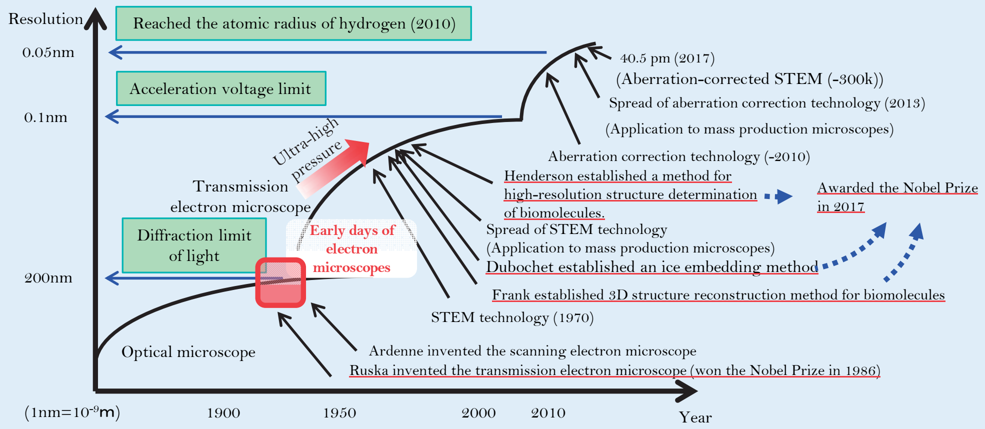
# Chapter 3 Technologies That Support and Drive Basic Research

This chapter introduces you to examples of important technologies that support basic research and how these technologies and basic research work like a pair of wheels to move science forward. Creating a hypothesis and testing it is part of basic research and it is aimed at discovering the laws behind various phenomena. This process is often expressed as the "search for truth" and many laws have been elucidated through it. In any field, basic research hits the wall when it reaches the limit of measurement and experimental methods. New technologies that lead to innovations in measurement and experimental methods are one of the important elements for breaking through such limitations. We have seen situations where new technologies were awaited for the solution of various challenges in basic research, and then the frontier of basic research was expanded, achieving dramatic, nonlinear development, as soon as such awaited new technologies appeared.

For example, if we look at the history of microscope, it started with the optical microscope, whose highest resolution was defined by the wavelength of visible light, which is about 200 nm. It was an electron microscope that broke this limit. An electron microscope uses an electron beam instead of visible light. The resolution was improved to 0.1 nm by increasing the acceleration voltage. In recent years, a new technique called aberration correction has been developed to further improve the resolution of electron microscopes to 0.05 nm. This way, the resolution of microscope has gone through two stages of nonlinear development, i.e. the development of an electron microscope and the new technique of aberration correction for electron microscopes (Figure 1-3-1).

Although Japan currently has a relatively low global market share for some of the latest measuring instruments and experimental equipment used at the forefront of basic research, its global share for advanced components and devices used in such equipment is relatively high. This means that Japan's technological capabilities have greatly contributed to the daily progress of basic research around the world. It is hoped that Japan will tap into its strength and expand its global share for the latest measuring instruments and experimental equipment into the future. Moreover, many of the latest technologies are becoming increasingly expensive, pushing up the costs of basic research. In addition, there are many advanced technologies that require high expertise in order to make full use of them. Therefore, in order to make effective use of these state-of-the-art technologies, it is important to create a system for sharing expensive equipment and to secure and train engineers for the future.

■Figure 1-3-1/History of improvement of microscope resolution




Source: JAIMA (prepared based on a material provided by TANAKA Michiyoshi at Tohoku University)

## 1 Detector That Opened a New Door to Physics (Photomultiplier Tube and Ultrapure Water)

In 2002, KOSHIBA Masatoshi, Professor Emeritus at the University of Tokyo, received the Nobel Prize in Physics for opening up a new door to neutrino astronomy, a science that explores the mystery of the universe from a new perspective. The existence of neutrinos had already been theoretically predicted in 1930, but their detection was extremely difficult. Various methods and equipment have been tested around the world to detect neutrinos. Kamiokande, which was built in 1983 at the Kamioka Mine in Gifu Prefecture, was one of such projects. Kamiokande was originally built for observing proton decay, but it also came into use for detecting neutrinos, as its photomultiplier tubes were capable of capturing rare, very weak light (Cherenkov radiation) that is produced when ultrapure water stored underground in the mine interacts with neutrons.

Photomultiplier Tube
Source: Institute for Cosmic Ray Research (ICRR), the University of Tokyo

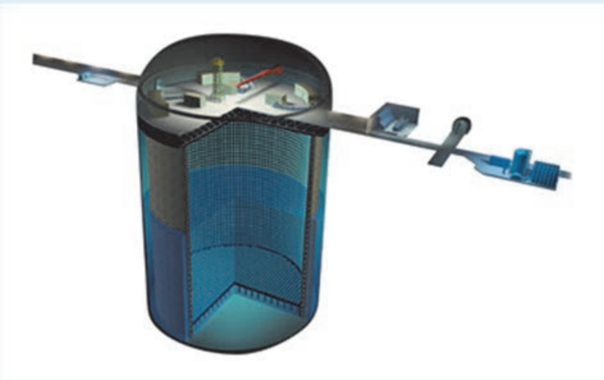
At the time when the designing of Kamiokande began, the largest photomultiplier tubes available from domestic and overseas manufacturers were 3 to 5 inches in diameter. However, it turned out that larger photomultiplier tubes were needed to achieve results that would outperform competitors. Therefore, a request was made to a manufacturer to develop custom-made photomultiplier tubes of a 20-inch diameter. The development of large photomultiplier tubes of this unprecedented size took over two and a half years until completion, with various innovative ideas produced along the way. In addition, in order to achieve good measurement accuracy, manufacturers needed to develop various kinds of filters and water purification equipment to create ultrapure water, which is water from which impurities were removed to the utmost extent. Thanks to these two technologies, it became possible to capture weak light, which led to neutrino detection.

Kamiokande started its operation to detect neutrinos in January 1987. About a month later, on February 23, the explosion of the supernova 1987A, 160,000 light years away from the Earth, was unexpectedly observed, and the neutrinos generated in this explosion were detected by Kamiokande. The number of neutrinos detected in this event was eleven in only thirteen seconds, but the team was able to identify their source to be 1987A based on their energy, direction, and detection frequency. This happened only about five weeks before Professor Koshiba retired. It was the first time in the history of humanity that neutrinos associated with a supernova explosion were detected, and it opened up the doors to a new frontier of physics, that is, neutrino astronomy.

Currently, the larger and more powerful version of Kamiokande, Super-Kamiokande, is in operation (Figure 1-3-2). The photomultiplier tubes have also been improved. Depending on the conditions, it can even detect light from a flashlight irradiated from the moon surface. Thanks to Super-Kamiokande, it was experimentally proven that neutrinos have mass, which brought the Director of the ICRR KAJITA Takaaki the Nobel Prize in Physics in 2015. Furthermore, the construction of the larger and upgraded version of Super-Kamiokande, Hyper-Kamiokande, is planned. Photomultiplier tubes are also used in IceCube (Figure 1-3-3), a neutrino observatory using abundant underground ice in the Antarctic that is operated under an international joint experiment project. Since it has much larger objects for neutrino to collide with than Super-Kamiokande, IceCube is expected to detect higher energy neutrinos.

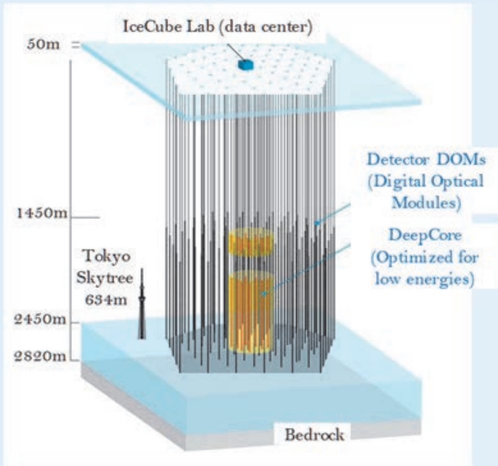
Photomultiplier tubes are also used for many purposes other than neutrino detection, such as the measurement of atmospheric nitrogen oxide and sulfur oxide concentrations, laser radar for automatic operation (LIDAR[1]), medical PET,[2] and X-ray diagnostic imaging. Photomultiplier tubes are expected to continue to be important equipment in a wide range of fields.

■Figure 1-3-2/Overview of Super-Kamiokande



Source: ICRR

■Figure 1-3-3/Overview of IceCube

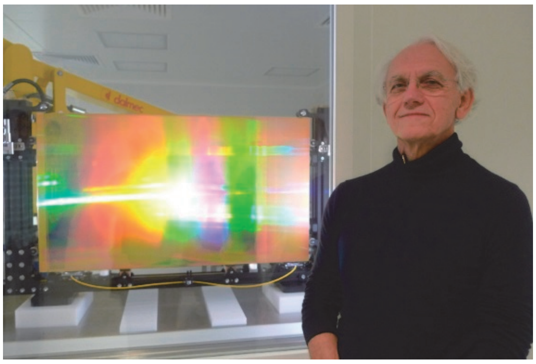



Source: Earthquake Research Institute, the University of Tokyo

1 Laser Imaging Detection and Ranging
2 Positron Emission Tomography

## 2 Essential Equipment for Optical Experiments (Diffraction Grating)

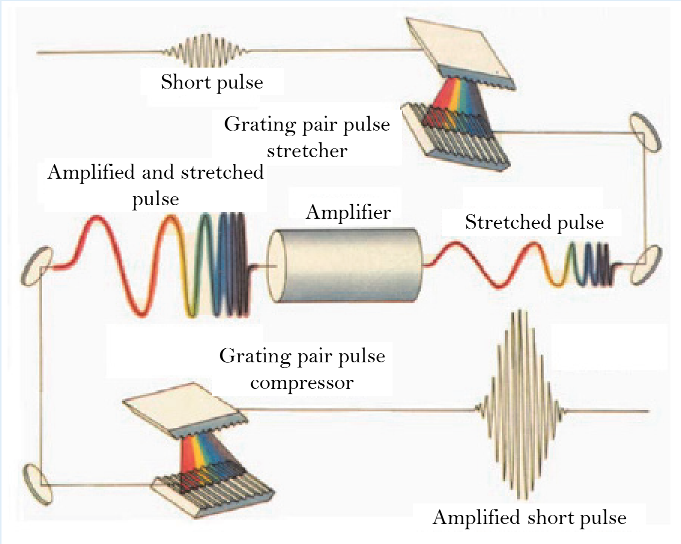
A diffraction grating is a key optical device used for the purpose of condensing and dispersing light in which a large number of parallel grooves are arranged at equal intervals on the surface of a substrate. In recent years, nanometer-precision microfabrication has also been used, while diffraction gratings with grooves arranged at non-uniform intervals has also been developed for higher performance. Grating was also an indispensable device in research by Prof. Gérard Mourou of the University of Rochester, US, who won the Nobel Prize in Physics in 2018 for the invention of chirped pulse amplification, a technology used to create high-intensity pulsed lasers. In this research, improvement of the durability of gratings was an issue as it involved high-intensity pulsed lasers. As a result of R&D efforts, the manufacturer succeeded in achieving the required durability, which helped high-intensity pulsed lasers come into reality. In commemoration of winning the Nobel Prize, Mourou donated the diffraction grating that he used in experiments over many years to the Nobel Museum.[1]



Diffraction grating and Prof. Mourou
Source: Horiba, Ltd.

In the chirp pulse amplification (CPA) scheme, first, the input pulse is spectrally separated by a grating to lower the energy per unit time. Next, the amplitude is amplified by an optical amplifier. Finally, the diffracted light is concentrated to create high-intensity pulsed lasers (Figure 1-3-4). Before the introduction of chirped pulse amplification, multiple oscillators and amplifiers were required to produce high-intensity pulsed lasers, and there was also a risk of damaging optical elements due to repeated amplification of high-intensity pulsed lasers. In addition, the overall size of the device was as large as a gymnasium. However, the chirped pulse amplification has made it possible to generate a laser pulse that is 1,000 times stronger than a conventional device using a desktop size device without damaging the optical elements.

■Figure 1-3-4/Scheme of chirp pulse amplification




Cited from HORIBA Technical Report Readout, No.27 (2003)

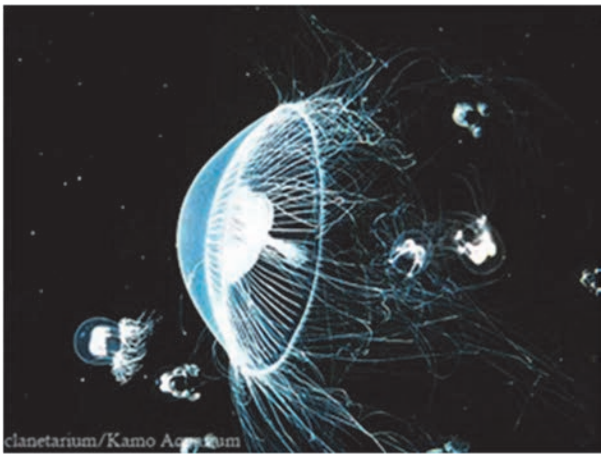
Currently, high-intensity pulsed lasers are used in various fields. In the material industry, they are used for microfabrication of substrate circuits in electronic equipment, which was an indispensable technology

1 http://www.horiba.com/jp/corporate-news/news/article/58453/

for the recent miniaturization of mobile phones and digital cameras. In addition, they are also used for processing CFRP[1] and in 3D printers. In the medical field, high-intensity pulsed lasers are starting to be used for cataract surgery and cancer treatment. Gratings are expected to become even more important in the future, as the use of high-intensity pulsed lasers expands in various fields.

## 3 Method to Make Proteins Glow While Keeping Subject Organisms Alive (GFP: Green Fluorescent Protein)

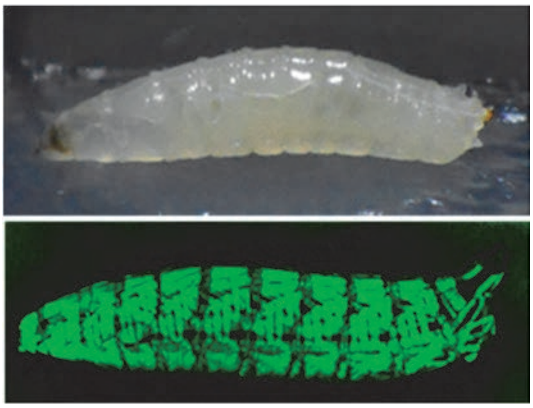
Proteins produced in cells are basic substances that constitute many organisms. The elucidation of their functions through observation is very important in basic research related to life science. However, since proteins are generally colorless and transparent, various visualization methods have been studied. Among them, green fluorescent protein (GFP)—which was discovered in the jellyfish Aequorea victoria by SHIMOMURA Osamu, Professor Emeritus at Boston University who won the Nobel Prize in Chemistry in 2008 for this discovery—has become a staple of basic research in life science. For his research on GFP, Shimomura and his whole family collected 850,000 Aequorea victoria over 19 years. It was his pure intellectual curiosity in exploring the mechanism of bioluminescence of Aequorea victoria that drove his unparalleled passion. GFP is a good example of a project that started as an academic research eventually contributing to the development of various fields.



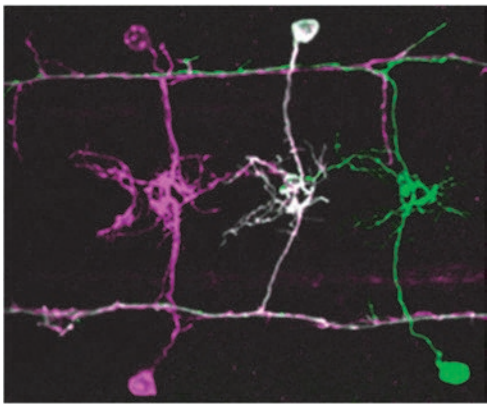
Aequorea victoria
Source: Kamo Aquarium, Tsuruoka City

Although there had already been many other visualization methods before GFP was put into use, they all required the subject organisms to be dead in exchange for visualization. GFP changed this norm. By linking GFP with subject proteins by genetic modification, it has become possible to observe the position and behavior of proteins while the subject organism is alive. This is the main reason why GFP is now an indispensable technology for basic research in life science. In addition, while the fluorescence emission color was typically green, it has also become possible to obtain many other colors by modifying a part of the structure of GFP, which has allowed for the simultaneous observation of different proteins.

1 Carbon Fiber Reinforced Plastics



Drosophila larva
Top: Appearance  Bottom: Fluorescence emission using GFP
Source: Graduate School of Frontier Sciences, The University of Tokyo



Drosophila neurons colored in three colors
Source: Graduate School of Frontier Sciences, The University of Tokyo

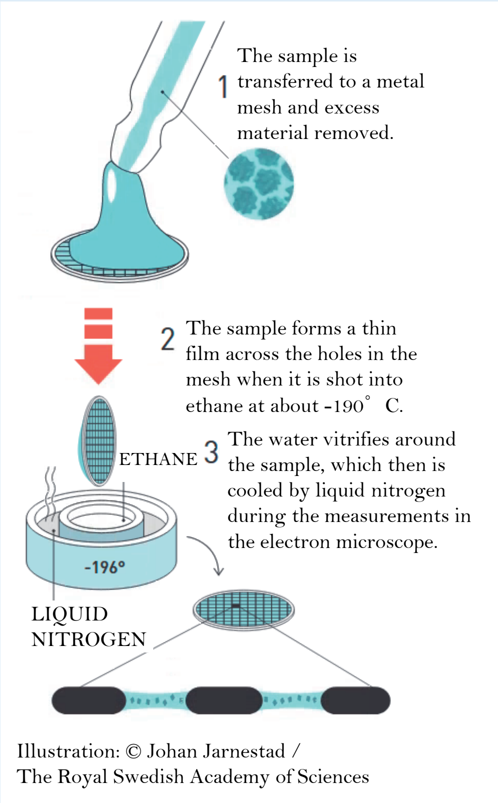
## 4 Technique for Observing Intact Proteins (Cryogenic Electron Microscopy)

Observing and understanding the molecular structures of proteins is important in basic research in life science. The results of such observation have been used for various purposes including drug development. This section looks at cryogenic electron microscopy, one of the technologies that have made this possible, which has greatly advanced recent basic research in life science.

The resolution of an electron microscope is sufficient for observing general proteins at the molecular level. However, there had been a problem that the state of the protein would change when observed with an electron microscope, because an electron microscope using electron beams requires the subject protein to be placed in a vacuum, which would cause the moisture in protein to evaporate. One of the methods that solved this issue was cryogenic electron microscopy. The three researchers involved in its development won the Nobel Prize in Chemistry in 2017. With the advent of cryogenic electron microscopy, the molecular structures of various proteins that had been unknown have been revealed one after another.

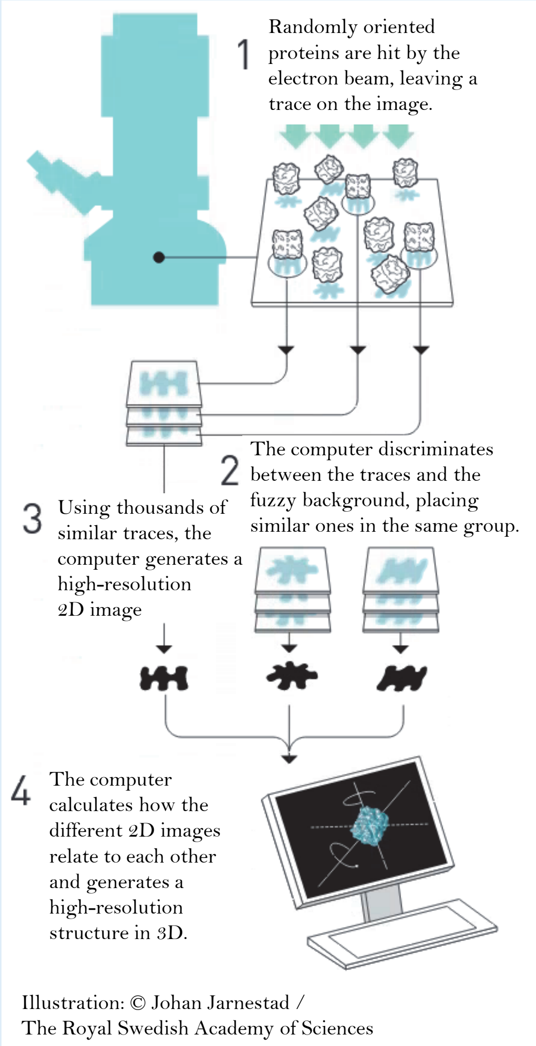
The observation process starts with pouring a protein solution onto a metal mesh and cooling it using a special method. Then, the water in the protein becomes vitreous, which prevents it from evaporating even in a vacuum. At the same time, the protein molecules are distributed almost two-dimensionally in the thin film between the grids, making it possible to observe individual molecules (Figure 1-3-5). Since each molecule is oriented in a random direction, the molecular structure of the protein cannot be determined by simply superimposing image data obtained by observation. However, cryogenic electron microscopy has made it possible to obtain information on the molecular structure of a protein in a higher resolution than the microscopic resolution by analyzing the correlations of tens of thousands of obtained image data using a special image processing algorithm (Figure 1-3-6).

■Figure 1-3-5/Sample cooling method




Source: The Nobel Foundation

■Figure 1-3-6/Method to obtain the 3D structure of a sample




Source: The Nobel Foundation

As described above, cryogenic electron microscopy is a measurement method using an existing electron microscope, and it is composed of a combination of a novel sample freezing method and an image processing method. The realization of this technique took not only the idea of the measurement method itself, but also high performance hardware, i.e. a highly sensitive electron beam detector, and software, i.e. a special image processing algorithm. These important elements were available because of other basic research efforts that brought about the miniaturization and high performance of electronic components and high functionality of computers. In other words, cryogenic electron microscopy, which supports basic research, is also supported by other basic research. This is another good example that well illustrates the importance of basic research.

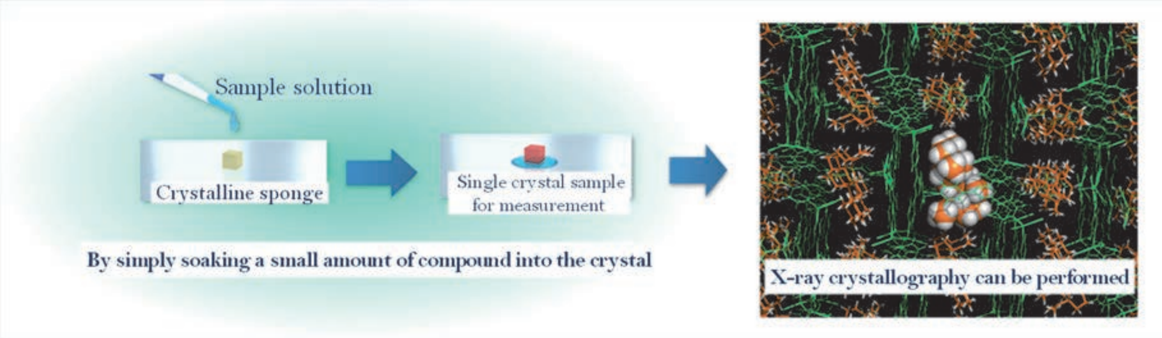
## 5 Measurement Method That Dramatically Improved the Efficiency of X-Ray Crystallography (Crystalline Sponge Method)

Accurately understanding molecular structures is one of the most important and indispensable basic processes in natural science research. The single crystal X-ray structure analysis method (x-ray crystallography) is the most reliable method to directly obtain information on three-dimensional molecule structures, but this measurement method required the process of sample crystallization. Many researchers have gone through many instances of trial and error in their attempts to create a single crystal. This challenge in x-ray crystallography was said to take a hundred years for researchers to solve. The crystalline sponge method that has recently been developed by FUJITA Makoto, Distinguished University Professor at the University of Tokyo, is a groundbreaking measurement method that solves this problem. In 2019, Fujita was awarded with the Imperial Prize of the Japan Academy and the Japan Academy Prize for the development of this method. In 2018 he also won the Wolf Prize, which has a reputation for predicting future winners of the Nobel Prize, for his achievement of developing a coordination-driven self-assembly material, a technology that underpins the crystalline sponge method.

The crystalline sponge method is a technique in which a sample solution is poured into a porous crystal called a crystalline sponge, and a periodic array of samples is created using the crystal as a template. This method makes it possible to perform X-ray crystallography and observe the molecular structure of the target compound without going through the crystallization process (Figure 1-3-7). By adopting the mechanism where a sample is poured into a space arranged beforehand, he solved the problem of X-ray structural analysis that was thought to take a hundred years to solve. In addition, the crystalline sponge method requires a far smaller amount of sample for measurement, as a diffraction experiment can be performed using one microcrystal grain. This feature has been proven to be a great advantage in the field of natural products chemistry where structures of components isolated from a small amount of natural products are analyzed. Thanks to the crystalline sponge method, the structures of more than dozens of natural compounds were determined in a short period of time. In addition, the combination of this technology and affinity screening with a mixture of many components has dramatically improved the efficiency of the workflow for determining structures of isolated natural products.

The use of the crystalline sponge method has rapidly spread not only in the academic world but also in pharmaceutical and other industries. The University of Tokyo has established a social collaboration course that provides a platform for research for the advancement of the crystalline sponge method, while technology transfer to related MEXT projects has been realized. The crystalline sponge method is expected to contribute greatly to the development of all natural science research involving molecules into the future.

■Figure 1-3-7/Overview of the crystalline sponge method




Source: School of Engineering, The University of Tokyo