develop LSI-MEMSs.

Kohki Okabe
Assistant Professor, Graduate School of Pharmaceutical Sciences, University of Tokyo; JST, PRESTO

Intracellular temperature imaging using polymeric thermometer and quantitative microscopy

Kohki Okabe received a B.S. from University of Tokyo in 2002, and his Ph.D. from University of Tokyo in 2007 (Prof. Takashi Funatsu's lab). Then, Kohki joined Dr Yoshie Harada's lab in the Tokyo Metropolitan Institute of Medical Science, where he started his project on intracellular thermometry. In 2008, he became Assistant Professor at Graduate School of Pharmaceutical Sciences, University of Tokyo. He is now also the PRESTO researcher of JST (project: Design and Control of Cellular Functions).

Kohki has made major contributions to the emergence of the field of intracellular thermometry and its application to cell biology. His team also pioneered the development of quantitative imaging of intracellular mRNA.

His honors include Early Research in Biophysics Award (Biophysical Society of Japan) and Young Researcher's Award (Bioimaging Society of Japan).

Madoka Suzuki
Principal Investigator, Principal Investigator, Waseda Biosciences Research Institute in Singapore (WABIOS), Waseda University

Thermal activation and temperature measurement of single living cells

Dr. Suzuki is an associate professor of Waseda University in Tokyo, Japan. He got a PhD on biophysics from Waseda University in 2005 and he also received MBA in Technology Management in 2008. Since 2009, he has been working in Waseda Bioscience Research Institute in Singapore (WABIOS) locating in Biopolis in Singapore as a principal investigator of Physical Biology Group. His research interests include the structural and functional reconstruction and molecular mechanism of muscle contractile system, and force measurement and functional analysis of molecular motors.

"Biomolecule Thermophoresis"

Dieter Braun, Ph.D.
Professor, Systems Biophysics, LMU Munich

[Abstract]
The movement of biomolecules in a temperature gradient is a sensitive and versatile way to probe protein interactions, including the important class of membrane receptors binding to its target molecule. The binding is detected all-optically in various biological fluids (www.nanotemper.de). We screened for drug-protein interactions without labeling the protein and were able to successfully commercialize the method. The physical basis of the movement was studied with DNA, RNA and polystyrene beads and could be understood by the energy in the shielding capacity in combination with the Seebeck effect. Recently, we demonstrated the thermophoresis of biomolecules in living cells.
Temperature gradients also move fluids by thermal convection. Combined with the thermal control of molecules, various molecule traps can be implemented. In hydrothermal pores of rock, thermal molecule traps occur naturally. They offer a compelling disequilibrium system to drive molecular evolution. We showed that a thermal gradient can drive DNA replication by thermal cycling and trap only the long nucleic acids. The combination bodes well to implement an autonomous Darwinian evolution of molecules. The first selection pressure is the physical molecule size.
Despite accumulating molecules in bulk water, thermophoretic traps enhance the polymerization of biomolecules. Thermal traps form metastable conglomerates from short oligonucleotides if they have matching end sequences. This implements a macroscale amplifier of nanoscale sequence information. Together with a hairpin replication scheme using tRNA, thermal traps can provide a roadmap from replication to translation without explicit backbone ligation chemistry.
Our aim is to create autonomous non-equilibrium systems in the lab to understand the transition from dead to living matter.

“Localized optothermal heating of nanoparticles for DNA melting”

Federico Buersgens, Ph.D.
Managing Director, GNA Biosolutions GmbH

[Abstract]

Heating and cooling has been the performance bottleneck of Polymerase Chain Reaction (PCR) since its invention about thirty years ago. All approaches so far require time-consuming repetitive heating and cooling of the entire sample. Usually, this is achieved by a heating block or stream of air that causes a heat transport in and out of the sample. However, the poor thermal conductivities of both the PCR solution and the reaction vessel severely limit the effective heating and cooling rates in conventional PCR to <10K/s.

We report on a novel approach for ultrafast DNA melting and its application for PCR. In this technique, after the elongation of primers functionalized onto gold nanoparticles a laser pulse locally heats up the nanoparticles. Thereby, the newly formed DNA double strands are denatured. For this purpose a microsecond optothermal excitation of the nanoparticles causes a localization of the heat field such that only a millionth of the solution is raised to the denaturation temperature. Thus, as soon as the excitation finishes, the thermal energy of the nanoparticles and their close environment is dissipated into the bulk of the solution cooling the nanoparticles to their initial temperature on a nanosecond time scale. This allows for up to one million times shorter PCR temperature ramps in comparison to conventional PCR with its global heating process. As the reaction remains at the productive temperature of the polymerase virtually the entire time, the cycle duration is fundamentally only limited by the processivity of the polymerase.

Surprisingly, it turns out that overcoming the heating and cooling restrictions of conventional PCR leads to a new strategy regarding the design and optimization of PCR protocols.

Single-molecule Raman mapping with sub-nm spatial resolution

Zhenchao Dong*

Hefei National Laboratory for Physical Sciences at the Microscale
University of Science and Technology of China, Hefei 230026, China
* zcdong@ustc.edu.cn

Visualizing individual molecules with chemical recognition is a dream target in catalysis, bio-science, nanotechnology, and materials science. Molecular vibrations provide a valuable "fingerprint" for this identification. The spectroscopy based on tip-enhanced Raman scattering (TERS) has opened a path to obtain enhanced vibrational signals thanks to the strong and confined plasmonic field in the proximity of the tip apex. In this talk, I shall demonstrate single-molecule Raman spectroscopic mapping with unprecedented spatial resolution down to about 0.5 nm, resolving even the inner structure of a single molecule and its configuration on the surface [1]. This is achieved by a delicate spectral-matching technique that invokes a double-resonance process and resultant nonlinear optical effect, thanks to the exquisite tuning capability provided by low-temperature ultrahigh-vacuum scanning tunneling microscopy. Our nonlinear TERS technique features the use of only a continuous wave laser rather than two pulse lasers. Our findings demonstrate that Raman spectromicroscopy goes intra-molecular and sub-nanometer, which opens up a new avenue to probe chemical identification, photochemistry, and even bio-imaging, all at the single-molecule scale.

[1] R. Zhang, Y. Zhang, Z. C. Dong*, S. Jiang, C. Zhang, L. G. Chen, L. Zhang, Y. Liao, J. Aizpurua, Y. Luo, J. L. Yang, J. G. Hou*, Nature **498** (2013) 82.

“Molecular fingerprinting and mapping of living cells by Raman microspectroscopy”

Hiro-o Hamaguchi, Ph.D.
Chair Professor, National Chiao Tung University

[Abstract]
Invention of a new microscopy always advances our understanding of life. Confocal Raman microspectroscopy, which is an alliance of Raman spectroscopy with confocal microscopy, is no exception. It is conferring biology with a new prospect for *in vivo* observations of dynamical biological processes at the molecular level. All biological processes, birth, life and death, result from dynamic interactions of ensembles of molecules in cells, tissues, organs and organ systems. Raman microspectroscopy provides time- and space-resolved Raman spectra or the “molecular fingerprints” that contain otherwise unobtainable information on molecules that take parts in these biological processes. Raman microspectroscopy offers the advantage of label-free monitoring of molecules, being suitable for exploratory studies of unknown molecular species that play important roles in fundamental biological processes. Application of Raman microspectroscopy to a wide range of biology/medicine will be discussed with the introduction of the sate-of-the-art linear and non-linear Raman microspectroscopy techniques developed in the author’s group.

“nano-FTIR spectroscopy of individual protein complexes”

Rainer Hillenbrand, Ph.D.
Ikerbasque Research Professor and Group Leader, CIC nanoGUNE

[Abstract]
We introduce the mapping of protein structure with 30 nm lateral resolution and sensitivity to individual protein complexes by infrared scattering-type scanning near-field optical microscopy (IR s-SNOM) and Fourier transform infrared nanospectroscopy (nano-FTIR). s-SNOM and nano-FTIR are based on recording the infrared light scattered by a metallized atomic force microscope tip probing the sample surface. We present and discuss local broadband spectra of individual viruses, ferritin complexes, purple membranes and insulin aggregates, which can be interpreted in terms of their alpha-helical and/or beta-sheet structure [1]. Applying nano-FTIR for studying insulin fibrils, we find clear evidence that 3-nm-thin amyloid-like fibrils contain a large amount of alpha-helical structure. Nano-FTIR spectra of one ferritin complex demonstrate extraordinary sensitivity to ultra-small amounts of material, about 1 attogram of protein, respectively 5000 C=O bonds. By further sharpening the tips and optimizing their antenna performance, we envision single protein spectroscopy in the future, paving the way to a new era in infrared bio-spectroscopy. We foresee manifold applications, such as studies of conformational changes in amyloid structures on the molecular level, the mapping of nanoscale protein modifications in biomedical tissue or the label-free mapping of membrane proteins.

[1] I. Amenabar, et al., Nature Commun. 4:2890 doi: 10.1038/ncomms3890 (2013)

Near-field microscopy of thermally excited waves

Yusuke Kajihara,

Abstract.

We demonstrate near-field microscopy of spontaneous surface waves with a sensitive near-field microscope, which does not utilize external illumination. Our “passive” near-field microscope is based on a home-made scattering-type scanning near-field optical microscope (s-SNOM), which is constructed by incorporating an atomic force microscope into a confocal microscope equipped with an ultra-highly infrared detector, named “charge sensitive infrared phototransistor” (CSIP, wavelength: 14.5 μm). In our s-SNOM, a sharp tungsten probe with a radius of 20 nm is vertically modulated at 10 Hz and spontaneous near-field signals are obtained after demodulating the signals at fundamental frequency. With the microscope, we achieve spontaneous evanescent waves from a room-temperature object with 5-μm pitch Au gratings deposited 100 nm on dielectric substrate. The spatial resolution now reaches 20 nm (λ/725). In this lecture, we show the microscope development, examples of signals, and detailed analyses for understanding the near-field signal origin. The distance dependence, the sample-size dependence, and the temperature dependence of the near-field signals confirm that the obtained signal should originate from thermally activated charge fluctuation strongly localized near the metal/dielectrics surfaces. We also demonstrate nano-thermometry by probing passive near-field signals on biased metal micro-patterns, which reveals local temperature distribution with nanoscale resolution. These results strongly suggest that our s-SNOM is very well suited for studying mesoscopic phenomena on biomolecules as well as hard materials.

"Novel Techniques for THz-wave measurement

Kodo Kawase[1,2], Kokuke Murate[1], Saroj R. Tripathi[1]
[1] Nogoya University, [2] Riken

Our group has been conducting research activities in several directions within the THz field. We introduced many types of widely tunable THz-wave sources using nonlinear optical effects, and we also suggested a whole range of real-life applications using THz-imaging techniques.

Among our studies, a high-resolution tomographic imaging was demonstrated using a reflection-type terahertz time-domain spectroscopy. To realize a practical system for general use, a robust all-fiber laser was used as the pump light source. Broadband terahertz waves were generated with the combination of ultrashort optical pulses using optical fibers and a nonlinear crystals. Using deconvolution signal processing, the wideband spectrum of the generated terahertz waves provided high-axial resolution of 5um leading to tomographic imaging of multilayered structure.

Also, we have started a research project on "influence of widely tunable ultra weak THz/MMW radiation on human skin cells" which is supported by the Ministry of Internal Affairs and Communications in Japan last year. The objective of this research project is to prove the Fröhlich hypothesis which predicts the possible resonant vibrations in cell membrane at a specific frequency around 0.1-1 THz. The cultured cell was irradiated with widely tunable THz wave in order to find the resonant frequency which causes the non-thermal effect combining with this vibration for cell membrane. The cell activity was monitored by AC impedance method and the MTT assay.

Infrared nanoscopy of biological and soft matter

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Infrared spectroscopic analysis rests on vibrations which label any chemical compound with a characteristic "fingerprint" spectrum in the 3-30 µm wavelength region—intrinsically. Combining IR with AFM allows to perform IR chemical recognition routinely at 20 nm spatial resolution.[1,2] Known as scattering-type scanning near-field optical microscopy (s-SNOM) this instrument has become commercially available (neaspec.com).

Recently, we have enhanced s-SNOM by a coherent broadband mid-infrared illumination[3] based on 100-fs pulses (thus even allowing nanoscale probing at ultrafast time resolution[4,5]); with this illumination, a complete IR spectrum is obtained at any sample position of the tip ("nano-FTIR"). By scanning the sample, an IR-hyperspectral line image or two-dimensional image is recorded.

The technique of nano-FTIR is being applied to thin-film organic conductors where it revealed coexisting structural phases.[6] Nano-FTIR hyperspectral imaging is likely valuable in all fields of nanoscience as has already been been demonstrated in studies of protein,[7] human bone,[8] and extraterrestric minerals.[9]

(1) Knoll, B.; Keilmann, F. *Nature* **1999**, *399*, 134.
(2) Huth, F.; Govyadinov, A.; Amarie, S.; Nuansing, W.; Keilmann, F.; Hillenbrand, R. *Nano Lett.* **2012**, *12*, 3973–3978.
(3) Keilmann, F.; Amarie, S. *Journal of Infrared, Millimeter, and Terahertz Waves* **2012**, *33*, 479.
(4) Wagner, M.; Fei, Z.; McLeod, A. S.; Rodin, A. S.; Bao, W.; Iwinski, E. G.; Zhao, Z.; Goldflam, M.; Liu, M.; Dominguez, G.; Thiemens, M.; Fogler, M. M.; Castro Neto, A. H.; Lau, C. N.; Amarie, S.; Keilmann, F.; Basov, D. N. *NanoLetters* **2014**, *14*, 894.
(5) Eisele, M.; Cocker, T. L.; Huber, M. A.; Plankl, M.; Viti, L.; Ercolani, D.; Sorba, L.; Vitiello, M. S.; Huber, R. *Nature Photonics* **2014**, *DOI:10.1038NPHOTON.2014.225*.
(6) Westermeier, C.; Cernescu, A.; Amarie, S.; Liewald, C.; Keilmann, F.; Nickel, B. *Nature Communications* **2014**, *5*, 4101.
(7) Amenabar, I.; Poly, S.; Nuansing, W.; E.H., H.; Govyadinov, A. A.; Huth, F.; Kruthokvostov, R. Z., L.; Knez, M.; Heberle, J.; Bittner, A. M.; R., H. *Nature Comm.,* **2013**, *4*.
(8) Amarie, S.; Zaslansky, P.; Kajihara, Y.; Griesshaber, E.; Schmahl, W. W.; Keilmann, F. *Beilstein Journal of Nanotechnology* **2012**, *3*, 11.
(9) Dominguez, G.; McLeod, A. S.; Gainsforth, Z.; Kelly, P.; Bechtel, H. A.; Keilmann, F.; Westphal, A.; Thiemens, M.; Basov, D. N. *Nature Communications* **in print 2014**, *DOI: 10.1038/ncomms6445*.

Basics and history of s-SNOM and Nano-FTIR

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By scattering focused VIS-IR-THZ radiation from an AFM tip the local optical response can be measured and mapped together with a sample's topography, at a spatial resolution down to 20 nm.[1]

This contribution will introduce the concept of optical near fields and its applications, starting with the Synge's[2] original ideas of both aperture-type and scattering-type optical near-field microscopies, along with the lecturer's own involvement in developing IR s-SNOM and nano-FTIR.

1. Keilmann, F.; Hillenbrand, R., Near-field microscopy by elastic light scattering from a tip. *Philosophical Transactions of the Royal Society A* **2004,** 362, 787-805.
2. Synge, E. H., Suggested method for extending microscopic resolution into the ultra-microscopic region. *Philosophical Magazine* **1928,** 6, 356-362.

“Hot Brownian motion”

Klaus Kroy, Ph.D.
Professor, University of Leipzig

[Abstract]

The classical theory of Brownian motion rests on fundamental laws of statistical mechanics, such as the equipartition theorem and the fluctuation-dissipation theorem, which do not hold under non-isothermal conditions. I will discuss the generalized fluctuation-dissipation relations and Langevin equations governing non-isothermal Brownian motion, including some explicit results for the frequency-dependent noise temperature and Brownian thermometry in dense liquids and rarified gases, far from equilibrium. I will also review recent experimental applications involving hot Brownian particles, such as photothermal correlation spectroscopy, photon nudging, and hot microswimmers.

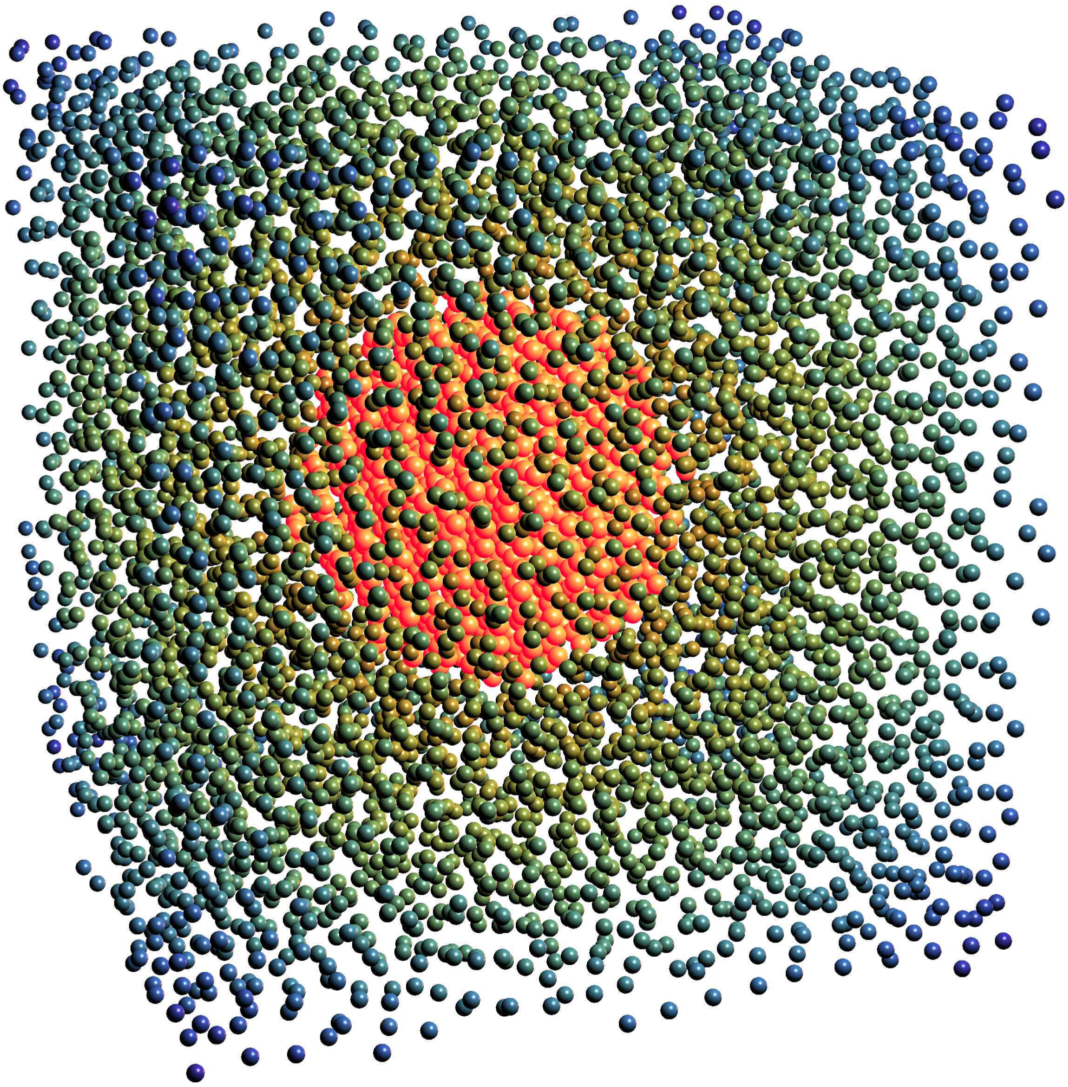


Figure: A hot nanoparticle in a Lennard-Jones fluid

Optothermal Manipulation of Cells with Plasmonic Nanoparticles

Theobald Lohmüller, Ph.D.
Group leader, Photonics and Optoelectronics Group, LMU Munich

[Abstract]
Light absorbed by plasmonic nanoparticles is very efficiently converted into heat. Gold nanoparticles, for example, can be used as a fine tool to apply heat to only a nanoscopic area which renders it possible to study temperature sensitive processes with unprecedented resolution.
Here, we demonstrate how plasmonic heating can be applied to control cell functions at the nanoscale. As a first example, we will discuss how gold nanoparticles that are bound to a free standing phospholipid membrane can be used to manipulate the membrane's resistance and permeability by adjusting the nanoparticle temperature. Shifting to living cells, we will show that gold particles can even be injected into a single cell by a combination of plasmonic heating and optical force.
In summary, these results illustrate how plasmonic heating can be employed to achieve nanoscale control over biological systems which paves the way for future biomedical applications in nanotheranostics and drug delivery.

Vibrational Spectroscopic Investigation of Molecular Mechanisms of Aerobic Respiration

Takashi Ogura (Graduate School of Life Science, University of Hyogo)

In the mitochondrial respiratory chain of aerobic organisms, three membrane protein complexes couple electron transfer with proton pumping. The proton motive force thus generated across the inner mitochondrial membrane is utilized to synthesize ATP, the energy currency of the cell.

Cytochrome *c* oxidase (CcO) is one of such molecular machineries and reduces dioxygen to water. This reaction is the reverse reaction of the photosynthetic oxygen evolution. The dioxygen reduction by CcO is coupled with proton pumping. X-ray crystallography has determined the three dimensional structures of bovine CcO at a resolution of 1.8Å. Vibrational spectroscopy, on the other hand, has revealed the reactivity of functional groups in the protein and also provided pieces of information with respect to the protein dynamics. Actually, time-resolved resonance Raman spectroscopy has been applied to study the dioxygen reduction reaction and the protein dynamics after ligand dissociation from the heme. This is based on the high selectivity of the technique to reveal vibrational spectra of the heme and its vicinity. A high-sensitivity infrared spectroscopy developed in our laboratory has enabled us to reveal dynamics of the metal centers and protein main chain after ligand dissociation. The results show the presence of “a conformational relay system”, between the dioxygen reducing site and proton gate site in the protein, by which an efficient proton pumping is realized.

Reference: Kubo, M., Nakashima, S. *et al.* (2013) *J. Biol. Chem. 288*, 30259 – 30269.

“Micro thermal sensor for heat detection of a biological cell”

Takahito Ono, Dr. Eng.
Professor, Tohoku University

[Abstract]
Resonant heat sensors are developed and applied to a calorimeter for the detection of heat from a brown fat cell. The measurement principle relies on the resonant frequency tracking of a Si resonator in temperature variation due to heat from a sample, and heat is conducted from the sample in water to the Si resonator in vacuum via a Si heat guide, as shown in Figure 4. A heat loss to surrounding and a dumping in water can be reduced by placing the resonant thermal sensor in vacuum. The fabricated resonant thermal sensor shows 1.6 mK of the temperature resolution, and 6.2 pJ of the detectable minimum heat. The heat from the single cell is detected in cases without any stimulation and with stimulation. As the results, pulsed heat production and continuous heat production are observed, respectively.

“Intracellular temperature imaging using polymeric thermometer and quantitative microscopy”

Kohki Okabe, Ph.D.
Assistant Professor, Graduate School of Pharmaceutical Sciences, University of Tokyo; JST, PRESTO

[abstract]
Temperature is a fundamental physical quantity that governs every biological reaction within living cells, and intracellular temperature reflects cellular thermodynamics and function. In medical studies, the cellular pathogenesis of diseases (e.g., cancer) is characterized by extraordinary heat production. Therefore, intracellular temperature imaging of living cells should promote better understanding of cellular events and the establishment of novel diagnoses and therapies. Here we report our thermometric methods for the monitoring and imaging of intracellular temperature based on a fluorescent polymeric thermometer and quantitative fluorescence imaging techniques. The fluorescent polymeric thermometer has several advantages in intracellular temperature measurement such as biocompatibility (i.e., size, sensitivity, and solubility) and functional independence (i.e., negligible interactions with cellular components), enabling intracellular temperature measurement in single living cells. The spatial and temperature resolutions of our thermometry were at the diffraction limited level (200 nm) and 0.2 °C, respectively. We first performed tracking of the averaged temperature of a single whole cell and showed that the averaged temperature of single COS7 cells significantly varied in association with biological processes such as apoptosis. Next, we developed a novel method to visualize intracellular temperature distribution, indicating non-homogeneous temperature distribution in steady-state living cells. Furthermore, we have investigated intracellular thermogenesis: both temperature monitoring and imaging showed that uncoupling of mitochondria allowed the local temperature increase, suggesting that mitochondria undertakes thermogenesis in living cells. Heating the cells with an external infra-red laser was also conducted and the visualization of intracellular temperature increase was detected with our thermometry. Therefore, our intracellular temperature imaging will contribute to uncover an important connection between intracellular temperature and cell functions.

"Temperature measurement and thermal activation of single living cells."

Madoka Suzuki, Ph.D.
Principal Investigator, Principal Investigator, Waseda Biosciences Research Institute in Singapore (WABIOS), Waseda University

[abstract]
'Temperature' can be considered as a local parameter even at the sub-cellular scale without theoretical consideration affecting macroscopic steady-state definition [1].
Requirements from biology, medical biology and electronics, thermometry at the small scale has been extensively studied [2]. There are two kinds of methods; luminescent and non-luminescent ones. The former is to measure the optical properties of materials as small as nanometer to micrometer sizes such as fluorophores and quantum dots. The latter includes, e.g., scanning thermal microscopy and the measurement of thermoreflectance. We have been focused on the optical methods as it is easy to combine them with other optical methodologies that are commonly used in biomedical fields [3-7].
We have also studied cellular responses to the microscopic temperature stimulus [1,4,5,8]. Local temperature gradients were created by focused infra-red laser beams either onto a metal aggregate or directly into the media. The temperature gradient created around a small heat source during laser illumination disappears immediately when the laser is terminated, as the heat quickly diffuses out through the surrounding medium.
I will introduce these methodologies developed as collaborations among multiple expertise, and new cellular insights found by using these methods.

1. Zeeb, V., Suzuki, M. and Ishiwata, S., J. Neurosci. Methods 139(1), 69-77 (2004).
2. Suzuki, M., et al., Luminescent nanothermometers for biological applications, In CRC Concise Encyclopedia of Nanotechnology. (ed. by B.I. Kharisov, O.V. Kharissova and U.O. Mendez), Taylor and Francis/CRC Press, (2015), in press.
3. Suzuki, M., et al., Biophys. J., 92, L46–8 (2007).
4. Tseeb, V., et al., HFSP J. 3(2), 117-23 (2009).
5. Oyama, K., et al., Lab Chip 12(9), 1591-3 (2012).
6. Takei, Y., et al., ACS Nano 8(1), 198-206 (2014).
7. Quinto-Su, P.A., Suzuki, M. and Ohl, C.-D., Sci. Rep., 4, 5445 (2014)
8. Oyama, K., et al., Biochem. Biophys. Res. Commun. 417(1), 607-12 (2012).

“OIST Seaside House”

General Information 一般用

1．Main Entrance Door 開館時間

The Main Entrance door is locked before 0800 and after 1800. The door can be opened with a security card provided at Information. For other late night enquiries, please use the videophone.

この施設の開館時間は、原則として午前８時から午後６時までで、時間外は出入口を施錠します。施錠中は、インフォメーションより入居時にお渡しするセキュリティーカードで出入りされるか、インターフォンでお問合せください。

2．Emergency Exit 非常口

There are emergency exits at the end of corridors on every floor.

非常口は、各フロアの廊下突当りにあります。

3．Smoking 喫煙

Smoking is not permitted inside the building. Please use the ashtrays provided on the first floor wood-deck.

喫煙場所は、１階テラスにあります。それ以外の場所での喫煙は他の宿泊者の迷惑になることや、防火対策上からも堅くお断りします。

4．LAN

The public spaces (lounge, meeting rooms and seminar room on the 1st floor, and Chura-hall on the 3rd floor) are equipped with wireless LAN and a wired LAN in each guest room and seminar room. To access the wireless LAN, an ID code must be obtained from Information. A LAN-cable is provided in every guest room. Non-staying guests may borrow a LAN-cable for use while at Seaside House.

公共スペース（１階ラウンジ、会議室、セミナールーム、３階ちゅらホール）では無線 LAN が、セミナールームおよび各宿泊室では有線 LAN がご利用可能です。無線 LAN での接続を希望される方はインフォメーションオフィスで ID の発行を受けてください。各宿泊室には LAN ケーブルを備え付けていますが、宿泊者以外でご希望の方にも LAN ケーブルの貸出が可能です。

5．The beach 海岸

Swimming is not permitted at the beach. There is no lifeguard on duty. No liability can be assumed for any accidents occurring on the beach, so please use caution. Due to local fishing grounds, please exercise good judgment and show respect for the environment.

海岸での遊泳は禁止されています。ライフガードは駐在していません。海岸での事故については責任を負いません。また、地元の漁場につき、分別をもって環境にご配慮ください。

6．Medical Facilities 周辺医療機関

Onna Clinic 恩納クリニック	Tel: 098-966-8115 6329 Onna, Onna-son 国頭郡恩納村恩納 6329	Internal, Pediatrics Close: Sunday, Public holidays, Tuesday and Thursday evenings 診療所 診療科目：総合内科、小児科 休診日：日、祝日、火・木午後
Nakagami Hospital 中頭病院	Tel: 098-939-1300 6-25-5 Chibana, Okinawa-shi 沖縄市知花 6-25-5	* also a designated emergency hospital 総合病院　救急センター有り
Okinawa Chubu Hospital 沖縄県立中部病院	Tel: 098-973-4111 281 Miyazato, Uruma-shi うるま市字宮里 281	* also a designated emergency hospital 総合病院　救急センター有り

7．Taxi タクシー

Ishikawa-musen Taxi 石川無線タクシー	Tel: 098-972-5406
Okito-kotsu Taxi 沖東交通 万座営業所	Tel: 098-966-2861

For Staying Guests 宿泊者の方へ

８.　Check-in and check-out チェックイン·チェックアウト

Please check-in at Information to receive a code number ‘key’ to the room. Check out time is 12:00 and please return the security card to the information office.

宿泊室の鍵は暗証番号式ですので、チェックイン時にインフォメーションオフィスでご案内します。チェックアウトは予定日に変更がなければ 12:00 までにご退室いただき、セキュリティーカードをご返却ください。

９.　Valuable articles 貴重品

There is no safe or vault for personal belongings or valuables. OIST assumes no liability for any lost articles or stolen items from Seaside House.

貴重品については、各自保管してください。宿泊室内での紛失・盗難等については責任を負いません。

１０.　Room Cleaning and Linen Service 清掃・寝具類

Each room is cleaned and supplied with fresh linens every other day. The cleaning schedule is from 9:30-16:30 hrs. A broom and rubbish bin are available in the laundry room on the 2nd and 3rd floors.

隔日午前９時 30 分～午後４時 30 分の間に、各宿泊室の清掃とリネン類の交換を行います。また、２階、３階のランドリールームに、清掃道具、分別ゴミ箱を配備してありますので、必要な方は自由にご利用ください。

１１.　Amenity Set アメニティ

Shampoo & rinse, body soap, a toothbrush set, a hair dryer, and slippers are provided in each room.

シャンプー、リンス、ボディソープ、歯磨きセット、ドライヤー、スリッパを宿泊室内に用意してありますので、自由にご利用ください。

１２.　Laundry, etc. ランドリー等

There are washer-dryers, detergent, a trouser press and an ironing board set in the 2nd and 3rd floor laundry rooms.

２階、３階のランドリールームに、洗濯乾燥機、洗剤、ズボンプレッサ、アイロンおよびアイロン台を用意してありますので、自由にご利用ください。

１３.　Kitchenette, Vending machine, Pay Phone 給湯、自動販売機、公衆電話

Soft drink, beer and noodle vending machines and a pay phone are located on the 1st floor. Hot water is available, next to the vending machines. No cooking is allowed in guest rooms.

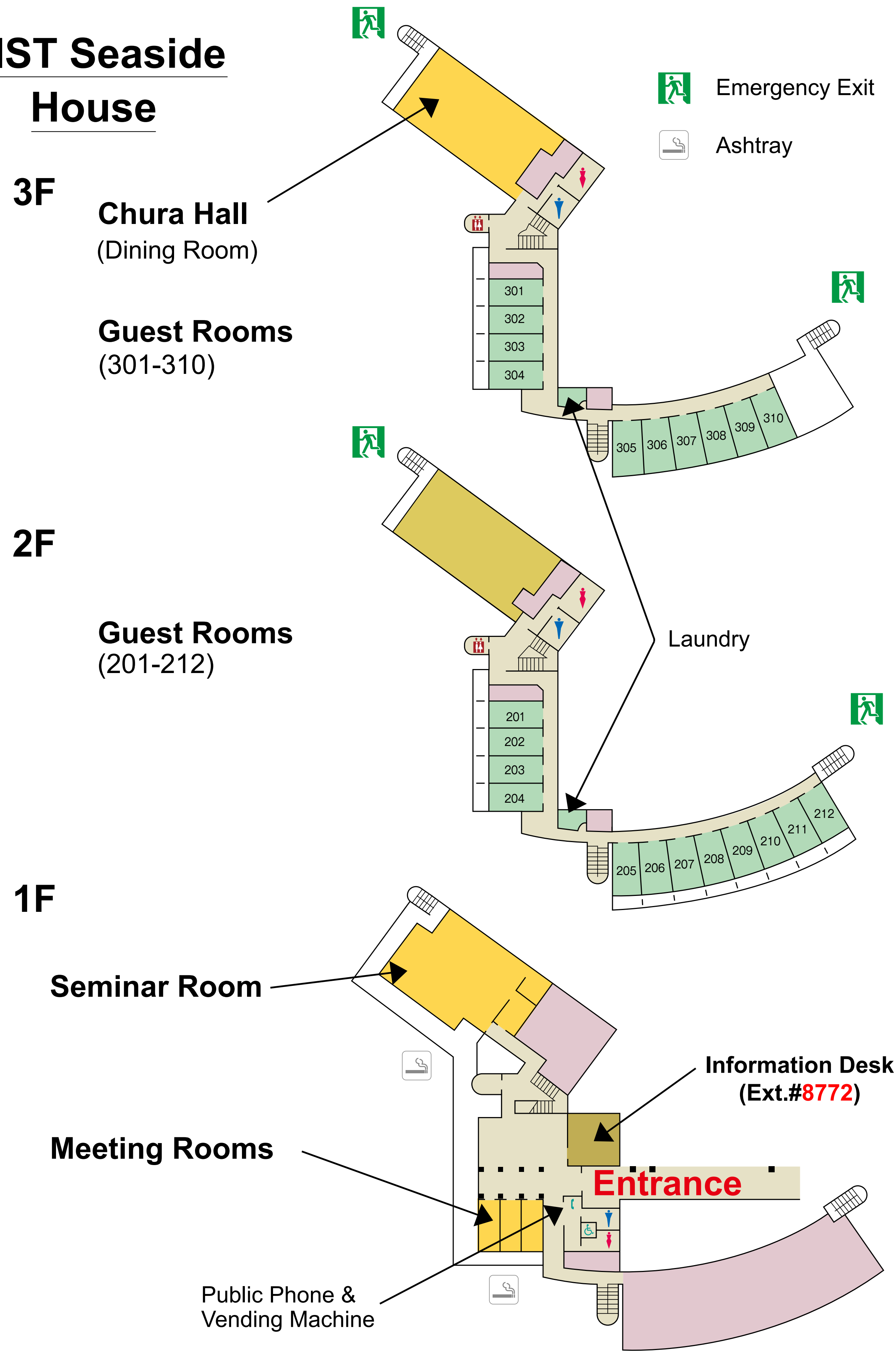
１階に自動販売機（ソフトドリンク、ビール、カップヌードル）および公衆電話が設置してあります。給湯コーナーを自動販売機隣に設置してありますので、自由にご利用ください。宿泊室内での調理は、防火対策上からも堅くお断りします。

１４.　General Enquiries お問合せ

Please ring up extension #8772 or from an outside line, 098-966-8772.

インフォメーションオフィス　内線電話 8772、外線電話 098-966-8772 まで。

OIST Seaside House



OIST Seaside House Guest Accommodation Regulations

宿泊施設利用規約

1. Check-in （利用開始日）

Those who stay at the Seaside House are required to check-in on the appointed date.

このSeaside House宿泊施設（以下「施設」という）の利用者は、指定された利用開始日に入居しなければならない。

2. Keeping the accommodation comfortable and safe（施設の維持）

Those who stay at Seaside House are required to observe OIST terms and conditions and general accepted manners in order to keep the accommodation comfortable and safe. Staying guests are required to acknowledge liability for all damages to the premises, equipment and furnishings made by negligence on his/her part.

利用者は、施設の利用に際し、当学の指示に従い、施設の正常な状態の維持に努めなければならない。利用者の責に帰すべき事由により、施設の設備・備品を毀損又は汚損した場合は、利用者の負担でこれを原状に回復しなければならない。

3. Prohibited activities（禁止行為）

The activities stated as below are prohibited by OIST guest regulations.

利用者は、次の事項に該当する行為、その他利用の目的に反する行為をしてはならない。

① Any alternation to the premises, equipments, furnishings and so on.
施設の設備・備品等の現状に変更を加えること。

② Careless use of fire or acts, which may cause damages to the premises of OIST or the adjoining.
施設内において火気その他危険物を粗略に使用し、又は施設に損害を及ぼす恐れの大きい行為をすること。

③ Any act that causes danger or nuisance to the neighborhood or the other guests of the accommodation or the adjoining.
近隣及び他の利用者に迷惑を及ぼす行為をすること。

④ Smoking in places other than the designated area located outside the building.
所定の場所以外で喫煙すること。

4. Entering the room（居室内への立入り）

Any person or agent authorized by OIST shall be allowed to enter the room to inspect or to repair the premises and every convenience shall be provided for that purpose.

利用者は、当学の指示を受けた者が施設の管理上必要により居室内への立入り、点検等を実施するときは、これに協力しなければならない。

５． Leaving （退室）

Staying guests are required to leave the accommodation in case of the following:

利用者は、次の各号のいずれかに該当するときは、施設を退室しなければならない。

① When the period assigned by OIST is over.
利用期間が満了したとき

② On the requirement of the guest’s departure by OIST in the course of its business.
当学が業務上の必要から退室を指示したとき

③ On the requirement of the guest’s departure by OIST because of any non-observance or non-performance of any of the agreements by Staying guest.
利用者がこの利用心得に違反したことにより当学が退室を指示したとき

6． Compensation（当学への請求）

OIST is not responsible for any compensation requests by staying guests for removal of a sort if and whenever staying guests are requested to leave the accommodation.

利用者は、施設退室を条件として、当学に対し立退料等その他の請求をすることはできない。

7． Beach（海岸）

Swimming is not permitted at the beach.
OIST disclaims all the responsibility for the accident occurring at the beach.

遊泳禁止。施設周辺の海岸において事故等が発生しても当学は一切の責任を負わない。

8． In case of emergency（緊急時の対応）

Staying guests are asked to immediately inform the Secretariat office in the following cases.

利用者は、次の各号のいずれかに該当する場合は、直ちに宿泊管理者に連絡し、当学の指示を受けるものとする。

① Any danger or damage to the premises, equipments and furnishings due to fire, typhoon, flood, earthquake, and so on.
火災、風水害、地震、その他の原因により建物その他付属設備に損害を及ぼし又は及ぼす危険が大きい場合

② Any sick persons on the premises or surroundings.
施設内若しくはその近辺において、病人が発生した場合