# JOINT STUDIES PROGRAMS

As one of the important functions of an inter-university research institute, IMS undertakes joint studies programs for which funds are available to cover research expenses as well as travel and living expenses of individuals. The proposals from domestic scientists are reviewed and controlled by an inter-university committee.

The programs are carried out under one of the following categories:

(1) Joint Studies on Special Projects (a special project of significant relevance to the advancement of molecular science can be carried out by a team of several groups of scientists).
(2) Research Symposia (a symposium on timely topics organized by collaboration between outside and IMS scientists).
(3) Cooperative Research (a research program carried out by outside scientists with collaboration from an IMS scientist).
(4) Use of Facility (a research program carried out by outside scientists at the research facilities of IMS except the UVSOR facility).
(5) Invited Research Project
(6) Joint Studies Programs using beam lines of UVSOR Facility.
(7) Use of Facility Program of the Computer Center (research programs carried out by outside scientists at research facilities in Computer Center).

In 2003 Oct.–2004 Sep., the numbers of joint studies programs accepted for the categories (1)–(7) were 5, 8, 92, 57, 1, 131, and 121, respectively.

# (1) Special Projects

## A. Ultrafast Time-Resolved Study on Photochromic Reactions in the Isolated State and Condensed Phase

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*([1]Kyushu Univ.; [2]Hokkaido Univ.; [3]Univ. Tokyo)*

In this joint project we investigated photochromic reactions of diarylethene derivatives and *N*-salicylideneaniline (SA). The cyclization and cycloreversion reactions of diarylethene derivatives have been studied with picosecond time-resolved Stokes and anti-Stokes Raman spectroscopy. We obtained information on the vibrational relaxation and the energy partitioning in the cycloreversion process in 1,2-bis(3,4-dimethyl-5-phenyl-2-thyenyl)perfluorocyclopentene. We also measured the fluorescence excitation and dispersed fluorescence spectra of the open-ring form of 1, 2-bis(3-methyl-2-thienyl)perfluorocylclopentene and SA in a supersonic jet expansion. The spectra reveals that an ultrafast internal conversion (IC) occurs from the photo-excited $^2$A state to $^1$A state that correlates to the $^1$A state of the closed-ring form. The vibronic pattern in the fluorescence excitation spectrum of SA suggested that a very fast occurs from the photoexcited $S_1(\pi\pi^*)$ state of the enol form in addition to the excited-state double-proton transfer. We have first to investigate the ultrafast excited-state dynamics in photochromic SA by femtosecond time-resolved REMPI spectroscopy. The excited-state dynamics of SA in both the enol and *cis*-keto forms has been clarified. There is a close similarity in the dynamics of diarylethene derivatives and SA; The initially photoexcited state is located above an electronic-dipole forbidden state, and IC plays an important role in photochromic reaction.

## A-1 Ultrafast Excited-State Dynamics in Photochromic *N*-Salicylideneaniline Studied by Femtosecond Time-Resolved REMPI Spectroscopy

Ultrafast processes in photoexcited *N*-salicylideneaniline (SA) have been investigated with femtosecond time-resolved resonance-enhanced multiphoton ionization (REMPI) spectroscopy. The ion signals *via* the $S_1(n,\pi^*)$ state of the enol form as well as the proton-transferred *cis*-keto form emerge within a few hundred femtoseconds after photo-excitation to the first $S_1(\pi,\pi^*)$ state of the enol form. This reveals that two ultrafast processes, excited-state intramolecular proton transfer (ESIPT) reaction and an internal conversion (IC) to the $S_1(n,\pi^*)$ state, occur on a time scale less than a few hundred femtoseconds from the $S_1(\pi,\pi^*)$ state of the enol form. The rise time of the transient corresponding to the production of the proton-transferred *cis*-keto form is within 750 fs when near the red-edge of the absorption is excited, indicating that the ESIPT reaction occurs within 750 fs. The decay time of the $S_1(\pi,\pi^*)$ state of the *cis*-keto form is 8.9 ps by exciting the enol form at 370 nm, but it dramatically decreases to be 1.5 ~ 1.6 ps for the excitation at 365 ~ 320 nm. The decrease in the decay time has been attributed to the opening of an efficient non-radiative channel; an IC from $S_1(\pi,\pi^*)$ to $S_1(n,\pi^*)$ of the *cis*-keto form promotes the production of the *trans*-keto form as the final photochromic products. The two IC processes provide opposite effect on the quantum yield of photochromic products: IC in the enol form may substantially reduce the quantum yield, but IC in the *cis*-keto form increases it. The time constants and reaction processes in SA obtained from present study are summarized in Figure 1.

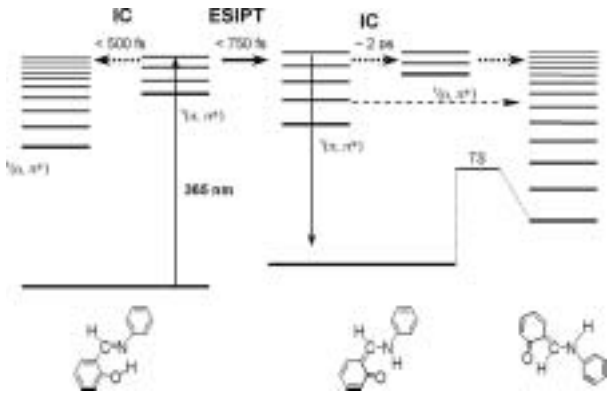


**Figure 1.** Excited-state dynamics in SA obtained from femtosecond time-resolved study.

## B. Structural Chemistry of High Oxidation State Intermediate of Terminal Oxidases

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*([1]Himeji Inst. Tech.; [2]Univ. Tokyo; [3]Keio Univ.; [4]JBIC; [5]Osaka Univ.; [6]RIKEN Harima Inst.; [7]Univ. Crete; [8]Hankuk Univ. Foreign Studies)*

Ogura's group have moved to Himeji Inst. Tech. and Takahashi was promoted to Osaka University during this program. The purpose of this project is to characterize the P intermediate of cytochrome *c* oxidase. Ogura's group carried out time-resolved resonance Raman as well as time-resolved absorption spectroscopy. Yoshikawa analyzed x-ray crystallographic structure of this enzyme. Okuno of Grad. Univ. of Adv. Stud. and Kitagawa examined IR spectra of the protein moiety of this enzyme to elucidate the proton pumping mechanism. Kim produced the P intermediate by incubation of oxidized enzyme with CO and investigated UV resonance Raman spectra. Varotsis brought bacterial terminal oxidases with him from Crete Univ. to Okazaki and measured its resonance Raman spectra. Prof. Shimada prepared a chimeric enzyme which consists of a bacterial subuit I and mammalian subunits II–VII. Prof. Pawel Kozlowski of Lousvile Univ. also joined this collaborative research to perform DFT calculations on the P intermediate. The all members got together on January 8, 2004 to discuss the structure of P intermediate.

It was pointed out that although the P intermediate generated by incubation of oxidized enzyme with CO exhibits the absorption and resonance Raman spectra quite similar to those of the P intermediate generated during the reduction of $O_2$ by fully reduced enzyme, the two P intermediates are distinct from each other, because the former becomes ferric state without showing the F intermediate but the latter shows it. The presence of an extra oxidation equivalent of the P intermediate on a heme was suggested from the DFT calculations and its compatibility with resonance Raman spectra became a highlight of discussion.

## C. Exchange and Spin-Orbit Interactions in Molecular Inner-Shell Excitation

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*([1]Univ. Hyogo; [2]Shizuoka Univ.; [3]IMS and Würzburg Univ.)*

In the orbital interaction theory,[1)] the electron-hole interaction between occupied and unoccupied orbitals is related to short-range interaction, delocalization (DL) or charge transfer (CT), and long-range interaction, polarization (PL) or local excitation. On the other hand, the electron-electron interaction in two occupied orbitals is related to short-range exchange repulsion (EX) and long-range electrostatic interaction (ES). The spin-orbit (SO) interaction is inherently of atomic character. EX and SO interactions involving core electrons of the first-row and second-row elements such as carbon 1s and sulfur 2p are relatively small in comparison with intra-valence EX and deep-core SO, but have been recently essential to understand fine structures newly found in high-resolution and sophisticated measurements of inner-shell phenomena using third-generation synchrotron radiation (SR) facilities.

There are some types of EX. EX in closed-shell electrons results in an *exclusion* effect on the electrons in the interacting region. This is completely different from electrons *sharing* in the interacting region and forming covalent bonds. The interatomic core-core and core-valance EX interactions[2)] are important in discussing core hole localization in the core ionization and resonant phenomena such as multi-atom resonant photoemission. The core excitation in the closed-shell system creates an open-shell valence and/or Rydberg electron. In this case, the intra-atomic core-valence and core-Rydberg EX result in large and small singlet-triplet (ST) exchange splittings, respectively, where the core electron is localized on an atom and the intra-atomic EX component is predominant. On the other hand, the Rydberg electron may have relatively large intermolecular EX interaction with surrounding molecules in cluster, liquid, solid, and adsorbate phases, because of its diffuse character. In the core excitation and ionization of the open-shell system, EX causes more complicated multiplet splittings (MS). The core-valence EX sometimes competes with the intra-valence EX in core excitations of open-shell molecules composed of first-row elements.[2–5)]

The SO splitting on the core electron is directly observed in X-ray photoelectron spectroscopy. Even in 2p photoabsorption spectroscopy, the 2p SO splitting of third-row and heavier elements is large and is easily distinguishable. The singlet and triplet (ST) 2p excited states are strongly and indistinguishably mixed with each other through SO or jj coupling. However, in second-row elements such as phosphor and sulfur, 2p SO is not satisfactorily analyzed due to a small and comparable splitting to the intra-atomic core-valence EX splitting. SO is still a major factor in the core-to-

Rydberg excited state with a small ST (EX) splitting, but is only one of some important factors in the core-to-valence excited state with a large ST (EX) splitting.[6] Recent high-resolution photoelectron spectroscopy is possible to reveal another small splitting in the $2p_{3/2}$ manifold due to the molecular filed (MF) effect.[7]

Now we have to consider SO, MF, and EX splittings in interpreting 2p excitation spectra of molecules involving second-row elements. Furthermore, de-excitation or resonant inner-shell spectroscopy may indicate additional features *via* triplet components in intermediate core-excited states; that is, triplet valence excitation in resonant inelastic X-ray scattering and quartet valence ionization in resonant photoelectron or Auger electron emission. In the present special project, we will discuss various types of EX and intermediate couplings between SO and EX to analyze experimental evidence in inner-shell spectra of some simple molecules.

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## D. Integration of Bio-Molecular Recognition Reaction System on Solid Surfaces and the Structure Analysis

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*([1]AIST; [2]Kyushu Univ.)*

Membrane protein biosensors and biochips are most important research targets in the post-genome. However, their developments are surprisingly delayed. One of the reason is due to the difficulty in integrating the membrane proteins on solid surfaces without loosing their physiological activities. They express their physiological activity only when they are reconstructed in the lipid bilayers under the water. In this research project, we are going to develop elementary technologies of constructing membrane protein/lipid bilayer systems on Si surfaces and conduct the analysis of their structures by AFM (Atomic force microscopy) and BML-IRRAS (Infrared reflection absorption spectroscopy using buried metal layer substrates).

### D-1 Lipid Membrane Formation by Vesicle Fusion on Silicon Dioxide Surfaces Modified with Alkyl Self-Assembled-Monolayer Islands

We have investigated the formation of the dipalmitoylphosphatidylcholine (DPPC) membrane by the vesicle fusion method on $SiO_2$ surfaces modified with self-assembled monolayer (SAM) islands of octadecyltrichlorosilane (OTS) with sizes comparable to those of the vesicles by means of atomic force microscopy. OTS-SAM islands with various size and coverage can be constructed on the $SiO_2$ surfaces prepared by thermal oxidation followed by partial hydroxylation in a $H_2O_2$/$H_2SO_4$ solution. When vesicles are sufficiently smaller than the $SiO_2$ domains, DPPC bilayers and DPPC/OTS layers form on the $SiO_2$ and OTS domains, respectively (Figure 1). However, the adhesion of larger vesicles onto $SiO_2$ is prevented by the OTS islands, therefore only DPPC/OTS layers form without formation of DPPC bilayers on the $SiO_2$ domains (Figure 1). On surfaces with domains in tens to hundreds nanometer scale, the relative size between the hydrophilic domains and the vesicles becomes important factor in the membrane formation by the fusion of vesicles.

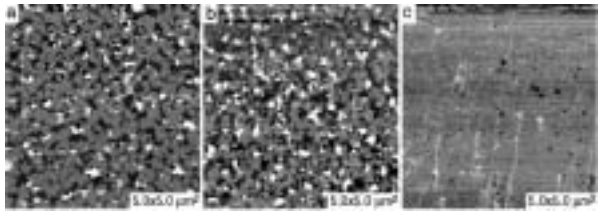

**Figure 1.** AFM images ($5.0 \times 5.0$ $\mu m^2$) of the OTS-modified $SiO_2$ surfaces ($\theta_{OTS} = 0.71$) obtained in the buffer solution (a) before and (b) after the deposition of 200-nm-filtered vesicles and (c) sonicated vesicles.

### D-2 Characterization of Dipalmitoylphosphatidylcholine/Cholesterol Langmuir-Blodgett Monolayers Investigated by AFM and FT-IR

The addition effects of cholesterol on the dipalmitoylphosphatidylcholine (DPPC) Langmuir-Blodgett (LB) monolayer have been investigated by atomic force microscopy (AFM) and infrared reflection absorption spectroscopy (IRRAS). The phase transformation from the pure DPPC to the DPPC/cholesterol phase proceeds through two stages: initial drastic changes in the surface morphology and the conformation of the DPPC acyl chains below 10% cholesterol, and the gradual homogenization of the morphology towards the liquid ordered phase up to 35% cholesterol (Figure 1). The IRRAS peak position indicates that the conformational disorder of the acyl chain becomes almost liquid level at the 10% cholesterol addition. In the homogeneous liquid-ordered phase at 35% cholesterol, the terminal methyl groups of the DPPC are aligned in good order as the solid-like gel phase, whereas the acyl chains have liquid level disordered conformation.

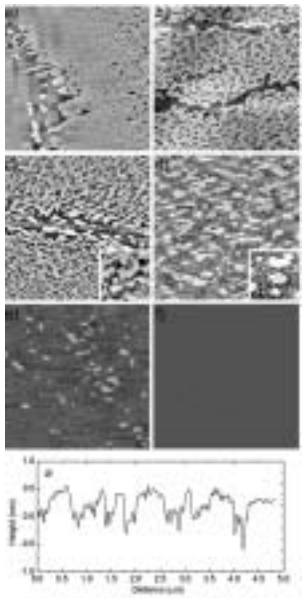

**Figure 2.** AFM images (5 × 5 $\mu m^2$) of DPPC/ cholesterol LB monolayers transferred onto the mica surface at the surface pressure of 10 mN $m^{-1}$. The concentrations of cholesterol are: a) 0% (pure DPPC), b) 10%, c) 20%, d) 30%, e) 35% and f) 100% (pure cholesterol) in the molar ratio. g) The profile of the line drawn in d). The scale of the inserts of c) and d) is 1.34 × 1.34 $\mu m^2$.

## D-3 Theoretical Analysis of the Oxygen Insertion Process in the Oxidation Reactions of $H_2O$ + H/Si(100) and 2H + $H_2O$/Si(100); Calculation of an Ab Initio Molecular Orbital Method and an Analysis of the Tunneling Reaction

The reaction paths were analyzed, by an ab initio molecular orbital method, for the surface reaction systems, 2H + $H_2O$/Si(100)-(2×1) and $H_2O$ + H/Si(100)-(2×1), in which $SiH_2$ species with one or two oxygen atom-inserted back bonds have been observed as new stable reaction products. It was found that common metastable states exist in both systems, and the initial energy is sufficiently higher than all transition state energies in the former system, while in the latter system, the energy of the highest transition state is much higher than the initial energy, and thus a tunneling effect plays an important role (Figure 1, and 2).

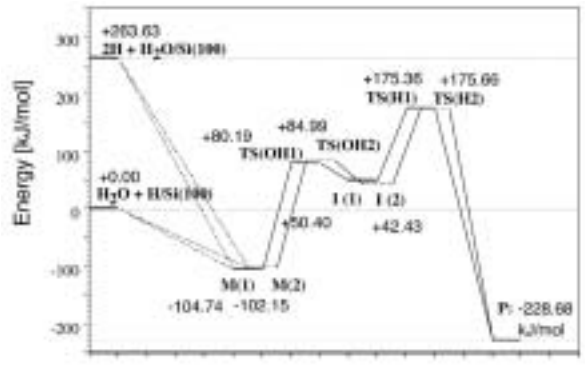


**Figure 1.** Energy diagrams for the oxygen insertion reactions, $H_2O$ + H/Si(100)-(2×1) and 2H + $H_2O$/Si(100)-(2×1). The dissociation limit $H_2O$ + H/Si(100)-(2×1) is set on the energy standard. The zero-point vibration energy correction is summed into the energies.

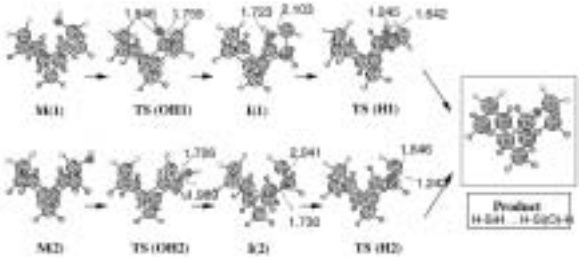

**Figure 2.** Fully optimized structures of the local minima and the transition states in the oxygen migration processes. Several bond lengths concerned with the migration reaction is expressed in Å.

## D-4 Integration of Membrane Protein on Si and Application to the Biosensor

Membrane proteins such as receptors and ion channels play an important in the regulation of the physiological condition and the signal transduction in the central nervous system, and they are considered to be the important target of the medical development based on the genome information. Concerning the membrane proteins, however, not only the structure analysis but also the development of the analysis method of the protein molecular recognition reaction are significantly delayed. It is considered to be due to that usual protein chip technologies of immobilizing the protein on the solid surface can not be applied for the membrane protein which shows the physiological activity only when reconstructed in the lipid bilayers. The supported membrane, the lipid bilayer with reconstructed membrane protein system on the solid surface, are attracting a significant attention as a new analytical method for analyzing a membrane protein molecular recognition interaction. It also provides an interesting tool for the study of the cell membrane surface reaction. The supported membranes are classified to the beads type[1)] and the planer type.[2),3)] Although the higher sensitivity is expected for the beads type, the planer type, however, has several advantages such as; (1) The precise structure control, which is important in the ion channel and the trans membrane receptor, can be made, and (2) Not only the membrane surface but also the inside information such as channel current can be obtained.

We are now developing the elementary process technologies necessary for the fabrication of the supported membrane structure shown in Figure 1. The important elementary processes are, (a) Surface structure control of the supporting substrate. Especially, it is pointed out that the decrease of the surface roughness is required.[2)] (b) Surface chemical modifications necessary for immobilizing the lipid bilayer and or proteins on the surface, and for depressing the edge leak current. (c) Formation of the planer lipid bilayer and the reconstruction of membrane proteins. In this sympo-

sium, I am going to introduce the recent results about each elementary process developments.
(a) Surface structure control of the supporting substrate: We have almost succeeded in developing the super flat surface Si substrate with buried AgCl/Ag/$CoSi_2$ electrode, for which the interface potential between buffer solution/electrode and electrode/Si(100) can be controlled.
(b) Surface chemical modifications: We have succeeded in deposition and patterning of OTS (octadecyltrichlorosilane) self-assembled monolayers, which we are going to use for the depression of the edge leak current. The surface modification by –COOH group has been attained keeping the surface roughness less than 1 nm. This was used for immobilization of avidin molecules on the substrate surface. The immobilization was certified by molecular recognition AFM and the IR absorption spectroscopy by BML-IRRAS.
(c) The tethered single lipid bilayers of DPPC and DMPC using avidin immobilized on the surface have been successfully deposited. The structure was certified by under-water-AFM. Several membrane protein molecules were successfully reconstructed in these lipid bilayers and their single molecule images were obtained by the same AFM technique.

We are going to continue these effort to attain the measurement of the function of the supported membrane systems in the succeeding research.

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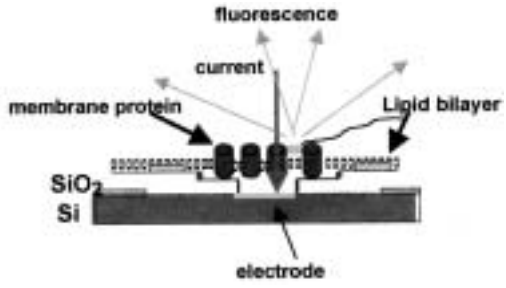


**Figure 1.** Supported membrane biosensor device.

### E. Development and Application of Short Wave Length Free Electron Laser

**KATOH, Masahiro; HOSAKA, Masato; MOCHIHASHI, Akira; TAKASHIMA, Yoshifumi[1]; HAMA, Hiroyuki[2]; GEJO, Tatsuo[3]; NISHINO, Hideo[4]; HARA, Toru[5]; COUPRIE, Marie-Emmanuelle[6]; BIELAWSKI, Serge[7]**
*([1]Nagoya Univ.; [2]Tohoku Univ., [3]Hyogo Univ.; [4]JST; [5]RIKEN; [6]LURE; [7]Univ. Sci. Tech. Lille)*

The free electron laser developed at UVSOR has some characteristics such as tunability in wave length, variability in polarization, high average power and natural synchronization with synchrotron light pulses. We achieved the average output power of 1.2 W in the visible region, which is the world highest record in storage ring free electron lasers. We have successfully carried out a two-photon double-resonant excitation on Xe atoms utilizing the SR pulses as a pump and the FEL pulses as a probe light.

As the next step, we are trying to realize a stable and high power oscillation in the UV region, hopefully shorter than 200 nm. The upgraded UVSOR, UVSOR-II, has a small beam emittance of 17 nm-rad in the FEL operating mode. This smaller emittance gives a larger FEL gain, especially in the shorter wave length. In December, 2003, we have succeeded in oscillating the FEL in visible wavelength region for the first time at UVSOR-II. We have confirmed the increase of the FEL gain. We are trying to oscillate the FEL at around 250 nm. The circular polarized FEL around 250 nm will be used for the experiment on absolute asymmetric photoreactions of amino acids.

## (2) Research Symposia

(From 2003 Oct. to 2004 Sep.)

1. Dynamics of Biomolecules in its Relation to Function and Structure
   (Dec. 22–24, 2003)
   Chair: **KITAO, Akio**

2. Size Effect in Nano-Scale Reaction Field
   (Jan. 19–20, 2004)
   Chair: **MAFUNE, Fumitaka**

3. Physical Chemistry of Molecular Functions—New Development in Theoretical/Computational Chemistry and Spectroscopy
   (July 21–23, 2004)
   Chair: **ISHIDA, Toshimasa**

4. Advanced Molecular Science and Its Frontiers
   (May 21–22, 2004)
   Chair: **SUZUKI, Toshinori**

5. Magnetic Structure and Magneto-Optical Effect of Chiral Molecule-Based Magnets
   (July 15–16, 2004)
   Chair: **INOUE, Katsuya**

6. Physical Chemistry Symposium for Young Researchers
   (June 2, 2004)
   Chair: **NAKAJIMA, Atsushi**

7. Particle Correlations in Elementary Reactions Involving Atoms and Molecules
   (June 3–4, 2004)
   Chair: **KOCHI, Noriyuki**

8. Conference on Molecular Electronics
   (April 8–10, 2004)
   Chair: **MATSUMOTO, Takuya**

## (3) Cooperative Research

This is one of the most important categories that IMS undertakes for conducting its own research of the common interest to both outside and IMS scientists by using the facilities at IMS. In 2003 Oct.–2004 Mar., 117 outside scientists from 45 research groups joined the Cooperative Research programs, and 128 outside scientists from 47 research groups in 2004 Apr.–2004 Sep. The names and affiliations of those collaborations are found in the Research Activities sections in this Review.

## (4) Use of Facility

The number of projects accepted for the Use of Facility in 2003 Oct.–2004 Mar. amounted 3, 23, and 0 for the Laser Research Center for Molecular Science (LRCMS), for the Research Center for Molecular-scale Nanoscience (RCMN) and for the Equipment Development Center (EDC), respectively. In 2004 Apr.–2004 Sep., the number of projects accepted amounted 1, 29, and 1 for LRCMS, for RCMN, and for EDC, respectively.

## (5) Invited Research

Under this joint-study program, several scientists were invited from other institutions of help for construction and improvement of instruments in IMS. The total number of the projects in this category was 1.

## (6) Use of UVSOR Projects

In the UVSOR Facility with the 750 MeV electron storage ring, there are sixteen beam lines available for synchrotron radiation research (see UVSOR ACTIVITY REPORT 2003). Under the Use of UVSOR Projects, many synchrotron radiation experiments have been carried out by outside scientists on eight beam lines in close cooperation with the UVSOR staff. The total number of the projects in this category was 131 (68 in 2003 Oct.–2004 Mar., and 63 in 2004 Apr.–2004 Sep.).

## (7) Use of Facility Program of the Computer Center

Computer Center provides three types of research programs for outside scientists: (a) Use-of-Facility Program; (b) Cooperative Research Program; (c) Advanced Research Program. The numbers of projects accepted for each programs during the fiscal year of 2003 were (a) 111 with 525 users, (b) 8 with 13 users and (c) 2 with 4 users. Computer time distributed for these projects amounted to 65% of the total annual CPU time available.