Principal Researcher	Yoshio Umezawa	**Number of Researchers**	3
Research Institution • Department • Title	Professor of Chemistry, Department of Chemistry, School of Science, The University of Tokyo	**Location of Institution**	Bunkyo-ku, Tokyo

Title of Project	"New Analytical Methods for Molecular Imaging in Single Live Cells and Interfacial Molecular Assemblies"
Abstract of Research Project	The principal research topics of the present project are directed to develop new analytical methods for molecular imaging. This is to implement "seeing what was unseen" in single live cells and interfacial molecular recognition chemistry. Methods of analysis for cellular molecular processes non-destructively in live cells have been explored extensively in our laboratory for the past several years. Our common approach for this was to develop genetically encoded fluorescent indicators to pinpoint each cellular process in single living cells. Chemistry-facilitated intermolecular electron tunneling is another approach to molecular imaging. The chemical imaging was obtained from a distinctive chemical affinity between the imaging tip of scanning tunneling microscopy (STM) and the substrate that alters the tunneling current. This was achieved by tailored chemical modification of the STM tips. For nondestructive analysis of chemical processes in living cells, we will develop novel intracellular fluorescent indicators for second messengers, protein phosphorylation, protein/protein interactions and protein localizations that work in single living cells. Key molecules and steps of cellular signaling pathways will be visualized under a confocal laser microscope in target live cells using developed fluorescent indicators. Another new approach to molecular imaging is also planned. When chemically modified tips are used for STM measurements, contrast enhancements at specific regions in STM images occur on the basis of hydrogen bond, charge-transfer and metal-coordination interactions, and as a result, allow to detect not only the distribution of specific chemical species and functional groups but also the orientation of functional groups. The contrast enhancements reflect the increase in a tunneling current due to the overlap of electronic wave functions induced by the chemical interactions between tip and sample.
References	1) T. Ozawa, Y. Sako, M. Sato, T. Kitamura and Y. Umezawa, "A Genetic Approach to Identifying Mitochondrial Proteins", *Nature Biotech.*, 21, 287-293 (2003). 2) M. Sato, T. Ozawa, K. Inukai, T. Asano, and Y. Umezawa, "Fluorescent Indicators for Imaging Protein Phosphorylation in Single Living Cells", *Nature Biotech.*, 20, 287-294 (2002). 3) R. Paulmurugan, Y. Umezawa and S. S. Gambhir, "Noninvasive Imaging of Protein-Protein Interactions in Living Subjects by Reporter Protein Complementation and Reconstitution Strategies", *Proc. Natl. Acad. Sci. USA*, 99, 15608-15613 (2002). 4) T. Nishino, T. Ito and Y. Umezawa, "Carbon Nanotube Scanning Tunneling Microscopy Tips for Chemically Selective Imaging", *Anal. Chem.*, 74, 4275-4278 (2002).
Term of Project	Fiscal years 2003-2007 . (5years)

Budget Allocation (in thousand of yen)	FY2003	FY2004	FY2005	FY2006	FY2007	TOTAL
	30.800	16,600	12,500	12,500	8,300	80,700

Homepage Address	http://www.chem.s.u-tokyo.ac.jp/~analyt/index.html