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Applications note
Bio Solutions

Solutions for Innovation

Bionote

Morphology observation / Elemental analysis
TEM / SEM / FIB / EPMA / XRF / Micro CT

Structural analysis of compounds
ESR / MALDI-TOFMS / NMR

Clinical chemistry analysis
Clinical Chemistry Analyzer

JEOL Ltd.

Bio Solutions

Bionote

Introduction

Instrumental analysis has contributed to the progress of biological science, through the observation of morphology, the chemical analysis of compounds, the elucidation of amino acid sequences of proteins, etc. Furthermore, this progress has been accelerated by the development of analytical methods, which are used in diverse fields like medical science, agriculture, food and biotechnology, as well as the basic research work like physiology, biochemistry and genetics.
This Bionote presents an overview of the basics, namely principles and features of various instruments, as well as application examples using numerous optional attachments. We hope that the Bionote will assist researchers and engineers who intend to perform analyses in finding and exploring new approaches.

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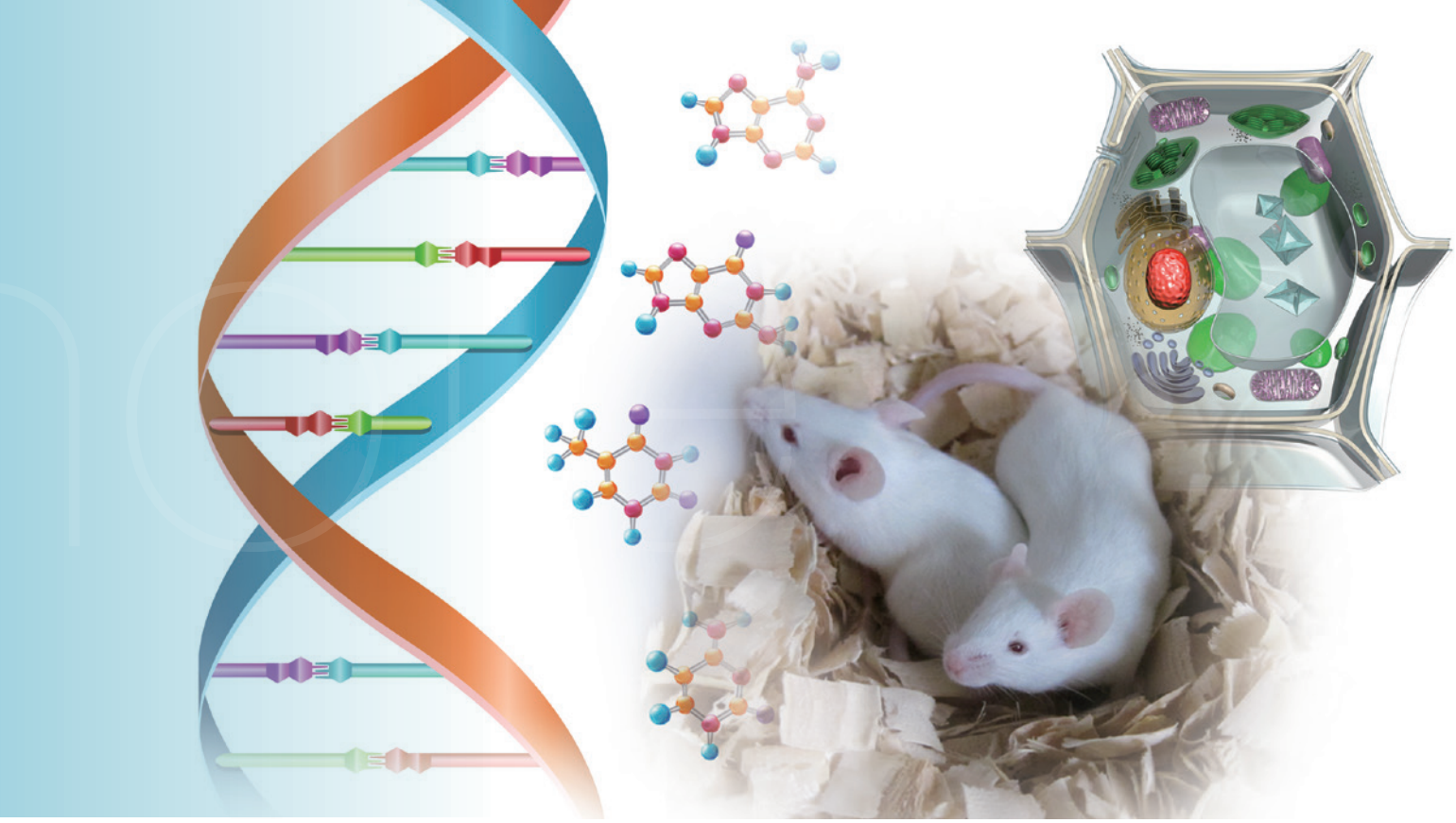


2. Structural analysis of compounds

3. Clinical chemistry analysis

1-1 Transmission Electron Microscope
TEM

The TEM is an instrument to observe a specimen using transmitted electrons. The TEM is suitable for observation from structures at the cellular size to macromolecular complexes to, finally, individual proteins and viruses. Various specimen preparation techniques allow for the elemental analysis of tissues thus expanding the knowledge gained using the TEM.

Transmission Electron Microscope : TEM

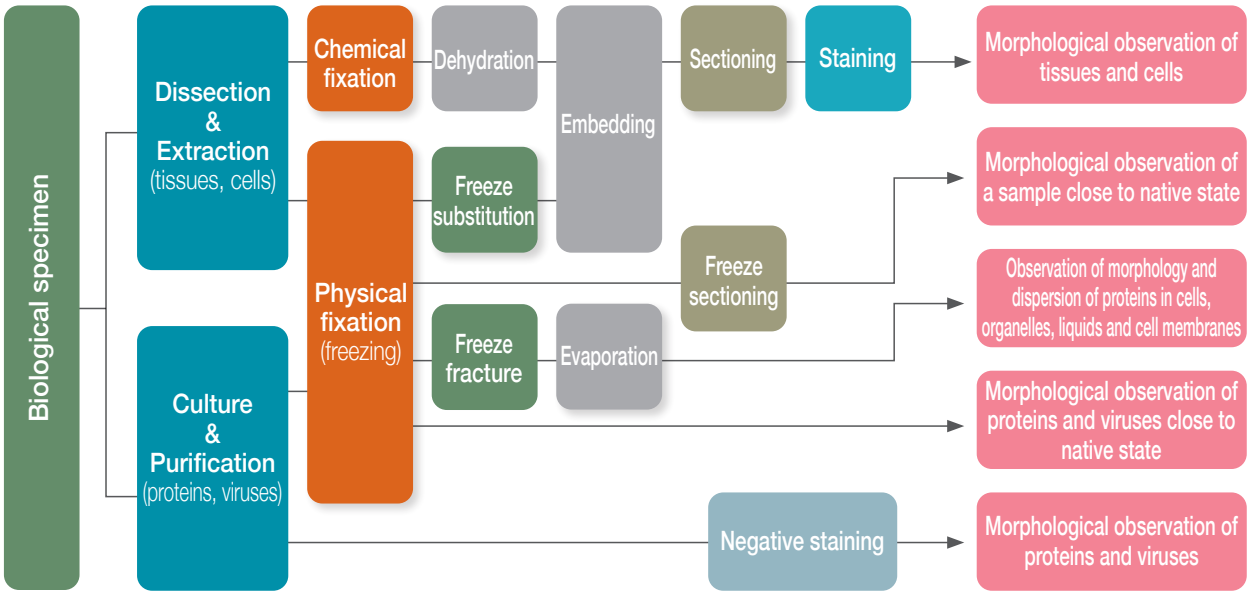
Observation of morphology
Wide range of magnification
Observing samples from tissues to proteins at various size scales.

Elemental analysis
Analyzing elements of samples using EDS.

Reconstruction of 3D structures by Tomography
Reconstructing 3D structures from a tilt series of sample images.

Specimen preparation

The structure of biological tissues start to degrade as soon as their cells are dead. Therefore, it is necessary to treat the sample in order to preserve their native structure. Additionally, the specimens must be thin enough, typically around 100 nm, to allow 120 keV electrons to transmitt the specimen. It is critical to select the appropriate sample preparation technique that preserves the information being required.



Chemical fixation

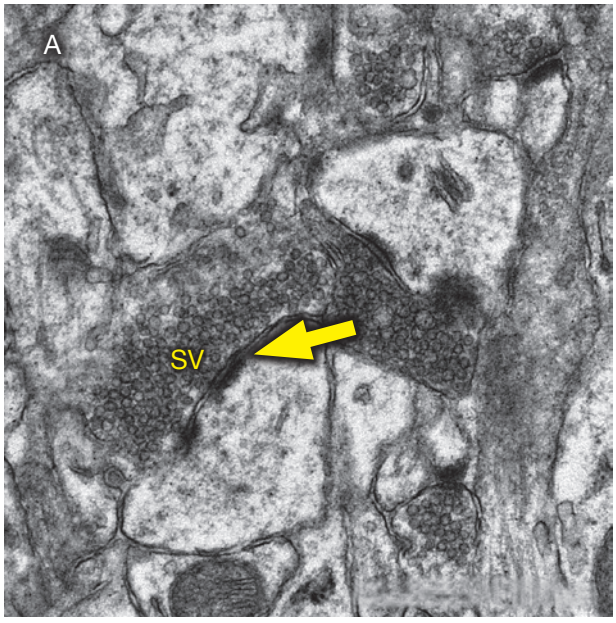
Chemical fixation is a standard technique for preserving nano structures of biological samples in TEM. Because the inside of electron microscope column is vacuum, we have to dehydrate a specimen in a way that preserves their morphology. Chemical fixation cross-links molecules of proteins and/or lipids with adequate chemical treatments to prevent decomposition and deformation of the sample. Specimens that are fixed are subsequently embedded in resin after which thin sections can be sliced using a microtome. Prerequisite is that the sections are thin enough to transmit the electron beam, typically in the range of 60 to 90 nm.

Procedures

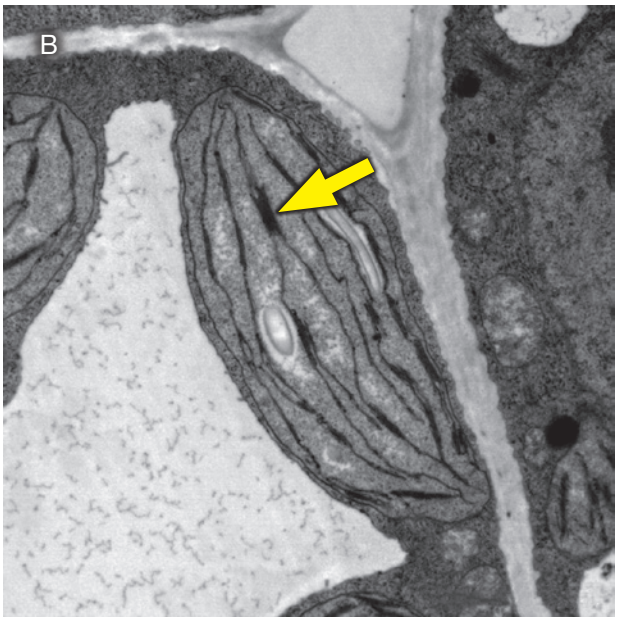
Tissue extraction	Cut an interested volume from a sample.
Chemical fixation	Crosslink with glutaraldehyde for proteins and osmium tetroxide for lipids.
Dehydration	Dehydrate the sample using a series of ethanol-water mixtures at increasingly higher ratios.
Resin embedding	Impregnate with resin by substituting ethanol for resin followed by polymerization sometimes in the presence of a staining agent.
Ultra-thin sectioning	After block trimming, specimens are sliced at about 60 to 90 nm using an ultramicrotome.
Electron staining	Apply post embedding staining to enhance specimen contrast.
TEM observation	

Application example

Two TEM images below show animal tissues (A: rat hippocampus) and plant tissues (B: spinach leaves) prepared by chemical fixation. Chemical fixation can be applied to all types of living organisms. In (A) synapses (indicated by the arrow) and synaptic vesicles (indicated by SV) are observed. In (B) chloroplasts and stacked thylakoid membranes (indicated by the arrow) are observed.



Specimen: Rat hippocampus — 500 nm



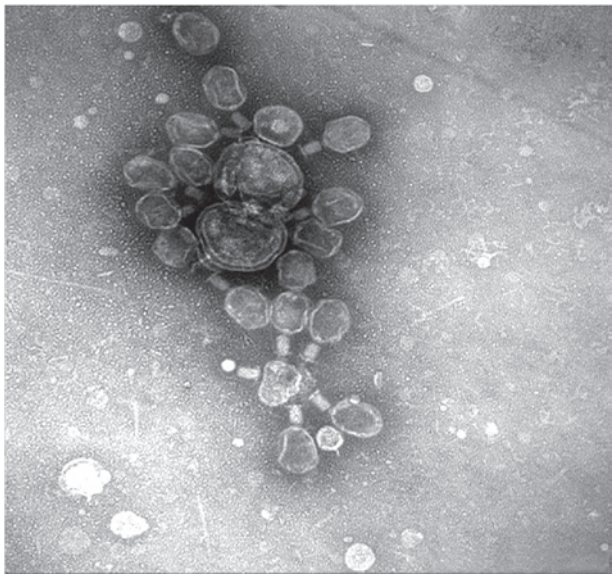
Specimen: Spinach leaves — 1 µm

Negative staining

Negative staining is a technique well suited to study the morphology of purified proteins or viruses. An aqueous solution of protein or viruses is applied to a support film followed by blotting with a filter paper to remove excess liquid. Immediately after this step, a solution of heavy metal salt (uranyl acetate, phosphotungstic acid, etc.) is applied to the specimen followed again by a blotting step to remove excess stain. This procedure only reveals the shape rather than internal details of a protein or a virus.

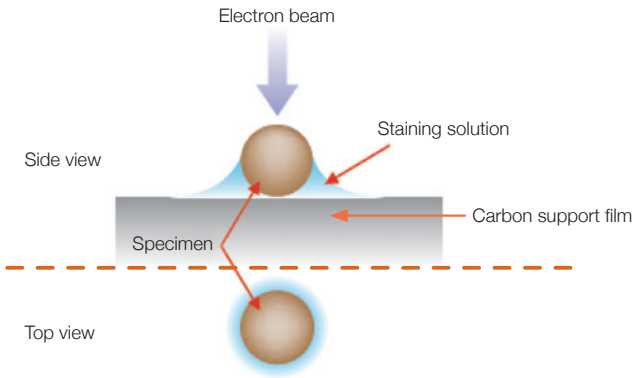
Application example

TEM image of bacteriophage T4 negative stained with an uranyl acetate solution



200 nm

Schematic of negative staining

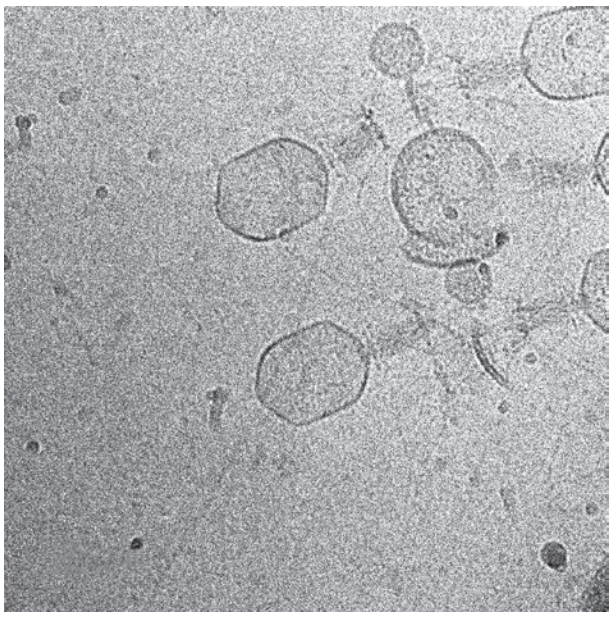


Cryo observation

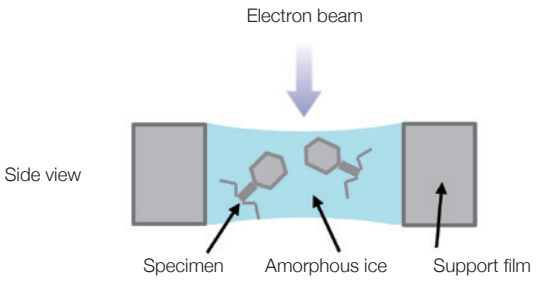
Cryo-TEM is a technique whereby thin films with specimen solution are vitrified using an appropriate plunging device and subsequently transferred and imaged in the frozen-hydrated state in the microscope. Cryo-TEM enables observation of specimens in the native state.

Application example

TEM image of bacteriophage T4 embedded in amorphous ice by the plunge freezing technique



100 nm

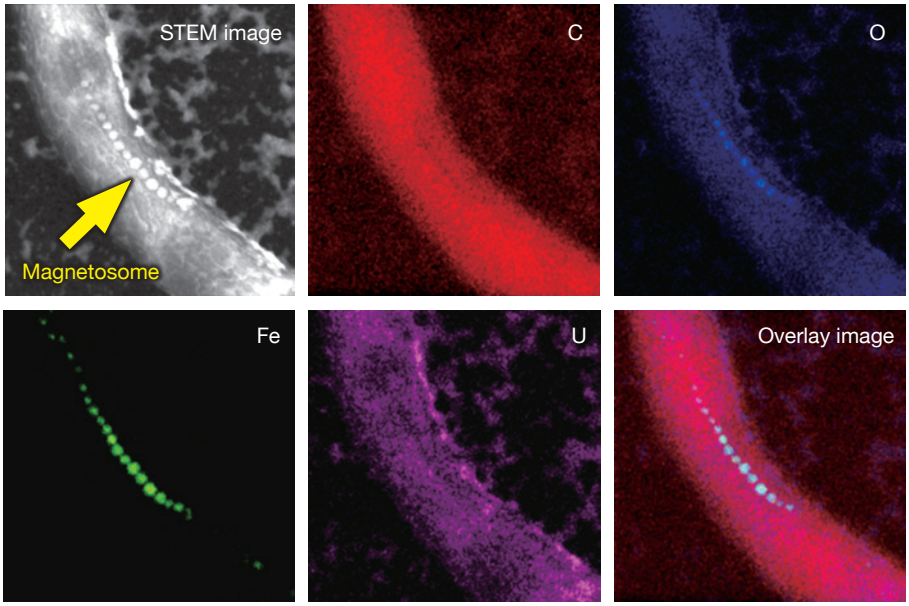


Elemental analysis

Energy Dispersive X-ray Spectroscopy (EDS) enables identification of elements ranging from Be to U. The EDS enables applications such as spot analysis and elemental mapping in combination with Scanning Transmission Electron Microscopy (STEM).

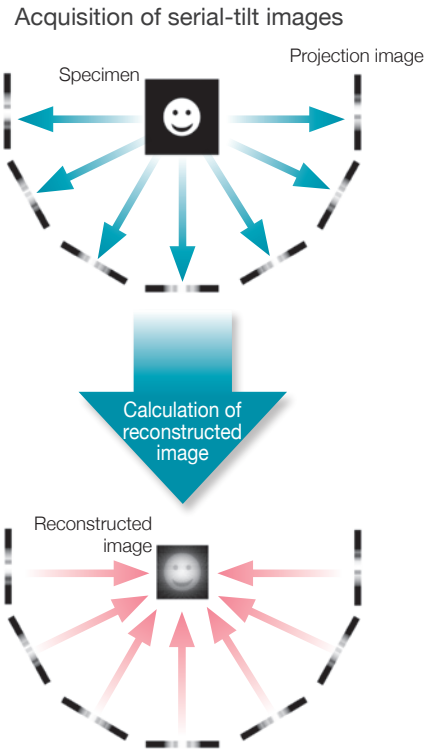
Application example

Specimen: Magnetotactic bacterium
EDS maps of magnetotactic bacterium which has special structures in cells (called Magnetosome) composed of magnetite.



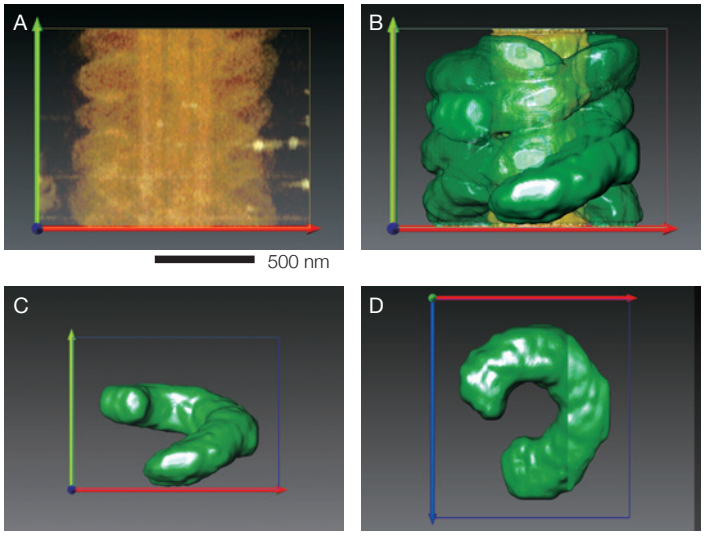
Reconstruction of 3D structures

Specimens observed in the TEM yield projection images thus losing all information about the 3D structure. To determine the 3D structure of a specimen, the principle of tomography is applied. Images of a specimen obtained at various tilting angles, a.k.a. Radon transforms, are combined in a reconstruction step using the inverse Radon transform to yield the original 3D structure as is used in for instance the weighted back projection.



Application example

Specimen: Mouse sperm
3D volume rendered image (A) of mouse sperm obtained by TEM tomography and its 3D image after color segmentation for mitochondria (green) and the flagellum (yellow). Bottom panels show side and top views of a single mitochondrion coiling the flagellum (C, D).



1-2 Scanning Electron Microscope SEM

The SEM is an instrument used to observe the surface morphology of a specimen. When the specimen surface is illuminated with the electron beam, various signals are emitted due to interactions between the specimen and electron beam. In the SEM, a finely-focused electron probe is scanned over the specimen surface, and then the signals generated from the specimen are detected by several detectors for morphological observation and elemental analysis of the specimen.

Schottky field emission SEM : FE-SEM

Surface observation
Observing the surface morphology of the bulk specimen.

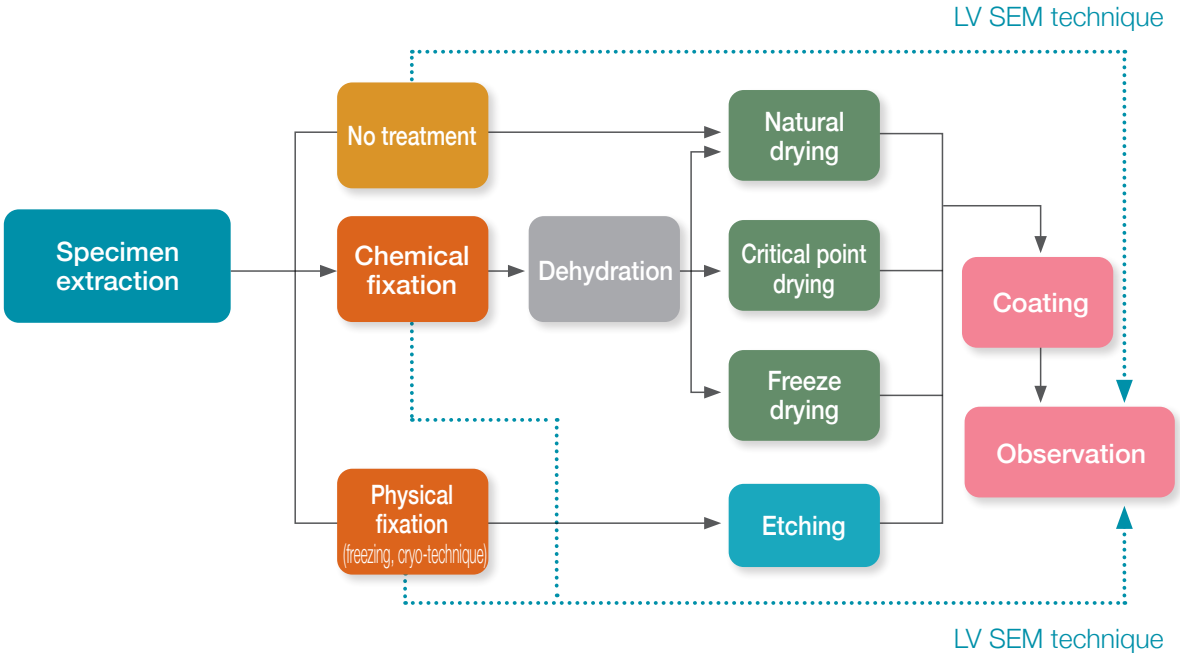
Compositional observation
Observing the compositional difference in the specimen through the backscattered electron image.

Elemental analysis
Analyzing elements of specimen using EDS.

Reconstruction of 3D structures
Reconstructing 3D structure by taking serial section images or serial block face images of the specimen.

Specimen preparation

When a hydrated specimen (biological tissue, etc.) is observed with the SEM, appropriate specimen preparation is required because the inside of the SEM column and specimen chamber is kept at high vacuum. To prevent deformation of the hydrated specimen under high vacuum, chemical fixation, physical fixation, or both of these techniques is generally used. In recent years, the low vacuum (LV) SEM technique with increased pressure inside the specimen chamber is also improved. It is necessary to choose an appropriate specimen preparation depending on examination requirements.



Chemical fixation

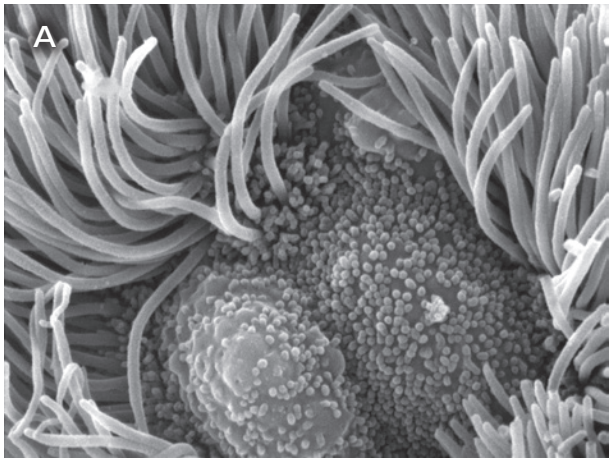
Chemical fixation is a technique that fixes proteins, lipids, etc., in a hydrated specimen (biological tissue, etc.) with chemicals to preserve structures of the specimen close to its native state. After the specimen is washed, proteins are fixed by gultaraldehyde whereas lipids are fixed by osmium tetroxide. Then, the specimen is dried using a freeze dry system or a critical point dry system. If necessary, thin (conductive) metal coating is applied to the specimen surface for SEM observation.

Procedures

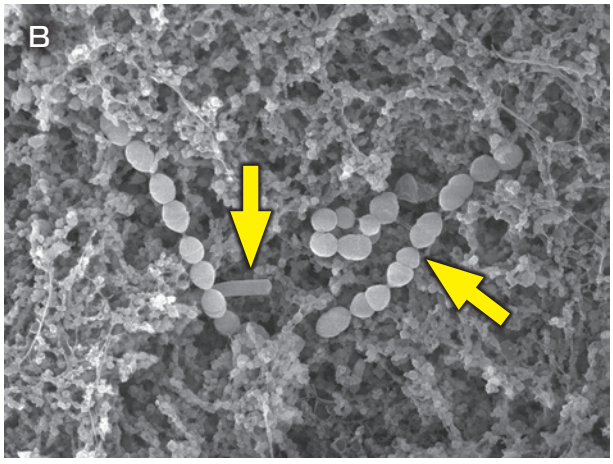
Specimen pickup	Cut a specimen.
Washing	Remove dust, mucus, etc.
Chemical fixation	Apply cross-linking to proteins and lipids where proteins are fixed by gultaraldehyde whereas lipids are fixed by osmium tetroxide.
Dehydration	To remove water from the specimen, substitute water for ethanol.
Substitution	Substitute ethanol for a solution suitable for drying.
Drying	Dry the specimen while preserving the morphology of the specimen.
Coating	Apply metal or carbon coating onto the specimen surface.
SEM observation	The specimen is illuminated with an electron beam under high vacuum.

Application example

A: Mouse tracheas, subject to general chemical fixation, were observed with the SEM. Flagella to remove foreign materials were found.
B: Freeze-dried yogurt, fixed only with gultaraldehyde, was subject to SEM observation. Two types of bacterium were observed.
Since fixation with osmium tetroxide was not applied, particles around bacteria are regarded mainly to be proteins.



Specimen: Mouse tracheas Coating : Pt
Accelerating voltage: 15 kV Direct magnification: x10,000
Secondary electron image

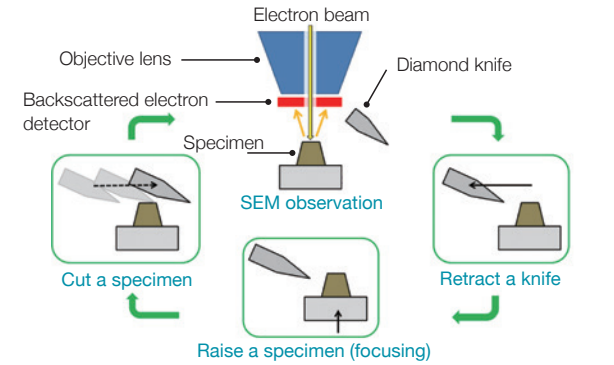


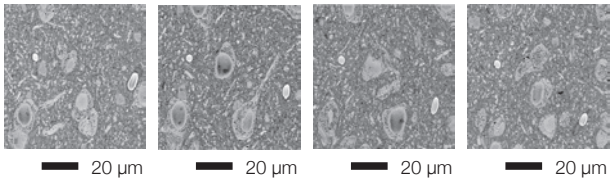
Specimen: Yogurt Coating : Pt
Accelerating voltage: 15 kV Direct magnification: x 5,000
Secondary electron image

Reconstruction of 3D structures

The surface of a resin-embedded block specimen of biological material is cut serially for SEM observation. Repetition of cut & imaging provides serial SEM images in the thickness direction. Then, a 3D reconstruction image is formed by stacking these serial images. Furthermore, extraction of the target objects (segmentation) reveals 3D structures over an area of several hundred micrometers.
A specimen is prepared by the NCMIR method #, which applies TEM specimen preparation techniques. “Cut & Imaging” can be made fully automatically with a dedicated system that is put a microtome into the SEM specimen chamber.

Deerinck, T. J., Bushong, E., Thor, A. & Ellisman, M.H.
NCMIR methods for 3D EM: A new protocol for preparation of biological specimens for serial block-face SEM. *Microscopy (Oxf),6-8.(2010)*

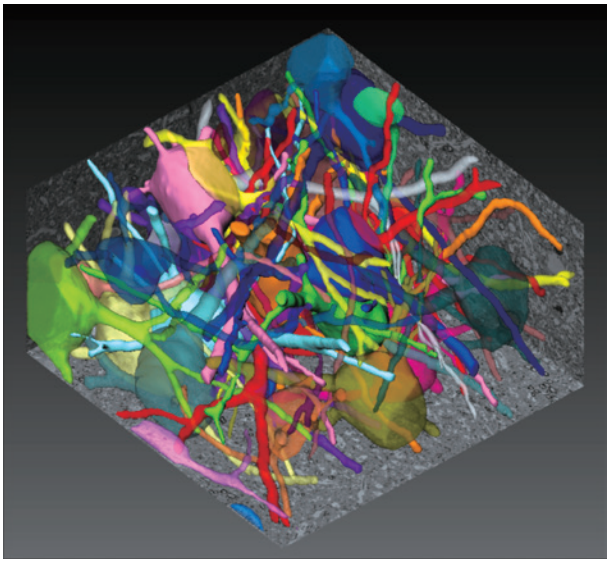




Work flow of cut & imaging and serially acquired 2D images.

Application example

Segmentation and coloring were conducted for the respective nerve cells of a hippocampus.



Nerve cells of a hippocampus

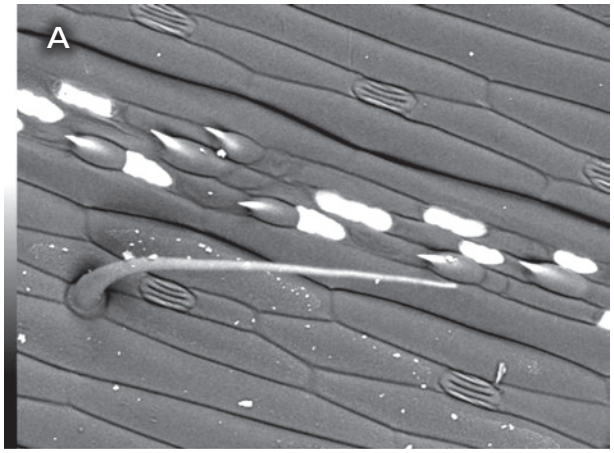
Specimen courtesy: Professor A. Mizoguchi (Dept. of Neural Regeneration and Cell Communication, Mie University Graduate School of Medicine.)

Elemental analysis

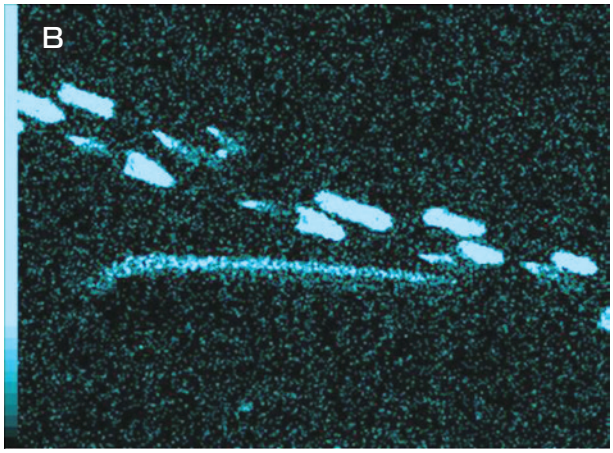
Energy Dispersive X-ray Spectroscopy (EDS) is used for elemental analysis by detecting characteristic X-rays generated from a specimen when illuminated with an electron beam. EDS, combined with the SEM, enables you to not only identify constituent elements through point analysis of areas with different contrast found by the backscattered electron image, but also confirm the 2D distribution of specified elements (elemental mapping result). When used with the LV SEM, it is easily possible to confirm the distribution of minerals contained in plants.

Application example

A: Backscattered electron image in LV mode (without coating) reveals that Si particles exist at the back of the leaf.
B: Elemental mapping result of Si taken from the same area in the left image. Large amounts of Si are confirmed to be contained in regions that correspond to white parts and a hair-shaped part appearing in the backscattered electron image.



100 µm



Si K

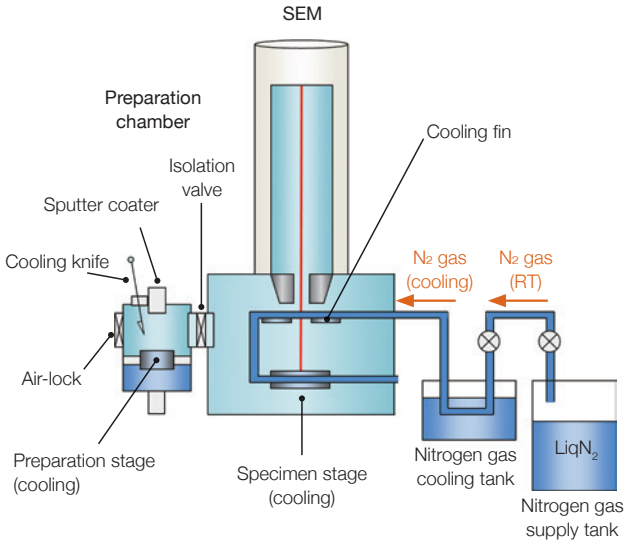
100 µm

Specimen: Leaf Accelerating voltage: 15 kV Direct magnification: x 300 Backscattered electron shadowed image

Physical fixation Cryo-SEM observation

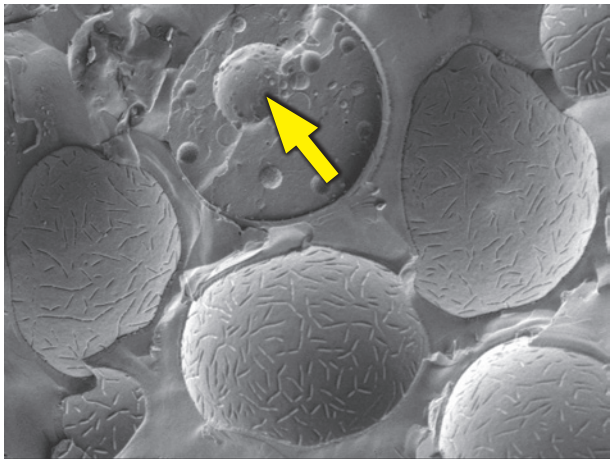
Physical fixation and subsequent cryo-SEM are techniques to freeze and observe a specimen while preserving the morphology of a hydrated specimen. If necessary, after the specimen is frozen, a fractured surface is created for a target site in the specimen. Then, the specimen is observed while keeping its frozen state, using the cryo-holder and cryo-stage.

Schematic of cryo-stage



Application example

Bread yeast was observed. This specimen was freeze-fractured to enable observation of surface structures and cross sectional structures. Nuclear pores of the yeast is seen near the center of the cross section (indicated by arrow).

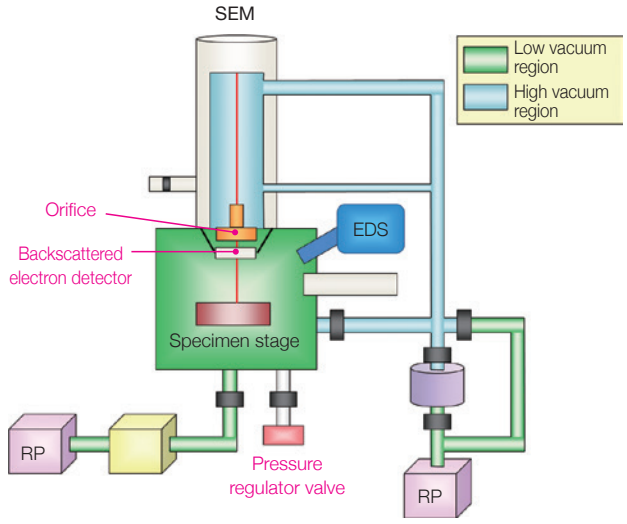


Specimen: Bread yeast Coating : Pt
Accelerating voltage: 1.5 kV Magnification: x10,000 Secondary electron image

Low vacuum SEM (LV SEM)

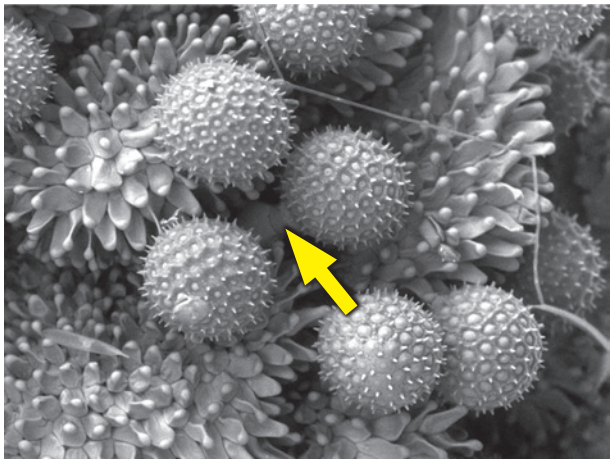
The Low vacuum SEM (LV SEM) is used to increase the pressure only in the SEM specimen chamber by differential pumping while maintaining high vacuum in the electron gun chamber and microscope column, to observe and analyze nonconductive or wet specimens. Since a low vacuum (1 Pa to several hundreds of Pa) in the specimen chamber is kept, vaporization of little water or fat content in the specimen is suppressed compared to the conventional SEM. Furthermore, the residual gas ionized by an incident electron neutralizes charging of a sample. Therefore it is unnecessary to apply coating on a sample. Combined use with the cryo-stage is also possible.

Schematic of LV SEM



Application example

Pistil of morning glory was observed. The pollination tube of the pistil (indicated by arrow) was seen without conductive coating.



Specimen: Pistil and pollens of morning glory.
Accelerating voltage: 15 kV Vacuum pressure: 27 Pa

1-3 Focused ion beam system
FIB

The FIB system irradiates a finely focused Ga ion beam onto a specimen while scanning the beam in the X and Y directions for milling and observation of the specimen. Owing to the sputtering effect of Ga ions, the system enables fine processing & cross section processing of the specimen and thin film preparation. Furthermore, the FIB system enables you to observe an SIM (scanning ion microscope) image formed by Ga irradiation, and also to prepare thin films of carbon, tungsten and platinum due to irradiation of organic metal gas. The Multi Beam system is also available, which is designed to effectively combine FIB and SEM capabilities. This powerful system simultaneously enables FIB cross-section milling and SEM imaging of the cross section.

Focused ion beam system: FIB

Cross-section & Thin-film preparation
Cross-section milling & thin-film preparation due to a Ga ion beam onto a specimen.

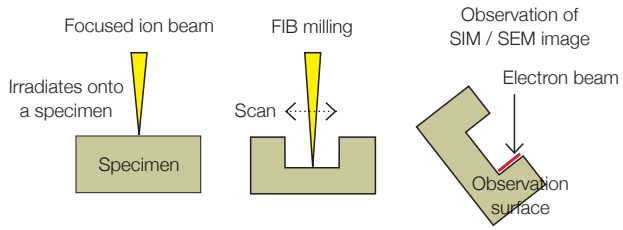
Observation
Forming SIM image and SEM image.

Cryo-observation
Processing and observing a frozen specimen with its frozen state kept, using Cryo-unit.

Reconstruction of 3D structures
Reconstructin 3D structures by repeating FIB milling and observed by SEM.

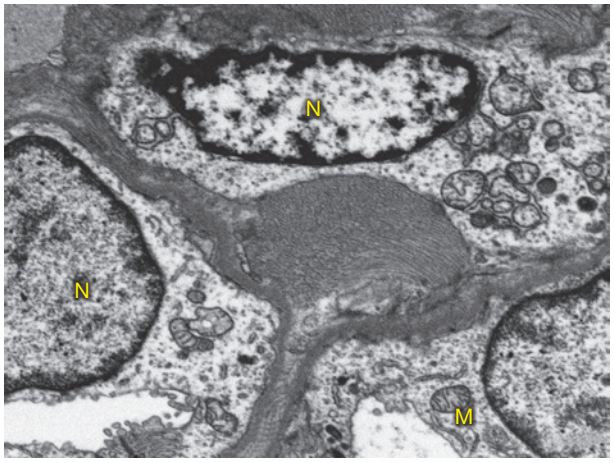
Principle

Basic procedures of FIB milling and observation



Application example

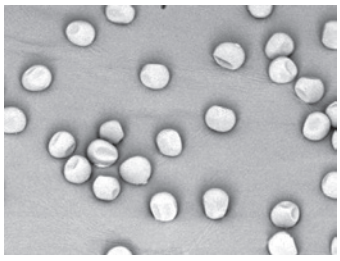
Specimen of rat cerebellum, subject to EPON-resin embedding, was milled by FIB, and then a prepared cross section was observed with the SEM. N : nucleus, M: mitochondrion



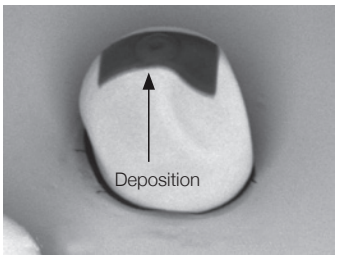
1 µm

Application example

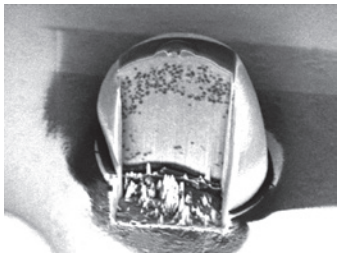
Pollen of raw corn was milled and its cross section was observed.



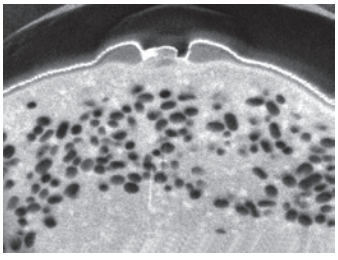
Low magnification image — 100 µm



Preparation for milling — 10 µm



FIB milling — 10 µm



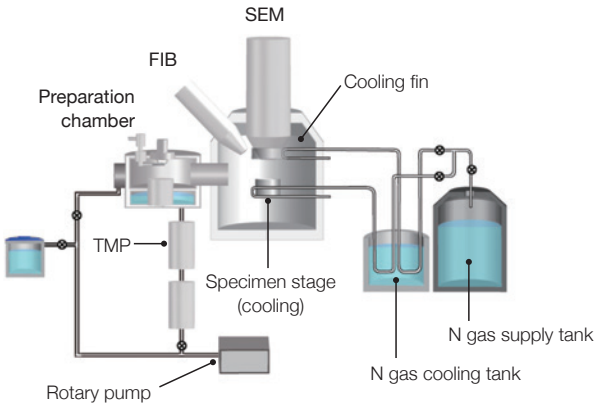
SEM observation — 5 µm

SEM image
(backscattered electron image)

Cryo observation

Cryo FIB-SEM is a technique to process and observe a frozen hydrated specimen while preserving the morphology of the hydrated specimen. The specimen is rapidly frozen without chemical treatment, and a specific region of the specimen (cellular tissue, food, cosmetics, etc.) is subject to cross sectional milling. These procedures enable observation and analysis of internal structures of the specimen.

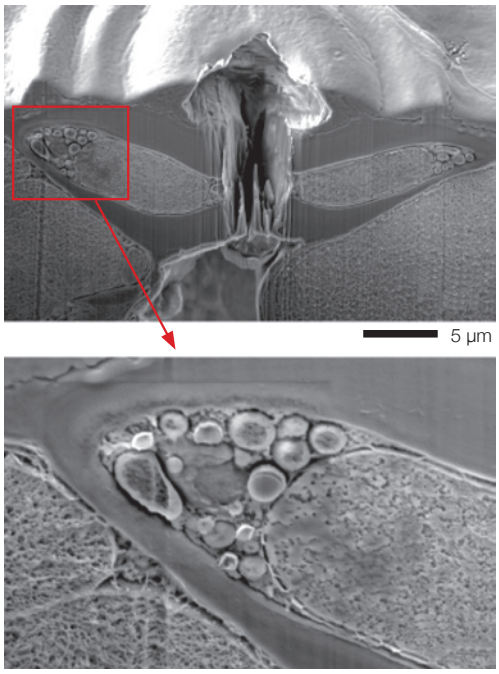
Schematic of FIB equipped with cryo-unit



The cryo-unit consists of the preparation chamber and the cooling stage. The preparation chamber incorporates a cooling knife, a sputter coater, etc., to perform fracturing and conductive coating of a frozen specimen. The cooling stage is cooled by liquid nitrogen gas. The unit enables FIB milling & observation and SEM observation.

Application example

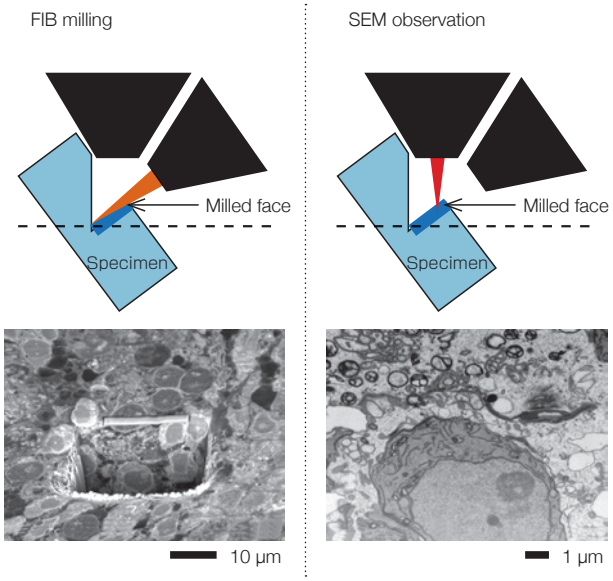
Stoma part of sasanqua leaf was subject to cross sections milling and observed by SEM. When using a freeze fracture technique, it is difficult to expose the cross section of a very small region such as pore. But the use of cryo FIB-SEM facilitates cross sectional milling of a small specific region.



Specimen: Cross section of stoma of sasanqua leaf

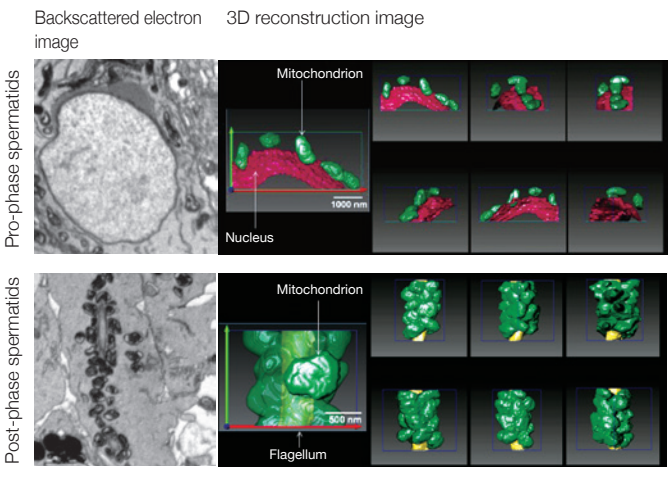
Reconstruction of 3D structures

In 3D reconstruction with FIB-SEM, cross sectional milling by FIB and acquisition of SEM image are serially performed. Then by stacking serial cross-section SEM images, 3D structures of the specimen are reconstructed. This technique is used for reconstruction of 3D structures of a biological specimen, which is fixed and resin-embedded for TEM observation. The features of 3D reconstruction by FIB-SEM are high positional accuracy and small distortion due to milling.



Application example

Rat spermatids, subjected to resin-embedding, were 3D-reconstructed. From the images below, the distribution and form of mitochondria are found to be different between the pro-phase spermatids and the post-phase spermatids.



Coloring by segmentation
Green: Mitochondrion Purple: Nucleus Yellow: Flagellum

1-4 Electron Probe Microanalyzer EPMA

The EPMA is an instrument used to perform elemental analysis in an area of micrometer order into the specimen. When the specimen surface is irradiated with the electron beam, various signals are emitted due to interactions between the specimen and electron beam. In the EPMA, a finely-focused electron probe is scanned over the specimen surface, and then characteristic X-rays whose wavelengths (energies) particular to the constituent elements are analyzed with a wavelength dispersive X-ray spectrometer (WDS) from the area of the order of micrometers.

Electron Probe Microanalyzer: EPMA

Surface observation
Acquiring the surface electron images of the sample.

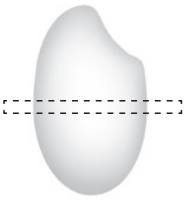
Trace-element analysis
Measuring the trace elements of only a few 10 to a few 100 ppm.

Qualitative & quantitative analysis
Analyzing the main components with accuracy of 1% relative error.

Elemental maps
Analyzing the element maps with sub-micron spatial resolution.

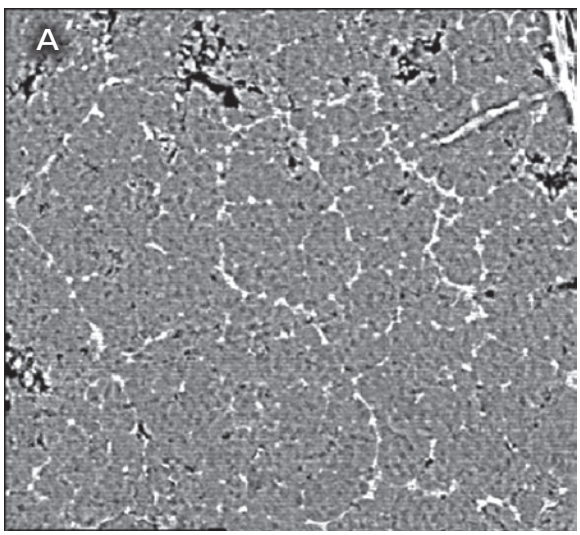
Thin film specimen analysis

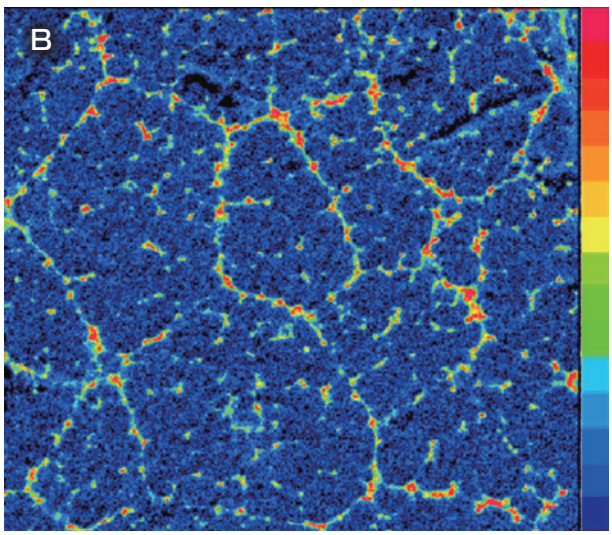
Generally, EPMA needs a very large probe current rather than the SEM-EDS work, sample damage (especially organic material) due to electron beam irradiation should be concerned. The rice grain (in the right image) was thinned down to a thickness of a few 100 nm with microtome and accelerating voltage is set to the voltage so that the irradiated electron beam can be transmitted through the specimen. This procedure suppresses thermal damage to the specimen during EPMA analysis.



Application example

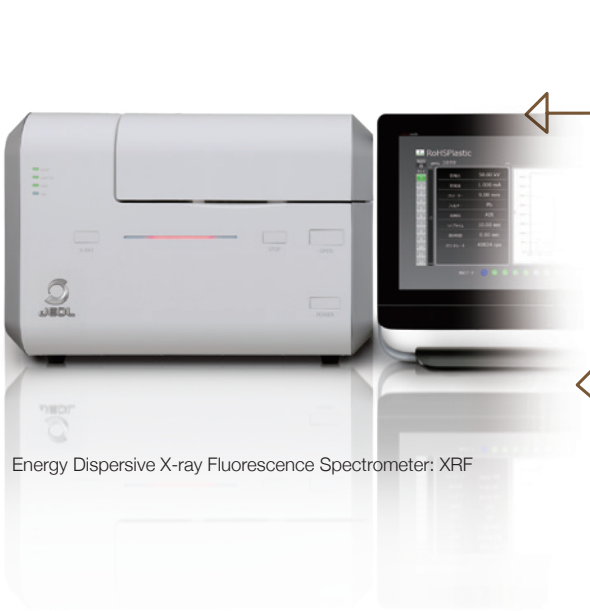
A: BSE image of the cross section of white rice.
B: Nitrogen map of rice grain which was acquired with BSE. X-ray intensity range shows as color level which is right side of this map. The nitrogen-rich regions are confirmed from the white part in BSE image.





1-5 X-Ray Fluorescence Spectrometer XRF

The XRF is an elemental analysis instrument that irradiates an X-ray beam onto a sample and detects fluorescence X-rays emitted from the sample for analyzing types of elements and elemental composition of the sample.
The XRF enables direct measurement of solid, powder or liquid samples. In addition, quantitative analysis, ranging from trace element to main element analysis, can be performed using by theoretical calculation (fundamental parameter (FP) method).



Energy Dispersive X-ray Fluorescence Spectrometer: XRF

Non-destructive analysis
Analyzing liquids (oil, emulsion, etc.) as well as solids.

Standardless quantitative analysis
Performing quantitative analysis, ranging from trace element to main element analysis, without standard samples.

Elemental analysis
Analyzing elements from C to U.

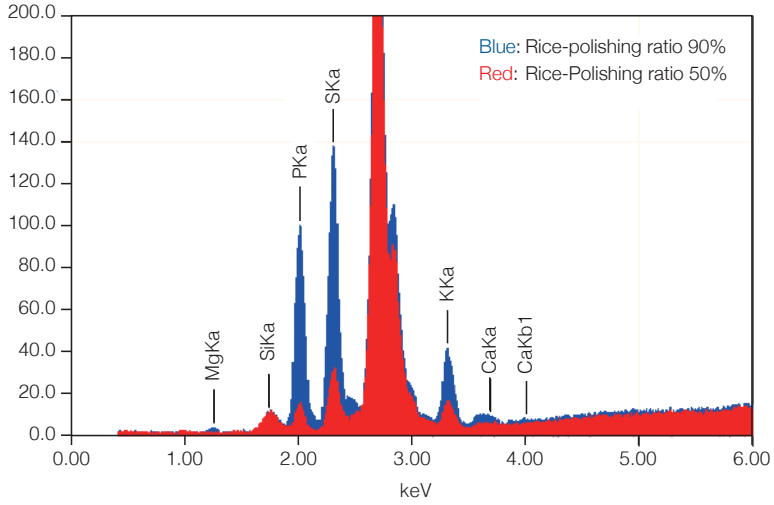
Deposit concentration analysis & thickness measurement
Analyzing deposit amount or thickness of deposited on the sample surface.

Non-destructive analysis

Application example

Relation of polishing ratio and mineral component of rice
The relation of polishing ratio and mineral component of rice, which is the principal food and also the material of Japanese Sake, was examined.

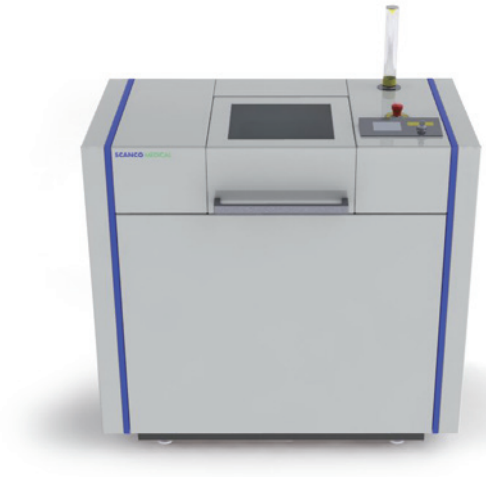
Comparison of minerals were carried out for white rice (polishing ratio: 90%) which Japanese people eat generally, and for another white rice (polishing ratio: 50%) which is used as the material for Japanese Sake. From the former rice (90% polishing ratio), Mg, P, S, K and Ca were confirmed. On the other hand from the latter rice (50% polishing ratio), very small concentrations of P, S and K were confirmed. This result indicated that the mineral content in the white rice was different depending on the polishing ratio.



White rice with 90% polishing ratio

1-6 Micro Focus X-Ray Computer Tomography
Micro CT

The MicroCT supports non-destructive 3D micro-structural analysis of a wide range of samples, including biological samples and industrial materials. Hardware and software (for measurement, 2D/3D structural analysis and finite element analysis (FEA)), designed based on the system source, are optimally integrated. This robust integration unifies the MicroCT for a seamless application.



Model: µCT50
SCANCO MEDICAL AG (Swiss)

Non-destructive 2D/3D structural analysis
Analyzing a large sample (max. 100 mm dia, 160 mm height) in air or liquid without pre-treatment.

High image resolution of 0.5 µm
Performing structural analysis at a resolution as high as 0.5 µm.

Automatic serial measurement of multiple samples
Performing automatic serial measurement of multiple samples under different measurement conditions.

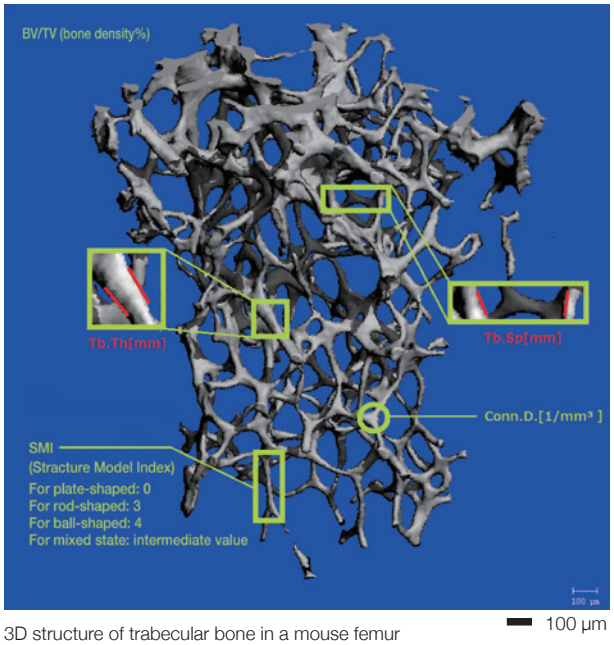
2D/3D stress simulation software
Executing all kinds of stress simulations with structural analysis software integrated with hardware.

Bone structural analysis

The MicroCT can perform a variety of structural analyses of bones, including modeling of osteoporosis diseases and quantitative analysis of fine structural changes in various bone diseases at the µm order. A narrow-angle cone beam eliminates the artifact and electrical noise that are characteristic of the MicroCT, thus enabling image analysis with high reproducibility quality. After finishing measurement and analysis, parameters of all of the bone analysis results, are automatically saved in the quantification database.

Application example

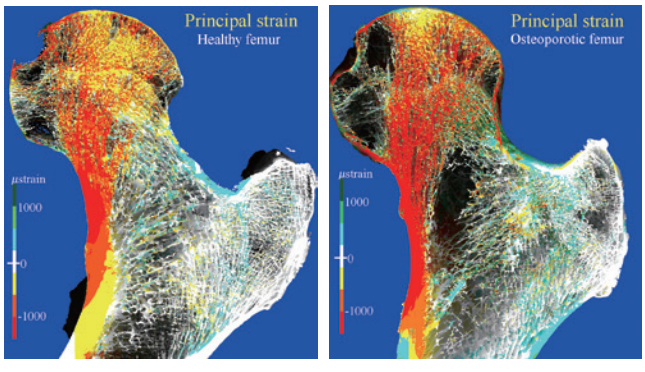
Bone parameter: TA, BV, BS, Tb.N, BV/TV, Tb.Th, BS/TV, Tb.Sp, TSL, Conn.Dens, TBPf, MIL, DA, SMI, Nd, TM, etc., plus all parameters of stress simulations.



3D structure of trabecular bone in a mouse femur

Left: Stress simulation 3D image based on a model of a femur of a healthy person
Right: Stress simulation 3D image based on a femur of a person suffering osteoporpsis

* Red color indicates a high stress part.



2-1 Electron Spin Resonance
ESR

In the living body, radicals are thought to be involved in a variety of reactions. ESR is a technique to selectively observe only radicals in a sample. It is similar in principle to NMR, although it is measured by sweeping a magnetic field while irradiating microwaves of a fixed frequency. The spectrum of ESR is given by the first derivative waveform and sensitivity is much higher than NMR.
Paramagnetic metals can also be measured and spectra reflecting their structure and environment can be obtained.

Electron Spin Resonance:ESR

Suitable for samples in various states including
liquids, gell, solids, gases, etc.

Metallic components are also observed
Structural analysis of the active center of metalloenzymes can also be evaluated.

Accurate correction with dedicated markers
Allows calculation of parameters important for radical identification.

Qualitative and quantitative analysis of radicals is possible
Generation and scavenging of reactive oxygen species, reactive nitrogen, and supersulfide can be evaluated.

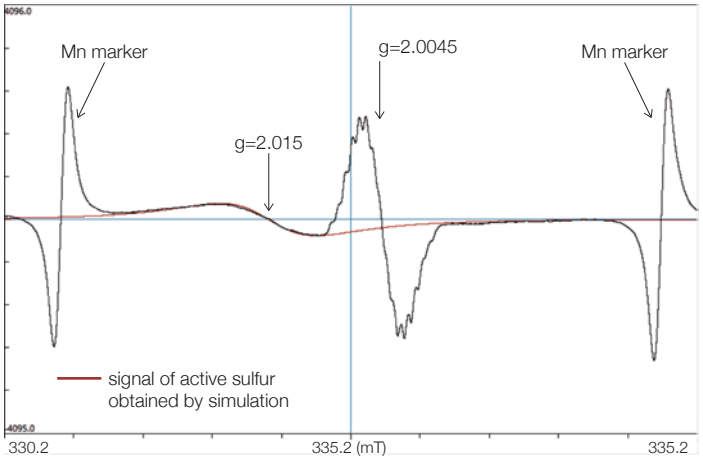
No pretreatment is required
because only radicals are Detected.

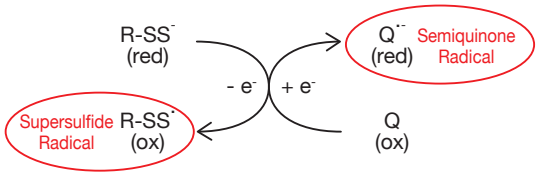
Measurement of supersulfide

In recent years, it has been reported that sulfur compounds have special functions in the living body. They are cysteine persufide(cys-SSH) , persulfide(R-SSH) and polisulfide(R-SSSH, etc). These have strong reducing properties and are called supersulfide. Below is an example of ESR measurement of the reaction of supersulfide (a model compound used in studies) with a quinone compound present in the living body*).

Application example

The spectrum obtained after 3 minutes of mixing Na_2S_2, a model compound of persulfide, and Coenzyme Q10, one of the quinone compounds, in a solution of acetone-50% / DMSO-10% / water is shown in the left figure. A broad signal with no splitting was obtained on the low-field side, and a sharp signal showing fine splitting was obtained in the center. ESR's own identification parameters, g-values, were g=2.015 and g=2.0045, respectively . The Mn marker signals at the edges of the spectrum are built into the instrument and allow calibration of the experimental data. The values indicate that the former is the supersulfide radical and the latter is the semiquinone radical derived from Coenzyme Q10. From these results, it was inferred that the following reactions were occurring.





Proposed reaction diagram of supersulfide (R-SS$^-$) and quinone compound (Q). ESR can detect ⬭ .

Supersulfide reacts with quinone compounds in the living body and is presumed to be involved in regulating function.

*) Abiko Y. et al. *Chem. Res. Toxicol*. 32(4):551-556, 2019

2-2 Matrix assisted laser desorption/ionization – Time-of-Flight Mass Spectrometer
MALDI-TOFMS

A MALDI-TOFMS is capable of analyzing a wide range of substances, from low molecular-weight compounds like amino acids to high molecular-weight compounds like proteins. This type of mass spectrometer is also utilized for Proteome analysis (Proteomics) that includes identification of proteins by analyzing peptides produced by the enzymatic digestion of the proteins.
The JMS-S3000 SpiralTOF™-plus2.0 incorporates JEOL's unique SpiralTOF™ ion optics system, achieving high mass resolving power, high mass accuracy and high ion transmission.


JMS-S3000 SpiralTOF™-plus2.0

Analysis of high molecular-weight samples
Analyzing a high molecular-weight sample (several tens of thousands Dalton or more) by the use of Linear TOF option.

Qualitative analysis by accurate mass measurement
Elucidating the composition formula by accurate mass measurement.

Structural analysis
Performing structural analysis of compounds using MS/MS.

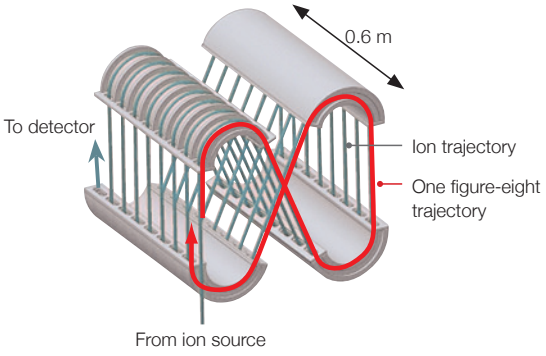
Mass imaging
Visualizing the distribution of organic compounds on the sample surface.

Spiral mode

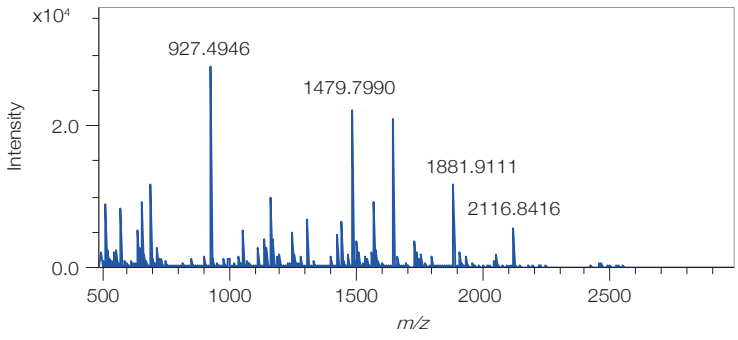
A spiral ion trajectory as long as 17 m, an order of magnitude longer than that of the conventional reflectron TOFMS, allows for mass analysis with high mass resolving power and high mass accuracy.

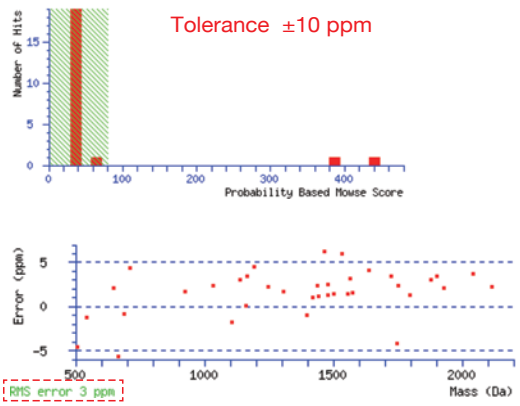
Application example

A tryptic digest of bovine serum albumin (BSA) was measured in SpiralTOF mode, and the resulting mass spectrum was subjected to protein identification by peptide mass fingerprinting (PMF). The JMS-S3000 SpiralTOF™-plus2.0 delivers high mass accuracy, enabling a very narrow mass tolerance for PMF, and provides confident protein identification with few false positive results.



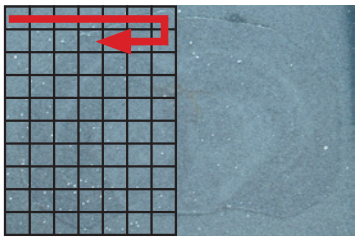
Sample: Tryptic digest of BSA



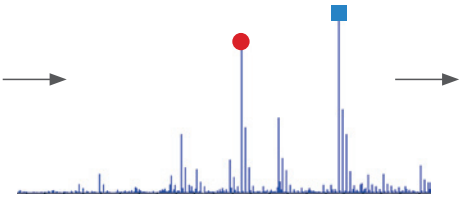


Mass imaging

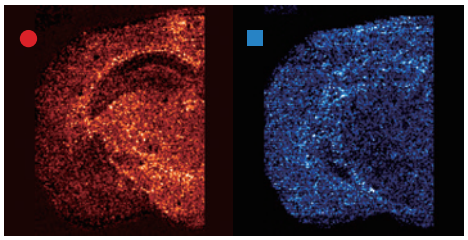
In MALDI imaging mass spectrometry, the sample is moved beneath the focused laser beam to create a time dependent series of mass spectra where each time corresponds to a specific spatial location. Analysis of the data allows the researcher to visualize the spatial distribution of specific compounds on the sample surface.



The sample is moved beneath the focused laser beam.



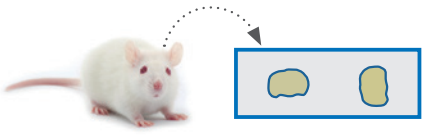
Acquire a series of mass spectra.



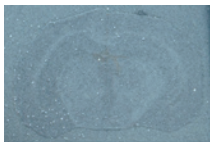
Mass images are created for each *m/z* of interest to visualize the compound distribution.

Application example

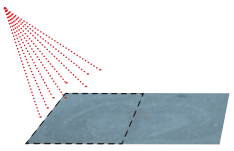
A matrix solution was sprayed to a mouse brain section and the left part of the section was subjected to mass imaging.
In the average mass spectrum, various kinds of lipids were detected. Mass images were created from the several observed lipids.
The unique ion optics of the JMS-S3000 SpiralTOF™-plus2.0 are capable of generating chemical images of compounds with m/z values separated by less than 0.1 u. This makes it possible to accurately determine the spatial distribution of compounds that would be difficult to distinguish by conventional reflectron TOF systems.



① Place a frozen section on a conductive slide glass (ITO* glass). * Indium Tin Oxide



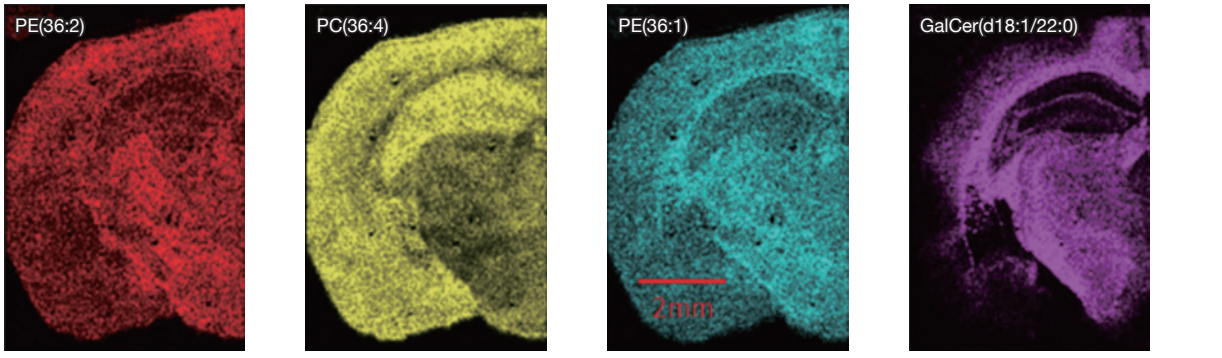
② Acquire an optical image for comparison with Mass images (scanner is used).



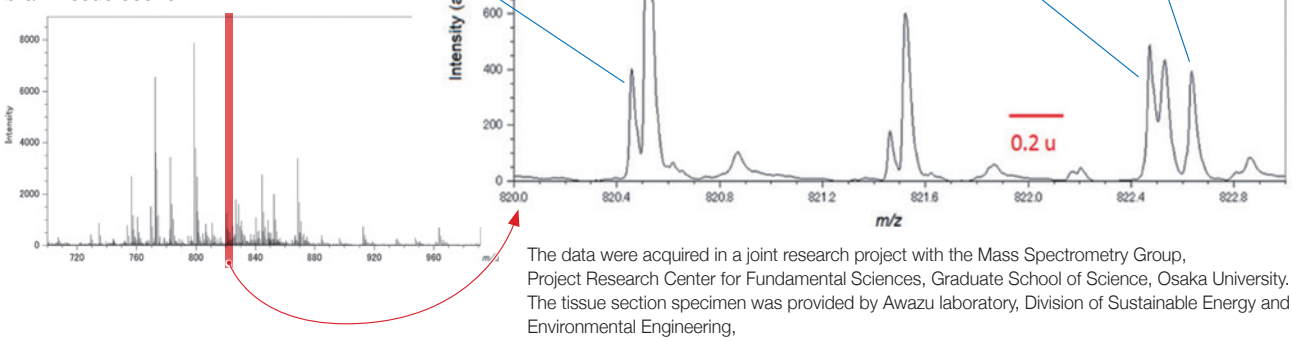
③ Spray the matrix solution.

④ Imaging analysis

Separation of isobaric icons and their MS images



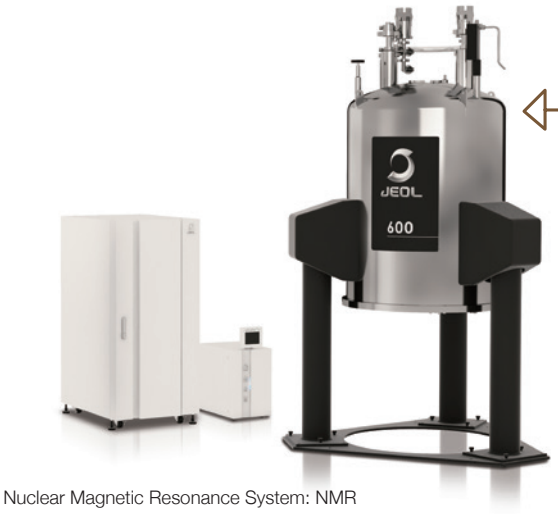
Averaged mass spectrum of a mouse brain tissue section



The data were acquired in a joint research project with the Mass Spectrometry Group, Project Research Center for Fundamental Sciences, Graduate School of Science, Osaka University.
The tissue section specimen was provided by Awazu laboratory, Division of Sustainable Energy and Environmental Engineering,
Graduate School of Engineering, Osaka University.

2-3 Nuclear Magnetic Resonance System
NMR

NMR is an analytical tool that focuses on specific atoms in molecules, and provides information on the environment around the observed nuclei and/or surrounding structure.
Non-destructive property of NMR measurement enables recovery of valuable samples, and avoids complicated pre-treatment of samples. NMR can handle various states of samples such as liquid and solid.
NMR observes signals from atomic nuclei, thus allowing the detailed 3D structure and inter-molecular interaction analysis at atomic resolution.



Nuclear Magnetic Resonance System: NMR

Various choices of sample conditions
Liquid-, gel-, solid-state samples available.
Crystallization is not necessary*.

Non-destructive measurement
You can use variable samples repeatedly.

Molecular interaction analysis
Providing information on various inter-molecular interactions (binding state, dissociation constant, etc.).

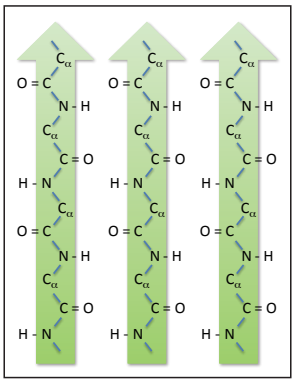
Atomic resolution
Providing information on molecular dynamics and higher-order-structure of biomolecules at atomic resolution.

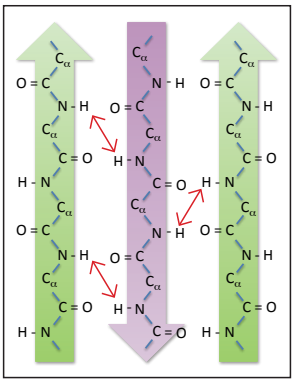
* Crystallization is required depending on the NMR experiments.

Secondary structural analysis

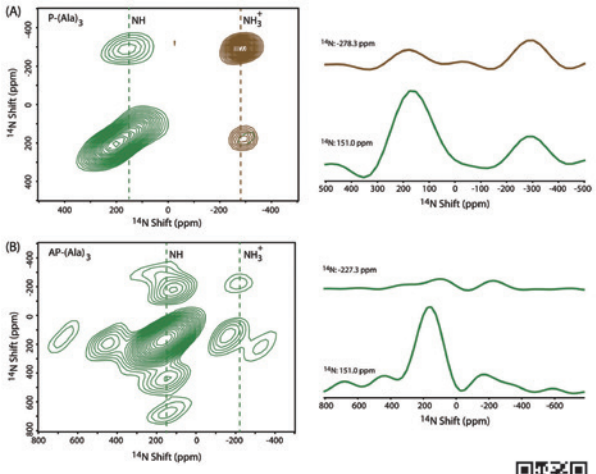
Application example

Secondary structural analysis of oligopeptides by solid-state NMR
Proteins or oligopeptides are known to form characteristic secondary structures. The β-sheet which is typical secondary structure, consists of the aligned β-strands. (The β-strand is a stretch of polypeptide chain.) The β-sheet is classified by the difference in the structural arrangement of the β-strands: parallel β-sheet and antiparallel β-sheet. By analyzing 3D $^{14}N/^{14}N/^{1}H$ correlation spectra measured with high sensitivity ^{1}H-observed ultra-fast MAS solid-state NMR, the structures of β-sheets are clearly distinguished.


Parallel β-sheet


Antiparallel β-sheet

$^{14}N/^{14}N$ correlation signals are efficiently observed in the antiparallel β-sheet due to short amide ^{1}H-^{1}H distances between the adjacent β-strands, and the antiparallel orientations of β-strands. Figures (A) and (B) show $^{14}N/^{14}N/^{1}H$ correlation spectra of oligopeptides; P-$(Ala)_3$ with a parallel β-sheet structure and AP-$(Ala)_3$ with an antiparallel β-sheet structure, respectively. In the 2D plane shown in (B), $^{14}N/^{14}N/^{1}H$ correlation peak between β-strands (around 450 ppm on the ^{14}N axis) is observed.



(A) $^{14}N/^{14}N/^{1}H$ correlation spectra of P-$(Ala)_3$ (parallel β-sheet)
(B) $^{14}N/^{14}N/^{1}H$ correlation spectra of AP-$(Ala)_3$ (antiparallel β-sheet)

[1]Pandey MK, Amoureux JP, Asakura T, Nishiyama Y. *Phys. Chem. Chem. phys.* 2016, 18, 22583-22589

Instrument used: JNM-ECZ600R(600MHz)

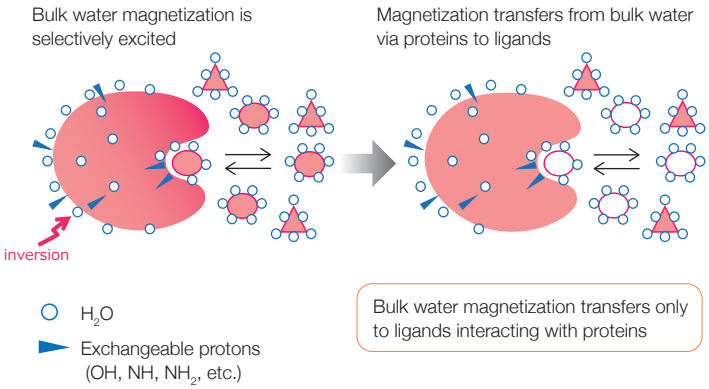
Analysis of inter-molecular interactions

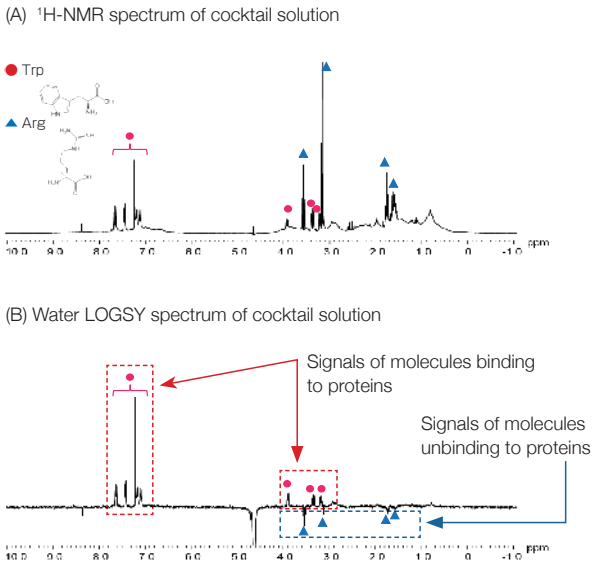
Application example

Analysis of interactions between proteins and small molecules by Water LOGSY

Solution-state NMR allows for analysis of interactions between molecules even if a sample mixture is under an equilibrium condition (binding – dissociation). Water LOGSY is used to analyze interactions between proteins and small molecules (drugs, amino acids, etc.).

Since Water LOGSY enables identification of molecules interacting with proteins even if a sample mixture composed of multiple molecules, it is known as a drug-screening technique.





A cocktail of human serum albumin (HSA), Trp and Arg was subjected to analysis of interactions by Water LOGSY (right figure).

In Water LOGSY, signals of molecules that interact with proteins are measured as positive (upward) spectra, whereas molecules that do not interact with proteins are measured as negative (downward) spectra. Thus, the molecules interacting with proteins can be distinguished.

Instrument used: JNM-ECZ400S(400MHz)

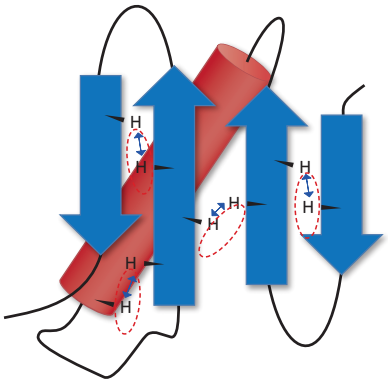
3D structural analysis

Application example

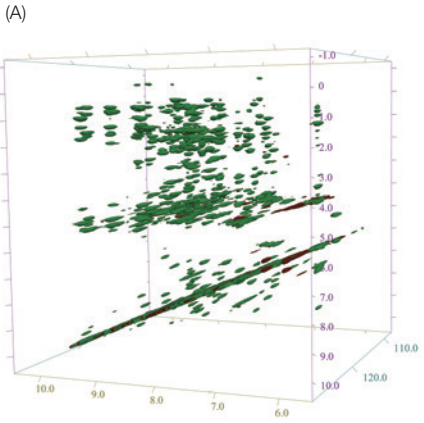
3D structural analysis of proteins by 3D ^{15}N-edited NOESY

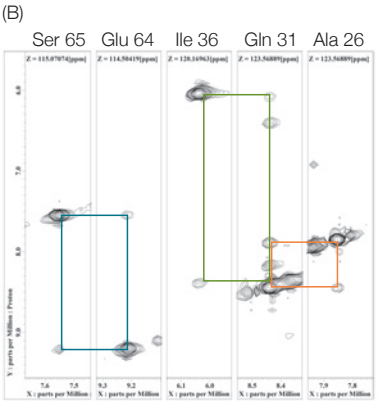
Solution-state NMR allows for acquisition of distance information between atomic nuclei by utilizing a phenomenon called NOE (Nuclear Overhauser Effect).

3D ^{15}N-edited NOESY experiment provides distance information between ^{1}H bonded to a ^{15}N (such as amide ^{1}H) of a sample which is labeled with stable isotope (^{15}N), and another ^{1}H which is spatially close to each other.



The distance information between ^{1}H-^{1}H which are close in space, can be obtained.





3D ^{15}N-edited NOESY spectra of ^{15}N-labeled human ubiquitin were analyzed. Figure (A) shows cubic display of 3D ^{15}N-edited NOESY spectra. Figure (B) presents an example of spectral analysis. From the figures it is observed that, the ^{15}N-edited NOESY spectra provide correlation peaks between the amino acid residues of human ubiquitin, Ser65-Glu64, Ile36-Glin31 and Gin31-Ala26. Thus, these amino acid residues are found to be spatially close to each other.

Instrument used: JNM-ECZ800R(800MHz)

3-1 Clinical Chemistry Analyzer

The Clinical Chemistry Analyzer is an instrument that measures various components of blood and urine, such as sugar, cholesterol, protein and enzyme. In clinical chemistry analysis, a sample of serum or urine is reacted with reagents for the subsequent analysis. These clinical tests are carried out in hospitals and medical institutions for medical checkup, disease identification, etc. Analysis results are utilized as important data for a wide range of purposes, including early detection of disease, and estimation of effects of medical treatment and after-effects of the treatment.

Clinical Chemistry Analyzer

Avoiding sample-to-sample carryover
New wash mechanism avoids sample-to-sample carryover, providing the ppb-order washing capability.

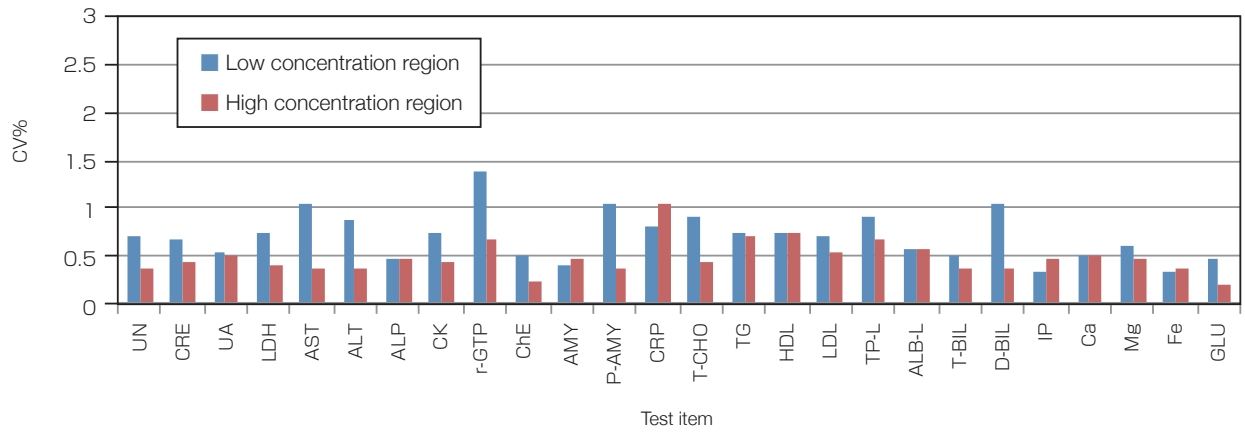
Micro-volume measurement
Micro-volume measurement with the µL order is enabled.

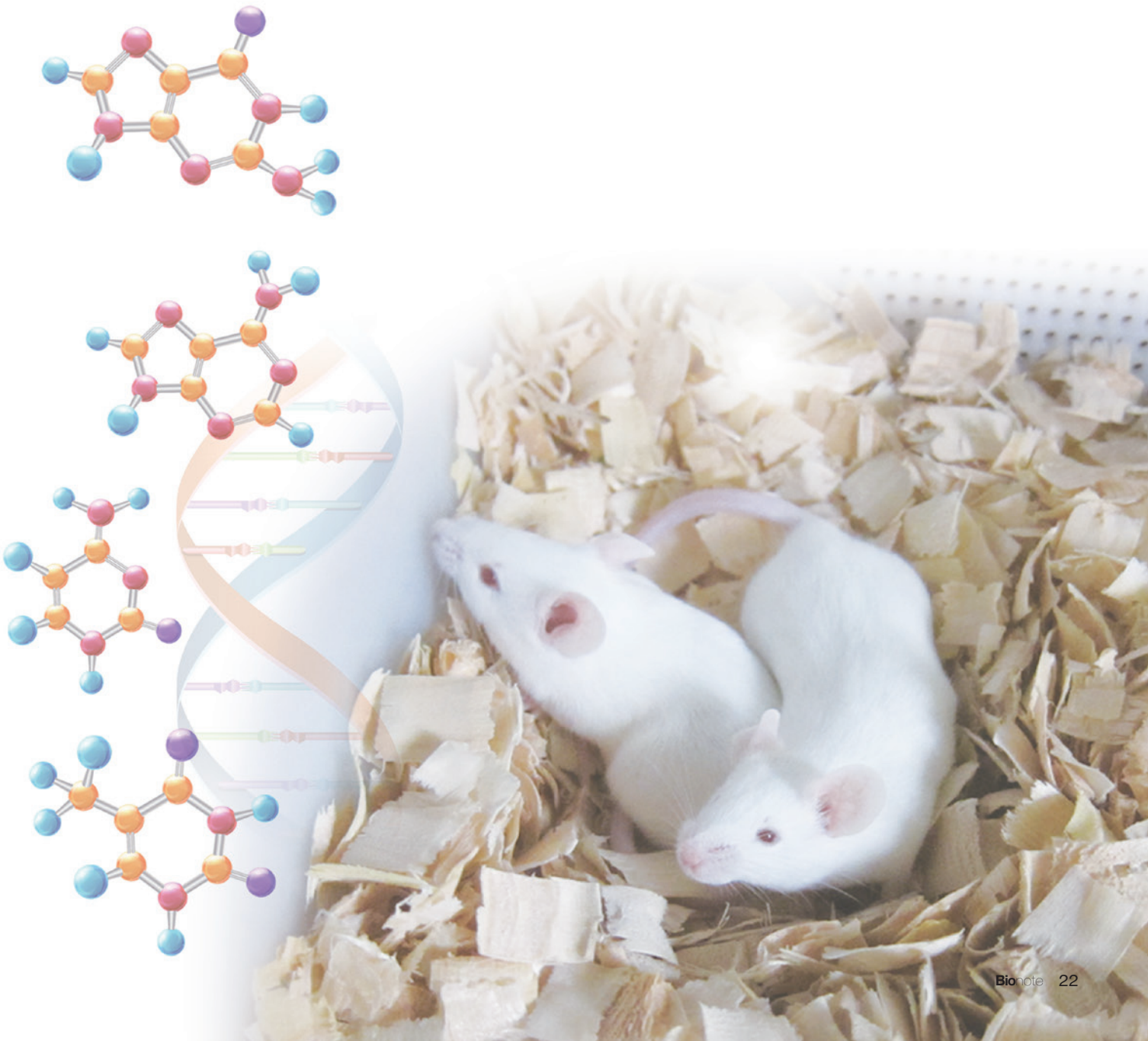
Integrated reagent management system
Exchange of reagents under working and automatic reagent Planck measurement are possible.

Blood & urine analysis

The Clinical Chemistry Analyzers are used in a wide range of institutions, such as small & medium-sized hospitals, medical test centers, and large-sized hospitals (university hospital, etc.).
The analyzer features a dramatically reduced reaction volume and superbly high throughput. Those features allow for measurement of more than 100 types of test items.
In addition, utilizing the feature of micro-volume measurement, the Clinical Chemistry Analyzers are used for animal (poison) testing in the non-clinical studies and medical checkup of animal pets.

Application example — Simultaneous reproducibility (n=20)





* Specifications and appearance of the instruments are subject to change without notice.

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