“Taking electron microscope images of biological objects in their natural state.”

“NanoSuit”

NanoSuit Inc.

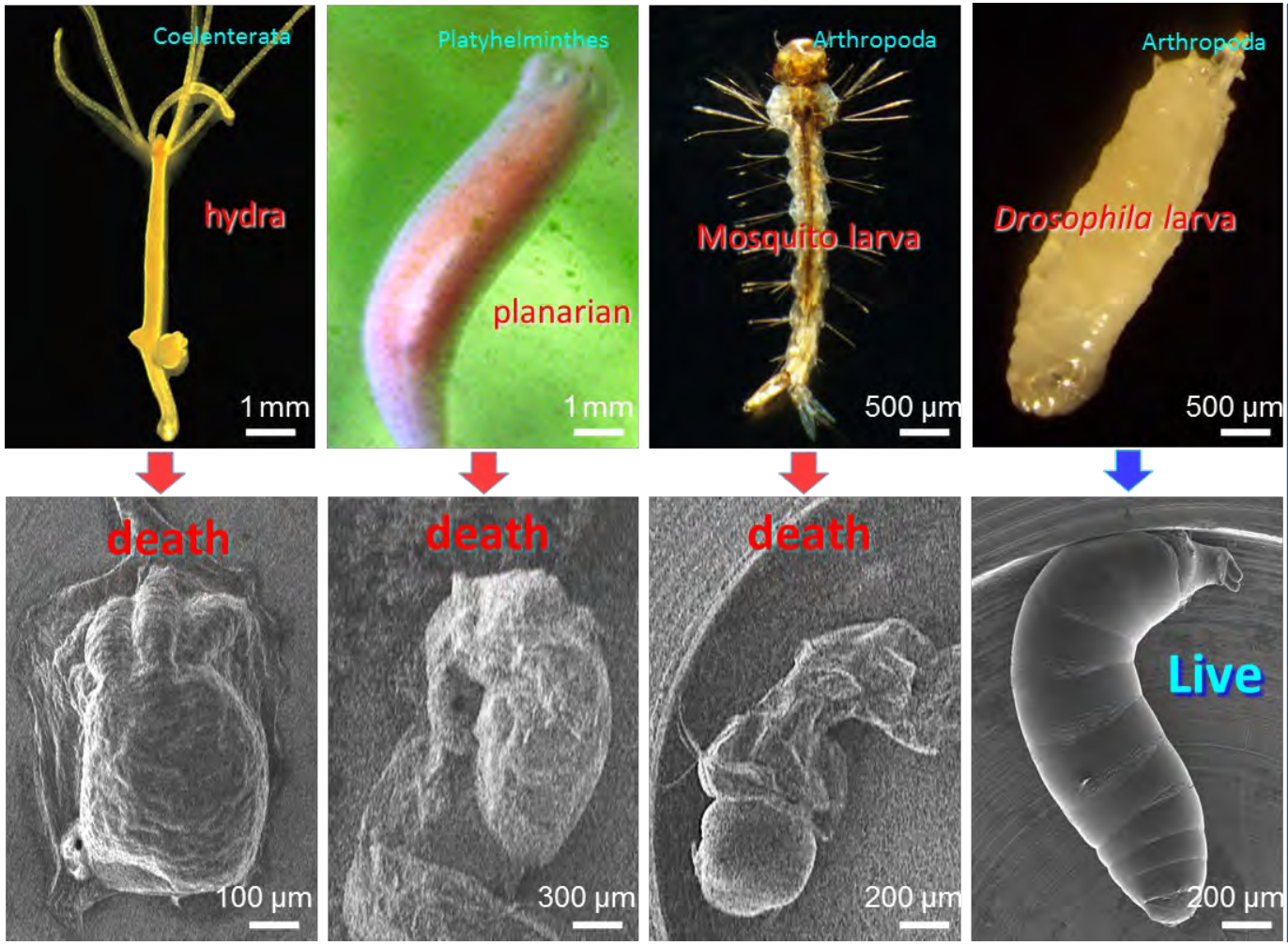
What is the “NanoSuit®” ?

- A novel technology which enables us to observe cells, microorganisms, etc. in a living state using scanning electron microscopy (SEM).

- “NanoSuit solution” forms a very thin barrier layer “NanoSuit” on the surface of an object and the barrier layer holds moisture in the object under vacuum condition in electron microscopy. Also, the barrier layer is electrically conductive.
- Therefore, “NanoSuit” makes it possible to observe biological objects with their natural image texture.

- ”NanoSuit” is very easy to use. Only dropping the “NanoSuit solution” onto the objects, then you can observe the objects using SEM.
  (You don’t need any other fixation procedures.)

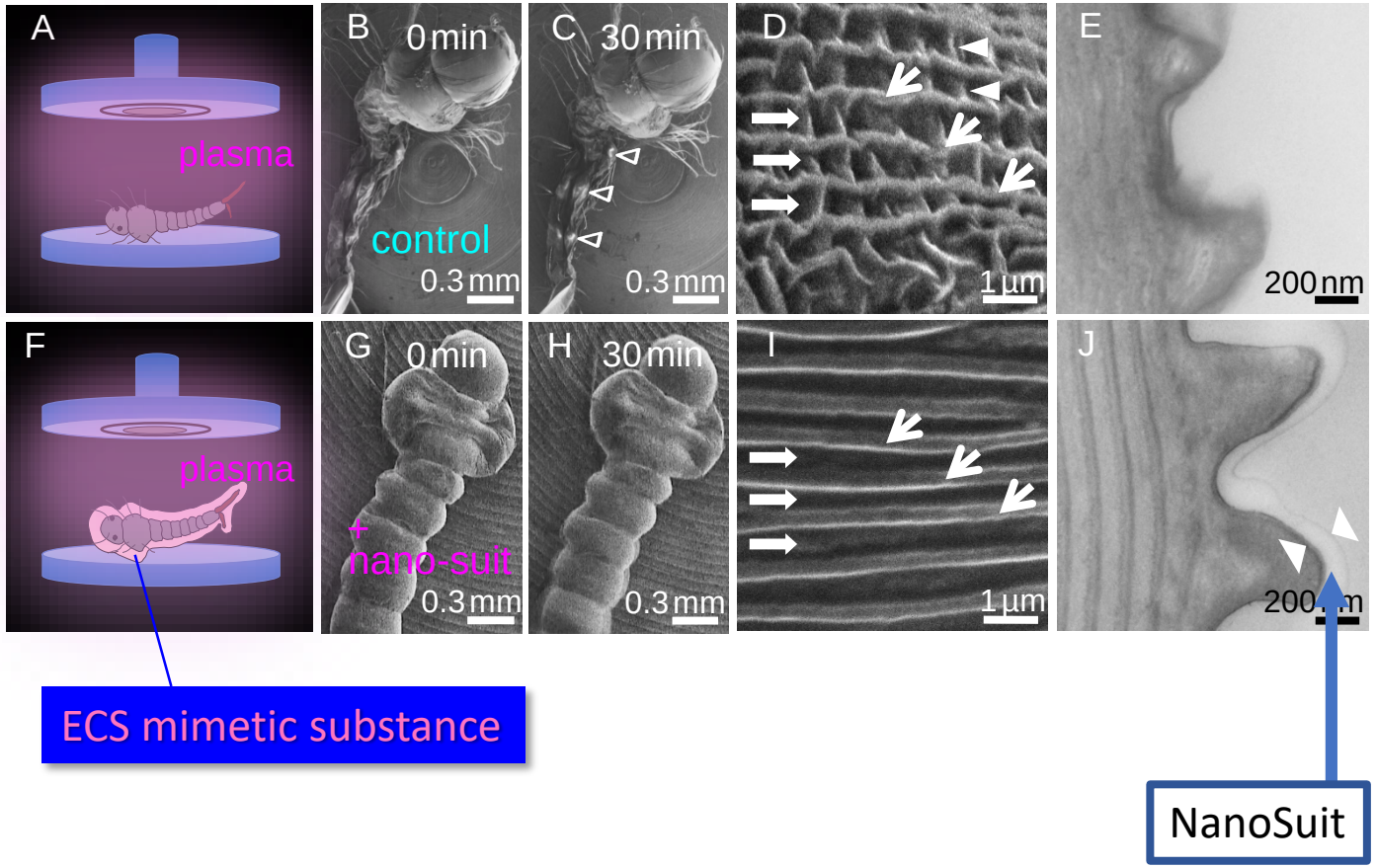
History



- Our founder Prof. Takahiko Hariyama had tried to observe various microorganisms using SEM, but almost all organisms died due to losing moisture under the vacuum condition in the electron microscopy.
- However, only a kind of fly larva was able to keep alive under the vacuum condition.

- Prof. Hariyama investigated that phenomenon and revealed that a very thin barrier layer was formed on the surface of the larva by electron beam irradiation and the barrier layer holds moisture and makes the larva alive.
- Hariyama and his colleagues had tried to mimic this phenomenon artificially and invented “NanoSuit” technology.

Technology/Science

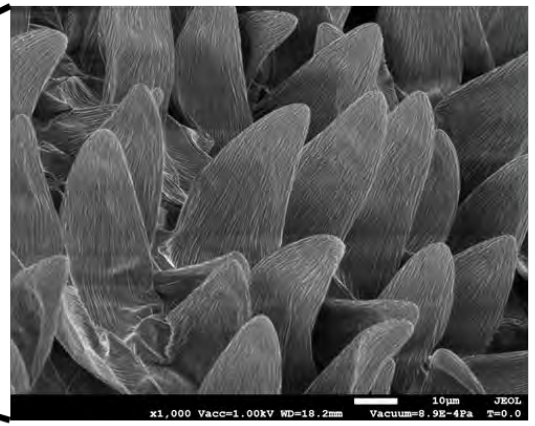


“NanoSuit®”

- Aqueous solution of the biocompatible polymer.
  (biologically safe material)
- Just drop onto the objects.
- Polymerize by electron beam or plasma irradiation, then a thin barrier layer is formed on the surface of the objects.
- The barrier layer holds moisture contained in the objects and its conductivity provides clear SEM images.

Example（flower petal）

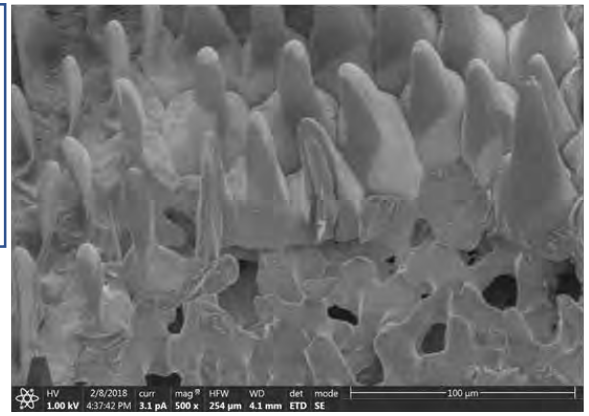
Control



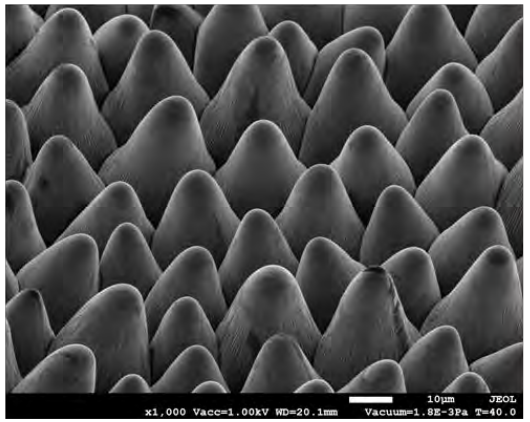
Under the vacuum condition, the petals are dried and not retain the original shape.

Cryo-SEM

The petals are frozen and not retain the original shape.



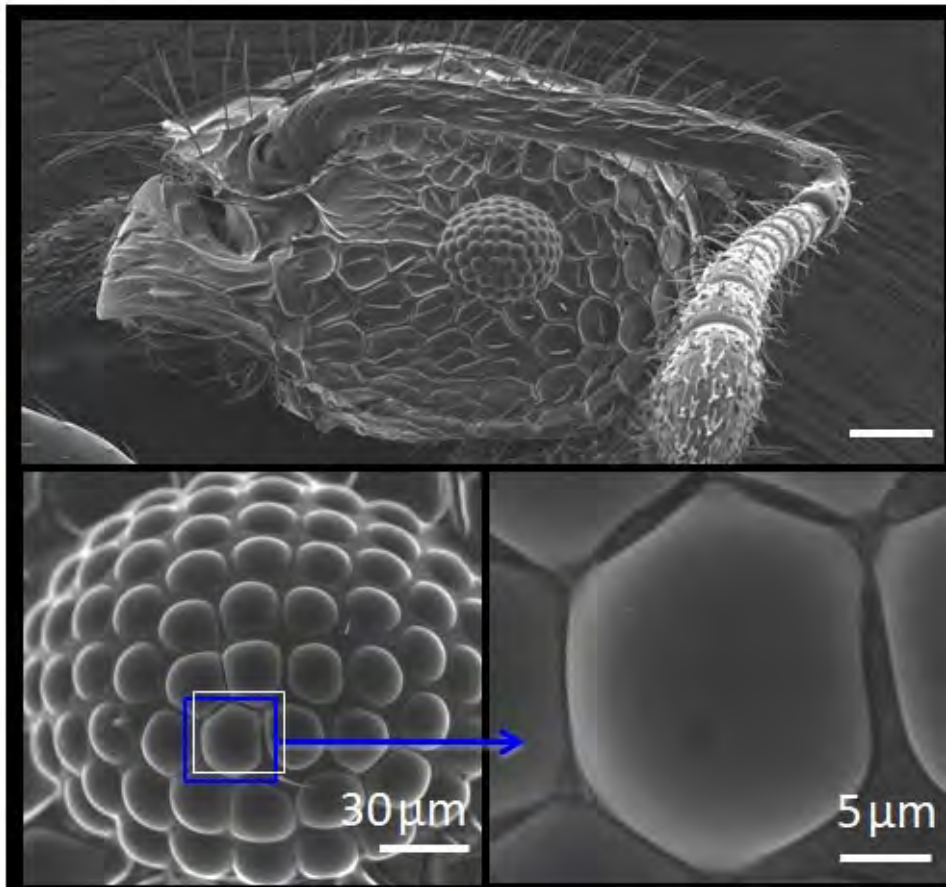
NanoSuit



“NanoSuit” keeps the petal moist under the vacuum condition and provides natural shape images of the fine structure of the petal surface.

Example（Insect）

Pristomyrmex punctatus

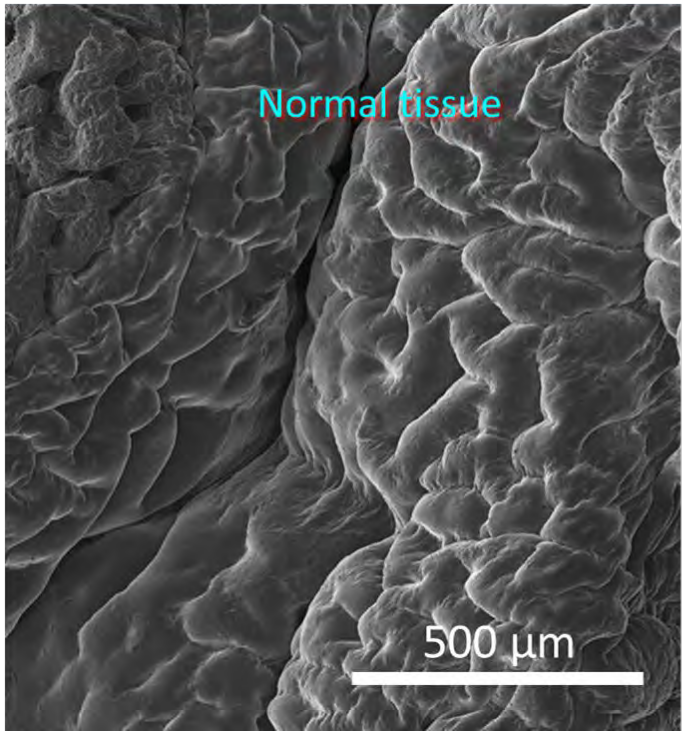


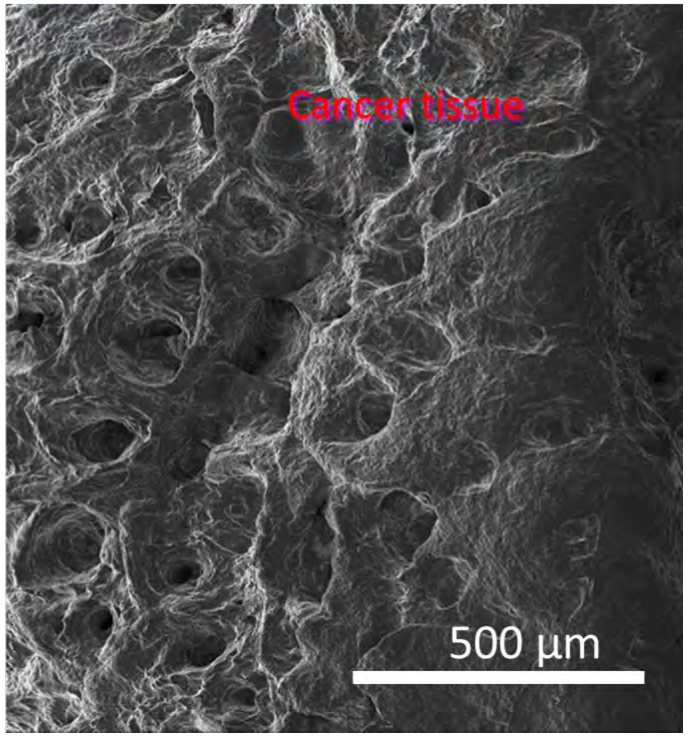
With “NanoSuit®”, you can observe the minute structure of the insect's body surface with a simple operation.

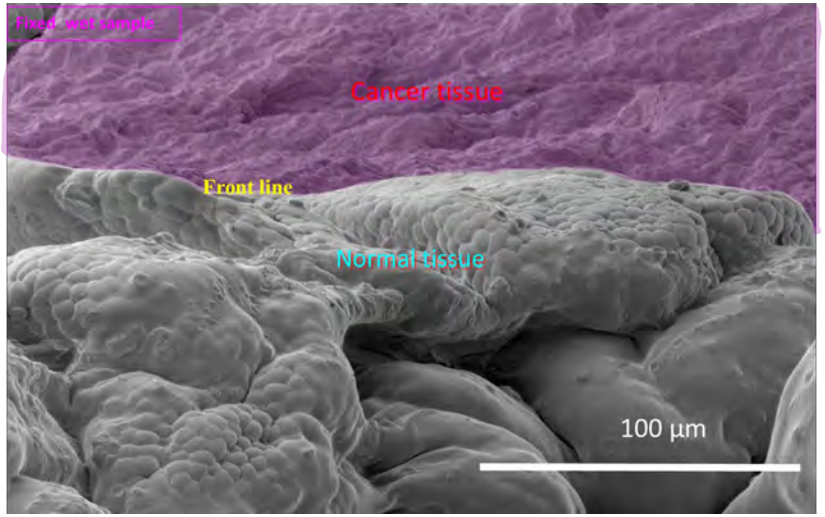
Example（living tissues）

Human Stomach Cancer （normal and cancer tissue）

SSE / nanosuit method

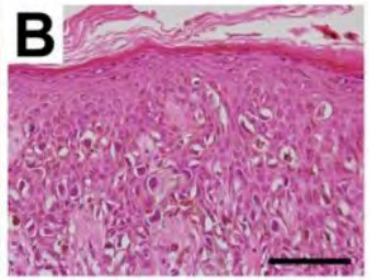






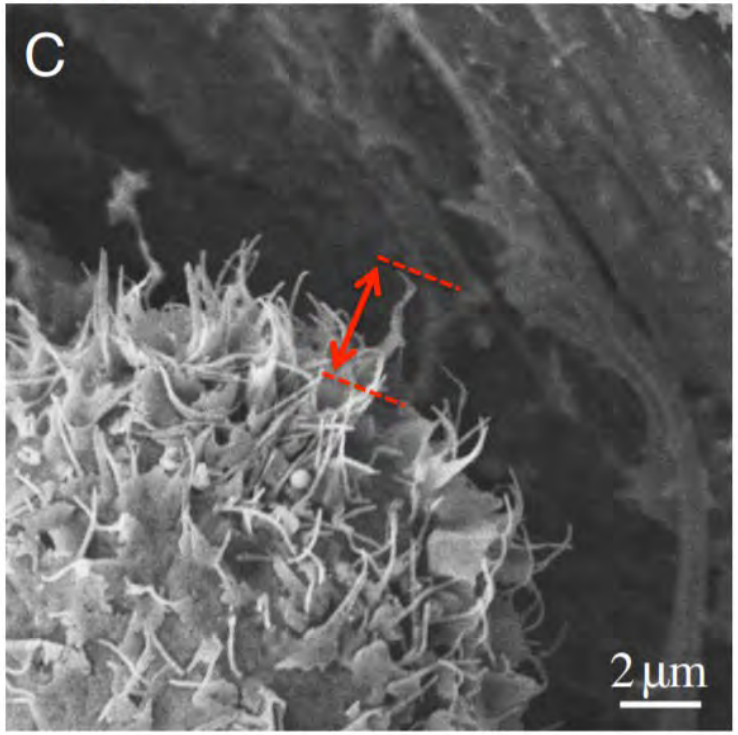
“NanoSuit®” keeps the texture of living cells and enables to distinguish cancer tissue and normal tissue.

Example（filaments of cancer cells; melanoma）

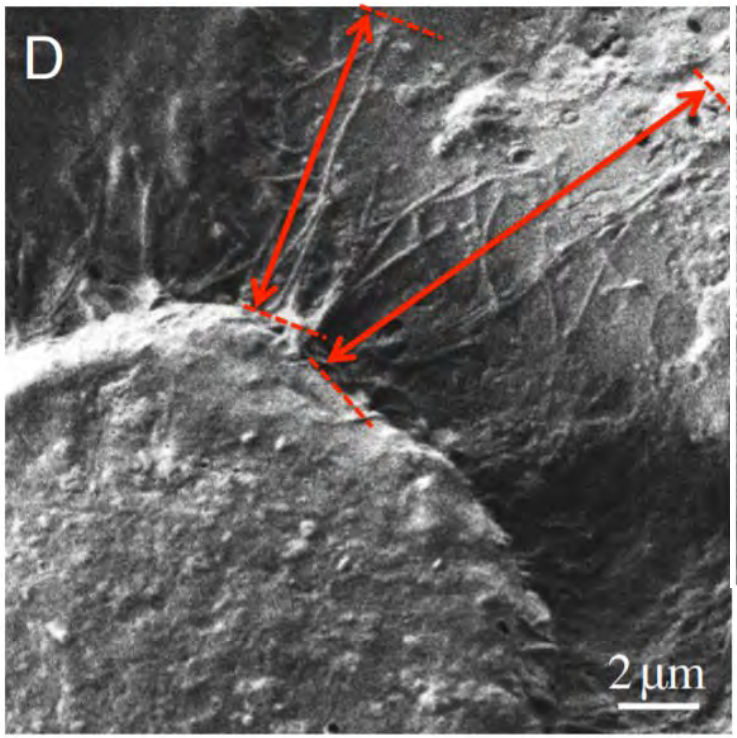


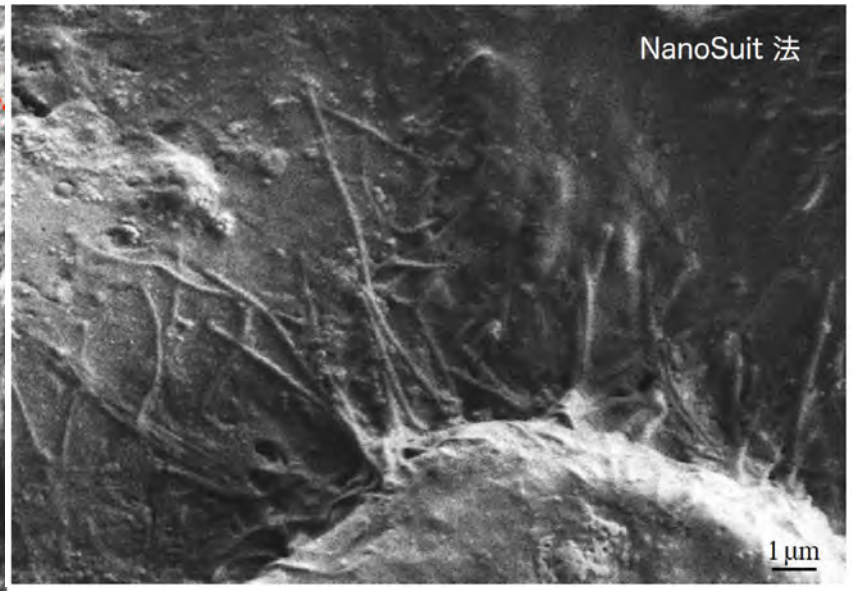
“NanoSuit®” enables us to observe the natural texture of microstructure of living cells. NanoSuit is useful for understanding the structure of “tumor-niche”.

Conventional method



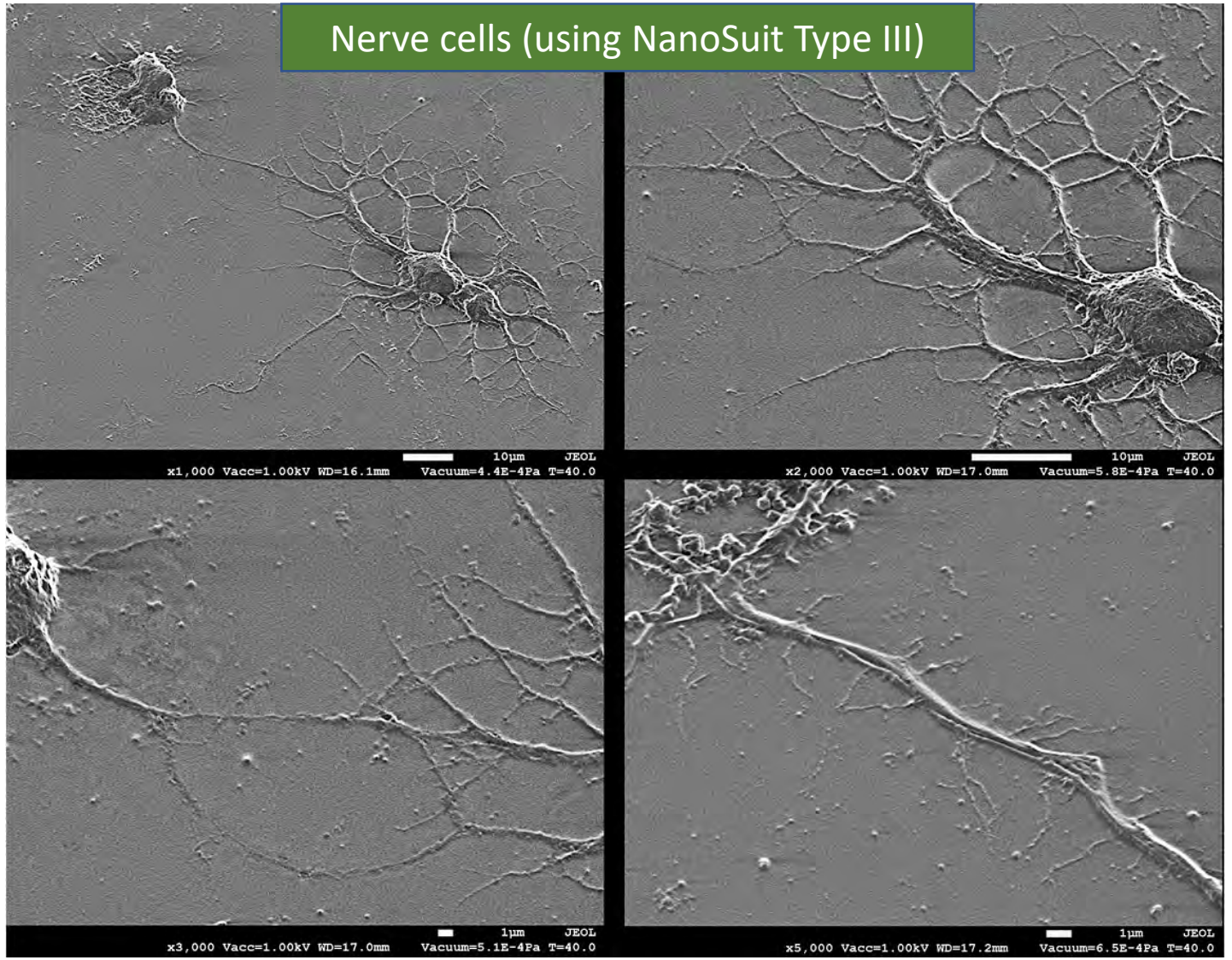
NanoSuit method





Example（Nerve cell）

Nerve cells (using NanoSuit Type III)

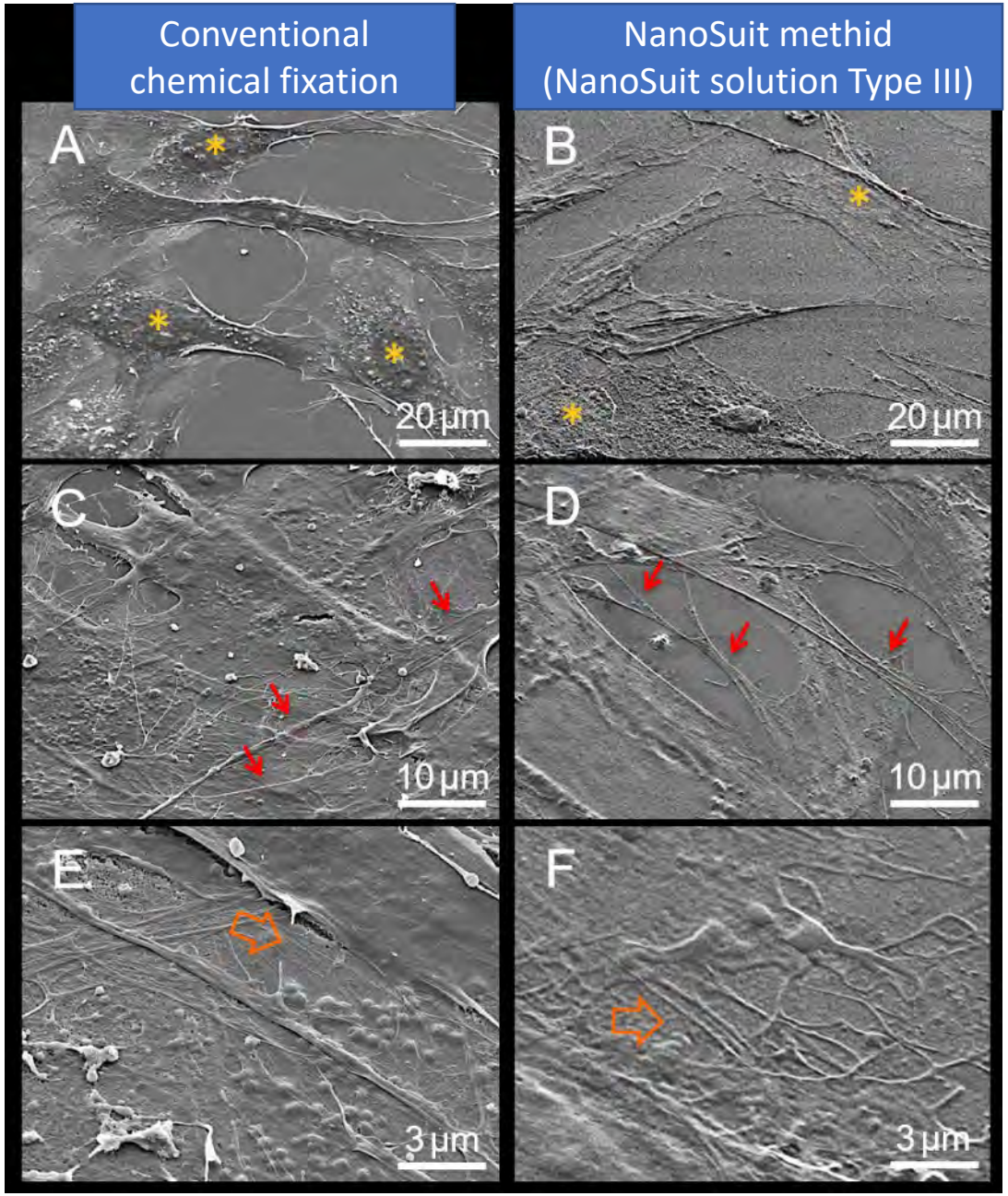


Nerve cells from the mouse cerebral cortex were observed alive using NanoSuit solution Type-III.
Since NanoSuit prevents drying, it is possible to analyze with high resolution without breaking fine neurites.

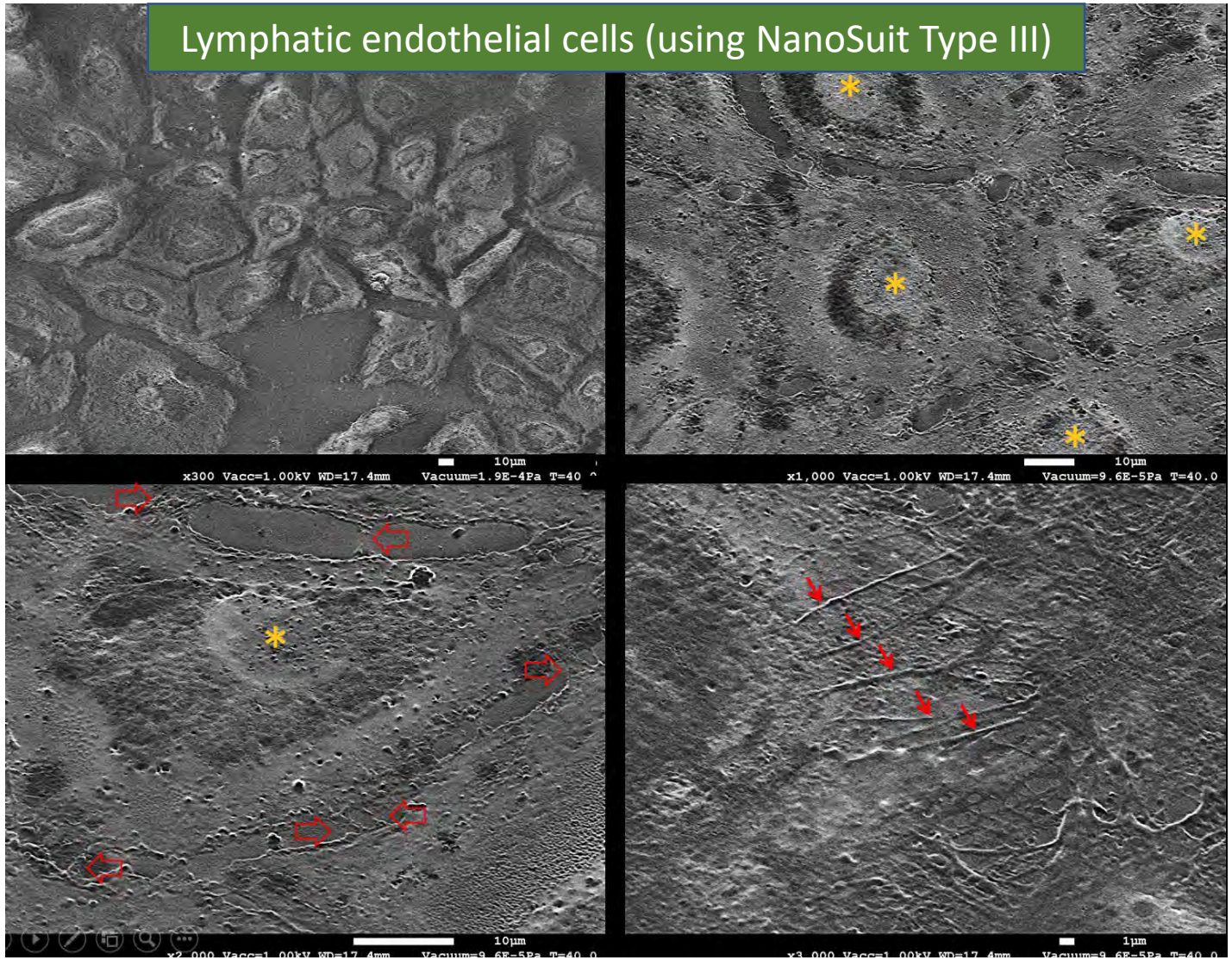
Example（Fibroblast)

This is the result of observing mouse-derived fibroblasts by the conventional method and the NanoSuit method. (Conventional method: chemical fixation / dehydration / drying / metal deposition) (A, C, E), NanoSuit method (using NanoSuit solution Type-III) (B, D, F).

Nuclei (*) are observed in cells (A, B). In addition, when strongly enlarged, filament-like microstructures (red arrows) are observed in the intercellular spaces (C, D). These filament structures are damaged and broken due to drying in the samples treated by the conventional method (E, orange arrow), while they are observed as they are in the NanoSuit method (F, orange arrow).

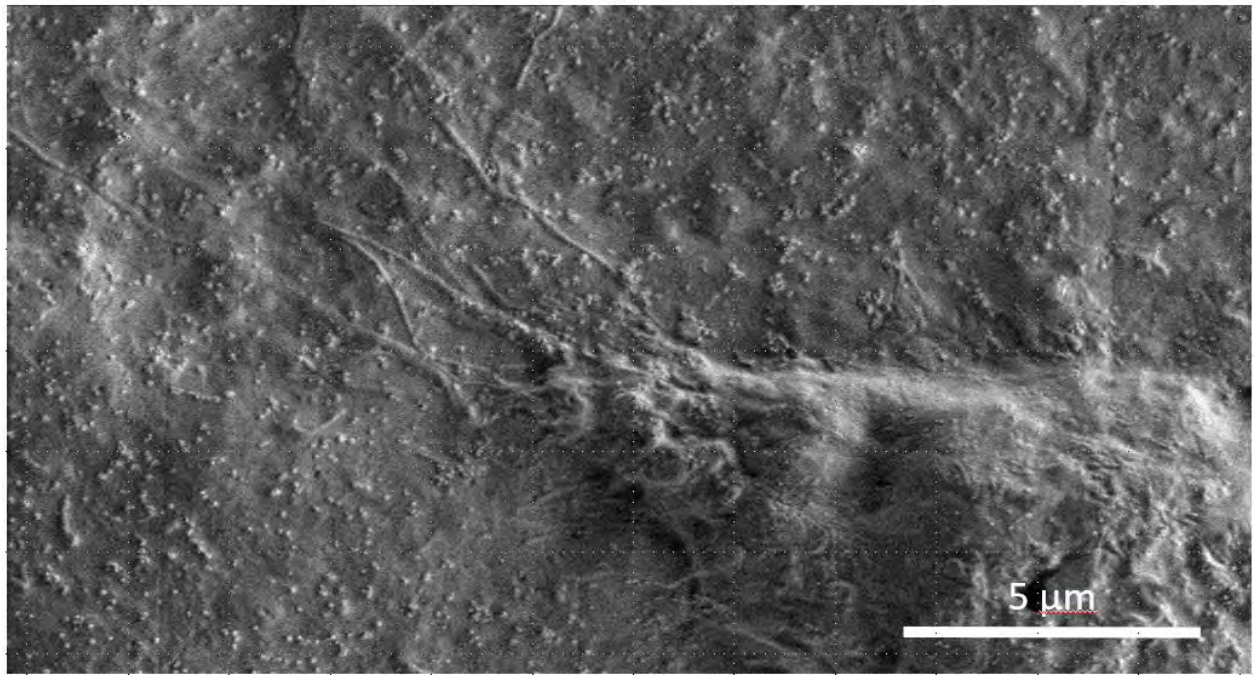


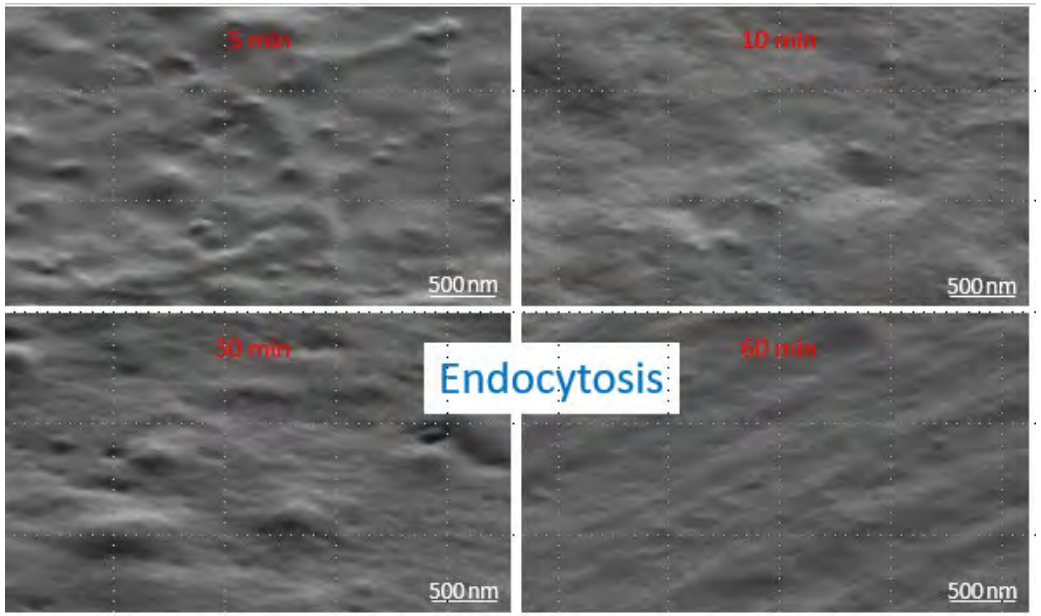
Example（Lymphatic endothelial cells）



Lymphatic endothelial cells from mice were observed alive using NanoSuit solution Type-III. Nuclei (*) are found in cells. In addition, when strongly enlarged, filament-like fine structures (red arrows) are observed in the intercellular spaces.

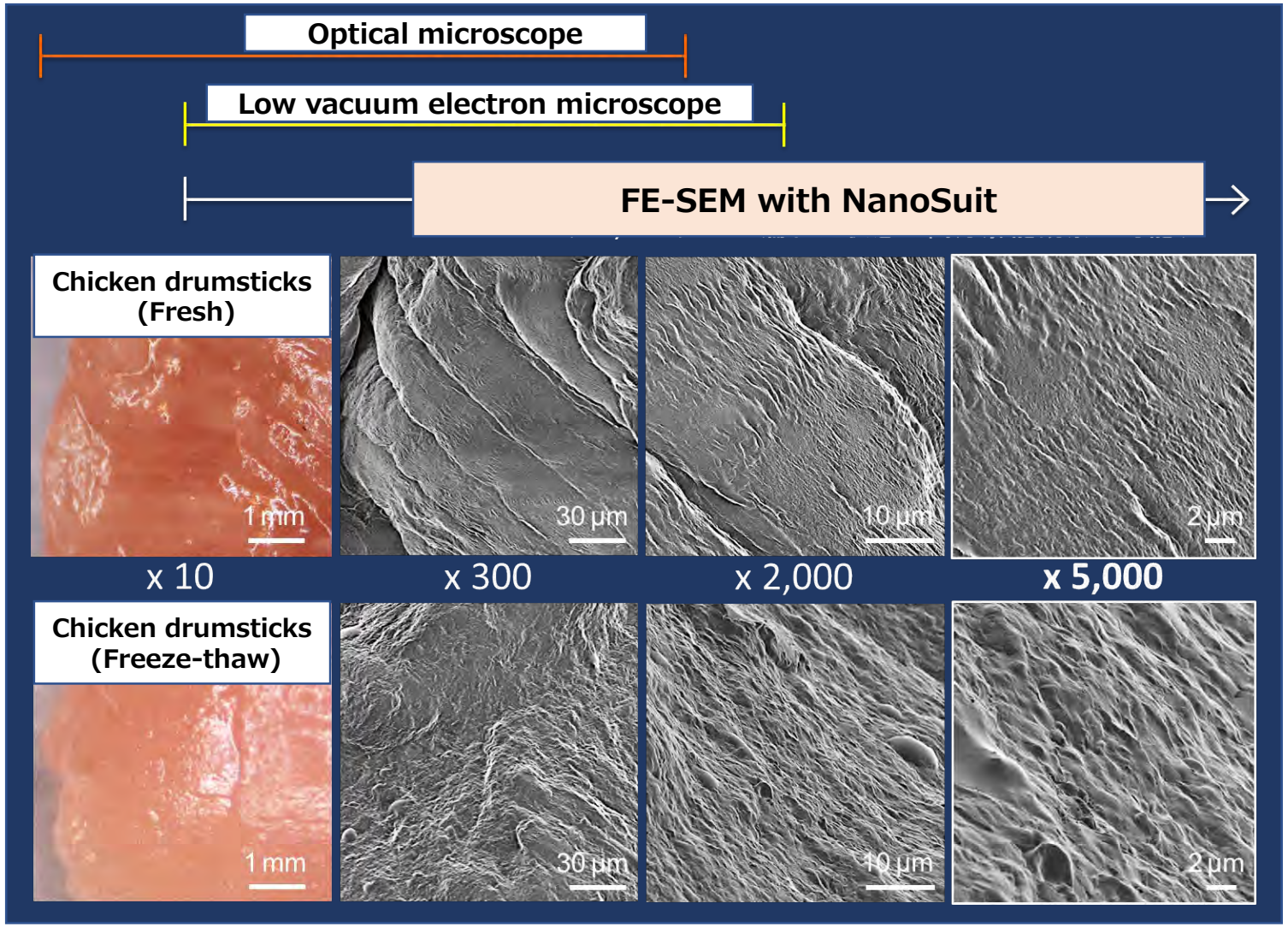
Example (Virus infection to living cultured cells (fibroblast / mouse)





Virus infection to living fibroblast cells is observed using NanoSuit method.
NanoSuit enables us to see the endocytosis of some viruses. At 5 min after dispersion viruses on the cell membrane, we found a lot of small particles, but following the time, its surface came smooth.

Example（Chicken drumsticks）

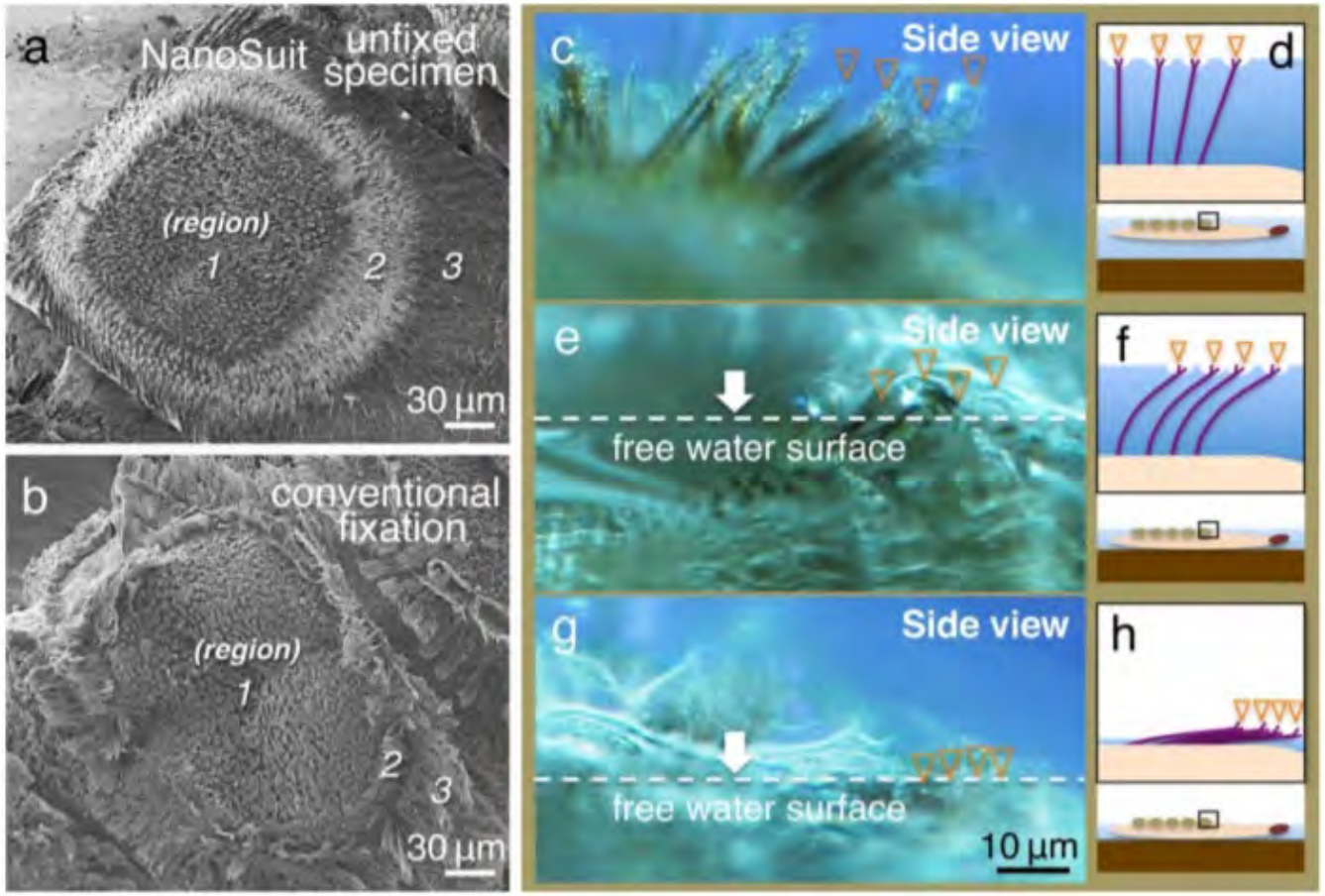


The surface of the “Chicken drumsticks (Fresh)" protected by NanoSuit solution and observed by FE-SEM with high resolution shows fibrous microstructures of uniform size.

On the other hand, in “(Freeze–thaw)" examined by the same method, it was observed that the fine structure of the surface was broken and liquid substances were seeping out to the surface.

These images suggest that such a difference in surface condition may be the cause of the dry feeling of the thawed food.

Example（Dixa longistyla)

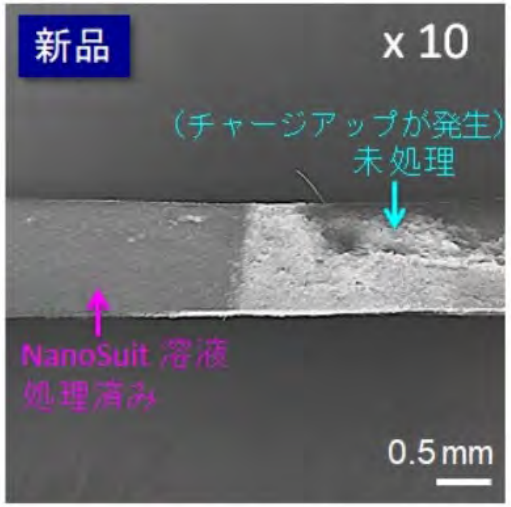


Published in Communications Biology (Nature)

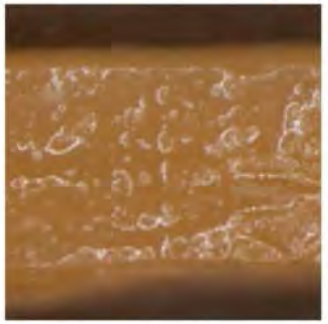
The larvae of Dixa longistyla were able to stay on the underwater side of the water surface, and it was known that the crown structure on the body surface contributed to this, but the mechanism has not been elucidated.
By observing the crown structure with the NanoSuit method, the true structure that could not be obtained with the conventional chemical fixation method, and the mechanism of the phenomenon of staying on the water surface was elucidated.

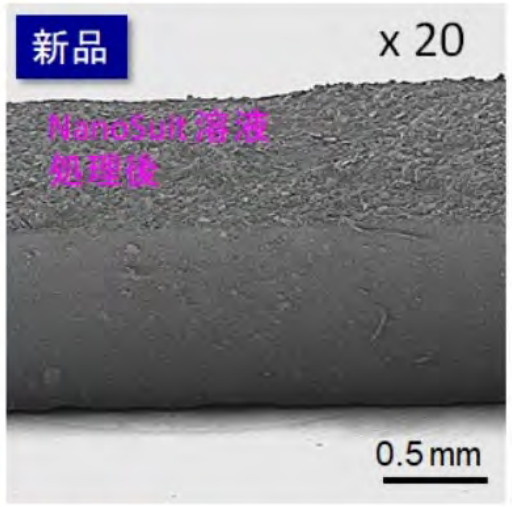
Example（Rubber band）

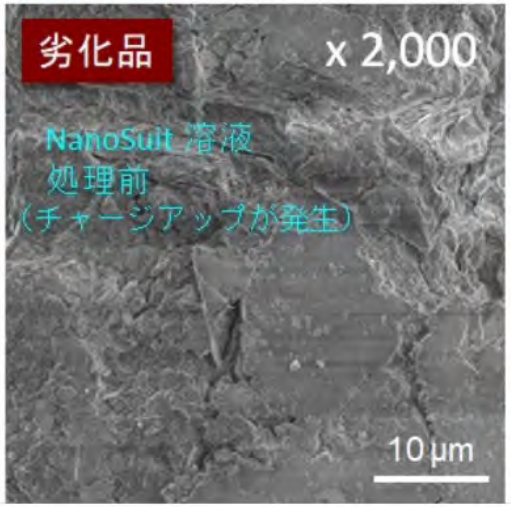
Thanks to its conductivity, NanoSuit makes it easy to observe insulating materials with erectron microscopy.



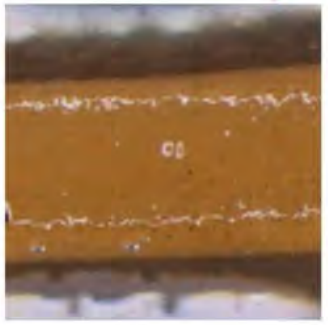
NanoSuit 溶液で処理

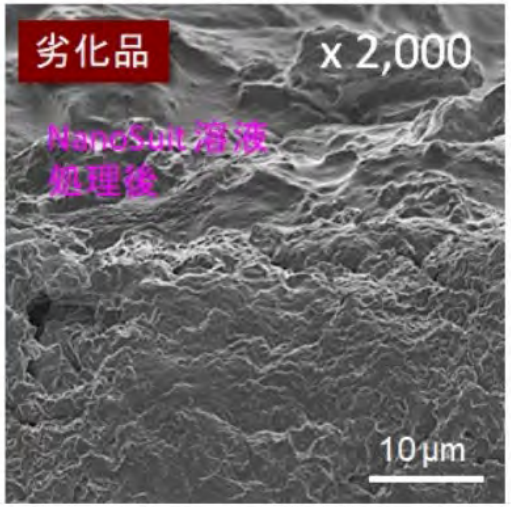


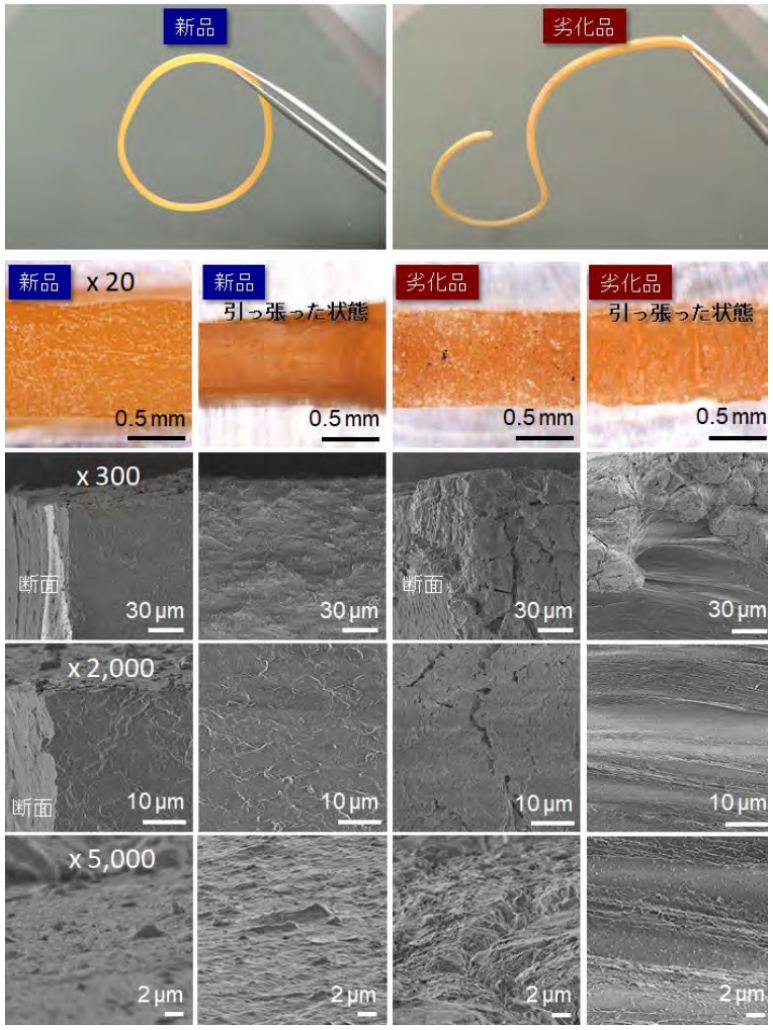




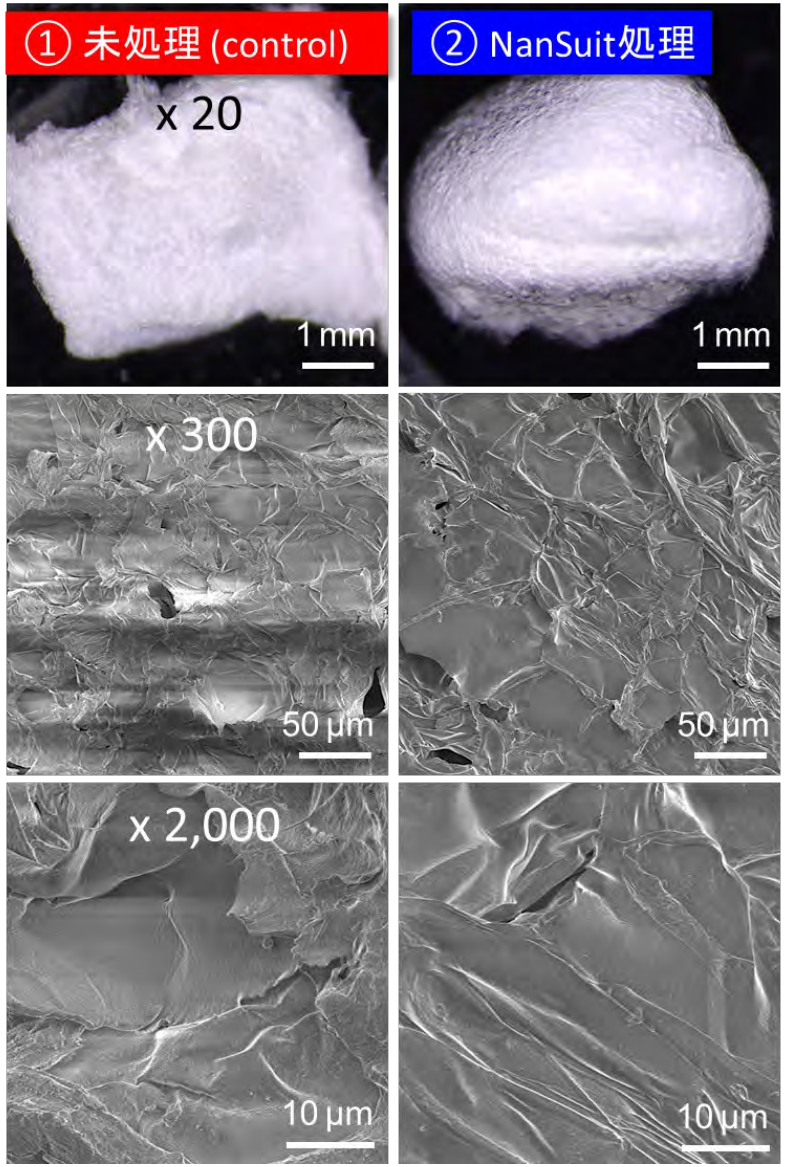
NanoSuit 溶液で処理





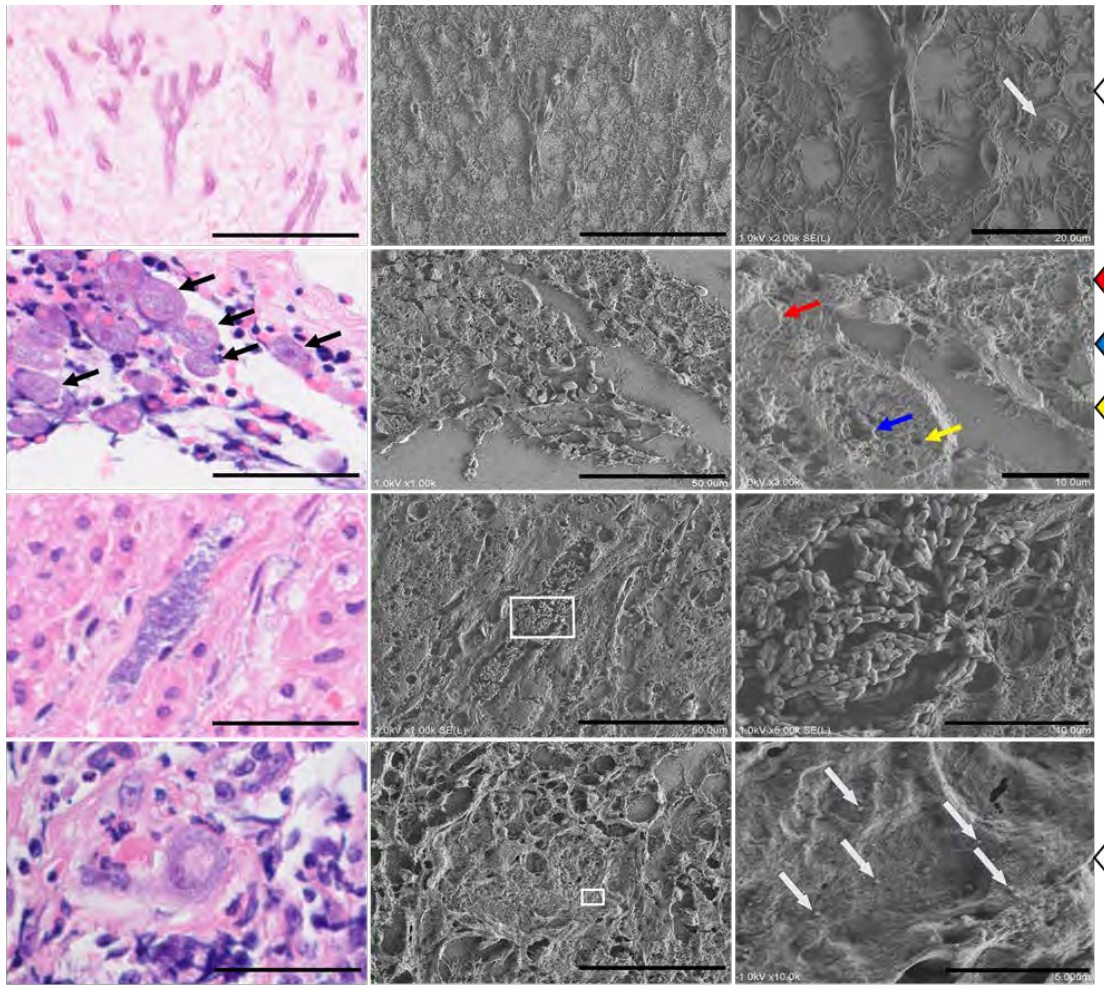


Example（Styrofoam）



Nanosuit makes it possible to clearly observe Styrofoam, which is easily charged with static electricity, with an electron microscope.

Example (CLEM; Correlative light electron microscopy)

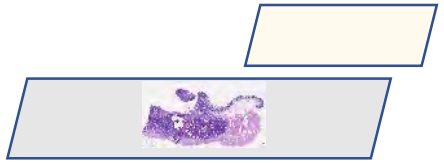


Prepare a glass slide using the conventional method for an optical microscopy. When you drop the tiny NanoSuit solution on the sample, you can observe with an electron microscope. By using this NanoSuit-CLEM method, you can observe the exact the same place of the sample with an optical microscope and an electron microscope. Since metal deposition is not required, you can store your important samples on slide glass again for an optical microscope. A lot of information can be obtained by combining the stained optical microscope observation results with the three-dimensional, high-magnification electron microscope observation.

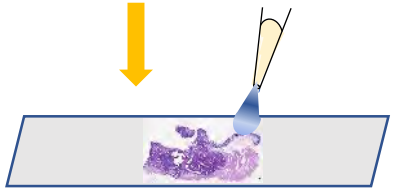
Procedure of “NanoSuit-CLEM”

Optical microscopy obsevation

HE specimens, immunostained specimens etc.



Remove the cover glass using appropriate solvent such as xylene.

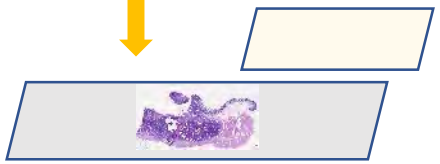


Apply NanoSuit Solution TypeⅡ onto the object

Spread the NanoSuit solution by spin coating or shaking the glass slide.

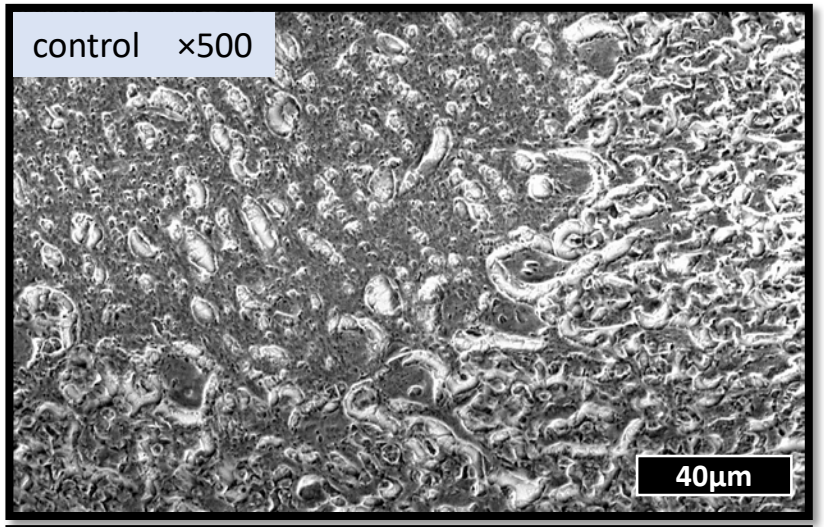
You can store the important samples after observing

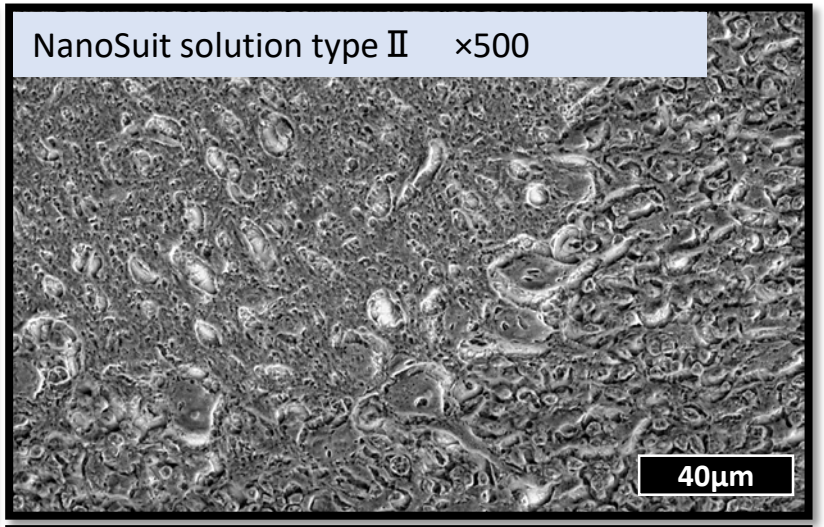
SEM observation

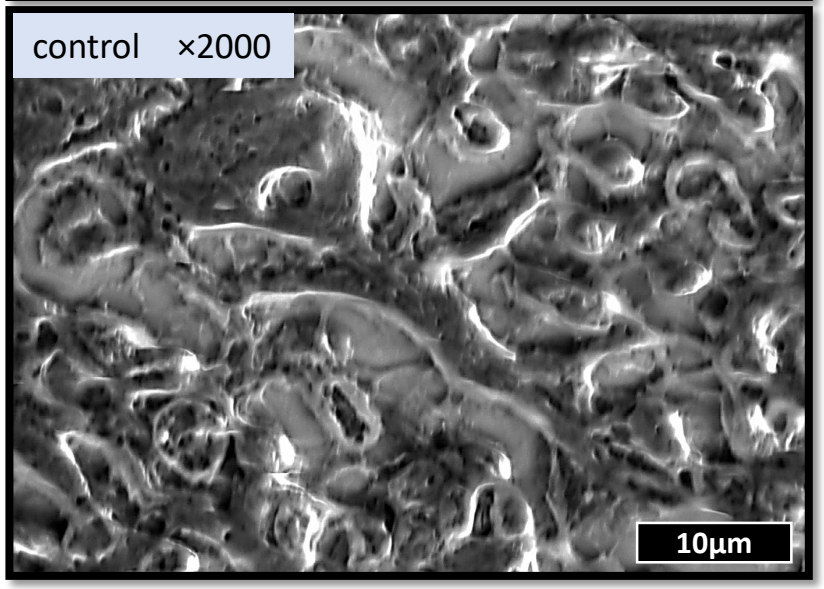


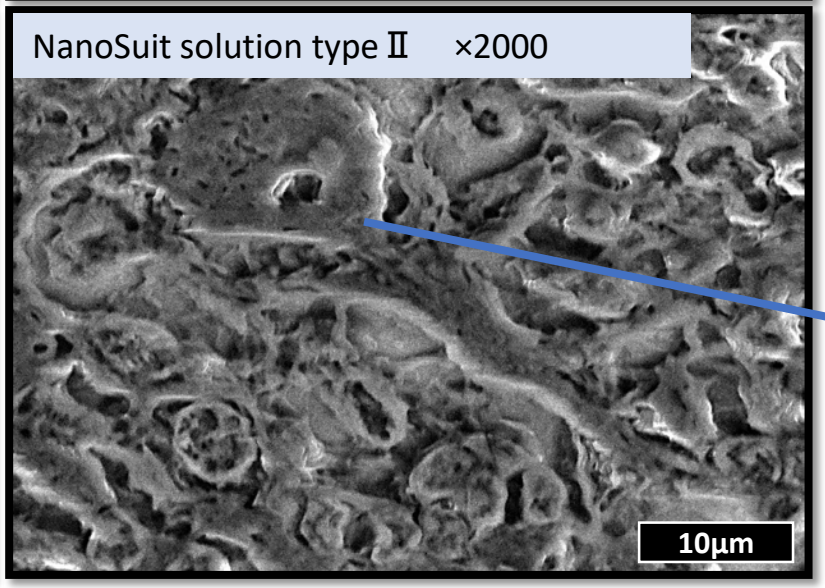
Possible to be re-stained
Reseal with cover glass

Charge-up reduction effect of NanoSuit (paraffin fixed specimen)









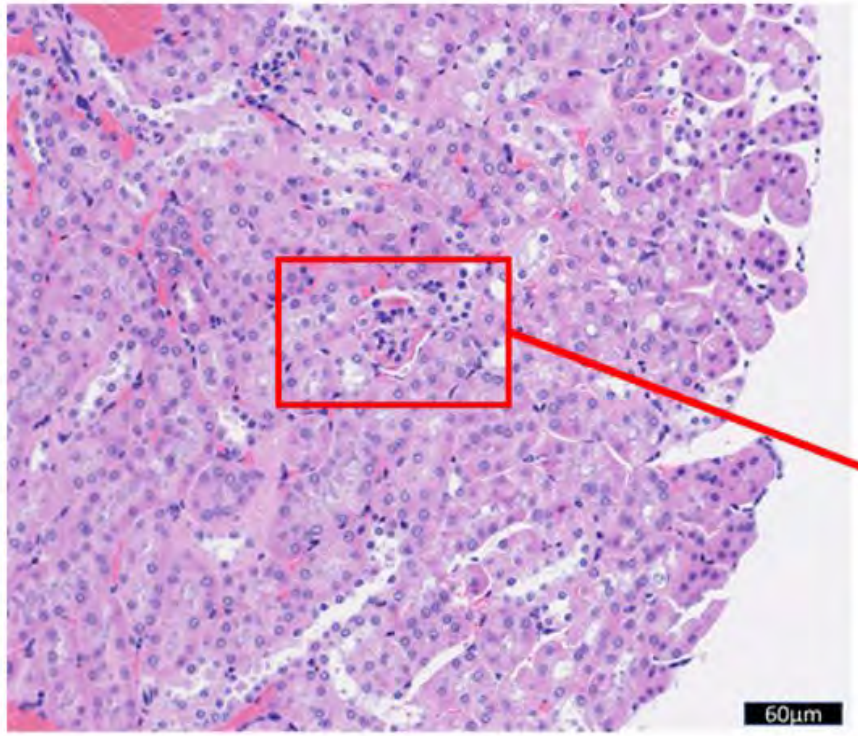
Sample: Mouse cerebellum
Observation conditions: Acceleration voltage 10kV, reflected electron mode

In the image without solution on the left, the image is glaring and distorted due to abnormal contrast caused by charge-up. This phenomenon gets worse as you increase the observation magnification.

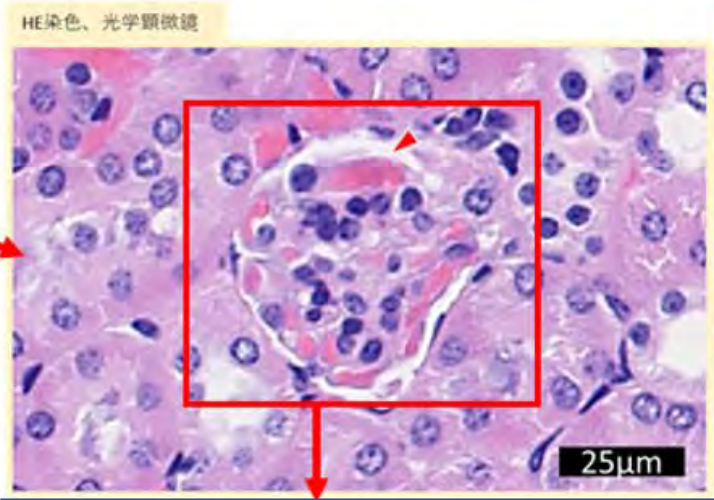
In the NanoSuit® solution type II coated image on the right, charge-up is reduced and fine structure is observed.

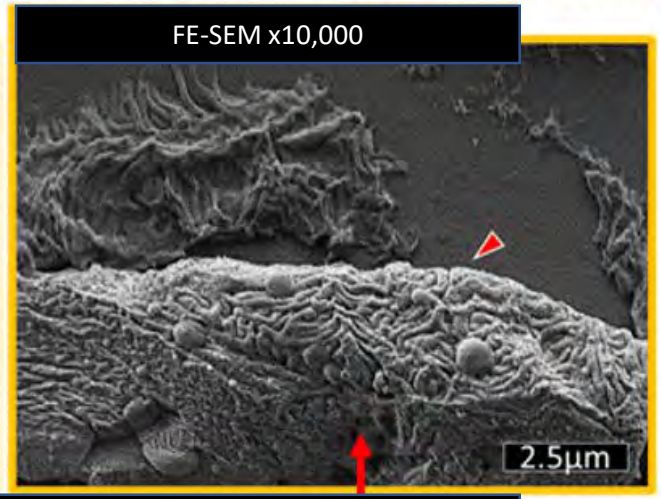
Cerebellum Purkinje cells

Mouse kidney glomerulus

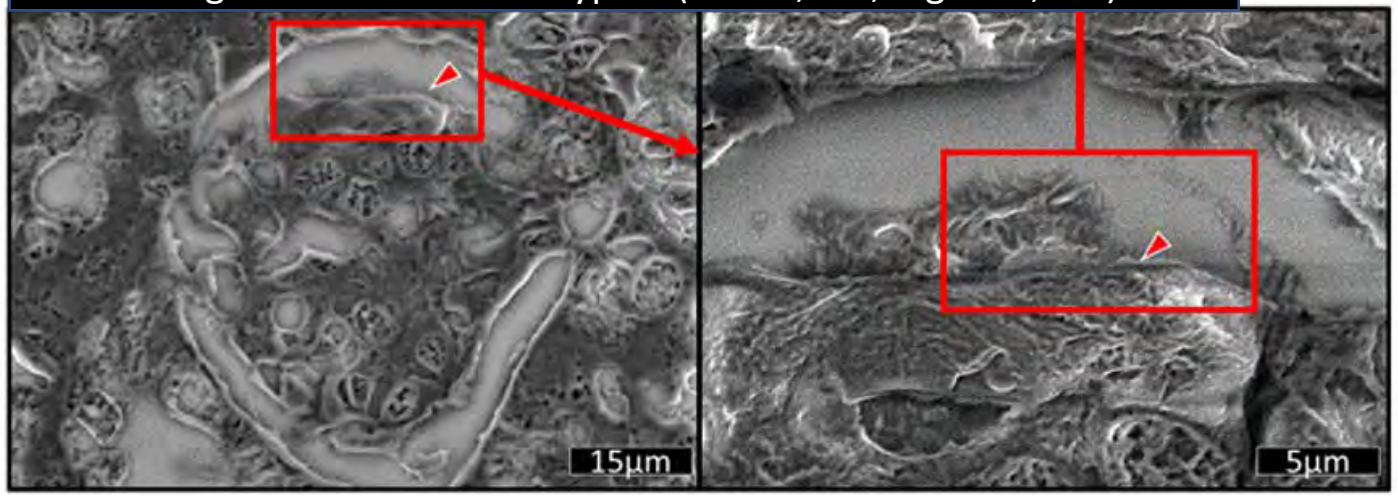


Observation with FE-SEM
Podocytes were clearly observed



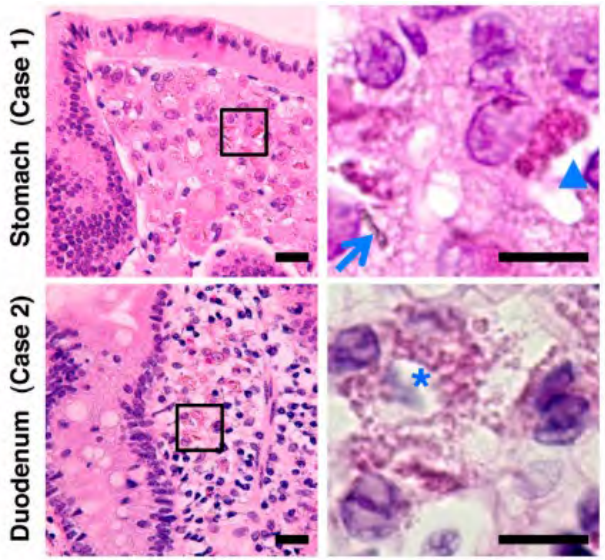


Using NanoSuit solution Type II (left x1,500, Right x5,000)

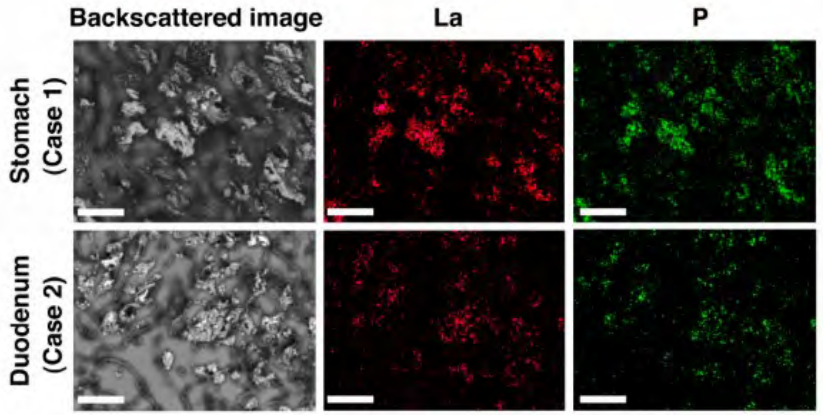


Observation with
desktop SEM

Example（SEM-EDS analysis using the NanoSuit-CLEM）



(a)



(b)

diagnostics

MDPI

Article

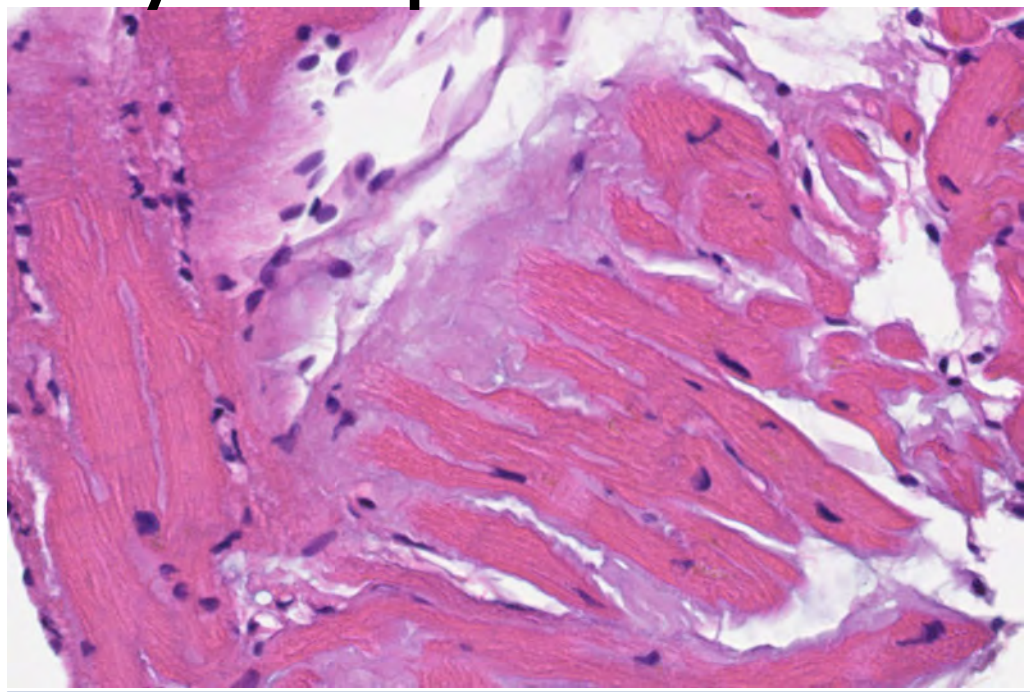
Utility of Scanning Electron Microscopy Elemental Analysis Using the 'NanoSuit' Correlative Light and Electron Microscopy Method in the Diagnosis of Lanthanum Phosphate Deposition in the Esophagogastroduodenal Mucosa

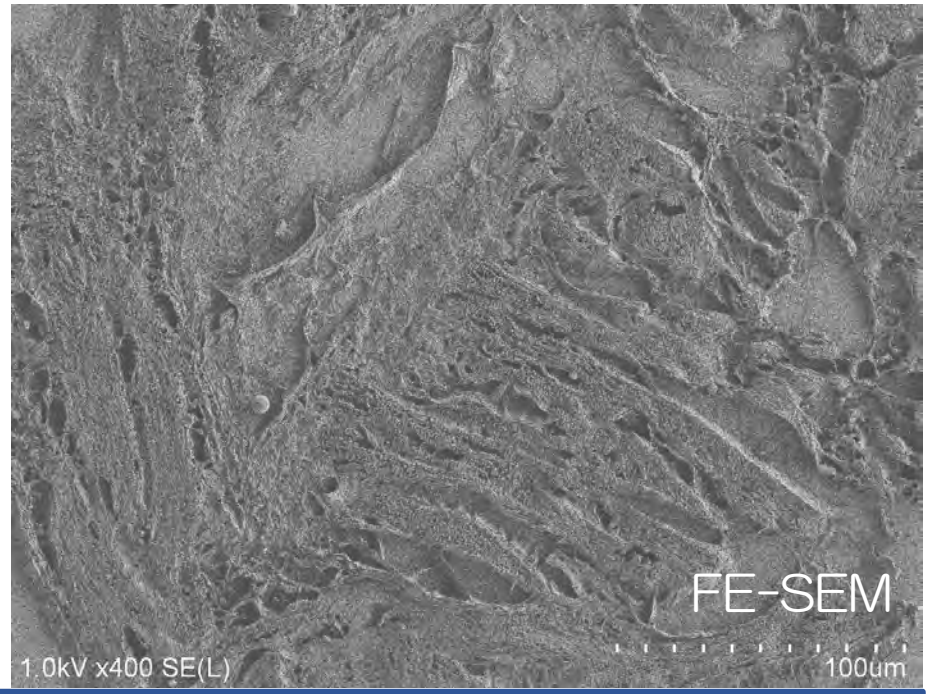
Kazuya Shinmura [1,*,†], Hideya Kawasaki [2,*,†], Satoshi Baba [3], Isao Ohta [4], Hisami Kato [1], Hideo Yasuda [5], Satoshi Yamada [6], Kiyoshi Misawa [6], Ken Sugimoto [5], Satoshi Osawa [7], Masanori Sato [8], Takahiko Hariyama [2] and Haruhiko Sugimura [1]

Figure 2. Lanthanum phosphate deposition in the stomach and duodenum. (**a**) Images by light microscopy of H&E-stained gastric and duodenal mucosa containing brown pigment deposition, which was suspected as lanthanum phosphate deposition. Images in the right column are a higher magnification of the boxed areas in the images in the left column. Depositions showing granular, needle-shaped, or amorphous structures are marked with an asterisk, arrow, or arrowhead, respectively. Scale bar = 20 μm (left); 10 μm (right). (**b**) Lanthanum phosphate deposition in the gastric and duodenal mucosa shown by SEM-EDS analysis using the NanoSuit-CLEM method. Images in the left column are backscattered SEM images showing a bright area in the mucosa. The middle- and right-column images are elemental mapping images using SEM-EDS analysis showing deposition of lanthanum (La) and phosphorus (P), respectively. Scale bar = 25 μm.

Example（Pathological specimen）

Amyloid deposition of cardiac muscle tissue

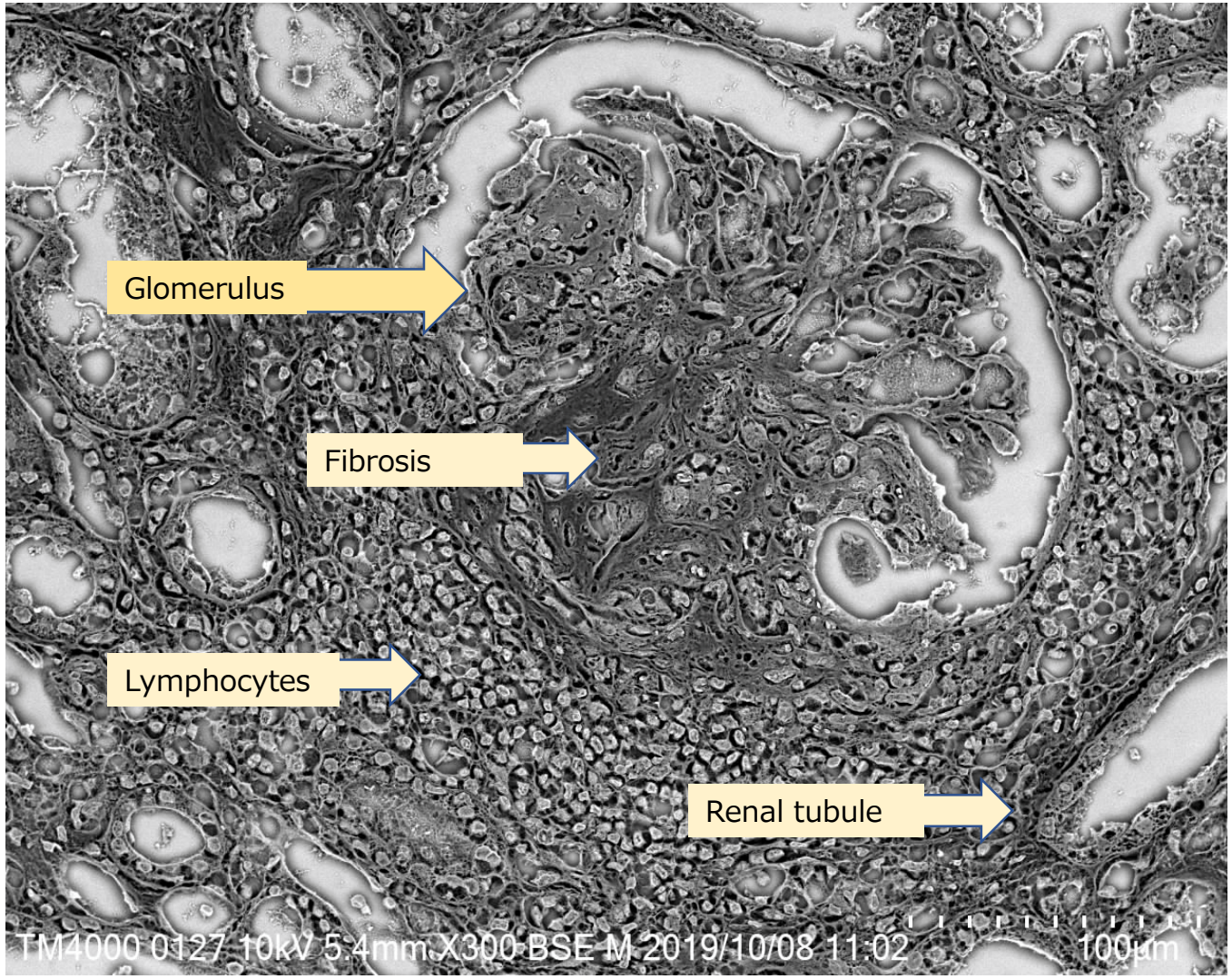




NanoSuit CLEM method

By CLEM method using “NanoSuit®”, it is possible to make a diagnosis by combining amyloid deposition in an image stained with an optical microscope and changes in the surrounding tissue morphology observed using electron microscopy.

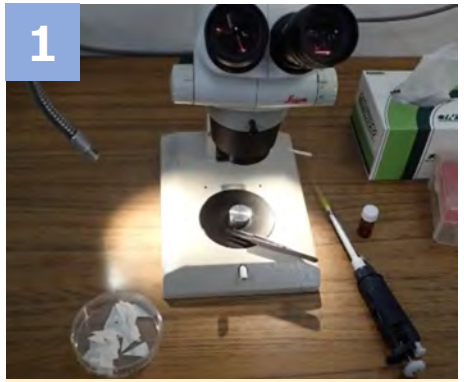
Example（Pathological specimen - Nephritis）



It is observed that the glomerulus is broken about half due to inflammation and lymphocytes are infiltrated.

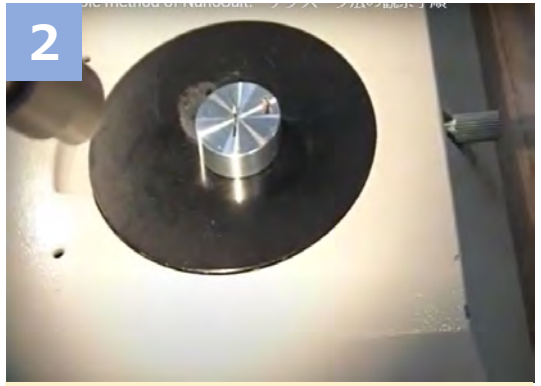
How to use “NanoSuit®”

1



Prepare microscope, pipet, water-absorbing sheet (filter paper), NanoSuit solution and the object

2

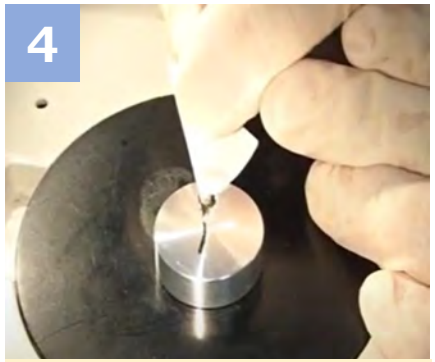


Place the object on the specimen table

3

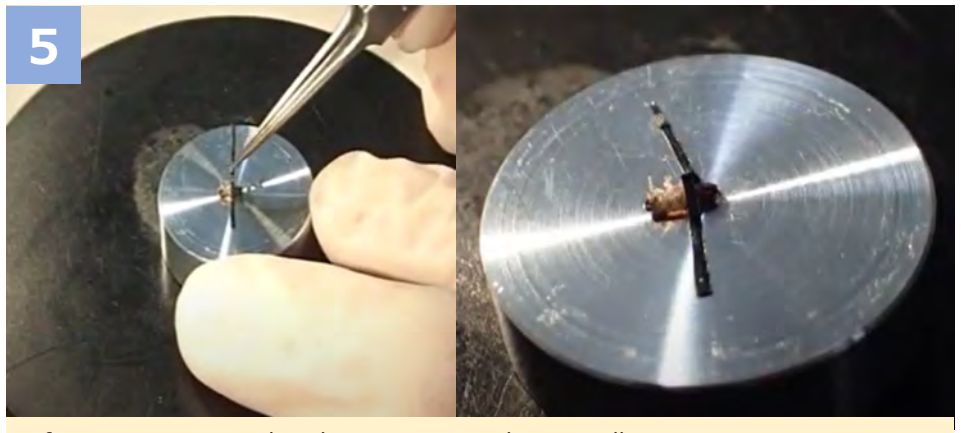
Drop NanoSuit solution using pipet and dip the object into NanoSuit solution

4



Absorb excess NanoSuit solution (In order to obtain clear image, this step is necessary)

5

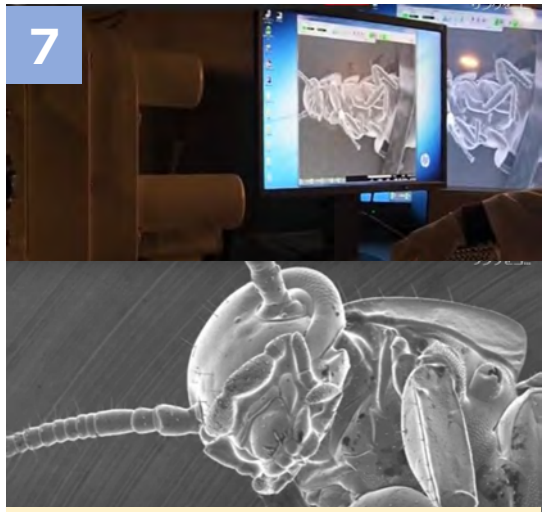


If necessary, strap the object using conductive adhesive tape

6

Place the specimen table in the sample room of the electron microscopy (SEM). Then start evacuation.

7



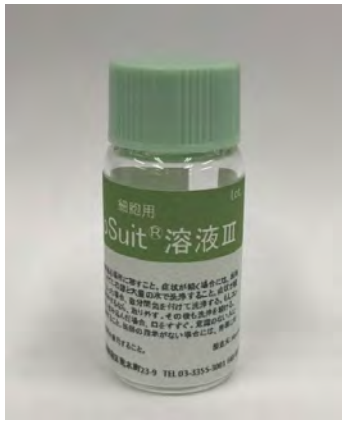
After evacuation, start observation immediately. (“NanoSuit” (barrier layer) is formed by electron beam irradiation.)

Tips

- To obtain a clear image, it is important to keep the amount of NanoSuit solution to the minimum necessary to wet the surface of the target object.
- After evacuation, start electron beam irradiation immediately. Otherwise, the object may be dried before forming “NanoSuit” (moist barrier layer).

NanoSuit® Solution I
(for micro-organisms/individuals/living tissues)

NanoSuit® Solution II
(for CLEM)



NanoSuit® Solution III
(for cells)

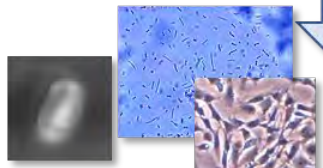
Outsourcing services
NanoSuit Inc. provides contract base services.

Contact: info@nanosuit.jp

NanoSuit Solution
- Product selection guide -

Small animals
plant
Food
Chemical materials,
etc.

Spheroids
Organoids



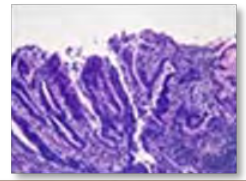
Cells
Bacteria

Exosomes
Virus

NanoSuit Solition I
(for micro-organisms/individuals/living tissues)

(May be applicable by diluting Nanosuit solution with water)

NanoSuit Solution III
(for cells)



NanoSuit Solution II
(for CLEM)

Only for paraffin-fixed pathological section specimens.

Patent

- NanoSuit technology is protected by several patents (granted) in the US, Europe, China and Japan.
- If you use “NanoSuit solution” for your business, please contact NanoSuit Inc.