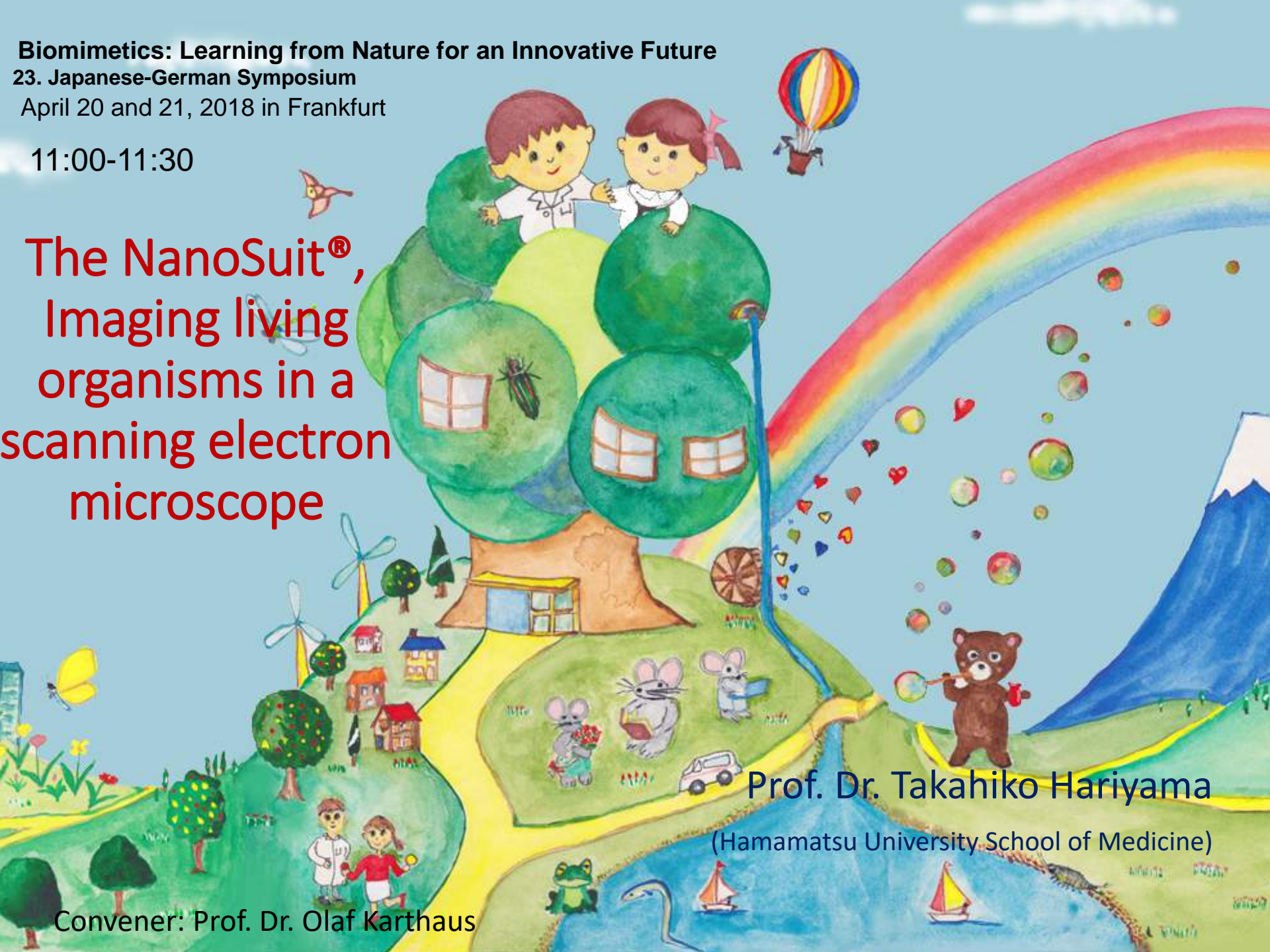


Biomimetics: Learning from Nature for an Innovative Future
23. Japanese-German Symposium
April 20 and 21, 2018 in Frankfurt

11:00-11:30

**The NanoSuit[®],
Imaging living
organisms in a
scanning electron
microscope**



Prof. Dr. Takahiko Hariyama
(Hamamatsu University School of Medicine)

Convener: Prof. Dr. Olaf Karthaus

Humans are called as a vision animal.

Paradigm shift by new innovation.



1600~ Light microscope Netherland

Paradigm shift life science (Organisms are composed of cells.)



1838

M.J.Schleiden



1839

T.Schwann

1930~ Electron microscope 1950~ popular equipment for science.

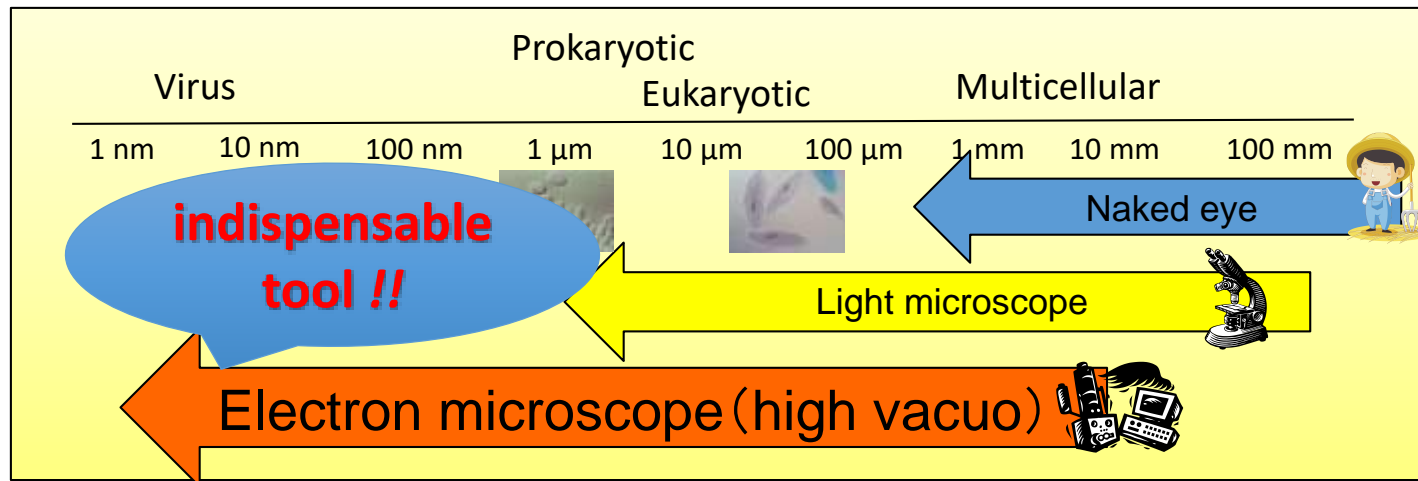
Paradigm shift life science (Virus, Organelles)



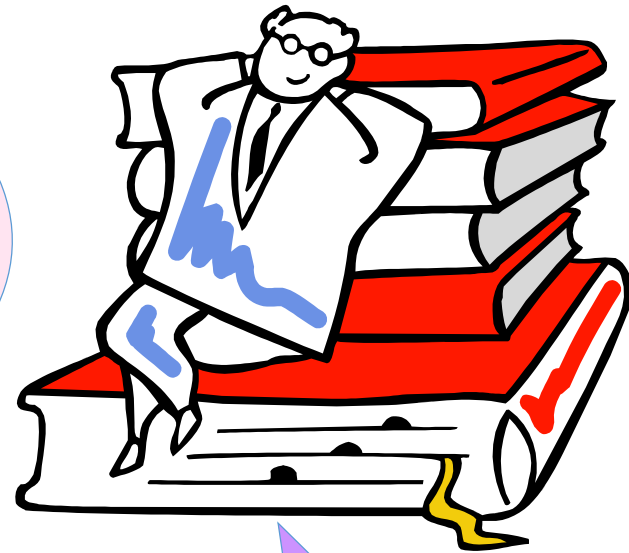
1936

Ernst Ruska

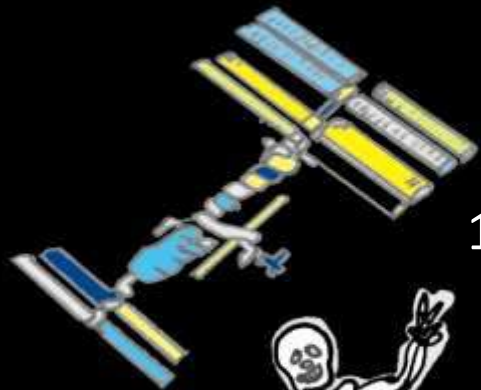
Paradigm shift



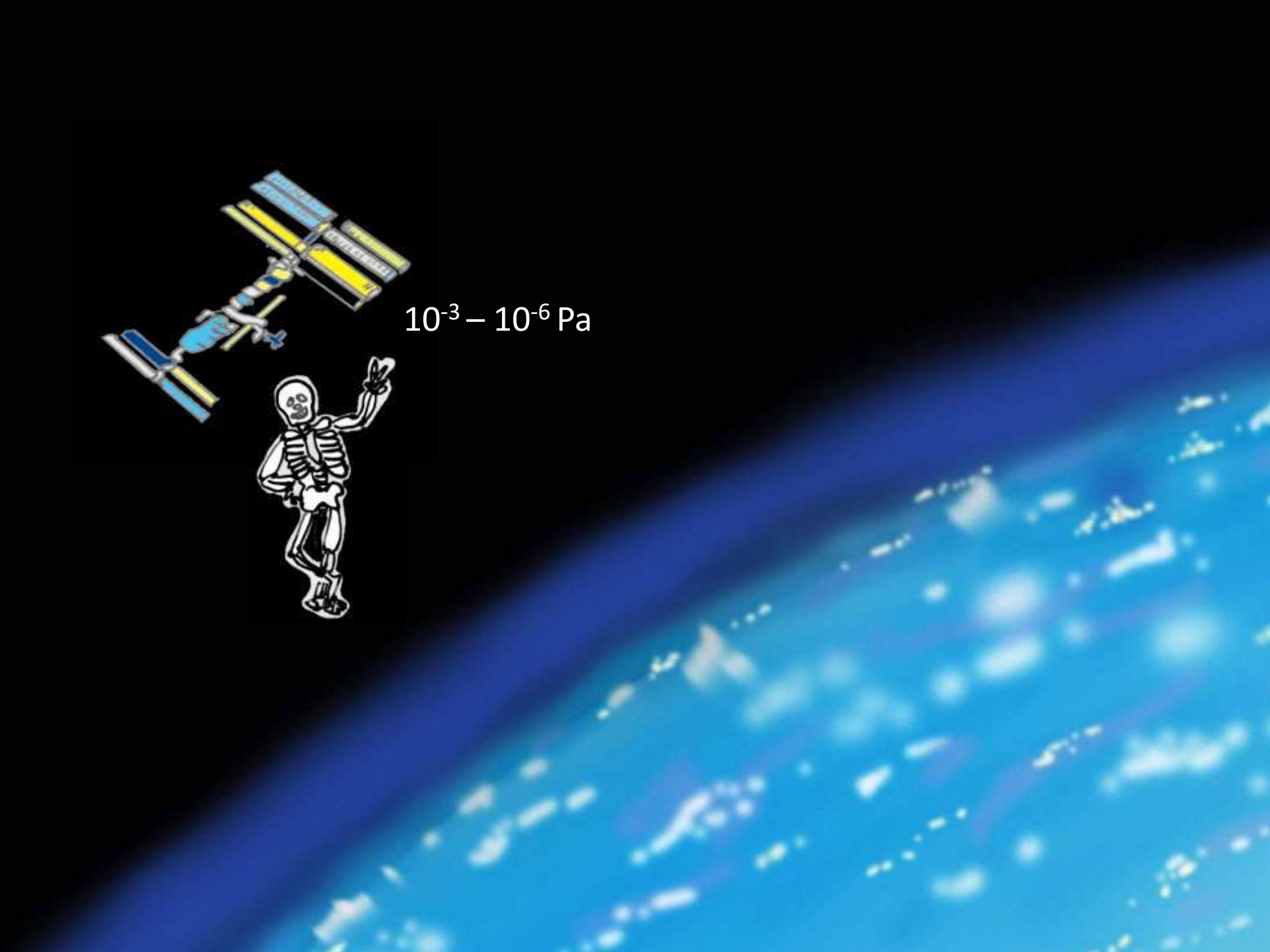
**I do want to observe
the real structure of
organism in an electron
microscope!**



**Absolutely
impossible!**

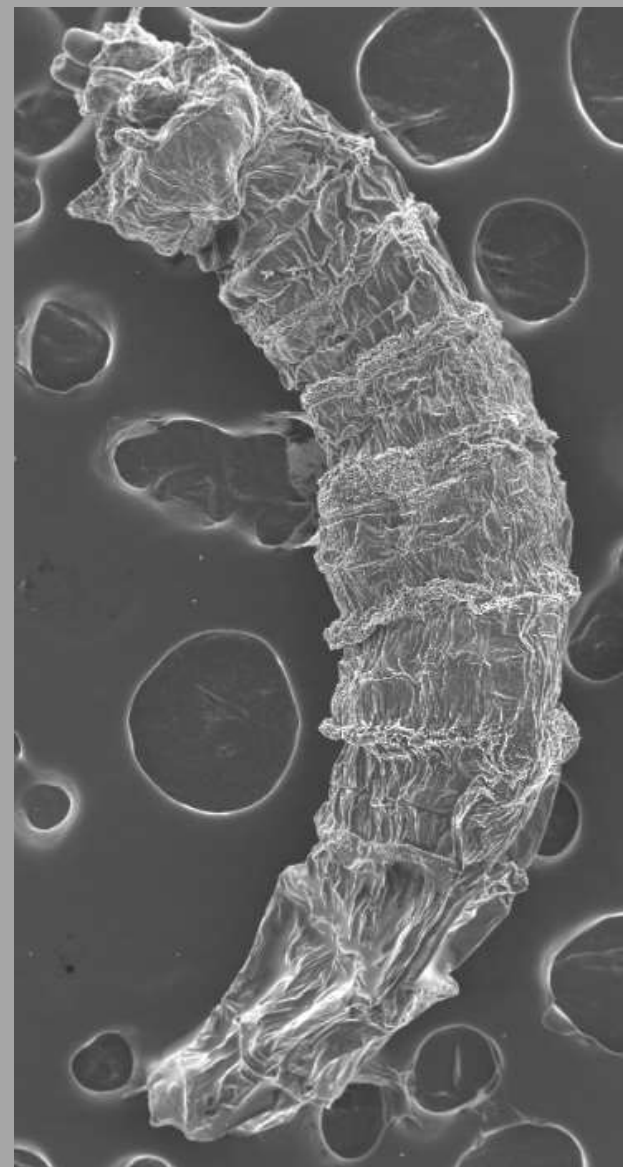


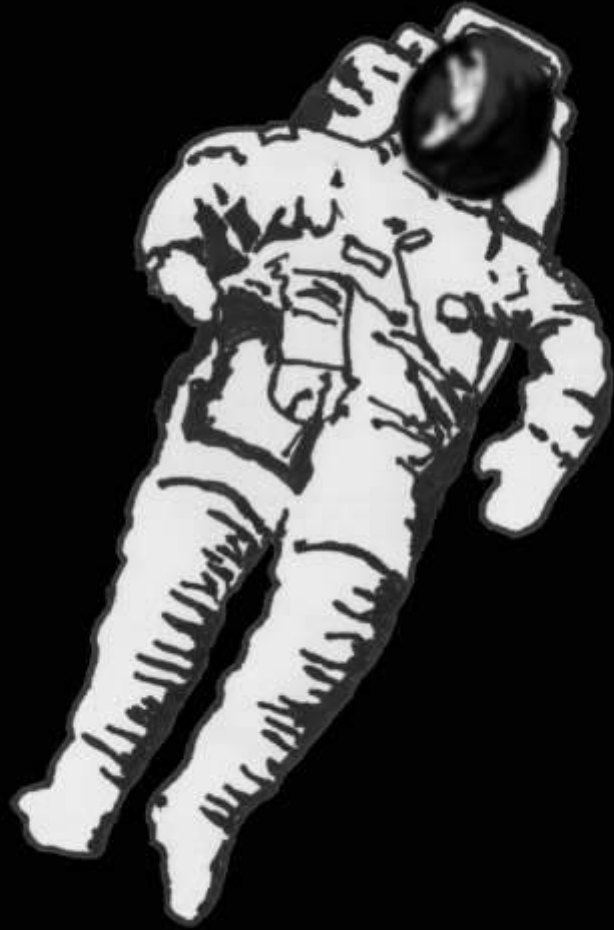
$10^{-3} - 10^{-6}$ Pa



Arthropoda

Drosophila larva





Pease R.F., T.L. Hayes, A.S. Camp and N.M. Amer (1966) Electron microscopy of living insects. *Science* 154, 1185–1186.

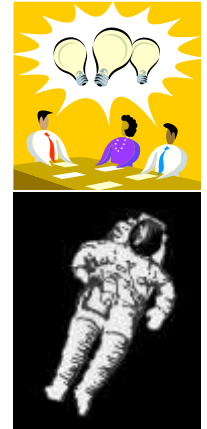
Electron micrographs of living specimens of the various developmental stages of the insect *Tribolium confusum* have been obtained with a scanning electron microscope. In most cases the specimens resumed their normal activity after being examined with the electron microscope and under went metamorphosis into the next stage.



Red flour beetle
Tribolium castaneum



Young scientists encouraged me!



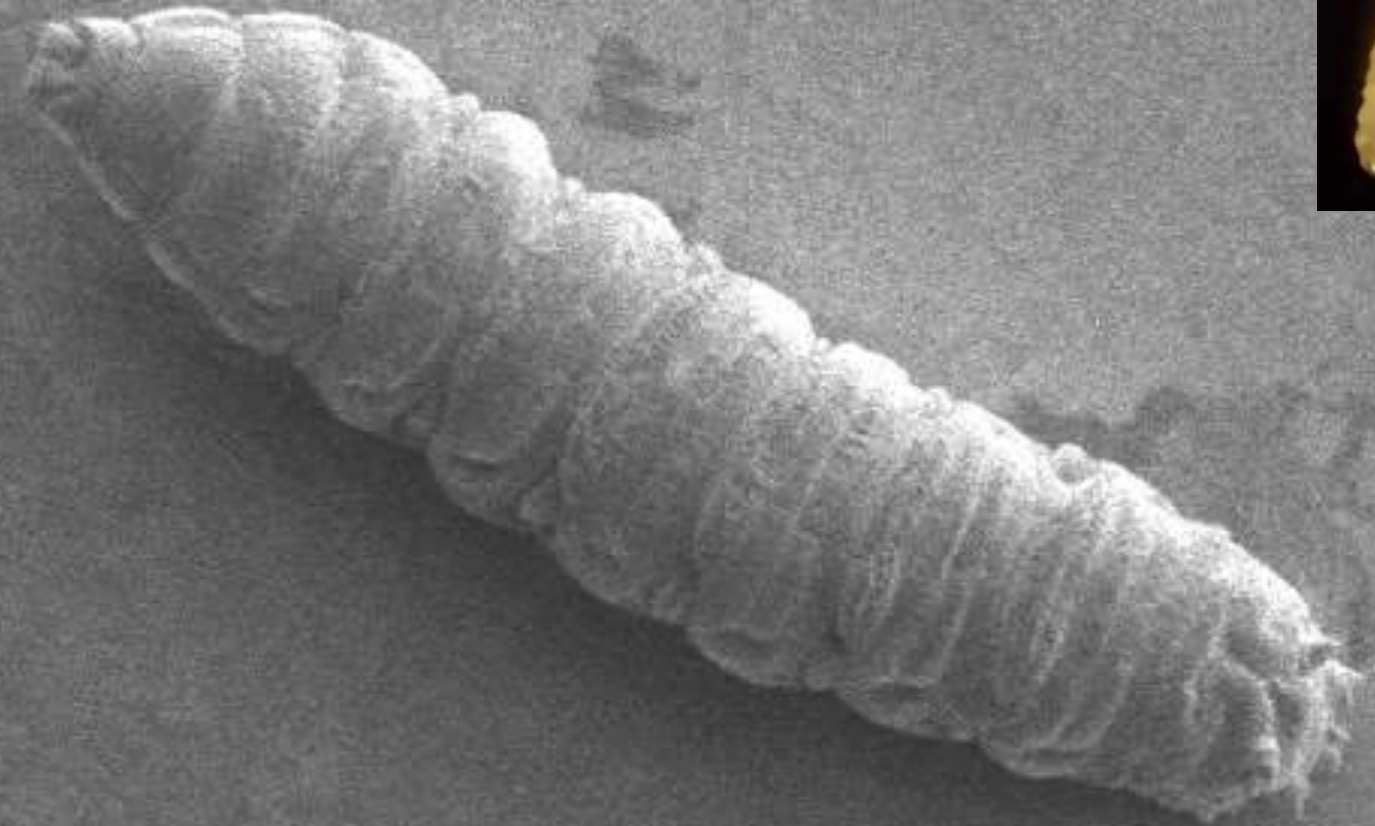
The pressure level of the SEM which used in the former report was 10^{-2} Pa.

We would like to observe several living specimens with an FE-SEM (10^{-3} - 10^{-7} Pa).

Our Aim

Observe several organism/tissues/cells in a FE-SEM.

Larva of *Drosophila melanogaster* in FE-SEM

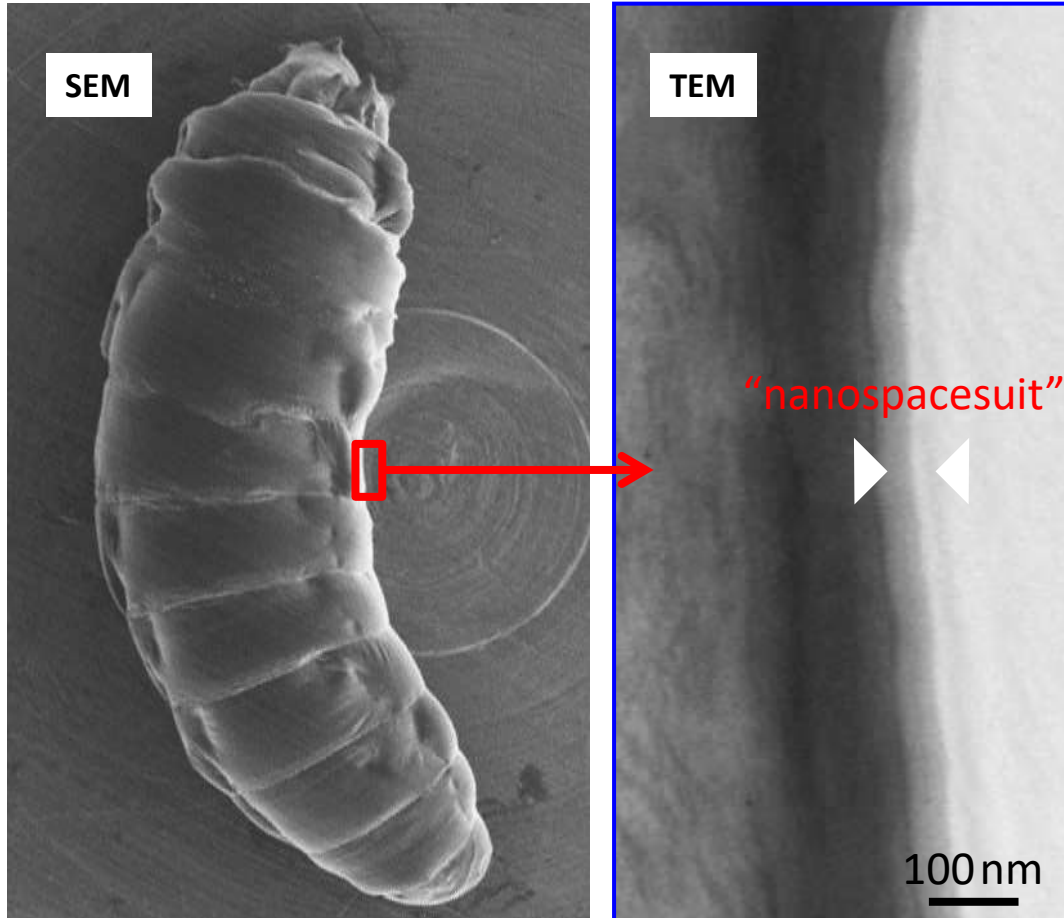


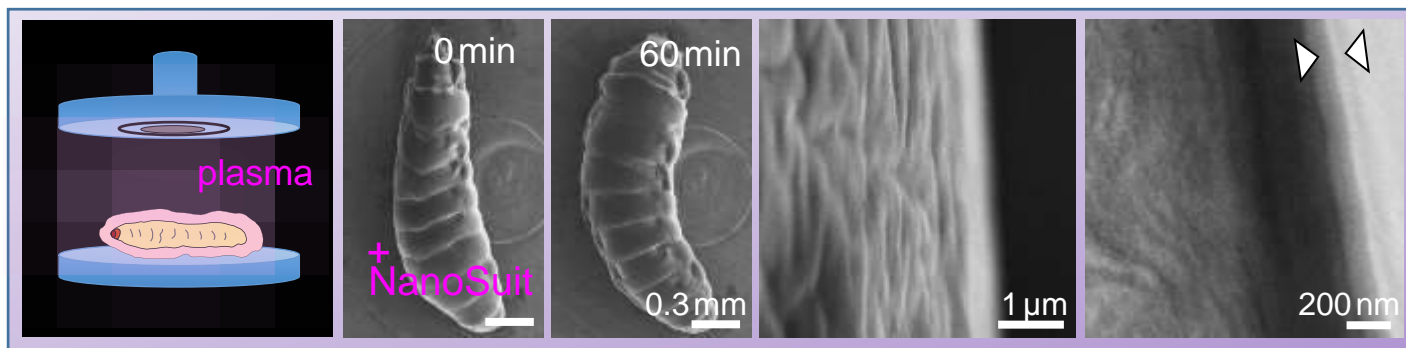
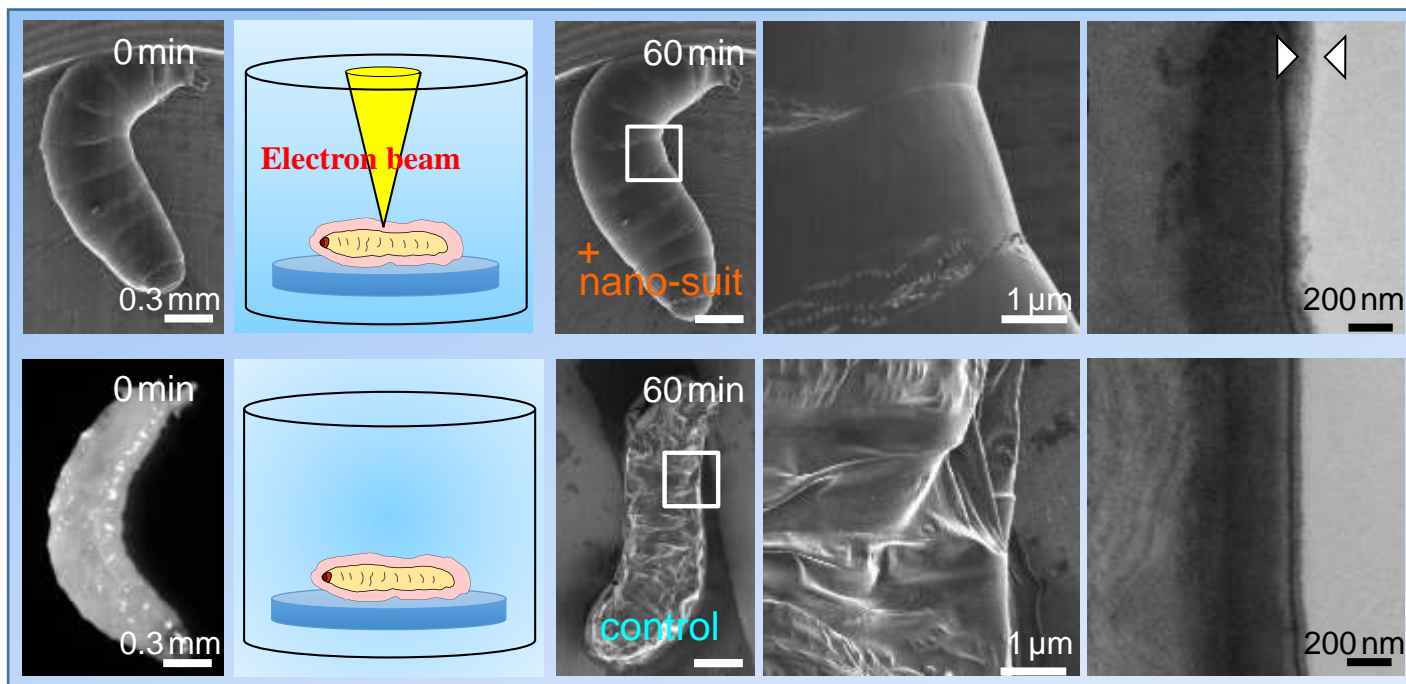
Arthropoda

Drosophila larva



Using ECS with electron or plasma irradiation,
we could make “nanospacesuit” . . .





When the animal possesses ECS and when irradiated by electrons or plasma, they can survive in high *vacuo*.

Next, we must keep living animal which do not possess ECS on their surface . . .



Biomimetics



ECS mimetic substance

**We chose amphiphilic molecules.
(1% Tween20) and irradiated.**

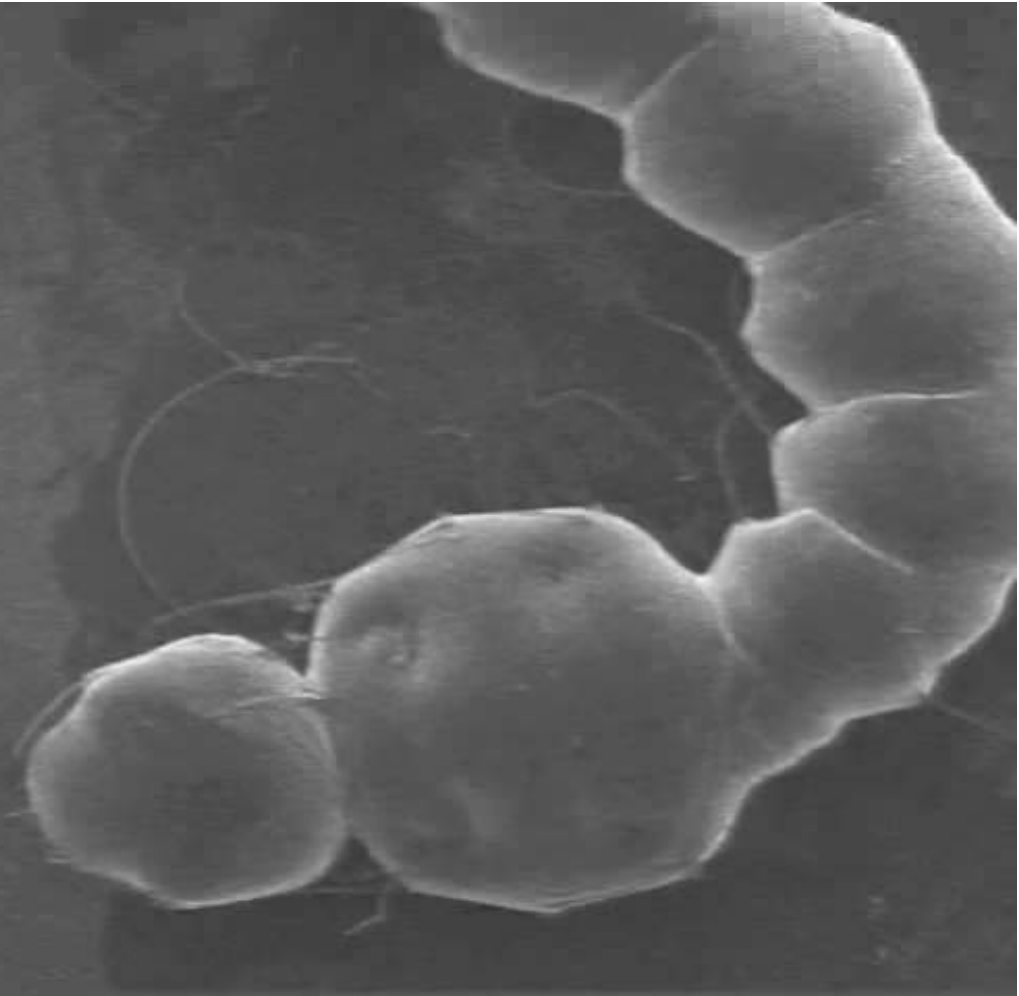


Polysorbate 20 (Tween 20)

larva of a mosquito, *Aedes albopictus*

FE-SEM

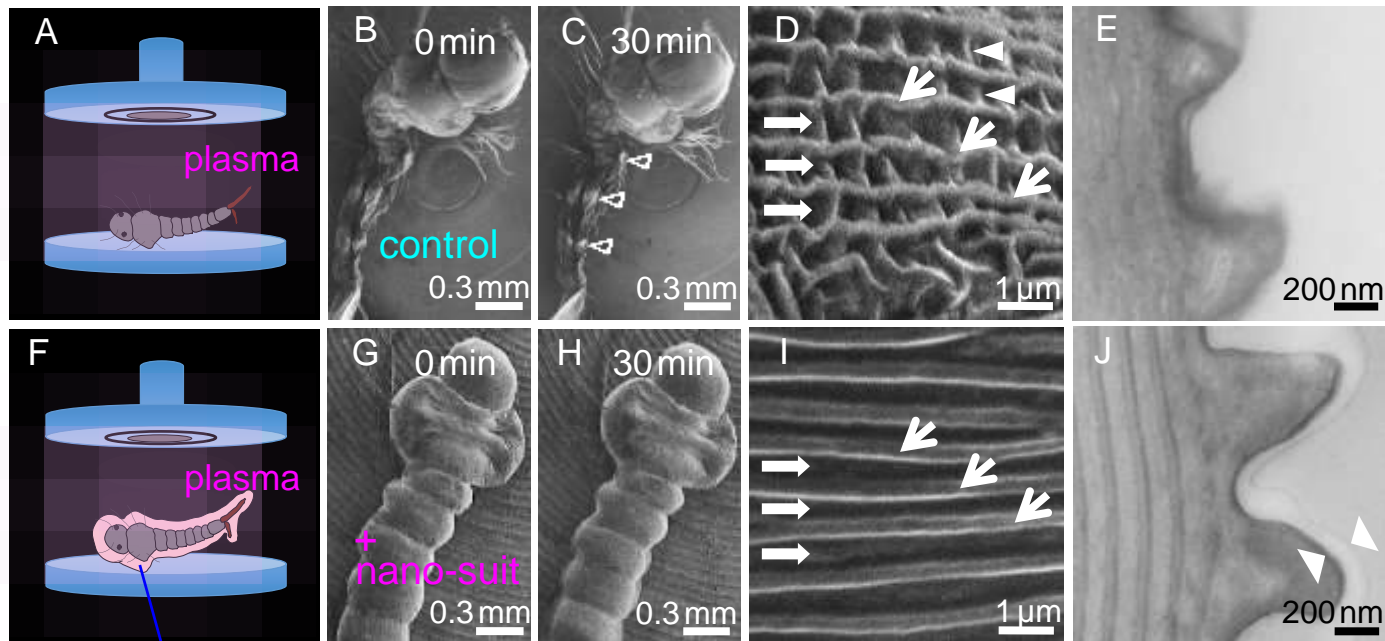
With nano-siut



Without nano-siut

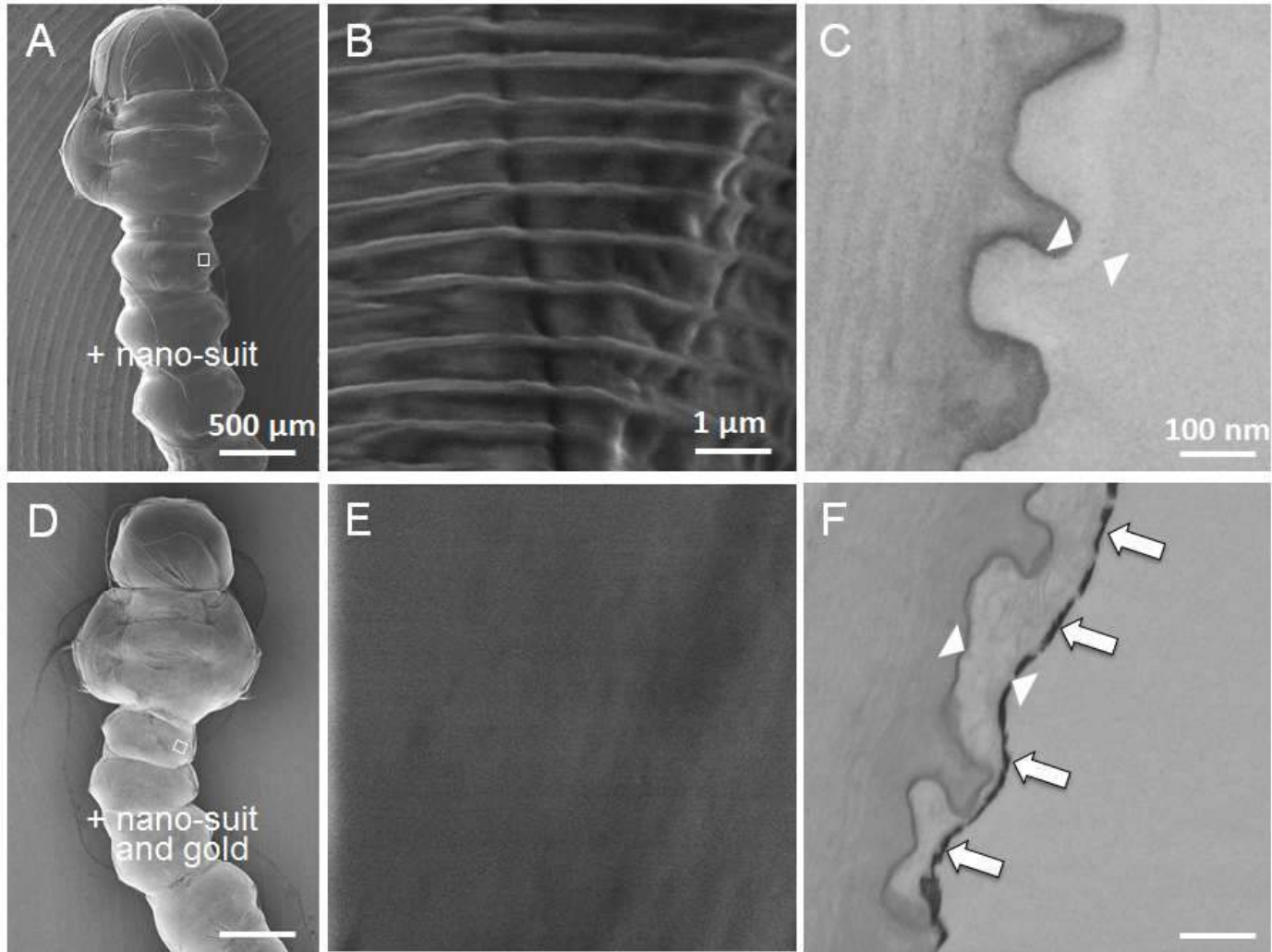


By only the plasma irradiation there was no NanoSuit, but with the additional amphiphilic molecules and the plasma irradiation, we found the NanoSuit on the surface of the cuticle, and the fine structure of the mosquito larvae were ordered..



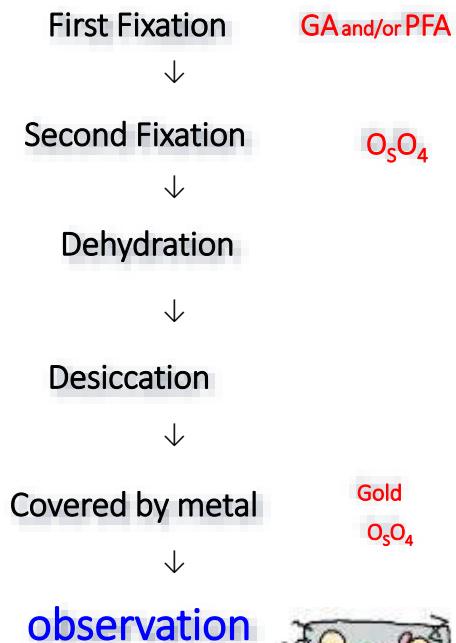
ECS mimetic substance

nano-siut



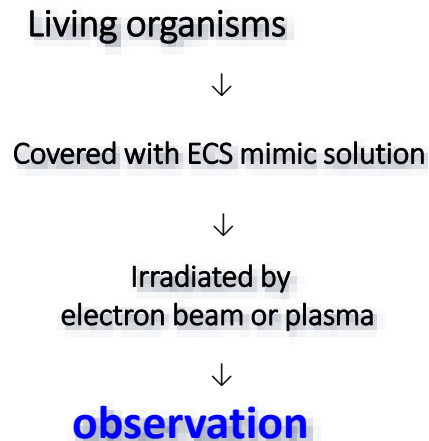
nanospacesuit

conventional SEM method



Need at least a whole day.

NanoSuit method



Need three minutes. カップラーメン Cup RAMEN noodle

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Nano-suit shields bugs in the void

Coating enables electron-microscope imaging of live organisms

Katherine Hamrick

16 April 2013



A thin polymer nano-suit allows multicellular organisms to tolerate high vacuums.

Walking in space SOURCE: AFP-T

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Science NEWS

Nano-Suit Protects Bugs From Space-Like Vacuums

Put a fruit fly larva in a space-like vacuum, and the results aren't pretty. Within a matter of minutes, the animal will collapse into a crumpled, featureless blob. Now, researchers have found a way to protect the bugs: bombard them with electrons, which form a "nano-suit" around their bodies. The advance could help scientists take high-resolution photographs of tiny living organisms. It also suggests a new way that creatures could survive the harsh conditions of outer space and may even lead to new space travel technology for humans.



Scientists at the University of Tsukuba in Japan and the University of California, San Diego (UCSD) have found a way to protect fruit fly larvae from the vacuum of space. They coated the larvae with a thin layer of polymer, which allowed them to survive in a vacuum for up to 30 minutes. The researchers used a scanning electron microscope (SEM) to take high-resolution images of the larvae while they were in the vacuum. The SEM's electron beam created a protective layer of polymer around the larvae, which prevented them from drying out. The researchers found that the nano-suit was made of a polymer called tetra-20, which is commonly found in detergents, soaps, and hair candy. The researchers then allowed each larva to breathe, so that the tetra-20 would polymerize and become a nano-suit, and coated the nano-suit larvae in the microscope's vacuum to watch what happened.

The team studied the layers "nano-suits." Most insects do not have natural layers on their surfaces that become nano-suits when exposed to an electron beam, however. So Hanyama and colleagues decided to create artificial nano-suits. They dunked mosquito larvae in a pool of water mixed with a chemical called tetra-20, which is useful because it's not toxic and is commonly found in detergents, soaps, and hair candy. The researchers then allowed each larva to breathe, so that the tetra-20 would polymerize and become a nano-suit, and coated the nano-suit larvae in the microscope's vacuum to watch what happened.

Mosquito larvae entering the artificial nano-suit could handle the vacuum for about 30 minutes, the team reports online today in the Proceedings of the National Academy of Sciences. Mosquito larvae without the nano-suits died swiftly and tortuously, as expected. "Within a few minutes, they were dehydrated," Hanyama says. "They're very cool experiments." The researchers replicated the experiment with other insects, including fallow deer, ants, and silver fishoppers, and the man-made nano-suit protected them all.

The finding is "exciting," says astrobiologist Lynn Rothschild of NASA's Ames Research Center in Moffett Field, California, who was not involved in the work, because it indicates that nano-suit-coated organisms might survive travel by a meteorite or comet through the extreme environments of space. She notes that it could also have applications for space travel. "Imagine a flexible space suit, roughly the diameter of a human hair that could protect against dehydration and radiation."

After the report of PNAS . . .

Takanori Hanyama, a biologist at the Niigata University School of Medicine in Japan, and his collaborators describe the results in the *Proceedings of the National Academy of Sciences*¹. The discovery builds on previous findings² that some organisms, including beetle larvae and toads, can survive short stints in the extremely low-pressure environment of scanning electron microscopes—and even, in the case of dormant tardigrades, or "water bears", in outer space.

The researchers made their discovery while testing how long various animals could survive in a high vacuum while being imaged inside a scanning electron microscope. Most organisms lose water rapidly in these conditions, leading to death by dehydration and physical distortion, but the larvae of the fruit fly *Drosophila* survived for 30 minutes and went on to develop normally after being returned to normal pressure.

The bodies of fruit fly larvae are naturally coated in a substance made of biological molecules such as proteins, and the researchers suspected that exposure to the electron beam caused molecules in the substance to lock together in long chains, or polymers. That would create a flexible, protective layer just 30–100 nanometers thick. Other organisms that have similar coatings, such as Japanese hemiptera (Aphididae japonica) and larvae of blue-bottle flies (*Photophilum leucostriatum*), survived in the high vacuum after being irradiated with plasma beams, which can generate a polymerization effect similar to that of an electron beam. "Plasma beams are already used for that purpose in some industrial applications³."

To further test their hypothesis, the team tried applying an artificial version of the coating—a detergent solution made from a surfactant—on organisms that did not have any, including a fallow deer (*Dipodomys japonicus*) and the larvae of the Asian tiger mosquito (*Aedes albopictus*). Under plasma irradiation, the coating formed a similarly effective nano-suit.

Structure survives
The researchers also found that many structural details of the surviving larvae were "completely different from that of untreated specimens and traditionally prepared specimens", suggesting that

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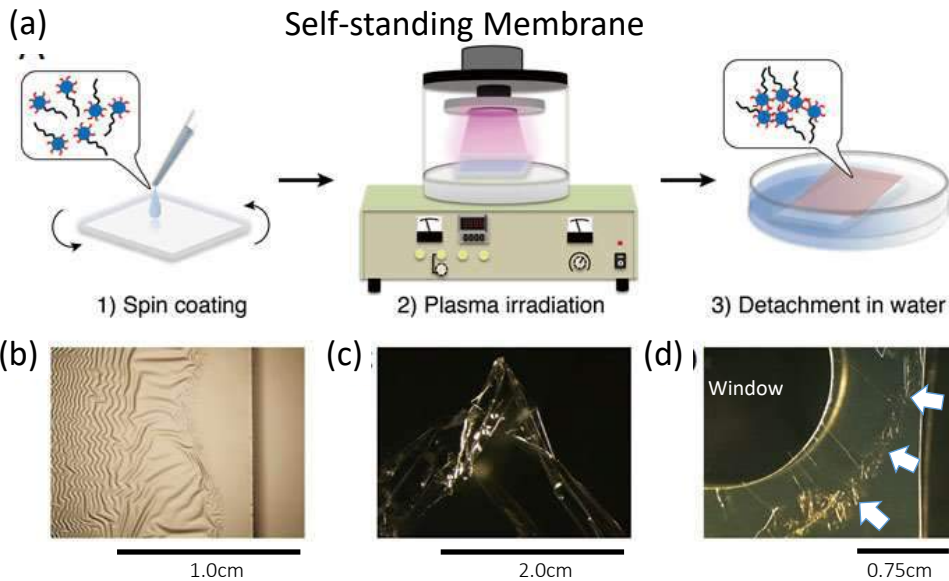
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After a Month, Animals, Great Migrate Home as Parasites
"Space Winger" Spook World Insects
Ferdinand University of Most Papers Are Tossed, But

We could make self-standing ultra-thin films from water soluble amphiphilic molecules.



Sample	Monomer	Polymerization site	Solvent	State
1	Tween 20 [®]	PEO chain	Water	⊙
2	Tween 40 [®]	PEO chain	Water	⊙
3	Tween 60 [®]	PEO chain	Water	⊙
4	Tween 80 [®]	PEO chain	Water	⊙
5	Deg 35 [™]	PEO chain	Water	⊙
6	Triton X-100 [™]	PEO chain	Water	⊙
7	Poly(ethylene oxide) [®]	PEO chain	Water	⊙
8	Pluronic F-127 [™]	PEO chain	Water	⊙
9	Pluronic F-68 [™]	PEO chain	Water	⊙
10	Lecithin (from soy bean) [®]	Multiple OH	Ethanol	⊙
11	Tannic acid [®]	Multiple OH	Ethanol	⊙
12	Tetraethoxysilane [®]	Multiple OH	Ethanol	⊙
13	Spin 20 [™]	Multiple OH	Ethanol	⊙
14	D-Maltose [®]	Multiple OH	Water	⊙
15	Trehalose C12 [®]	Multiple OH	Water	⊙
16	D-Glucose [®]	Multiple OH	Water	⊙
17	n-Doxyl-β-D-maltoside [™]	Multiple OH	Water	⊙
18	MEGA-8 [™]	Multiple OH	Water	○
19	CHAPS [™]	Multiple OH	Water	○
20	D-Trehalose [®]	Multiple OH	Water	○
21	Sodium cholate [™]	Multiple OH	Water	○
22	n-Octyl-β-D-glucoside [™]	Multiple OH	Water	○
23	Inulin [®]	Multiple OH	Water	△
24	Pullulan [®]	Multiple OH	Water	△
25	D-Sorbitol [®]	Multiple OH	Water	△
26	L-Tyrosine [®]	Multiple OH	Water	△
27	L-Glutamic acid [®]	Multiple OH	Water	△
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29	Lauric acid [®]	Single OH	Ethanol	○
30	Stearic acid n-dodecyl ester [®]	Single OH	Ethanol	○
31	Decanoic acid [®]	Single OH	Ethanol	○
32	L-Proline [®]	Single OH	Water	△
33	L-Lysine [®]	Single OH	Water	△
34	L-Histidine [®]	Single OH	Water	△
35	Linoleic acid [®]	OH & C=C double bond	Ethanol	⊙
36	Linoleic acid [®]	OH & C=C double bond	Ethanol	⊙
37	Oleic acid [®]	OH & C=C double bond	Ethanol	⊙
38	Erucic acid [®]	OH & C=C double bond	Ethanol	⊙
39	Methacryloylcholine chloride [®]	OH & C=C double bond	Ethanol	⊙
40	L-Glutamine [®]	OH & C=C double bond	Water	○
41	L-Arginine [®]	OH & C=N double bond	Water	○
42	1,3-Diallylimidazolium bromide [®]	C=C double bond	Ethanol	⊙
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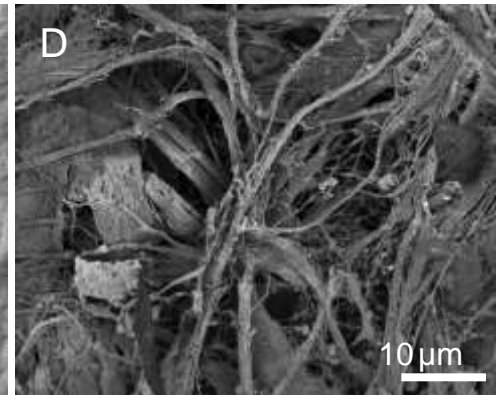
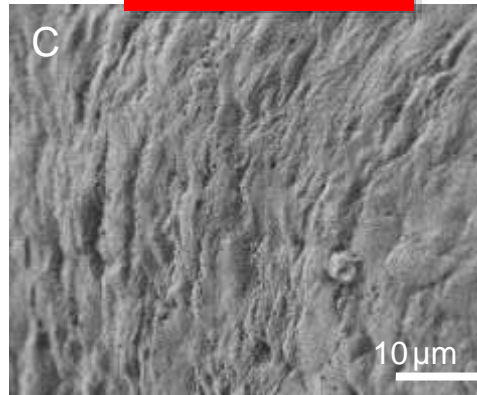
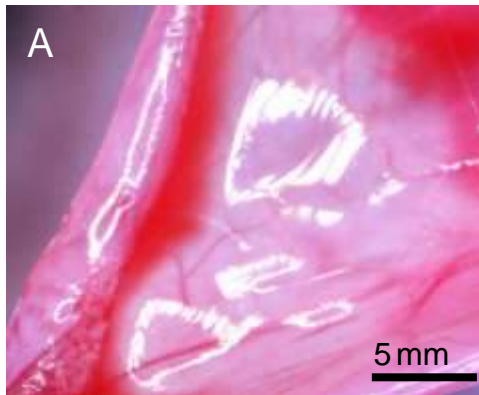
⊙: Large, thick, stable film ○: Small, thin, stable film, △: Unstable film
[®] Wako Pure Chemical Co., [™] Tokyo Kasei Kogyo Co., [®] Sigma-Aldrich Japan Co., [®] Kanto Chemical Co., [™] Dainippon Co.

Tissue and Cells

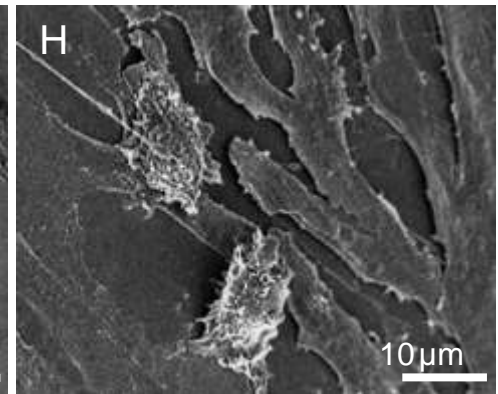
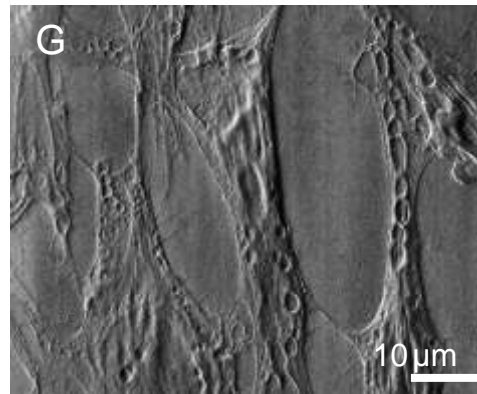
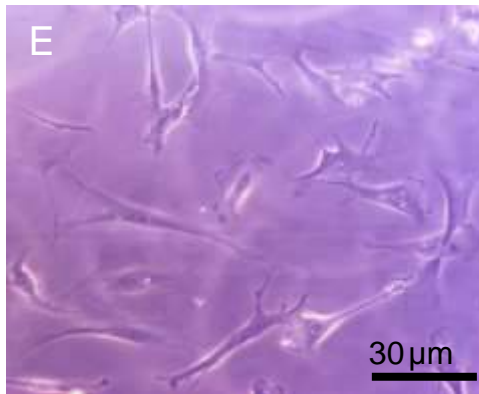
NanoSuit
Method

Traditional Method

Tissue
Mouse
(Peritoneum)

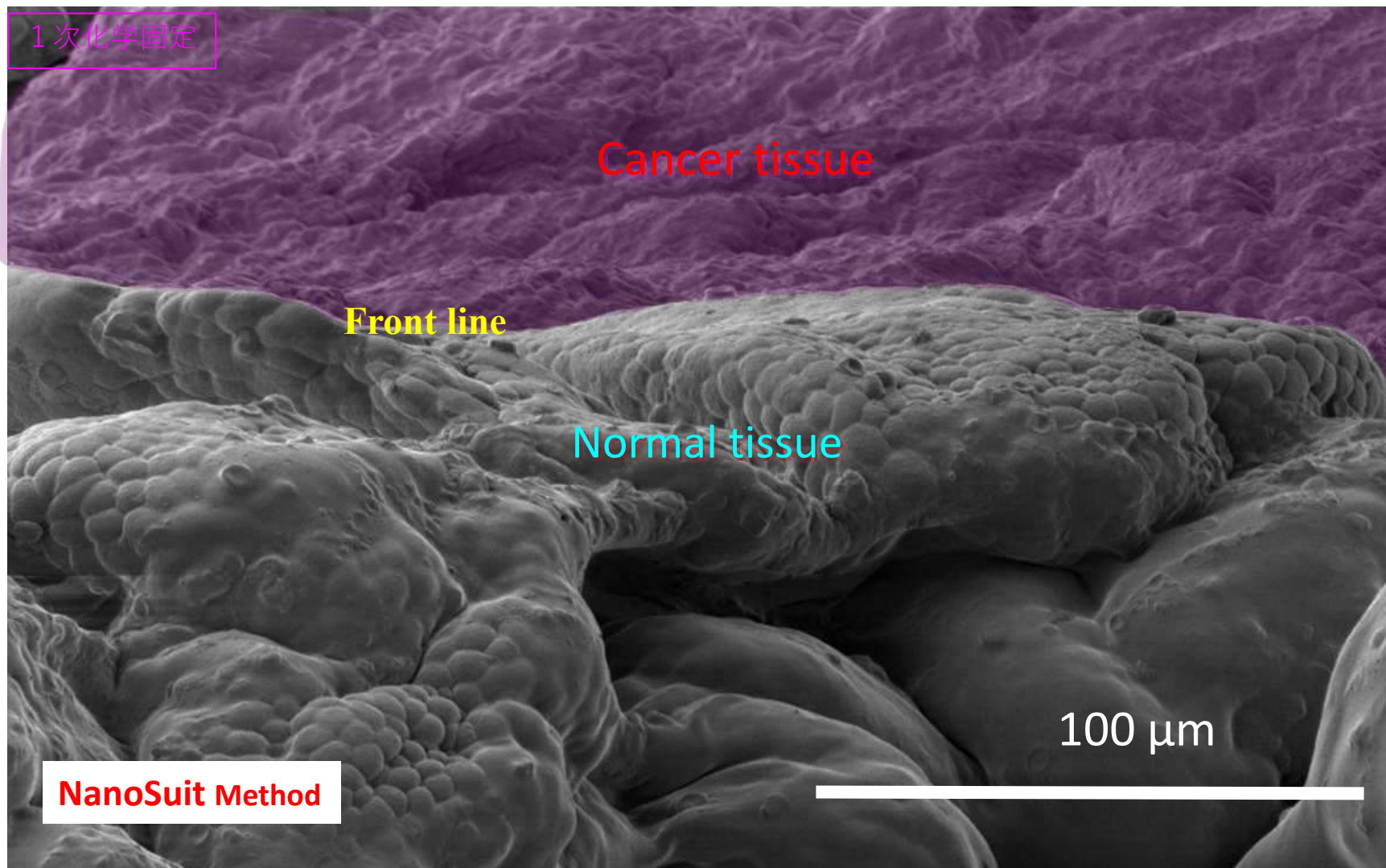


Cell
Human
(fibroblast)



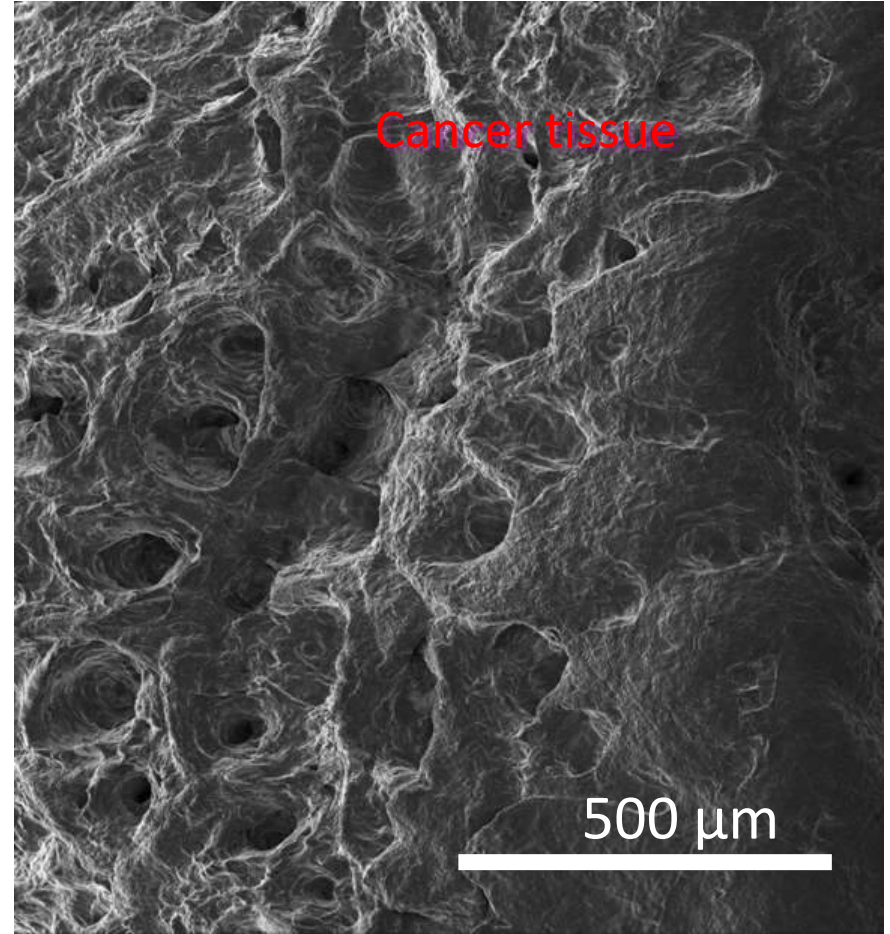
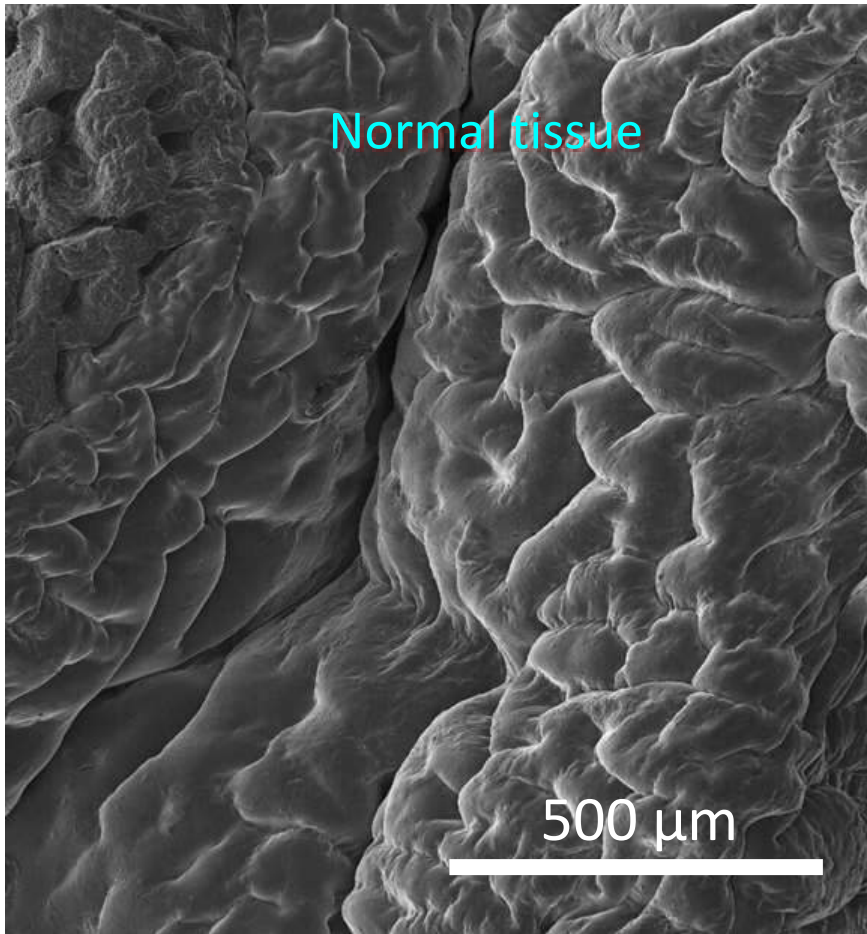
Human Stomach Cancer (Front line between cancer and normal tissue)

1次化学固定



Human Stomach Cancer (normal and cancer tissue)

SSE / nanosuit method



Human Stomach Cancer

Fixed wet sample

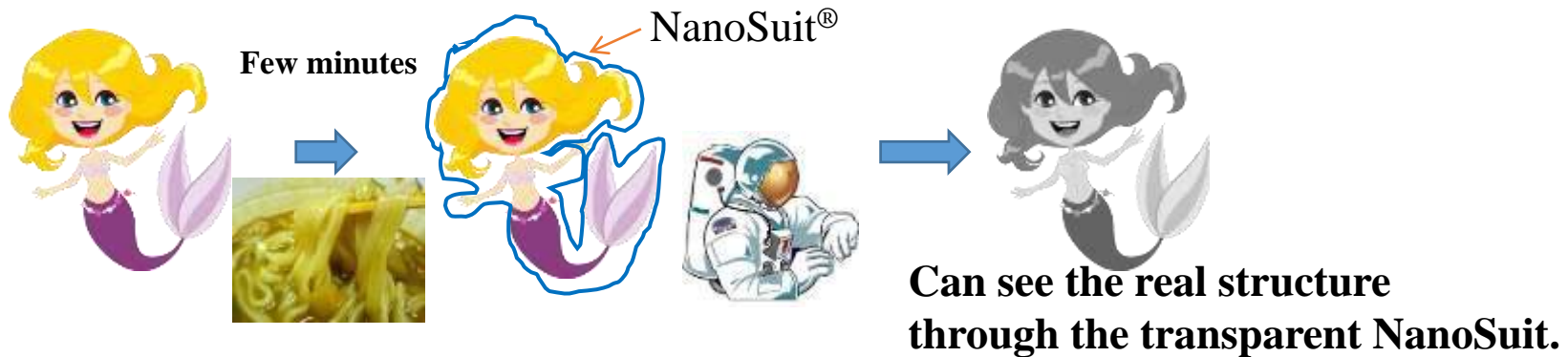
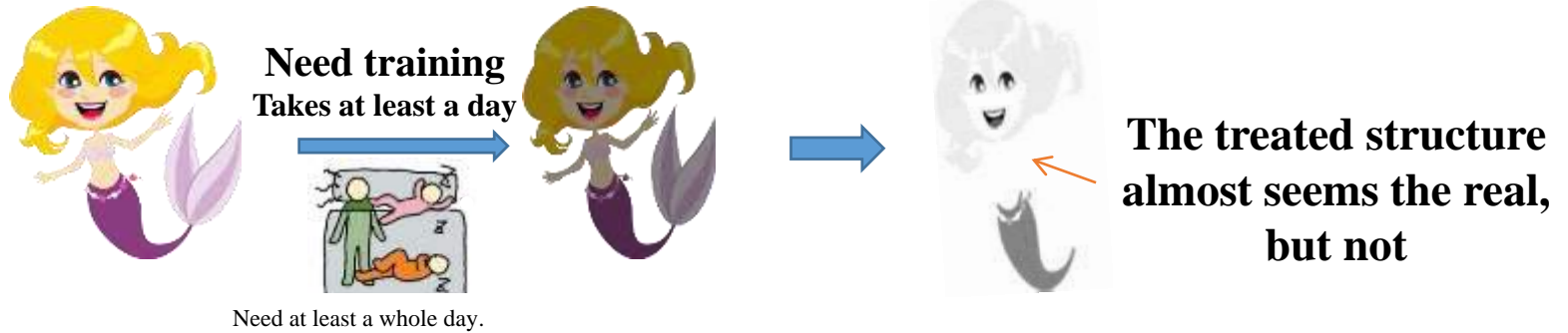
NanoSuit 法

After the observation of the surface, we can peeled
otbecause they are wet ! !

20 μm



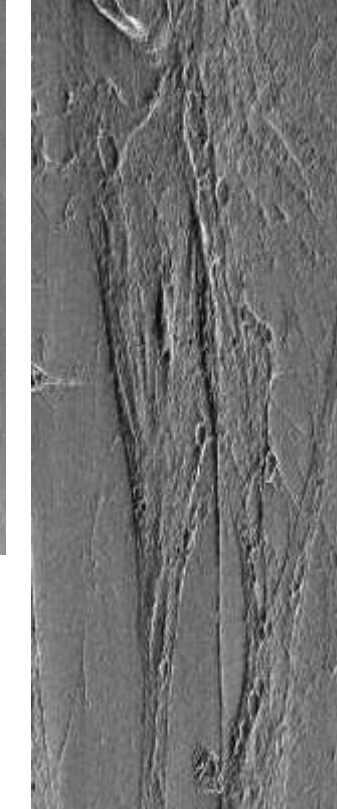
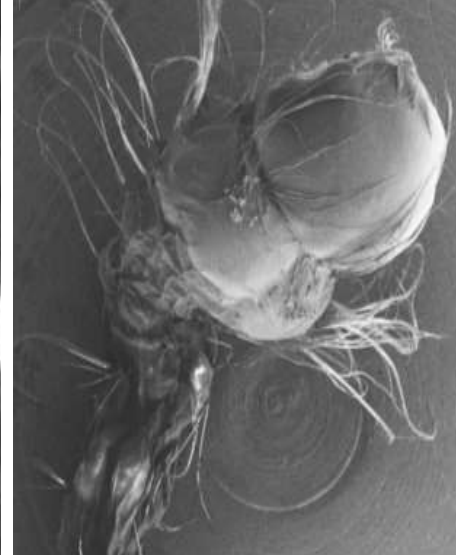
The “NanoSuit®” enabled the direct observation of hydrous biological samples.



**Don't you want to see
what you cannot see?**

**We can now observe
subcellular size
organic substances
as it is.**

For Biomimetics!



4800 Vac=1.01kV WD=14.8mm 20um
Vacuum=3.1E-4Pa T=0.

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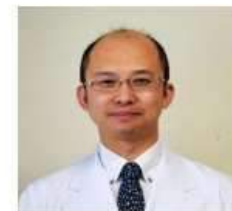
Hiroyuki Konno



Hirotoshi Kikuchi



Hiroshi Watanabe



Takanori Hiraide



Daisuke Ishii



Masatsugu Shimomura



Chihiro Suzuki



Sayuri Takehara



Hideya Kawasaki



Tomohiro
Matsumoto



Yusuke Ozaki



Yuji Hirai



Yoshinori Muranaka



Takahiko Hariyama



Satoshi Hirakawa



Takeshi Nokuo

Vielen Dank!

