3. Molecular Imaging Center

Yasuhisa Fujibayashi, Ph.D., D.Med.Sc.
Director, Molecular Imaging Center

Outline of Research Career
Yasuhisa Fujibayashi graduated from the Department of Radiopharmaceutical Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, then started his professional career in 1983 at Kyoto University Hospital as an assistant professor of the Radioisotope Research Laboratory. Ten years later, he moved to the Graduate School of Pharmaceutical Sciences, Kyoto University, as an associate professor of Genetic Biochemistry. In 1999, he became Professor of Molecular Imaging at the Biomedical Imaging Research Center, University of Fukui (former Fukui Medical University), and then Director of the Center. In 2010, he joined NIRS in his present position and he continues his research career.

Objectives
The question “What is Life?” has been a universal issue. One of the most famous attempts to answer the question is in the book entitled What is Life? by Erwin Schrödinger (1944), in which he argues that life is based on the interactions between molecules. Recent progress in molecular and cellular biology has in principle clarified this point. However, it is still difficult to observe the behavior and role of a single molecule in living systems.

Molecular imaging (MI) is a relatively new research field created to investigate and visualize molecular and cellular processes in biological systems. The field is based around various imaging technologies such as positron emission tomography (PET), magnetic resonance imaging (MRI), and optical imaging. It is anticipated that combining these technologies will bring new insights into what life is, because each modality has different characteristics in terms of sensitivity, time and spatial resolution and what type of subjects it can be applied to.

The Molecular Imaging Center at the National Institute of Radiological Sciences (MIC-NIRS) is funded by the Japanese Government as the “Japan Advanced MI Research Program (J-AMP). We are working on a wide range of projects from basic to clinical research, and our main target is to become a core center promoting translational research in the MI community and related areas.

Overview
At MIC-NIRS, there are four research groups, namely the Diagnostic Imaging Group, Molecular Neuroimaging Group, Molecular Probe Group, and Biophysics Group. Under the supervision of the Board of Executive Directors, the Planning and Promotion Unit works to support these groups.
The Molecular Neuroimaging Group is directed toward understanding the neurobiology of neuropsychiatric disorders such as schizophrenia, depression and Alzheimer’s diseases, and identifying optimal treatments of these disorders. Clinical and basic approaches are integrated using in vivo and in vitro imaging technologies. This program aims to identify diagnostic molecular markers for neuropsychiatric disorders, leading to drug discovery and novel therapeutic treatments.

To quantify the neurotransmission function, optimal quantification methods and the PET scanning protocol for various PET ligands in the human brain were established. Based on them, the normal database of neurotransmission function and higher brain function was constructed. Using these data, focuses of emotion in the brain could be visualized in combination with PET and MRI. It was also clarified that schizophrenia patients showed increased dopamine synthesis in the striatum and activation of microglia in the brain.

In basis studies, comparative PET and autoradiographic imaging of mice and humans indicated the importance of disease animal models for understanding molecular pathophysiology of neuropsychiatric disorders. Using similar techniques, the mechanism of motivation and its dysfunction were studied in primates as well as rats. These results are applied for screening and evaluation methods for therapeutics such as anxiolytic drugs, anti-schizophrenia drugs and so on.

Main tasks of the Molecular Probe Group are the development and routine production of useful PET probes for clinical diagnosis and bio-functional analysis. To achieve these tasks, a versatile automated synthesis system to produce safe PET probes with less radiation exposure to personnel has been developed. Using this system, new labeling techniques are applied to the synthesis of novel PET probes which can quantitatively visualize biological functions. The standard production method for clinical application of molecular PET probes is being established and related technology transfer to other international as well as domestic PET facilities is underway.

In biomarker research, basic structures of radio-labeled probes for the multi-drug resistance protein MRP4, organic anion transporter OAT1, and metabolic glutamate receptor mGluR was clarified. Also the glutathione/GST reox function was visualized in monkey brain using a newly developed F-18-ligand. A new imidazoline-2 receptor probe, 11-C-FTIMD with ultra-high specific radioactivity was successfully synthesized and its improved accumulation in the brain was confirmed. Development and application of new synthesis procedures for C-11-carbamate, urea and other compounds were achieved. As for radionuclide production, targets and an automation system for Zr-89, Br-76 and Tc-99m production were developed.

The Biophysics Group aims at developing instruments and methodologies for quantitative measurements of in vivo molecular functions using PET, MRI and optical imaging. This group consists of four teams, namely the Imaging Physics Team, Image Analysis Team, Biosignal Physiology Team, and Magnetic Resonance Molecular Imaging Team.

PET is a promising methodology for molecular medicine. The Imaging Physics Team proposed Open-PET, a new concept for the design of PET with higher resolution at reasonable sensitivity and cost, and X’tal-Cube, a next-generation DOI (depth of interaction) detector, and developed their prototypes as proof-of-concept. The Image Analysis Team developed algorithms for the compartment model estimation for quantitative PET imaging. Combination of anatomical MRI images with PET images realized quantitative PET image construction with lower noise. An automatic blood sampling system for small animals was developed and commercialized, which allows precise evaluation of pharmacokinetics and pharmacodynamics. The Biosignal Physiology Team has developed new MRI methods for the evaluation of water diffusion, tissue elasticity, tissue oxygen tension, and so on. Among them, the team found that the water diffusion method allowed evaluation of the degenerative brain damages. Using an Alzheimer model mice brain, it was clarified that amyloid deposition decreased blood vessel response. The Magnetic Resonance Molecular Imaging Team developed nano-probes as theragnostic DDSs based on thermo-sensitive liposome. Redox imaging with nitroxide probes using a combination of MRI and ESR was performed. As well, noninvasive evaluation of radiation therapy using Mn-enhanced MRI and high-speed quantitative MRI was successfully performed.

In the Planning and Promotion Unit, the Research Promotion and Administration Section performs fundraising, mounts public relations activities, is responsible for external affairs of collaborative research, and coordinates arrangements associated with intellectual property. The Clinical Research Support Section has clinical research coordinators (CRCs) and doctors at the core and it maintains the support system for clinical research. Each section has experts whose professional backgrounds lie in a variety of fields and they support the activities of the Center.
3.1. Research on Molecular Imaging of Cancer

Tsuneo Saga, M.D., Ph.D.
Director, Diagnostic Imaging Group

Outline of Research Career
Dr. Saga received a Ph.D. from Kyoto University in 1991 for his investigations on cancer targeting of radiolabeled monoclonal antibodies. He continued his research on antibody targeting at the National Institutes of Health (1991-1993) and at Kyoto University (1995-2006). In addition, for the last 9 years, he has been conducting clinical and basic research covering the wide area of cancer imaging. Since 2006, he has been the leader of the Diagnostic Imaging Group at NIRS; this group works to further advance basic and clinical research on molecular imaging of cancers.

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Objectives
The Diagnostic Imaging Group is conducting research on functional cancer imaging by PET and other modalities to clarify the characteristics of an individual cancer such as its malignancy grade and responsiveness to treatment. Although several PET probes are available for clinical studies to characterize cancers, development of new imaging probes is necessary for more comprehensive evaluation of cancers and to further contribute to the management of cancer patients.

The Clinical Diagnosis Team focuses on clinical research of PET for the contribution to the management of cancer patients including those considered for carbon-ion radiotherapy (CIRT) conducted at NIRS. In addition to FDG and $^{11}$C-methionine, we are evaluating newly developed cancer-imaging probes, such as $^{18}$F-FLT, $^{62}$Cu-ATSM and $^{18}$F-FAZA, to determine their clinical usefulness.

The Molecular Diagnosis Team conducts basic molecular imaging research focusing on designing and evaluating imaging probes that capture the changes of biomolecules specifically associated with cancers and other diseases to realize effective non-invasive diagnoses. We also are developing novel in vivo reporter gene imaging systems to facilitate the establishment of new therapies such as gene therapy and regenerative therapy.

The Biomolecule Team focuses on elucidating genetic and molecular events occurring during carcinogenesis, searching for suitable targets of molecular imaging of cancers. By using functional screening of genes and proteome analysis of the blood and tissue samples, we select the genes and proteins specifically expressed in cancers. Through the exploration of cancer targets, we are aiming for the development of novel molecular imaging methods which can depict the characters of each cancer.

Progress of Research in the 2nd Mid-term Plan

1) Clinical studies on cancer imaging using various PET probes
   We conducted clinical PET research studies using $^{18}$F-fluorothymidine (FLT), a marker of cellular proliferation and proved that FLT is a significant prognostic indicator of lung cancer patients receiving CIRT and a useful marker of bone marrow activity. We have also started an initial clinical study of $^{13}$C-thiotimidine (4DST), a novel proliferation marker, to confirm the safety and whole body distribution. Clinical PET studies using hypoxia PET probes, $^{18}$F-FAZA and $^{62}$Cu-ATSM, are ongoing to evaluate whether tumor uptake of these probes can be an indicator of responsiveness to treatment.

2) Exploration of new therapeutic and diagnostic targets of mesothelioma
   In the search for a specific molecular target of mesothelioma, we found that the manganese (Mn) content is increased in various mesothelioma cell lines compared to mesothelial cells, reflecting the Mn-superoxide dismutase (SOD) expression, suggesting biological significance of Mn in mesothelioma formation and/or progression. As Mn is a signal enhancer in magnetic resonance imaging (MRI), we performed Mn-enhanced MRI and succeeded in visualizing small (~1 mm) pleural tumors expressing Mn-SOD.

   To identify a new therapeutic target of mesothelioma, we conducted a large-scale functional screening of mesothelioma cells using siRNAs against 8,589 human genes. Knockdown of 39 genes significantly suppressed mesothelioma cell proliferation, including 7 genes having an anti-apoptotic function, among which COPA was highly expressed in mesothelioma cell lines, but not in a normal
mesothelial cell line. COPA depletion induced apoptosis and suppressed tumor growth, indicating that COPA would be a promising therapeutic target of mesothelioma.

3) Development and application of animal models for researches on preclinical imaging and treatment

We established a subcutaneous and pleural dissemination model of epithelioid and sarcomatoid mesothelioma in mice and compared tumor uptake and PET imaging of three PET tracers, one glucose analog (FDG) and two thymidine analogs (FLT and 4DST). The two thymidine analogs were highly accumulated in epithelioid mesothelioma, while the glucose analog was highly accumulated in sarcomatoid mesothelioma, suggesting that the suitable PET tracer is different depending on the histological subclass of mesothelioma (Fig. 3.1).

Acute rejection remains a major complication after liver transplantation. We conducted FDG-PET in a rat liver transplantation model. FDG uptake significantly increased in liver allografts indicating acute rejection, in which high FDG signals were localized in the peri-portal area showing inflammatory cell infiltration. Furthermore, immunosuppressive treatment induced a marked decrease in hepatic $^{18}$F-FDG uptake. $^{18}$F-FDG-PET would be a promising imaging method for detecting acute rejection and also for monitoring immunosuppressive treatment.

4) Development of antibody and peptide probes for cancer imaging and treatment

Radiolabeled cancer-specific antibodies are powerful tools to visualize cancers. We developed antibody-based PET/SPECT imaging probes for three cancer-related antigens, ERC/mesothelin, c-kit, and EGFR, and succeeded in visualizing xenografted tumors by PET/SPECT. These antibodies can also be applied to molecular-targeted internal radiotherapy by labeling with cytotoxic radionuclides such as $^{90}$Y, and we showed that $^{90}$Y-labeled antibodies suppressed tumor growth in mice.

Neovascularization is important in tumor growth, invasion and metastasis. Integrin $\alpha_\beta_3$, expressed on the surface of endothelial cells of neovasculature and some tumor cells, can be targeted by RGD peptide. In collaboration with Dr. Dumy’s group at Joseph Fourier University, we conducted small animal PET imaging of integrin $\alpha_\beta_3$ overexpressing tumors with $^{64}$Cu-labeled RAFT-c(RGD)$_4$, that has very high specificity and affinity, and succeeded in clear visualization of the tumors with the accumulation well correlated to integrin $\alpha_\beta_3$ expression (Fig. 3.2).

5) Preclinical studies using reporter imaging technique

By using a human Na$^+/I^-$ symporter (hNIS) reporter gene, we evaluated therapeutic effects of a novel angiogenic gene therapy using hepatocyte growth factor (HGF) gene in a rat myocardial infarct model by reporter imaging using $^{99m}$TcO$_4^-$ as a reporter probe. We have also established a colon cancer cell stably expressing hNIS and succeeded in imaging spontaneous liver metastasis after orthotopic transplantation of this cell.

6) Characterization of intratumoral region of high Cu-ATSM accumulation

Cu-ATSM is a PET probe for hypoxic tissues. Studying intratumoral distribution of Cu-ATSM, we found that the regions of high Cu-ATSM accumulation had quite unique characteristics: low vessel density, low proliferation, low pimonidazol accumulation, and high ratio of cancer stem cell marker positive (CSCM+) cells. Treatment of tumor xenograft with $^{64}$Cu-ATSM was more effective than X-ray treatment in decreasing the ratio of CSCM+ cells.
MAJOR PUBLICATIONS


3.2. **Research on Molecular Neuroimaging**

Tetsuya Suhara, M.D., Ph.D.  
*Director, Neuroimaging Group*

**Outline of Research Career**
Dr. Suhara received the Ph.D. from the Jikei University School of Medicine in 1991 for his study of dopamine receptor binding *in vivo*. He began working at NIRS in 1989. From 1992-1993, he studied in the PET group of the Department of Clinical Neuroscience, Karolinska Hospital, Sweden. He has researched brain functional imaging for many years. He has served as a visiting professor at the Department of Neuropsychiatry, Nippon Medical School since 2004, at the Graduate School of Medicine, Yokohama City University since 2006 and the Graduate School of Medicine, Osaka University since 2010.

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**Objectives**

1) Clinical Neuroimaging  
   a) Development of methods to quantify the neurotransmission function  
   b) Construction of the normal database of the neurotransmission function and higher brain function  
   c) Clinical research for elucidation of pathophysiology of neuropsychiatric diseases  
   d) Estimation of drug treatment effect  

2) Molecular Neurobiology  
   a) Application of animal models to R & D of diagnostic and therapeutic indices for dementia  
   b) Mechanistic elucidation of *in vivo* interactions between imaging biomarker probes and their target molecules  
   c) Analysis of dialogs between different neurotransmitter systems with animal models of neuropsychiatric disorders

3) System Neurochemistry  
   a) Brain mechanism of motivation and its dysfunction in primates  
   b) Research studies on an animal model of brain developmental disorders  
   c) Developing a multidisciplinary approach for primate brain function

**Progress of Research (Achievements, Prospects) in the 2nd Mid-term Plan**

1) Clinical Neuroimaging  
   a) Development of methods to quantify the neurotransmission function  
   The optimal quantification methods and PET scanning protocols were established for various radio ligands in healthy human subjects. A new graphic plot analysis was developed which could determine the total distribution volume and nondisplaceable distribution volume independently, and therefore the binding potential.  
   b) Construction of the normal database of the neurotransmission function and higher brain function  
   The normal database of the dopaminergic neural system using several radioligands for dopaminergic functions was constructed. The relation between regional densities of dopamine D1 and D2 receptors and cognitive functions were investigated. The inverted U-shaped relation between prefrontal dopamine D1 receptor and the cognitive function (WCST performance) in normal volunteers was found in healthy subjects. With the functional MRI technique, it was revealed that the emotion of “envy” induced neural activation in the anterior cingulate cortex. Dopamine D1 receptor binding in the amygdala was positively correlated with amygdala signal change in response to fearful faces, but not in dopamine D2 receptor. Dopamine D1 receptors might play a major role in enhancing amygdala response when sensory inputs are affective.  
   c) Clinical research for elucidation of pathophysiology of neuropsychiatric diseases  
   PET studies with [11C]DOPA demonstrated that patients with schizophrenia showed an increase in dopamine synthesis rates (ki) in the striatum. A significant correlation between ki in thalamus and the score of severity of symptoms was also observed. The widespread accumulation of [11C]DAA1106 was observed in the brain of patients with Alzheimer’s disease, indicating the expression of PBR due to an activation of microglia.  
   d) Estimation of drug treatment effect  
   The measurement of dopamine D2 receptor occupancy using PET was optimized for accurate evaluation of the therapeutic effect of antipsychotics.

2) Molecular Neurobiology
a) Application of animal models to R & D of diagnostic and therapeutic indices for dementia

We provided the first demonstration that hallmark pathologies of Alzheimer’s disease (AD), senile plaques and neurofibrillar tangles composed of amyloid-beta peptide (Aβ) and tau protein, respectively, in animal models can be captured by PET. Promotion of tau pathogenesis and neuronal loss by inflammatory microglia expressing 18-kDa translocator protein (TSPO) was shown by imaging of TSPO in tau transgenic mice.

b) Mechanistic elucidation of in vivo interactions between imaging biomarker probes and their target molecules

Our comparative PET and autoradiographic imaging of mice and humans indicated roles of N-terminally truncated and modified Aβ, AβN3pE, as a major component of AD-like plaques enriched with binding sites for amyloid PET probes. In vivo binding of a novel PET probe to agonistic binding sites on D2 dopamine receptor and sensitive detection of changes in synaptic dopamine release with this radiotracer were demonstrated by PET of awake rats and monkeys.

c) Analysis of dialogs between different neurotransmitter systems with animal models of neuropsychiatric disorders

A new PET probe for substance P receptor (NK-1 receptor) was developed and evaluated to visualize neurotransmissions associated with neuropsychiatric disorders. PET and autoradiographic analyses also revealed abnormalities of monoamine and glutamate receptors in mice heterozygously deficient in CaMKα, which modeled mental illnesses. Glutamate-mediated molecular mechanisms of synaptic elasticity in the amygdala potentially implicated in fear learning were clarified by our electrophysiological studies.

3) System Neurochemistry

a) Brain mechanism of motivation and its dysfunction in primates

We developed methods to evaluate a low motivational state in the monkey model of depression (hypothyroidism) through behavioral task performance (patent pending). Using the methods, we demonstrated that there were two behavioral factors for low motivation: decrease in reward sensitivity and increase in cost sensitivity. Application of SSRIs rescued the cost sensitivity, suggesting that increase of cost sensitivity is associated with low serotonin level in the brain. A PET activation study with motivational task performance revealed that the ventral striatum and ventromedial prefrontal cortex signal internal drive information. We clarified the functional dissociation in motivational valuation between lateral and orbital prefrontal cortices. Furthermore, the ability of rapid learning of visual categorization based on motivational value is reserved after bilateral ablation of the lateral prefrontal cortex.

b) Research studies on an animal model of brain developmental disorders

We have shown that synaptic dysfunction in CA1 hippocampus in juvenile rat model of maternal immune activation developed as having risk for schizophrenia in offspring. We found a decrease in dopamine D2 receptor bindings in the ACC and a loss of interneurons in the corresponding area in the model. We developed a primate model of maternal immune activation in offspring (patent pending).

c) Developing multidisciplinary approach for primate brain function

We developed the primate slice preparation for neural recording in vivo using marmoset monkeys. In collaboration with the Tokyo Metropolitan Institute for Neurology, the dopaminergic neuron protecting effect of vector injection against MPTP-induced dopaminergic neuronal degeneration was proofed in vivo by PET measurements with dopaminergic transporter ligands. We developed an evaluation system for emotion by vocalizations in marmosets. Using this system, we established screening and evaluation methods for anxiolytic drugs (patent pending).

MAJOR PUBLICATIONS


5. Ito H, Takano H, Takahashi H, Arakawa R, Miyoshi M,
3.3. RESEARCH ON MOLECULAR PROBES AND RADIOPHARMACEUTICALS

Toshimitsu Fukumura, Ph.D.
Director, Molecular Probe Group

Outline of Research Career
Toshimitsu Fukumura started his professional career in the Faculty of Pharmaceutical Sciences, Kyushu University Hospital in 1985 as an Assistant Professor of Radiopharmaceutical Chemistry and then moved in 1986 to the Department of Radiology, Kyushu University. In 1998, he joined Japan Steel Works, a major supplier of cyclotron and automated radiopharmaceutical production systems, to develop new automated synthesis apparatuses. In April 2006, he came to NIRS and in 2008, he was appointed group leader of the Molecular Probe Group.

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OBJECTIVES
Molecular probes play essential and important roles in the rapidly developing field of molecular imaging. The purposes of the molecular probe group are: 1) to develop novel probes assessing in vivo biological and physiological functions; 2) to develop new labeling methods to expand the possibility of producing a wider variety of probes at high yield and high quality; 3) to develop a new integrated system for the production of safe probes considering the GMP standard, without radiation exposure to personnel by implementing automations; and 4) to establish the production methods and quality control methods of the developed probes for clinical applications.

1) The Probe Research Team
Aims of this team are to develop novel probes for quantitative assessment of biological functions.

2) The Radiochemistry Team objectives are to develop new labeling methods and labeling precursors with PET radionuclides, to achieve ultra higher specific activity for various kinds of PET probes.

3) The Production System Team and Radiopharmaceutical Production Team not only have the above objectives but also have missions to support research activities for PET. Research by these teams is intended to establish routine production and quality assurance methods for new PET molecular probes. This includes the development and validation of satisfactory regular production and quality control methods for safe administration into human subjects as well as the evaluation of toxicity and radiation dosimetry for clinical applications. The research activities performed in FY 2010 are described below.

PROGRESS IN RESEARCH IN THE 2ND MIDTERM PLAN
New PET probes
1) A promising candidate compound for molecular probes for measuring the functional activity of MRP4 and organic anion transporter (OAT) was picked from the results of an examination using knock-out mouse.

2) A promising candidate for PET molecular probes which enables quantitative measurement of glutathione/GST reduction function labeled with $^{18}$F was found to show excellent radioactive kinetics in vivo.

3) Several PET probes for mGlu1 were developed and evaluated. From this research a promising compound that has high in vivo specific binding ability was found.

4) During the past 5 years, more than 102 PET probes that are potent candidate compounds for the imaging of brain and tumor imaging were prepared and evaluated. From this activity, we developed manufacturing and quality control processes for 11 PET probes for clinical research and got approval of them.

Suitability of an ultra-high specific activity labeled $[^{11}$C]PET probe
A novel PET probe for $I_2$ imidazoline receptor of $[^{11}$C]FTIMD has been labeled with ultra-high specific activity greater than 100 mCi/nmol. An animal PET study demonstrated that ultra-high specific activity labeled $[^{11}$C]FTIMD showed a significant increase in specific binding ability in the brain compared to specific activity labeled $[^{11}$C]FTIMD (2 mCi/nmol) that has been achieved at other PET centers. The present study demonstrated that animal PET with ultra-high specific activity $[^{11}$C]FTIMD is a powerful tool for the imaging of $I_2$ imidazoline receptor quantitatively.
New method and labeling procedure
1) A labeling method for the \(^{11}\text{C}\)labeled carbamate and asymmetric \(^{11}\text{C}\)urea by intermolecular coupling using \(^{11}\text{C}\)phosgene was established.
2) Using the C-\(^{11}\text{C}\) formation reaction, a simple and effective labeling method for amino acids was established and the reaction was utilized for the synthesis of \(^{11}\text{C}\) AIB.
3) A synthesis apparatus for producing \(^{11}\text{C}\) cyanide was developed and used for the development of PET molecular probes.

Non-standard PET radio nuclide
A production system producing \(^{76}\text{Br}\) was developed and the production method for \(^{76}\text{Br}\) is being optimized. Using the same system, a production method for \(^{89}\text{Zr}\) was also developed. Furthermore, a basic study for the production method of \(^{99m}\text{Tc}\), an important radionuclide in nuclear medicine, by proton induced nuclear reactions was carried out.

Application of the PET probe
1) Utilizing a PET probe for peripheral benzodiazepine, the probe suitability for some disease was evaluated using an animal disease model.
2) Functional imaging was successively obtained in brain astrocyte by measuring \(^{11}\text{C}\)benzylacetate using micro-dialysis in an animal disease model.

Contribution to the quality of clinical PET in Japan
The chemical purity tests of \(^{18}\text{F}\)FDG preparations produced in other PET facilities in Japan were conducted.

Major Publications
3.4. Research on Biophysics

Iwao Kanno, Ph.D.
Director, Biophysics Group

Outline of Research Career
Dr. Kanno graduated in Electrical Engineering from Tohoku University in 1970. He worked at the Akita Research Institute of Brain and Blood Vessels after graduation of Tohoku University. He obtained a Ph.D. (Tohoku University) in 1977. In 1978 he had a short stay at Bispebjerg Hospital in Copenhagen, Denmark, during which he developed methods to measure regional cerebral blood flow using $^{133}$Xe and dynamic tomography. He then developed his first PET scanner on returning to Akita in 1979. After a short time working at Hammersmith Hospital in London in 1982, he set up a PET research laboratory in Akita in 1983. Since then he has designed and built four commercial PET scanners, developed an $^{15}$O PET system for measuring brain circulation and metabolism. In 2006, he moved to the Molecular Imaging Center at NIRS as the Center Director and also as Group Leader of the Biophysics Group of the Molecular Imaging Center.

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Objectives
The Biophysics Group aims to develop instruments and methodologies for quantitative measurements of in vivo molecular functions using PET, MRI and optical imaging. The group consists of four teams whose progress in FY 2010 is described below.

Progress in Research in the 2nd Mid-Term Plan

1) Magnetic Resonance Molecular Imaging Team
This team newly proposed and developed “Molecular Magnetic Resonance Imaging”. This new concept, which consisted of functional contrast agents and hybrid nanoparticles, was tested in an animal study. Manganese contrast agent is a useful functional probe. The team proved that manganese-enhanced MRI can provide good image contrast for studying reactive gliosis in a rat chronic stroke model, anoxic depolarization in a rat super-acute stoke model, and in a layer structure of the brain. The team also developed a multimodal therapeutic contrast agent using nitroxyl radical as a novel nonradioactive methodology. A visible anti-cancer drug “SLENU” was developed for in vivo noninvasive, real-time MR imaging of blood-brain barrier (BBB) permeability. The nitroxyl radical probes were tested in an in vivo tumor model and the results were published. A drug delivery imaging technique using temperature-sensitive liposome was developed and applied in vivo. The multimodal and multifunctional liposome was synthesized as an MRI contrast agent for optical imaging and as an anti-cancer drug with tumor targeting capability. The drug kinetics, including accumulation in a tumor and drug release using thermo-triggering, and the anti-tumor effects were visualized in mice. A multimodal quantum-dot nano-probe was developed for both MR and optical imaging. Quantum-dots have more suitable fluorescence properties than conventional organic dyes. The fluorescence properties were protected by a hydrophobic structure around the nanoparticle core, and the inclusion of MRI contrast agents was facilitated by adding a further amphiphilic silica shell structure. In vivo application was tested using both MRI and optical imaging.

2) Biosignal Physiology Team
This team succeeded in extracting a slowly diffusing water (SDW) signal using a new compartment model, and the SDW compartment signal showed a neural-activity correlated time course more clearly than conventional functional MRI. The results indicate that diffusion functional MRI (DfMRI) has good potential as a new brain functional imaging method. The team also clarified details of the brain diffusion property in a study with regional heterogeneity and age-related change in sub-regions of an internal capsule (IC) evaluated by diffusion tensor imaging. The results may provide important information towards understanding age-related changes and may also be useful for clinical diagnosis of a diseased IC. The team measured brain metabolites in the medial prefrontal cortex of schizophrenic patients and healthy controls by proton magnetic resonance spectroscopy ($^1$H MRS), and obtained a significant relationship between prefrontal cortex-related neurocognitive functions and brain metabolites in the medial prefrontal cortex. These data suggest that specific metabolites of the medial prefrontal cortex are associated with the neurocognitive deficits in schizophrenia. This was a collaboration study with researchers at Chiba University.

Collaborative studies with active clinical sites have been widely performed using evidence-based molecular...
imaging methods such as MR spectroscopy (MRS), DW imaging, susceptibility imaging, and target-specified enhanced MRI. Proton MRS was applied to pediatric radiology in cooperation with the Kanagawa Children’s Medical Center, and ^13^C MRS was used for diagnosis of liver function with the Institute for Adult Diseases. Tumor structures were visualized by diffusion tensor imaging in a collaboration study with the NIRS Hospital. Glycosaminoglycan specific MR contrast enabled evaluation of the dysfunction of cartilages around the knee joints; this was done in a collaboration study with Chiba University and Teikyo Chiba Medical Center researchers.

Experiments with a two-photon laser microscope and closely related instruments were performed in order to better understand brain microcirculation. A chronic experimental protocol was developed where the cortical microcirculation of an awake mouse can be longitudinally observed with the two-photon laser microscope through a cranial window. The system allows clarification of cellular and molecular communication between vessels, glia and neurons.

3) Image Analysis Team

PET can visualize various functionalities of living tissues such as receptor density. For fully quantitative functional imaging, the behavior of an administered radiopharmaceutical in target tissues is modeled with a compartment model, and the model parameters are estimated using a time history of radioactivity concentration in arterial plasma and tissue. This team developed algorithms for the compartment model estimation. One approach was based on MAP estimation. The Logan plot is a widely utilized algorithm for neuroreceptor imaging, but its quantitative performance suffers largely from noise in the PET data. The proposed algorithm was found to be robust for the noise and it realized fast and quantitative neuroreceptor imaging. A new algorithm for partial volume correction was also proposed. Anatomical information was acquired from MRI and wavelet transformation was applied to incorporate the brain structure into PET images. The team members also focused their interests on applying the algorithms to actual experimental data derived from both humans and small animals. A reference tissue model was evaluated to quantify the dopamine transporter. The imidazoline subtype-2 receptor was also evaluated as a new target ligand in the brain with rat experiments.

The team developed a new experimental apparatus to measure radioactivity concentration in the arterial plasma of mice. A permitted volume of blood sampling from mice is limited to only around three μL, and a plasma separation and volume measurement should be conducted on this small volume. The team developed a new system, in which sampled blood was dripped onto a specially designed disc with U-shaped channels etched on the surface. The blood was centrifuged, and its volume and radioactivity were measured in the apparatus. The system has been evaluated, and it is going to be commercialized.

4) Imaging Physics Team

PET is a promising method to promote molecular imaging research as well as cancer diagnosis. However, there are still strong demands for higher resolution, higher sensitivity and lower cost. Therefore this team carried out basic studies on instrumentation, image reconstruction and data corrections to improve image quality and quantity in nuclear medicine.

For PET, it is essential to arrange detectors close to the object in order to increase sensitivity and avoid loss of spatial resolution due to the angular deviation effect. In practice, however, the parallax error caused by the thickness of the crystals degrades spatial resolution at the peripheral regions of the field-of-view. Therefore we invented a novel depth-of-interaction (DOI) capable detector and developed a prototype brain PET scanner, jPET-D4. Toward practical use, we developed component technologies, such as a modified design for higher resolution and an image reconstruction method to make full use of DOI information.

Based on our core technology for DOI measurement, we proposed a new equipment concept, OpenPET, which is an open-type geometry for PET to visualize a physically opened space between two detector rings. Axial spatial resolution, which was degraded with the extended gap due to the parallax error, was recovered by use of DOI detectors. OpenPET is expected to enable PET image-guided radiation therapy by letting the beams pass though the gap, and extension of an axial field-of-view with a limited number of detectors. At this stage, a small prototype has been developed to show a proof-of-concept.

On the other hand, the recent development of small semiconductor photo-detectors such as Geiger-mode avalanche photodiodes (GAPDs), that can replace conventional photomultiplier tubes, is leading researchers to make available new PET detectors. Therefore we developed a next generation DOI detector, X’tal cube. The challenging aspect of this work is implementing effective detection of scintillation photons by optically covering all 6 faces of a segmented crystal block with GAPDs. At this stage, a prototype detector with 2 mm isotropic resolution has been developed.

**Major Publications**

1. Zhelev Z, Bakalova R, Aoki I, Matsumoto K, Gadjeva V,


