文部科学省科学技術試験研究委託事業 分子イメージング研究戦略推進プログラム
岡山分子イメージング高度専門人材育成事業
総括国際シンポジウム

International Symposium on Bio-imaging and Gene Targeting Sciences in Okayama

Okayama University The 50th Anniversary Hall
February 15, 2015

Organizers:
Okayama University (President: Prof. Kiyoshi Morita)
Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Dean: Prof. Mitsune Tanimoto)
Okayama University Dental School (Dean: Prof. Takuo Kuboki)

Co-Organizer:
RIKEN, Institute of Physical and Chemical Research

Supporter:
Japan Science and Technology Agency (JST)

International Symposium on Bio-imaging and Gene Targeting Sciences in Okayama

Contents

Date:
Sunday, February 15, 2015

Venue:
The 50th Anniversary Hall, Okayama University
(1-1-1 Tsushima-naka, Kita-ku, Okayama, 700-8530)

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Program Committee:
Satoshi Kubota (Chair)
Toshitaka Oohashi
Hiroshi Kamioka
Takuya Matsumoto

This symposium is supported by a trust fund for Molecular Imaging Research Strategies by MEXT-Japan, titled “Center of excellence for molecular imaging specialists education in Okayama”, and a Grant-in-Aid for the COE projects by MEXT-Japan, titled “Center of excellence for molecular and gene targeting therapies with micro-doze molecular imaging modalities.”

文部科学省科学技術試験研究委託事業
分子イメージング研究戦略推進プログラム

岡山分子イメージング高度専門人材育成事業
総括国際シンポジウム

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【開 催 日】
平成 27 年 2 月 15 日（日）

【会　　場】
岡山大学創立五十周年記念館
（〒700-8530　岡山市北区津島中 1-1-1）

【主　　催】
国立大学法人岡山大学（学長：森田　潔）
岡山大学大学院医歯薬学総合研究科（研究科長：谷本光音）
岡山大学歯学部（学部長：窪木拓男）

【共　　催】
独立行政法人理化学研究所

【後　　援】
独立行政法人科学技術振興機構

【実行委員】
久保田聡（委員長）
大橋俊孝
上岡　寛
松本卓也

このシンポジウムは，文部科学省科学技術試験研究委託事業　分子イメージング研究戦略推進プログラム「岡山分子イメージング高度専門人材育成事業」, および, 文部科学省概算要求特別経費（プロジェクト分）－国際的に卓越した教育研究拠点機能の充実－「分子イメージング・マイクロドーズ（第０相）臨床試験体制を擁する分子標的治療研究・教育拠点の構築－（独）理化学研究所との連携による教育研究基盤の確立－」事業の一環として開催されます.

Welcome to the International Symposium on Bio-imaging and Gene Targeting Sciences in Okayama, announcing our current achievement and future prospects on life sciences

Prof. Mitsune Tanimoto

Dean
Okayama University Graduate School of Medicine,
Dentistry and Pharmaceutical Sciences

Prof. Takuo Kuboki

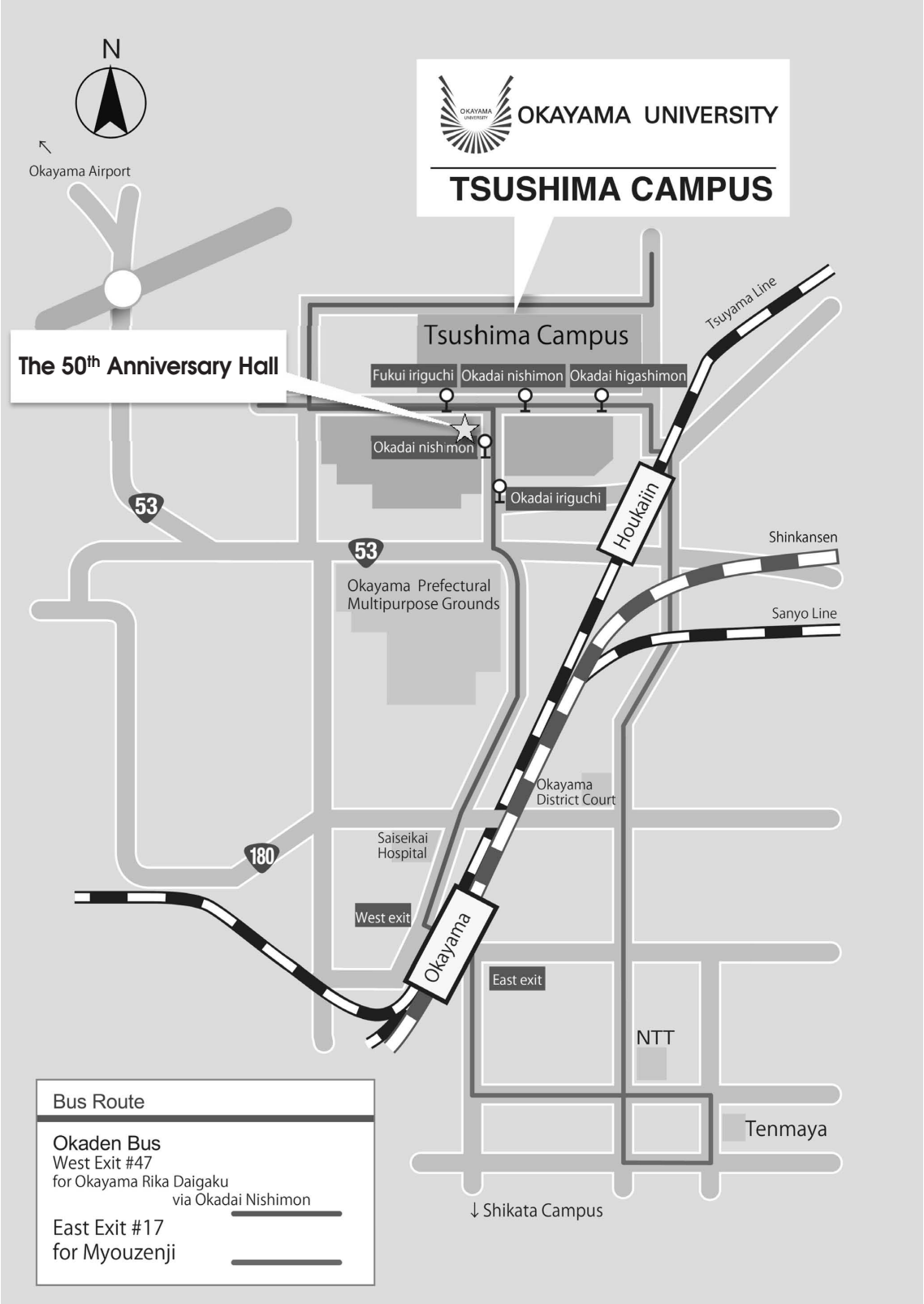
Dean
Okayama University Dental School

On behalf of the organizer and program committee, we hereby sincerely welcome you to our Okayama University. Based on the long tradition as a medical school, Okayama University graduate School of Medicine, Dentistry and Pharmaceutical Sciences was born in 2005. Since then, this Graduate School has fostered advanced medical care and advanced research, which plays an active role not only in Chugoku-Shikoku region, but also in Japan towards the world.

Under the support of the Ministry of Education, Culture, Sports, Science and Technology-Japan (MEXT), Okayama University has developed an educational project entitled the Program for the Development of Highly Specialized Professionals on Bio-Imaging Medicine since 2011. In order to conclude this grand project, the final symposium is going to be held under the title of International Symposium on Bio-imaging and Gene Targeting Sciences in Okayama in February, 2015. Key note lectures will be given by Dr. Takanori Saido, Senior Team Leader in RIKEN Brain Science Institute and Prof. Eiji Matsuura, Vice Director of OMIC in Okayama University. In these lectures, current knowledge on the aging process of neurons leading to Alzheimer's disease and novel therapeutic strategies against atherosclerosis and cancers, which are being explored in our OMIC projects will be presented. Furthermore, 3 symposium sessions will introduce research frontiers that critically represent the direction for our future life sciences through interdisciplinary research collaborations. In these sessions, leading scientists in the world will be invited and will present hot topics on Nuclear Architecture Imaging Nano-bioengineering; and Bone and Cartilage Molecular Imaging, followed by active scientific discussion. We hope this symposium is enjoyable, and fruitful for all of the attendees in exploring the life sciences for the next generation.

Location

The 50th Anniversary Hall, Okayama University
(1-1-1 Tsushima-naka, Kita-ku, Okayama, 700-8530)



The 50th Anniversary Hall Layout

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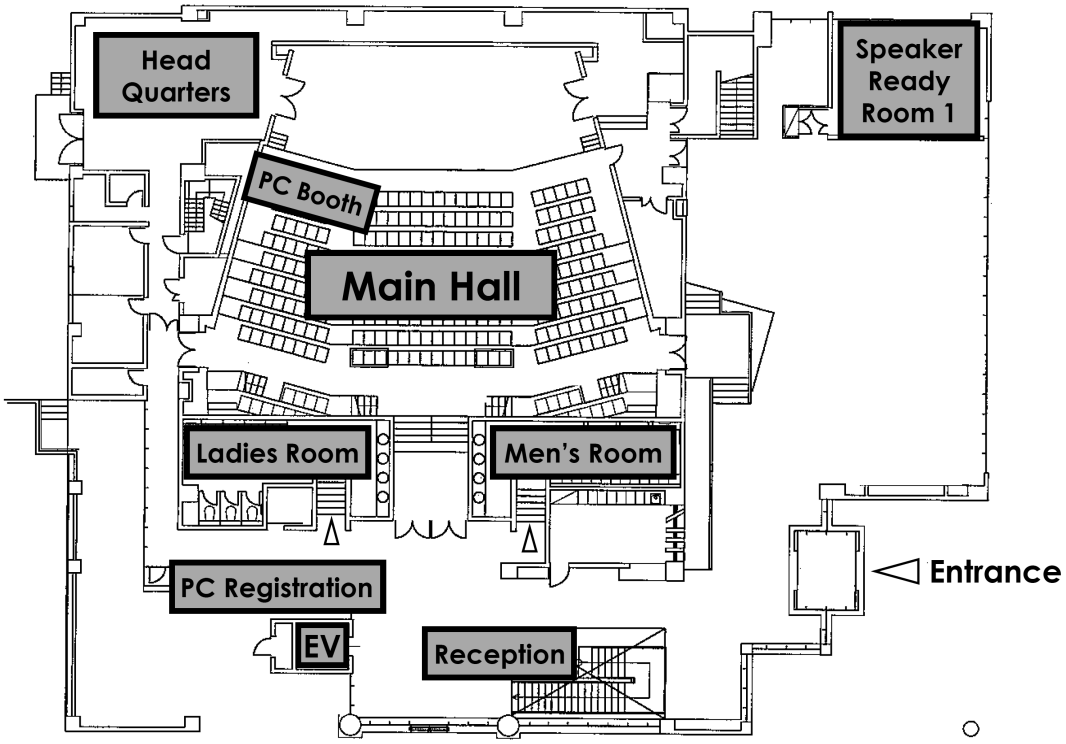

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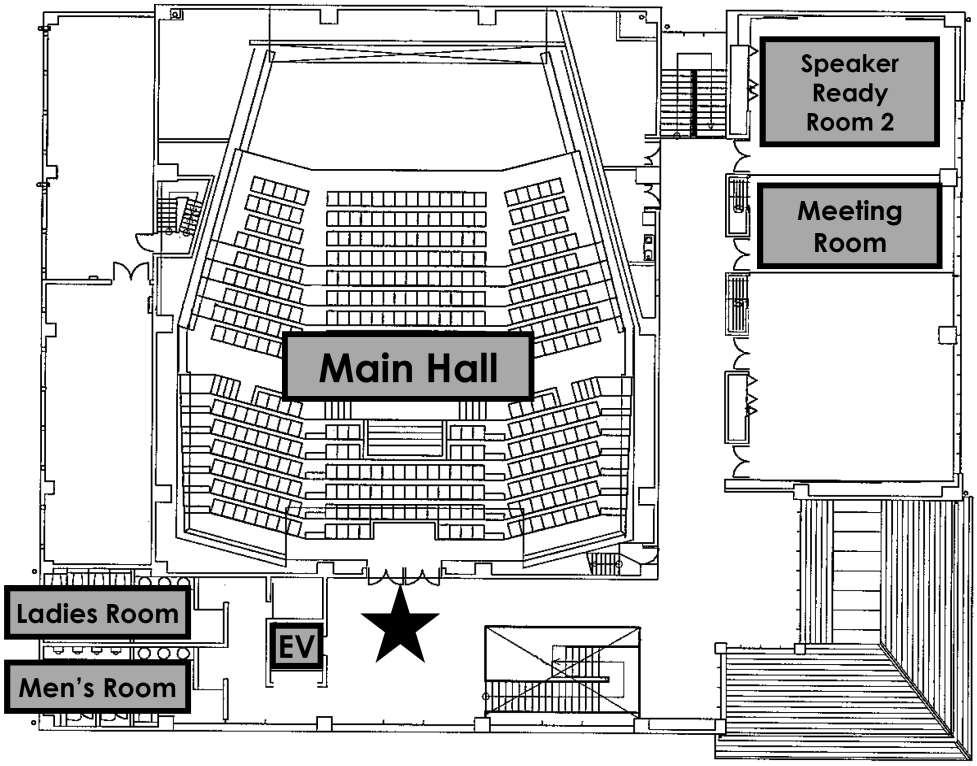

*Complementary lunch boxes will be served in the 2nd-floor hallway (★).

Time Table

		Main Hall
8:00 AM		
9:00 AM	9:00	**Opening Remarks**
	9:10	**Special Lecture 1**
10:00 AM	9:50	**Special Lecture 2**
	10:30	**Break**
	10:40	**Symposium** -Session 1-
11:00 AM		
12:00 PM		
	12:10	**Break**
	12:20	**Luncheon Seminar**
	12:50	**Break**
1:00 PM	1:00	**Symposium** -Session 2-
2:00 PM		
	2:30	**Break**
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3:00 PM		
4:00 PM		
	4:45	**Closing Remarks**
5:00 PM		

Program

Sunday, February 15, 2015

9:00 - 9:10 a.m. Opening Remarks

9:10 - 10:30 a.m. Special Lectures

Special Lecture 1 *Chair: Prof. Hideki Matsui (Okayama University)*

"Stop Preclinical Alzheimer's Disease!"

Dr. Takaomi C. Saido (RIKEN Brain Science Institute) ····· p.10

Special Lecture 2 *Chair: Prof. Takuo Kuboki (Okayama University)*

"Okayama Medical Innovation Center (OMIC) and Molecular Targeting Technology"

Dr. Eiji Matsuura (OMIC, Okayama University) ····· p.11

10:30 a.m. Break

10:40 a.m. - 12:10 p.m.

Symposium -Session 1- *Chair: Prof. Satoshi Kubota (Okayama University)*

Characterization of Cellular Status by Nuclear Architecture Imaging

1. **"Mechanisms of Heterochromatin Positioning in the Nucleus"**

   Dr. Irina Solovei (Ludwig Maximilians University of Münich (LMU)) ····· p.16

2. **"Role of Spatial Positioning of Chromosome Territories: Evolutionary Views and Characteristics in Cancer Cells"**

   Dr. Hideyuki Tanabe (The Graduate University for Advanced Studies (SOKENDAI)) ····· p.18

3. **"Visualization of Dynamics of Methylated DNA in living cell and animal"**

   Dr. Kazuo Yamagata (Osaka University) ····· p.20

12:10 p.m. Break

***Complementary lunch boxes will be served in the 2nd-floor hallway**

12:20 - 12:50 p.m. Luncheon Seminar *Chair: Prof. Masaharu Takigawa (Okayama University)*

"Single Cell Transcriptome Analysis Dissects Cell Fate Determination from iPS Cells to Cardiomyocyte"

Dr. Akira Watanabe (CiRA, Kyoto University) ····· p.14

12:50 p.m. Break

1:00 - 2:30 p.m. Symposium -Session 2- *Chair: Prof. Takuya Matsumoto (Okayama University)*

Current Topics in Nano-Bioengineering

1. **"Imaging and Gene Expression Patterning with Micro- and Nanofluidics"**

   Dr. Shu Takayama (University of Michigan) ····· p.21

2. **"Nanobiomaterials for Diagnosis and Treatment of Vascular Disease"**

   Dr. Hyun Joon Kong (University of Illinois) ····· p.22

3. **"Genetically-encoded Tools to Optically Control and Image Ca^{2+} Dynamics"**

   Dr. Takeharu Nagai (Osaka University) ····· p.23

2:30 - 2:45 p.m. Break

2:45 - 4:45 p.m. Symposium -Session 3- *Chair: Prof. Toshitaka Oohashi (Okayama University)*
Prof. Hiroshi Kamioka (Okayama University)

Hot Topics in Bone and Cartilage Imaging

1. **"Details in the Nano-world: Assessing Structure-function Relationship of Cartilage by Atomic Force Microscopy"**

   Dr. Attila Aszodi (Clinical Center University of Münich (LMU)) ····· p.24

2. **"A Feasible Study of Molecular Bio-imaging of Articular Cartilage Proteoglycans"**

   Dr. Toshitaka Oohashi (Okayama University) ····· p.26

3. **"Quantitative Illumination on Bone Histology and Cell Biology by Fluorescence Imaging"**

   Dr. Ji-Won Lee (Ehime University) ····· p.28

4. **"Bioimaging of Osteocytes *in vivo* and *in vitro*"**

   Dr. Hiroshi Kamioka (Okayama University) ····· p.29

4:45 - 5:00 p.m. Closing Remarks

Special Lectures

9:10 - 9:50 a.m. Special Lecture 1 *Chair: Prof. Hideki Matsui (Okayama University)*

Stop Preclinical Alzheimer's Disease!

Dr. Takaomi C. Saido
RIKEN Brain Science Institute

Deposition of amyloid ß peptide (Aß), the primary cause of Alzheimer's disease (AD), in the brain precedes the disease onset approximately by 25 years. This silent state, accompanied by Aß pathology without the major symptoms, is referred to as preclinical AD. Because one 3rd of Japanese population is already over the age of 65, approximately one 3rd of Japanese people are in the state of preclinical AD. To minimize the number of patients and thus of care givers, it is necessary to establish diagnosis/prognosis and prevention of AD in the preclinical state. Based on our achievements on metabolism of Aß and on generation of the most relevant mouse AD models, I will describe the present status and future perspective of AD research.

Academic Career:

2009 – present	Visiting Professor in Japan Women's College
2008 – present	Group Director in RIKEN Brain Science Institute
	Visiting Professor in Waseda University
2006 – present	Visiting Professor in Graduate School of Agricultural and Life Sciences
2005 – present	Visiting Professor in Institute for Frontier Medical Sciences, University of Kyoto
2004 – present	Visiting Professor in University of Nagoya School of Medicine
1999 – 2000	Visiting Professor in University of Tsukuba School of Medicine
1997 – present	Visiting Professor in Tohoku University School of Medicine
1997 – present	Visiting Professor in Yokohama City Medical School
1997 – present	Laboratory Head in RIKEN Brain Science Institute
1992	Visiting Scientist in Scripps Institute
1988 – 1997	Research Scientist in Tokyo Metropolitan Institute of Medical Science

9:50 - 10:30 a.m. Special Lecture 2 *Chair: Prof. Takuo Kuboki (Okayama University)*

Okayama Medical Innovation Center (OMIC) and Molecular Targeting Technology

Dr. Eiji Matsuura

Collaborative Research Center for OMIC, and Department of Cell Chemistry,
Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences

Okayama Medical Innovation Center (OMIC), which has fully equipped molecular imaging research facilities, was established at Okayama University (Medical Campus) as a collaborative research center for industry-academia-government teams in 2009 by the Japanese Science and Technology Agency (JST) with the aim of revitalizing regional industries and started operating since April, 2011. In addition, the MEXT Project for Creation of Research Platforms and Sharing of Advanced Research Infrastructure promotes the joint usage (of OMIC) of advanced research facilities and equipment possessed by universities and independent research institutes for industry, academia, and government organizations (since 2013). The project could contribute to achieving vital issues through technological innovation. The center also assists educational programs on molecular imaging researches at the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, in corporation with Riken (Kobe).

Up to date, we have been promoting several original research programs supported by JST, MEXT, and other governmental grants at the center. In those studies, we are now establishing a novel molecular targeting technology, namely, Theranostics (Thera- Diagnostics), of which concept is that the combination technology simultaneously providing both of “Therapeutic” and “Diagnostic” effects to the patients with cancer, atherosclerotic, and other diseases. For the technology, we can utilize humanized and a shorten antibody variant (single chain Fv; scFv) that is labeled with ^{64}Cu or ^{89}Zr (a novel PET nuclide), and a drug delivery system (DDS) consisted of novel bio-degradable polymers.

In the lecture, I would like to introduce the OMIC molecular imaging center and to overview our ongoing research projects.

Academic Career:

2011 – present	Professor, Collaborative Research Center for OMIC, and Department of Cell Chemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
2004 – 2011	Associate Professor, Department of Cell Chemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
2001 – 2004	Associate Professor, Department of Cell Chemistry, Okayama University Graduate School of Medicine and Dentistry
1997 – 2001	Assistant Professor, Department of Cell Chemistry, Institute of Cellular and Molecular Biology, Okayama University Medical School
1995 – 1997	Research Associate, Department of Biochemistry, Hokkaido University School of Medicine
1988 – 1995	Senior Research Scientist, Diagnostics Division, Yamasa Corporation
1986 – 1988	Research Associate, Department of Pediatrics/Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO, USA
1984 – 1986	Research Associate, Department of Immunochemistry, Faculty of Pharmaceutical Science, University of Okayama

Luncheon Seminar

12:20 - 12:50 p.m. Luncheon Seminar *Chair: Prof. Masaharu Takigawa (Okayama University)*

Single Cell Transcriptome Analysis Dissects Cell Fate Determination from iPS Cells to Cardiomyocyte

Dr. Akira Watanabe

Core Facility of Genome and Epigenome Analysis, Center for iPS Cell Research and Application (CiRA), Kyoto University

During human development, a single-cell fertilized egg generates hundreds of different types of the cell. This cell fate specification is finely regulated by epigenetic mechanism. However, there is little understanding of the mechanisms by which a particular gene network at a branch of the cell differentiation defines direction to specific cell type. For the clinical application, it is also important to generate a specific cell type with a high efficiency, by precise control of differentiation. Continuous supply of cardiomyocyte is required for cell-based therapy and drug screening to evaluate cardiotoxicity. We aim to understand the detailed dynamics of transcription during differentiation from induced pluripotent stem (iPS) cells to cardiomyocyte and increase the efficiency of cardiac differentiation.

We performed single cell RNA-seq using iPS and cardiomyocyte-directed cells. We differentiated human iPS cells into cardiomyocyte by changing the growth factors at each time point, and harvested the cells at day 1, 3, 5, 7, 9, 21 and 30 after induction of directed differentiation. Twenty-four of the singlet cells harvested at each time point were then applied into C1 Single Cell Auto-Prep System for cDNA synthesis, followed by library preparation using NexteraXT DNA Sample Prep Kit (Illumina). Massively parallel sequencing was performed by HiSeq2500, and sequencing data was processed by mapping by Tophat and calculating relative expression value RPKMforgenes. Principle component analysis showed heterogeneity of gene expression in each day point, and enabled data to sort samples into differentiation status. Our newly developed method modified from Weighted Correlation Network Analysis (WGCNA) identified core gene expression modules of differentiated and iPS cells. In addition, we could detect several groups of genes whose expression were dynamically changed during the differentiation process. We propose time-information-free analysis as a powerful approach for unveiling the dynamics of transcriptome in reprograming and differentiation.

Academic Career:

2009 – present	Assistant Professor in the Core Facility of Genome and Epigenome Analysis, Center for iPS Cell Research and Application (CiRA), Kyoto University
2003 – 2009	Postdoctoral fellow in Research Center for Advanced Science and Technology, the University of Tokyo

Symposium

10:40 a.m. - 12:10 p.m. Symposium -Session 1- *Chair: Prof. Satoshi Kubota (Okayama University)*

Characterization of Cellular Status by Nuclear Architecture Imaging

1. Mechanisms of Heterochromatin Positioning in the Nucleus

Dr. Irina Solovei
Human Biology and Bioimaging group,
Ludwig Maximilians University of Münich (LMU)

Spatial segregation of transcriptionally active euchromatin and silent heterochromatin is an important factor regulating nuclear functions. Majority of the eukaryotic nuclei have conventional architecture with transcriptionally active euchromatin residing in the nuclear interior and heterochromatin abutting the nuclear periphery and the nucleolus. Recently we found a unique exception from the above rule, nuclei of rod photoreceptor cells of nocturnal mammals. For optical reasons, heterochromatin is concentrated in the center of these nuclei whereas euchromatin lines the nuclear periphery, thereby forming an inverted nuclear organization in comparison to conventional nuclei. In both conventional and inverted nuclei, chromosomes acquire a complex folded structure which adapts to the shape of the nucleus and secures correct intranuclear positioning of eu- and heterochromatin regions.

To elucidate possible mechanisms of establishing of inverted versus conventional nuclear architecture, we carried out a detailed study of epigenetic landscape in both nuclear types. We showed that major epigenetic factors associated with eu- or heterochromatin remain similar in conventional and inverted nuclei. Moreover, depletion of methylation code writers (e.g., Suv3-9, Suv4-20, G9a) or readers (e.g., MECP2) does not affect global nuclear architecture in both cases.

Next we analyzed spatial arrangement of heterochromatin in tissues from wild type and mice with mutations in the lamin B receptor (Lbr) and lamin A/C (Lmna) genes. We identified two mechanisms tethering peripheral heterochromatin to the nuclear envelope, an LBR-dependent and lamin A/C-dependent, which are sequentially used at early and late stages of differentiation, respectively. Tethers have opposite effects on the expression of tissue-specific genes: selective disruption of lamin A/C downregulates whereas absence of LBR upregulates muscle gene expression. Importantly, the absence of both LBR and LA/C leads to loss of peripheral heterochromatin and inversion of nuclear architecture with heterochromatin localizing to the nuclear interior in non-rod cells.

Taken together, our data suggest that the major epigenetic factors do not play a crucial role in the choice between inverted and conventional nuclear architecture. Conventional mammalian nuclei relay on strong redundancy of epigenetic code itself and its writers, whereas the inversion in rods relies on absence of specific readers, LBR- and lamin A/C-dependent peripheral heterochromatin tethers.

Academic Career:

2009 – present	Principle Investigator in Ludwig Maximilians University of Munich (LMU), Human Biology and Bioimaging group
1996 – 2009	Senior Research Scientist in Ludwig Maximilians University of Munich (LMU), laboratory of Prof.T.Cremer
1990 – 1996	Senior Research Scientist in the Biological Research Institute of the St-Petersburg University and Wellcome Trust Fellow in University of Leicester (UK), laboratory of Prof.H.Macgregor
1984 – 1990	Research Scientist in the Biological Research Institute of the St-Petersburg University
1984	Doctor Degree of Biological Science (PhD) from Department of Cell Biology and Histology, Biological Faculty, University of St-Petersburg

10:40 a.m. - 12:10 p.m. Symposium -Session 1- *Chair: Prof. Satoshi Kubota (Okayama University)*

Characterization of Cellular Status by Nuclear Architecture Imaging

2. Role of Spatial Positioning of Chromosome Territories: Evolutionary Views and Characteristics in Cancer Cells

Dr. Hideyuki Tanabe

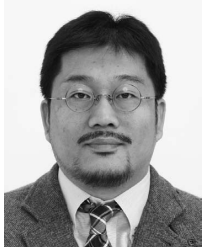
Department of Evolutionary Studies of Biosystems, School of Advanced Sciences,
The Graduate University for Advanced Studies (SOKENDAI)

Chromosomes are discretely, highly compartmentalized within the cell nucleus in eukaryotes forming so-called “chromosome territories (CTs)”. It has been studied for nearly two decades, firstly in humans, other mammals, and chickens by utilizing 3D-FISH techniques. How do CTs occupy the cell nucleus? What kind of regulations can be applied to arrange their positioning? From previous studies for this decade, it has been revealed that radial positioning of CTs from center to nuclear rim has the following characteristics. 1) Radial positioning of CTs depends on the physical size and gene density of each CT; larger gene-poor CTs are located toward periphery and smaller gene-dense CTs are located into interior of the nucleus. For example, human 18 and 19 CTs are gene poor and gene dense, respectively, which localize in periphery or in interior regions discretely in human lymphocytes. 2) Evolutionarily syntenic regions of CTs are inclined to localize the same radial positioning among species. It has been demonstrated that the topology of syntenic CTs with human 18 and 19 chromosomes is evolutionarily conserved in chickens as well as in primates. Especially, gibbon syntenic CTs with human 18 and 19 chromosomes are divided into several pieces but their radial positioning is highly conserved. 3) Radial positioning of CTs depends on the region of Lamin Associated Domains (LADs); LADs are located near the nuclear rim, which are tethering mainly gene-poor chromosomal regions corresponding to G/C-bands roughly consisting of heterochromatin. 4) Actin related protein 6 (Arp6), which is one of the ubiquitous components of chromatin remodeling complexes conserved from yeast to human, has affected to global nuclear radial distribution of CTs. Arp6-knock out chicken DT40 cells have shown disturbed global nuclear architecture. 5) Cancer cells have shown almost same characteristics with normal cells, however, in glioblastoma cells radial positioning of CTs has been disturbed intensely revealed by peripheral versus interior localizing combined CTs as probes for 3D-FISH techniques.

Collectively, spatial radial positioning of CTs could be affected strongly with physical properties such as gene density, LADs association, and Arp6 related mechanisms, whereas less affected with the status of gene expression or epigenetic factors. Organization of nuclear architecture from evolutionary views will be discussed.

Academic Career:

2006 – present	Associate Professor in Department of Evolutionary Studies of Biosystems, School of Advanced Sciences, The Graduate University for Advanced Studies (SOKENDAI)
2003 – 2006	Associate Professor in Department of Biosystems, School of Advanced Sciences, The Graduate University for Advanced Studies (Sokendai)
2001 – 2003	Senior Staff Scientist in Division of Genetics and Mutagenesis, National Institute of Health Sciences
1999 – 2001	Long Overseas Scientist (STA fellow): Prof. Thomas Cremer's laboratory, Institute of Anthropology and Human Genetics, Ludwig Maximilians University, Münich, Germany
1998	Doctor Degree of Science (Thesis Doctor) from Graduate School of Science, Hokkaido University
1993 – 1998	Staff Scientist: JCRB Cell bank, Division of Genetics and Mutagenesis, National Institute of Health Sciences
1991	Master Degree of Science from Graduate School of Science, The University of Tokyo
1989	Graduation from Department of Anthropology, Faculty of Science, The University of Tokyo



10:40 a.m. - 12:10 p.m. Symposium -Session 1- *Chair: Prof. Satoshi Kubota (Okayama University)*

Characterization of Cellular Status by Nuclear Architecture Imaging

3. Visualization of Dynamics of Methylated DNA in living cell and animal

Dr. Kazuo Yamagata
Research Institute for Microbial Diseases,
Osaka University

In mammals, DNA is methylated at CpG sites, which play pivotal roles in gene silencing and chromatin organization. Furthermore, DNA methylation undergoes dynamic changes during development, differentiation, and in pathological processes. The conventional methods represent snapshots; therefore, the dynamics of this marker within living organisms remains unclear. To track this dynamics, we made a knockin mouse that expresses a red fluorescent protein (RFP)-fused methyl-CpG-binding domain (MBD) protein from the ROSA26 locus ubiquitously; we named it MethylRO (methylation probe in ROSA26 locus). Using this mouse, we performed RFP-mediated methylated DNA immunoprecipitation sequencing (MeDIP-seq), whole-body section analysis, and live-cell imaging. We discovered that mobility and pattern of heterochromatin as well as DNA methylation signal intensity inside the nuclei can be markers for cellular differentiation status. Thus, the MethylRO mouse represents a powerful bioresource and technique for DNA methylation dynamics studies in developmental biology, stem cell biology, as well as in disease states.

Academic Career:

2010 – present	Associate professor in Research Institute for Microbial Diseases, Osaka University
2007 – 2010	Research scientist in RIKEN Center for Developmental Biology
2003 – 2007	Assistant professor in Graduate School of Life and Environmental Sciences, University of Tsukuba
2000 – 2002	JSPS postdoctoral research fellow at Genome Information Research Center, Osaka University
2000	Ph.D. from University of Tsukuba Graduate School of Agriculture

1:00 - 2:30 p.m. Symposium -Session 2- *Chair: Prof. Takuya Matsumoto (Okayama University)*

Current Topics in Nano-bioengineering

1. Imaging and Gene Expression Patterning with Micro- and Nanofluidics

Dr. Shu Takayama
Biomed Eng and Macromolecular Science & Engineering,
University of Michigan

This presentation will present work from our laboratory that combines micro- and nanofluidics with imaging and gene expression. The specific nanofluidic technology to be described include use of fracture-fabricated tunable nanochannels for single chromatin fiber linearization and multi-color imaging of histone modifications along the fiber. In microfluidic topics, I will describe efforts in our lab in studying GPCR signaling in response to computer-controlled microfluidic pulsed stimulation. Live cell imaging of cells with genetically engineered protein reporters and analysis of the cell response with computer models of the signaling pathway enable non-invasive dissection of cell signaling pathways. We also demonstrate that there is an optimal stimulation frequency at which transcription factor activation is maximized and is larger than can be obtained with continuous stimulation. Finally, use of aqueous two phase system micropatterning to perform patterned gene expression and knockdown of genes in select regions of a monolayer of cells with be described. Time permitting some of our latest results in microfluidic *in vitro* fertilization studies will be presented.

Academic Career:

2013 – present	Associate Director in Michigan Center for Integrative Research in Critical Care
2013 – present	Director in NIH Microfluidics in Biomedical Sciences Training Program
2010 – present	Professor, Biomed Eng and Macromolecular Science & Engineering, U. of Michigan
2010 – present	WCU (till 2013) then Adjunct (from 2013) Professor, UNIST, Korea
2010 – 2014	Associate Chair for Translational Research, Biomed Eng Dept, U. of Michigan
2006 – 2010	Associate Professor, Biomed Eng and Macromol Sci. & Eng., U. of M.
2000 – 2005	Assist Professor, Biomed Eng and Macromol Sci. & Eng., U. of M.
1998 – 2000	Leukemia and Lymphoma Society Postdoctoral Fellow, Harvard University

1:00 - 2:30 p.m. Symposium -Session 2- *Chair: Prof. Takuya Matsumoto (Okayama University)*

Current Topics in Nano-bioengineering

2. Nanobiomaterials for Diagnosis and Treatment of Vascular Disease

Dr. Hyun Joon Kong

Departments of Chemical and Biomolecular Engineering, Bioengineering, Pathobiology, Center for Biophysics, University of Illinois at Urbana-Champaign

The human body is highly vascularized to transport oxygen, nutrients, and hormones to and from cells residing in tissue and organ systems. A variety of intrinsic and extrinsic factors can cause occlusion, leakage, or rupture of vasculature, thus leading to tissue ischemia, necrosis, and ultimately death. To date, extensive efforts have been made to detect and treat these vascular diseases with diverse bioactive molecules, imaging probes, and stem cells. These diagnostic and therapeutic modalities are often integrated with various engineering technologies to further improve their performance. In order to advance these efforts, we have developed several implantable nanobiomaterial systems to elevate the quality of vascular imaging, repair and regeneration by integrating materials chemistry and characterization with biotransport phenomena. In this talk, I will highlight a few of the diagnostic and treatment tools we have developed, including (1) a gadolinium-coated nanoparticle designed to enhance the quality of magnetic resonance imaging (MRI) of ischemic tissue (Fig. 1a), (2) nanomaterials for delivery of stem cells to inflamed blood vessels (Fig. 1b), (3) a “living” microvascular stamp to control the organization of blood vessel during regeneration (Fig. 1c), and (4) a self-folding hydrogel to modulate vascular drug release (Fig. 1d).

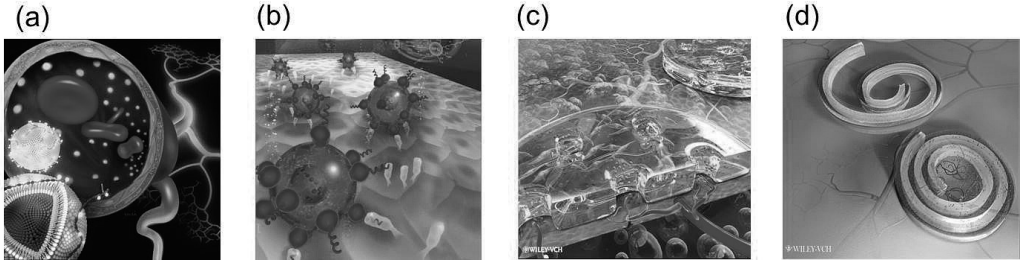


Fig.1 Materials for imaging and treatment of vascular diseases

Academic Career:

2013 – present	Associate Professor, Departments of Chemical and Biomolecular Engineering, Bioengineering, Pathobiology, Center for Biophysics, University of Illinois at Urbana-Champaign
2007 – 2013	Assistant Professor, Departments of Chemical and Biomolecular Engineering, Bioengineering, Pathobiology, Center for Biophysics, University of Illinois at Urbana-Champaign
2004 – 2006	Research Associate, School of Eng. and Applied Sci. (Bioengineering) Harvard University
2001 – 2004	Post-Doctoral Research Fellow, Dept. of Biological and Materials Sci. Univ. of Michigan
1997 – 2001	Research Assistant, Dept. of Chemical Eng. & Civil & Environmental Eng. Univ. of Michigan

1:00 - 2:30 p.m. Symposium -Session 2- *Chair: Prof. Takuya Matsumoto (Okayama University)*

Current Topics in Nano-bioengineering

3. Genetically-encoded Tools to Optically Control and Image Ca^{2+} Dynamics

Dr. Takeharu Nagai
The Institute of Scientific and Industrial Research,
Osaka University

In living organism, Ca^{2+} is one of the most versatile second messenger to control biological processes such as muscle contraction, hormonal secretion and apoptosis induction. Its spatial and temporal dynamics has key roles to regulate these physiological phenomena. To reveal such dynamics, variety of Ca^{2+} indicators had been developed. They enabled noninvasive visualization of Ca^{2+} dynamics, provided meaningful information for research in wide range of biological field. However, for deeper understanding of relationship between the spatiotemporal Ca^{2+} dynamics and the following response, development of tools to manipulate intracellular Ca^{2+} level have been desired. In current methods, Ca^{2+} concentration can be controlled by light through Ca^{2+} binding compounds with photocleavable moieties. However, they require irradiation of toxic ultraviolet wavelength light and/or cell loading associated with disruption of the cell membrane. These properties which have possibility to impair cells become big problem especially in the case of *in vivo* measurement. In addition to this, Ca^{2+} release from such compounds is irreversible. To overcome this, we developed a genetically-encoded photoactivatable Ca^{2+} releaser called PACR (PhotoActivatable Ca^{2+} Releaser). That is composed of Ca^{2+} binding protein and light-sensitive protein. Affinity of PACR for Ca^{2+} was decreased during irradiation of blue light. Thus reversible and repeatable increasing of Ca^{2+} concentration in cell is possible without damage to living specimens. By using PACR, we succeeded nucleus specific temporal Ca^{2+} increase in HeLa cells and excitation of specific neuron in freely moving C. elegans by blue light irradiation. This useful tool is expected to contribute on researches to reveal the role of Ca^{2+} dynamics in complex biological phenomena. In addition to this manipulation tool, I would like to introduce color variants of super-duper luminescent protein that we developed recently, which can be used compatibly with optogenetic actuators.

Academic Career:

2014 – present	Vice Director, The Institute of Scientific and Industrial Research, Osaka University
2012 – present	Visiting senior chief researcher, Quantitative Biology Center, RIKEN
2012 – present	Professor, The Institute of Scientific and Industrial Research, Osaka University
2008 – 2014	Researcher, PRESTO, JST
2005 – 2012	Professor, Research Institute for Electronic Science, Hokkaido University
2001 – 2005	Researcher, PRESTO, JST
1998 – 2001	Researcher, RIKEN
1995 – 1998	Research fellow, JSPS

2:45 - 4:45 p.m. Symposium -Session 3- *Chair: Prof. Toshitaka Oohashi (Okayama University)*
Prof. Hiroshi Kamioka (Okayama University)

Hot Topics in Bone and Cartilage Imaging

1. Details in the Nano-world: Assessing Structure-function Relationship of Cartilage by Atomic Force Microscopy

Dr. Attila Aszodi

Experimental Surgery and Regenerative Medicine, Department of Surgery,
Clinical Center University of Münich (LMU)

The performance of most tissues in vertebrates crucially depends on their structural-mechanical properties, which is determined by matrix-matrix, cell-matrix and cell-cell interactions. Connective tissues of the skeletal system accommodate for recurring mechanical stress by building up hierarchical macromolecular structures that provide biomechanical stability. Cartilage is a macromolecular fiber (collagen)/gel (proteoglycan) composite material which withstands compressive, shear and tensile forces. The growth plate (GP) and the articular cartilage (AC) are specialized structures which drives the longitudinal elongation of the skeletal elements and dissipate load in joints, respectively. Mutations in genes coding for extracellular matrix proteins and their receptors often affect the organization of these structures leading to skeletal pathologies such as chondrodysplasia and osteoarthritis.

Atomic force microscopy (AFM) offers a unique opportunity to simultaneously assess the structural and mechanical properties of biological tissues in their physiological environment. AFM operated either on the native surface or cryo-sections of cartilage provides a non-destructive way for quantification of morphological and mechanical data at the nanometer scale. Recently, we have applied AFM-based imaging and nano-indentation (IT) techniques to characterize structural and biomechanical properties of the developing, normal and diseased mouse cartilaginous tissues. Our data implicates that nanoscale IT-AFM is a sensitive tool to monitor structural and functional properties of the mouse GP and AC in growing endochondral bones and to distinguish between healthy and diseased cartilage. AFM-imaging is capable to capture overview and ultrastructural details on tissue slices. IT-AFM measurements enable the analysis of matrix components such as collagen II fibrils or the proteoglycan moiety. The combination of AFM imaging and indentation measurements demonstrates that a decrease in fibril density is accompanied by reduced nano-stiffness of the proteoglycan gel likely due to lower osmotic swelling pressure exerted by the composite material. Thus, the structural architecture can be directly correlated with the compressive stiffness of various matrix compartments at the level of collagen fibrils and proteoglycans. IT-AFM measurements of chondrodysplastic mutant mouse strains suggest that the main cartilage matrix protein collagen II and its ß1 integrin receptors have a pivotal role in the control of morphogenesis and in the regulation of the mechanical properties of the GP. The dramatic reduction of the elastic modulus in Col2a1-null GP cartilage evidences that collagen II is the major protein which determines the compressive stiffness of the cartilage. ß1 integrins, besides playing a key role

for proliferative chondrocytes to adopt their elongated shape and columnar arrangement, also modulate cartilage biomechanics likely via the control of collagen matrix assembly.

Academic Career:

2011 – present	Chair, Group leader, Experimental Surgery and Regenerative Medicine, Department of Surgery, Clinical Center University of Münich (LMU)
2002 – 2010	Group leader, Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany
1998 – 2001	Assistant professor, Department of Experimental Pathology, Lund University, Lund, Sweden
1995 – 1997	Postdoctoral fellow, Department of Protein Chemistry, Max-Planck Institute for Biochemistry, Martinsried, Germany
1990 – 1994	PhD student, Agricultural Biotechnology Center, Gödöllö, Hungary
1988 – 1989	Scientific co-worker, Institute for Drug Research, Budapest, Hungary

2:45 - 4:45 p.m. Symposium -Session 3- *Chair: Prof. Toshitaka Oohashi (Okayama University)*
Prof. Hiroshi Kamioka (Okayama University)

Hot Topics in Bone and Cartilage Imaging

2. A Feasible Study of Molecular Bio-imaging of Articular Cartilage Proteoglycans

Dr. Toshitaka Oohashi
Department of Molecular Biology and Biochemistry,
Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

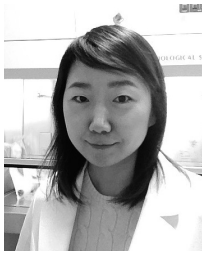
In the process of cartilage degeneration seen in osteoarthritis, loss of proteoglycan from articular cartilage has been widely accepted as a critical early event, followed by collagen degradation designated as a point of no return. Loss of articular cartilage in osteoarthritis is indirectly evaluated by radiography as a joint space narrowing in the most common diagnosis, but early lesions of cartilage damage cannot be detected. Although several negatively charged contrast agents were employed, they provided indirect images of the articular cartilage based on the electrostatic repulsion with anionic glycosaminoglycans (GAGs) in the extracellular matrices (ECMs). Recent advance in the development of targeted molecular probes and new imaging modalities enabled the detection of qualitative and functional change of articular cartilage. In this session, we report the recent progress of bio-molecular imaging of articular cartilage including our molecular probes targeting articular GAGs.

Chondroitin sulfate, a major component of articular cartilage, has negative charge caused by sulfates. Thus we designed lysine oligomers (monomer - pentamer) which were connected with the a-carboxylic acid and e-amino group. These lysine oligomers possess a-amino groups as cationic moieties. NBD was used as a fluorescent group. Triiodobenzene (TIB) was used for a X-ray contrast medium. Estimation of the length between periodic sulfates in chondroitin sulfates using X-ray data predicted that lysine tetramer derivative Kε4-NBD and lysine pentamer derivative Kε5-NBD possess potent affinity to chondroitin sulfates. Evaluation of the affinity of the lysine oligomers to chondroitin sulfate by fluorescence polarization showed that Kε5-NBD possesses most potent affinity to chondroitin sulfates of all. In addition, Kε4 and Kε5-NBD stained articular cartilage ECM efficiently. Then, we created a novel articular cartilage imaging X-ray probe Kε4-TIB and demonstrated an *ex vivo* imaging of articular cartilage in rat osteoarthritic models. The imaging could quantitate loss of proteoglycan from osteoarthritic cartilage.

Kε4-TIB may have a potential of *in vivo* X-ray imaging in animal arthritic models, which contribute to drug discovery research for osteoarthritis.

Academic Career:

2014 – present	Professor, Department of Molecular Biology and Biochemistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University
2005 – 2014	Associate Professor, Department of Molecular Biology and Biochemistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University
1998 – 2005	Lecturer, Department of Molecular Biology and Biochemistry, Okayama University Medical School
1996 – 1997	Research Fellow, in Max-Planck Institute of Biochemistry (Prof. Fässler), JSPS Postdoctoral Fellow for Research in COE Abroad
1992 – 1997	Assistant Professor, Department of Molecular Biology and Biochemistry, Okayama University Medical School (Leave of absent: 1996-1997)



2:45 - 4:45 p.m. Symposium -Session 3- *Chair: Prof. Toshitaka Oohashi (Okayama University)*
Prof. Hiroshi Kamioka (Okayama University)

Hot Topics in Bone and Cartilage Imaging

3. Quantitative Illumination on Bone Histology and Cell Biology by Fluorescence Imaging

Dr. Ji-Won Lee

Division of Bio-Imaging, Proteo-Science Center (PROS),
Ehime University,

Fluorescence microscopy has revealed considerable detail in cellular structures and functions by specific labeling with a high resolution. This fluorescence imaging system is also attractive tool as the complementation of the biochemical “quantitative” analysis and histological “spatial” analysis in the cellular events. In this symposium, we will introduce about our current applications of fluorescent imaging to bone research. We first observed spatially distinct distributions of specific protein from osteocytic lacunae in rat femurs, demonstrating the relative expression levels of these specific proteins exhibited reciprocally reversed patterns in midshaft cortical bone through three-dimensional immunofluorescence morphometry and quantification. These observations guided us to investigate mechanistic study of cellular signal. We next found the dynamic rearrangement of actin cytoskeletal affect to osteoclast functions such as cell locomotion by using real-time imaging and super-resolution microscopy. Our recent findings and several approaches for visualization and quantifying the cellular and molecular signal suggest that fluorescence imaging enables us to distinguish the cellular functions from genetic mutations as well as satisfying with a much better resolution. Quantitative approaches of fluorescence imaging, as introduced here, will provide us further unprecedented insights in bone biology

Academic Career:

2013 – present	Assistant Professor, Division of Bio-Imaging, Proteo-Science Center (PROS), Ehime University,
2011 – 2013	Post-doctoral fellowship (supported by Japan Society for the Promotion of Science), Department of Oral Pathology, Tokyo Medical and Dental University
2009 – 2011	Post-doc fellowship, Research Institute for Biological Functions, Chubu University
2009 – 2010	Visiting Researcher, Section of Molecular Craniofacial Embryology, Tokyo Medical and Dental University
2008 – 2009	Visiting Researcher, Institute for Oral Science, Matsumoto Dental University
2008	Visiting lecturer, Institute for Oral Science, Matsumoto Dental University
2007 – 2008	Lecturer, Department of Food Science and Technology, Keimyung University – Microbiology
2006 – 2007	Lecturer, Department of Food Science and Technology, Daegu University – Microbiology
2005 – 2007	Lecturer, Department of Food Science and Technology, YoungNam College – Biochemistry, Food Processing and preservation
2002 – 2008	Researcher, TMR (Traditional Microorganism Research) center, Keimyung University

2:45 - 4:45 p.m. Symposium -Session 3- *Chair: Prof. Toshitaka Oohashi (Okayama University)*
Prof. Hiroshi Kamioka (Okayama University)

Hot Topics in Bone and Cartilage Imaging

4. Bioimaging of Osteocytes *in vivo* and *in vitro*

Dr. Hiroshi Kamioka
Department of Orthodontics,
Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

In a variety of scientific fields, it is a worthwhile topic to visualize natural phenomenon. Newly developed visualizing method often leads breakthrough in the scientific fields. Especially, in the biological field, it is significant to reveal temporal-spatial response happened in the cells with visualizing molecular level phenomenon. Such visualization could provide information to understand cellular behavior to their extracellular stimulus *in vivo* and *in vitro*. Although osteocytes are the most abundant cells in bone, it has been difficult to study their biological feature because they are embedded in hard bone tissue. So, even the real 3D structure of the osteocyte was not uncovered till lately. On the other hand, newly developed technique of visualization was recently introduced in the bone cell biology. In this presentation, we will introduce our application of confocal laser scanning microscopy to visualize osteocyte morphology in bone, calcium imaging of osteocyte *in vivo*/*in vitro* to show real time response of osteocytes, and the combination of ultra-high voltage electron microscopy and computer simulation of fluid flow to reveal mechanosensitivity of osteocytes in bone.

Academic Career:

2014 – present	Professor in Dept. of Orthodontics, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University
2011 – 2015	Visiting Lecturer in Dept. of Biomechanics, Research Center for Nano Medical Engineering, Institute for Frontier Medical Sciences, Kyoto University
2005 – 2013	Associate Professor in Dept. of Orthodontics, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University
1999 – 2005	Lecturer in Dept. of Orthodontics, Okayama University Hospital
(1995 – 1998)	Post-doctral fellow in Dept. of Anatomy, Indiana University Medical School
1993 – 1999	Assistant Professor in Dept. of Orthodontics, Tokushima University Dental School

Note

文部科学省科学技術試験研究委託事業 分子イメージング研究戦略推進プログラム
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総括国際シンポジウム

International Symposium on Bio-imaging and Gene Targeting Sciences in Okayama