Microscopy and Biomimetics: the NanoSuit? Method and Image Retrieval Platform

Microscopy and Biomimetics: the NanoSuit® Method and Image Retrieval Platform

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Abstract

This review aims to clarify a suitable method towards achieving next-generation sustainability. As represented by the term “Anthropocene”, the Earth, including humans, is entering a critical era, therefore science has a great responsibility to solve it. Biomimetics, the emulation of the models, systems, and elements of nature, especially biological science, is a powerful tool to approach sustainability problems. Microscopy has made great progress with the technology of observing biological and artificial materials and its techniques have been continuously improved, most recently through the NanoSuit® method. As one of the most important tools across many facets of research and development, microscopy has produced a large amount of accumulated digital data. However, it is difficult to extract useful data for making things as biomimetic ideas despite a large amount of biological data. Here, we would like to find a way to organically connect the indispensable microscopic data with the new biomimetics to solve complex human problems.

Key word: scanning electron microscope, NanoSuit®, SDGs (Sustainable Development Goals), Biomimetics, BX (Biological Transformation research strategy)

Introduction

Various environmental issues, such as the recent pandemic disaster caused by SARS-CoV-2, global warming, deterioration of biodiversity, marine pollution with microplastics, and food wastage are on the agenda of discussions around the world concerning the question of the limits of the Earth. The terms "planetary boundaries" and "Sustainable Development Goals" (SDGs) have become widely used around the world [1].

One notable symbolic event concerning the limits of the Earth was perhaps the coining of the term "Anthropocene" by Paul Crutzen of the University of Mainz, who won the Nobel Prize in Chemistry in 1995 for his research on the ozone hole. It has been over 20 years since Crutzen advocated establishment of the Anthropocene to the International Geosphere-Biosphere Program (IGBP), and the debate about exactly when the Anthropocene began continues today [2]. Behind this concept is the explosive growth of human activity and its effects in the second half of the 20th century of the "Great Acceleration," including such phenomena as warming, species extinction, plastic production, the human population explosion, and radioactive waste [3]. Recently, Crutzen's concept of human activity as a permanent trace, exemplified by the presence

of artificially created radioactive materials, microplastics, and heavy metals is globally recognized [4].

Under these circumstances of facing huge problems in the relationship between human beings and nature, the Fraunhofer Institute in Germany has put into advocating what is calls the Biological Transformation Research Strategy (BX) [5], which is to increase the application of materials, structures, and principles of living nature to technology and management with the goal of creating a sustainable world. The BX is driven by progress in the life sciences to enable a better understanding of biological processes. Combined with advances in digitization and materials and production sciences, entirely new technological solutions and high-tech markets can arise as BX integrates biotechnology, biomimetics, and information technology (IT).

The broad concept of biotechnology encompasses a wide range of procedures for modifying living organisms according to human purposes, beginning with such early fundamentals as the domestication of animals, cultivation of plants, and improvements to these through artificial breeding. In the 20th century, biotechnology could provide solutions to social crises, such as food and energy shortages. Ereky coined the term "biotechnologie" to describe the process by which raw materials could be biologically upgraded into socially useful products with the aid of living organisms, and he is

regarded as the “father” of biotechnology [6]. However, biotechnology first arose around the time when the wolf was tamed as a domestic dog, more than 10,000 years ago, by the humans who abandoned nomadic life and created settlements. In the late 20th and early 21st centuries, biotechnology has expanded to include new and diverse sciences, such as genomics, recombinant gene techniques, and applied immunology. Another form of biotechnology using the properties of genes is the recent widespread use of real-time reverse transcriptase–polymerase chain reaction (RT-PCR) for detecting SARS-CoV-2 (the virus of COVID-19). The term "biotechnology" has been included in a high school biology textbook to describe contributions to improving agriculture, industry, and medicine for human life.

Despite attempts to address the serious problems currently affecting society, research tools and techniques are limited. Of the various techniques we can use, observation is the most basic and indispensable research tool for humans. In the life sciences, the concept of cell theory depended on the light microscope (LM) and we could later progress to understanding organelles, bacteria and viruses only with the aid of the electron microscope (EM). Since those basic discoveries, microscopic techniques have progressed in both fields (light and electron microscopy) in order to better observe biological materials. Microscopy is, therefore, an essential technology for many

disciplines, although strong cooperation between biology and other scientific disciplines has not been achieved. It is necessary to reconsider how to use this fundamental and useful technology to effectively solve global problems. This review discusses the importance of observations with a microscope to underpin biomimetics and solve the major problems of our time as we proceed through the Anthropocene.

A short History of Biomimetics

Like biotechnology, biomimetics has been a way of thinking since the beginning of human history. The concept is a simple one: observe how nature overcomes a problem and mimic that technique, structure or substance. This concept was apparent in some of the products of Leonardo da Vinci, such as his observation of the flight of birds, which enabled him to speculate that air travel could be modelled on the mechanics of avian flight. Throughout his life, da Vinci produced many theoretical works dealing with the mechanics of flying and the nature of air. The only reason he failed in inventing successful flight is probably the lack of sufficiently light-weight materials at that time, compared with those available in modern times which now allow us to fly.

The term biomimetics was coined by Otto Herbert Schmitt in the 1950s for the transfer of ideas and analogues from biology to technology [7]. Schmitt designed and built a successful electronic device, now called the Schmitt trigger, to mimic the propagation of action potentials along the giant nerve fibers of a squid. Swiss engineer George de Mestral, who lived in the same era as Schmitt, took out patents for Velcro (the hook-and-loop fastener). During a hunting trip in the Alps, his dog became covered in the bur-covered seeds of burdock. He was inspired by the tiny hooks on the prickly seeds to conceive the possibility of binding two materials reversibly in a simple fashion, once he could devise a way to manufacture the hooks and loops, and so he began to explore the use of synthetic fibers until he succeeded in making Velcro®. His Velcro® became widely known in the 1960s when NASA used it in spaceships to prevent astronauts themselves, foods, pieces of equipment, and other items from floating away in zero gravity.

The Schimitt trigger and Velcro® fasteners are symbols of the dawn of biomimetic research (Fig. 1). Hints can be found in many places by looking carefully at nature. An example is the scanning electron microscope (SEM) image in Fig. 2, which appears to be a man-made industrial spring. This is actually the hook to connect the front and rear wings of a carpenter bee. Both wings have the same hook but the hook on one

wing turns in the opposite direction to that on the other wing, and they can connect firmly but separate easily.

Following the nanotechnology that emerged at the beginning of the 21st century, research on biomimetics has reignited, mainly in Europe and the United States, to utilize subcellular-sized structures of living organisms. Biomimetic mechanics and materials have also contributed to robotics. In Japan from 2012, “Engineering Neo-Biomimetics” was introduced as a new academic field supported by Grants-in-Aid for Scientific Research on Innovative Areas, with the aim of developing new biomimetics centred on cooperation between biology and engineering. Thus, biomimetics is both a historical and a young marriage of technology and study which embraces the practical application of the mechanisms and functions of biological science to fields such as engineering, design, chemistry, physics, electronics, and optics. Therefore, pursuing biomimetics requires strong interdisciplinary collaboration.

Currently, the biomimetics research globally has grown to incorporate large-scale biology including ecology, aiming at both urban design and agriculture and nature conservation, and trying to achieve sustainability of the entire Earth (Fig.1). In Japan, a research group led by Professor Naoki Mori of Kyoto University erected a new academic field which has succeeded in gathering interdisciplinary collaboration centred

on biomimetics research in agriculture [8]. The aim of the group is “Biomimetic Optimization for Symbiotic Sustainable Agriculture inNOVAtion (BOSSANOVA)”: the phrase *bossa nova* means ‘new sensation’ in Portuguese. In the Anthropocene epoch, it is essential to build new sensations, new ways of life, and new scholarship, especially in an attempt to resolve the dilemma of agriculture versus nature conservation. New Biomimetics will contribute greatly to the SDGs, not only at nano-level, micrometre, subcellular-size, but also at macro-scales from meters to kilometres.

A short History of the Electron Microscope and the NanoSuit®

The invention of the transmission electron microscope (TEM) by Max Knoll and Ernst Ruska in 1931 overcame the barrier to obtaining higher microscopic resolution than that imposed by the limitations of the LM. Ernst Ruska produced the first commercial TEM in 1938 at Siemens, and his brother Helmut Ruska tried to photograph several submicroscopic biological structures, such as bacteria, parasites, and viruses [9]. Pease and Nixon started to combine several improvements of scanning electron microscopy into one instrument, and this was the prototype for the first

commercial SEM called “Stereoscan” in 1965 [10]. Soon after this development, Pease et al. observed several stages of a living insect in an SEM (1966) without any pre-treatment, and in most cases the specimens resumed their normal activity after being observed with SEM and underwent metamorphosis to the next stage [11]. The Nobel Prize in Physics 1986 was awarded to Ernst Ruska "for his fundamental work in electron optics, and the design of the first electron microscope", and to Gerd Binnig and Heinrich Rohrer "for their design of the scanning tunneling microscope." In Japan, scientists gathered to decide to build a TEM, and the team from this group evolved into the Japan Electron Optics Laboratory (JEOL). Hitachi and Toshiba also played a major role in the early development process, and now Japan is renowned for its advances in electron microscopy. In view of the reality that there are few electron microscope manufacturers outside Japan, it is clear that Japan must continue to take responsibility for the development of electron microscopes.

Nowadays, without EM technology, we would not have our current knowledge of living things. However, organisms are viewed by placing them in high-vacuum conditions because electron microscopes use a beam of electrons instead of rays of light. Biological specimens are chemically fixed, dehydrated, and embedded in a polymer resin to stabilize them sufficiently to allow ultrathin sectioning for TEM, and

dehydrated specimens are coated with metal by sputter coaters to yield good contrast in the SEM [12]. These complex procedures preclude the observation of living organisms and often produce unwanted artifacts but provide a beautiful contrast. Dehydration always results in shrinkage, and freeze-drying (FD) and critical point drying (CPD) also give rise to shrinkage [13, 14] Therefore, researchers have tried to modify the SEM design to require lower levels of vacuum to circumvent such problems [15-18]. However, all these microscopes (such as low-vacuum SEM or environmental SEM) require reduced vacuums ($<10^{-3}$ Pa) and result in inferior resolution. Recently, the idea has emerged to avoid machine modifications by, instead, using ionic liquids, which are in a liquid state salt under 100 °C so their vapour pressures are negligibly small, and they do not easily evaporate, even under vacuum, and they have electrical conductivity for SEM observation [19]. However, ionic liquids are toxic and viscous, and the ultrafine structure of a sample is difficult to observe when its surface is covered with an ionic liquid. The ionic liquid must also infiltrate and replace the liquid of the living body, so it is unknown how closely the resulting surface resembles the actual living body as it is in reality. Despite these problems, this is excellent technology for which there are high expectations, and it is now commercially available [20].

Despite the early pioneering experiments performed by Ruska, Pease and others,

and several significant recent improvements, it was still considered that we cannot observe any living things using an EM. We recently devised an important method to cover organisms with a thin nano-film, coined a "NanoSuit®", which can maintain structure under wet/alive conditions in a high vacuum, and provide the electrical conductivity for good-quality SEM observation because of the chemical composition of the NanoSuit® [21].

Familiar examples of a biofilm include the slime that gathers in a poorly cleaned kitchen sink and the layer of plaque formed on teeth. Many eukaryotes release extracellular secretions for protection of individual cells. Humans, too, release secretions onto the skin surface to keep it moist. Copious exocrine secretions are released onto the surface of Dipteran larvae, extracellular substances (ECS) to maintain their immediate external environment, particularly to avoid desiccation. Many organisms can withstand large environmental changes by the use of these secreted biofilms and/or ECS, presumably as a strategy of drought tolerance for organisms that have evolved to live away from the sea, where all life originated.

To investigate the mechanism of tolerance of living organisms for high vacuum conditions, we introduced several living organisms of various taxa directly into an SEM to see whether or not they could survive under high vacuum conditions, as reported by

Pease and Hayes [11]. We found that larvae of the dipteran fruit fly *Drosophila melanogaster* (Oregon-R strain) could tolerate high vacuum conditions when they were irradiated by electron beam immediately in an SEM. The larvae possess a soft cuticle, but they maintained their form and continued to move around actively for 60 min in high vacuum conditions. A nanometre-thin coating was detected outside the cuticle on the surface of the surviving larvae when observed by TEM. The result led to the hypothesis that electron-beam-enhanced cross-linking had occurred within the ECS to form a durable polymer on the surface and that this polymer enhanced survival under vacuum conditions [21]. Applying this principle of polymerization of natural ECS on the outer surface of dipterans by electron-beam or plasma irradiation gave rise to development of the NanoSuit® membrane, which can keep small animals alive under the high vacuum of an SEM.

We have also examined the possibilities of this effect in plants, to see whether or not they have any natural ECS which can be used to make a NanoSuit® membrane tolerant to high vacuum conditions (Fig.3). Cherry blossom petals (Fig.3 A) were chosen as experimental specimens and their behaviour was examined under high-vacuum conditions. Experiments on healthy living petals (Fig.3 C-F) demonstrated that without any pre-treatment the overall morphology of specimens is well preserved and intact

after imaging in an SEM, suggesting that natural substances on the petal surface behave like animal ECS and form a NanoSuit® membrane following irradiation with an electron beam. When the surface of the petal was washed with ethanol (Fig.3 G-J), the structure and colours showed no apparent change, but it was destroyed after washing with chloroform (Fig.3 K-N). In contrast, the petal treated by the conventional method showed the apparent shrinkage of both the petal and cell sizes (Fig.3 O-Q). We went on to show that the surface material of the petal can be extracted with chloroform and polymerized into a free-standing membrane by plasma irradiation. From our results, we conclude that surface materials, which have the ability to prevent water loss under natural conditions, increase the natural environmental barrier properties at the surface and can protect plants under high vacuum conditions [22, 23]. These findings [21-23] enable the development of a whole new suite of techniques for observing a broad range of living/wet organic samples under high-resolution SEM.

From a biomimetic point of view, we have tried to mimic the properties of the ECS layer and developed the non-toxic NanoSuit Type-I solution. To test the barrier properties of the NanoSuit® membrane made by this solution, the surfaces of several different animals, previously unable to survive SEM exposure, were provided with an exogenous layer by immersing them briefly in the solution before electron or plasma

irradiation. When live mosquito larvae were observed under the SEM without any additional treatment, they quickly shrank and ceased to move. Larvae that were treated with a NanoSuit Type-I solution but not irradiated in the SEM showed the same collapsed structure when observed by SEM after a delay of 30 min since introducing them to the SEM chamber. However, larvae covered with NanoSuit Type-I solution and observed by SEM *ab initio* retained their morphology and exhibited active movements for more than 30 min. Subjecting them to plasma irradiation before introducing them into an SEM also produced the same results (Fig.4). These results indicate that irradiation with electrons or plasma can polymerize the natural outer layer to form a NanoSuit® membrane, which protects living organisms from high vacuum conditions [21-26], and can be manufactured as a self-standing membrane [21, 22, 27, 28].

NanoSuit® for Tissue Observation – NanoSuit Type-I & Type-III solutions

Usually, the excised tissues or single cells of organisms are not protected by natural ECS, so an alternative barrier is necessary for optimal preservation of microscopic

structure.

Recently, three types of NanoSuit® solutions are commercially available (Nisshin EM Co., Ltd., Tokyo, Japan). NanoSuit Type-I solution can be used to observe large fresh samples such as plants, small animals, and chemical materials, and Type-III solution to observe small samples such as eukaryotic and prokaryotic cells, exosomes, and viruses. The Type-II solution is fit to correlative light and electron microscopic observations. The NanoSuit® membrane which consist of those three types possess both barriers against gases and solutions, and electrical conductivity.

Fig. 5 shows the effects of freezing on fish using the NanoSuit Type-I solution. Transverse slices of cherry salmon (the salmonid fish, *Oncorhynchus masou masou*, called amago in Japan) were treated with NanoSuit Type-I solution. Low-magnification LM observations show different reflections on the surface of the three materials, hinting at qualitative differences, and those frozen in a household freezer (Fig. 5B, B1) feel watery. With high-magnification and high-resolution SEM, a regular striped appearance (presumably a property of the sarcomeres in the muscle) can be seen on the surface of the wet slices (Fig. 5A2-A4 and Fig. 5C2-C4), but the sample that was frozen in a freezer has no such appearance (Fig. 5B2-B4). It was therefore possible to easily and rapidly distinguish the qualitative difference between the sample frozen in a -20°C

household freezer and the rest of the two methods. The mechanism producing those differences is well-known to be caused by the freezing methods. When the water inside the muscle slowly freezes, the ice crystals inside the food grow larger and damage the food tissue, but not the case of flash freezing.

NanoSuit® Type-III has been improved to better serve as a surface shield enhancer (SSE) solution [29]. To increase the barrier effect protecting underlying tissues, a thin liquid film is polymerized over the sample. In some cases, this treatment yielded an effective NanoSuit® thin membrane and permits imaging in the SEM (the following example made use of a Field Emission-Scanning Electron Microscope (FE-SEM). The clear surface structure of pathological tissue (Fig.6A, B) and cells (Fig.6 C) are easily observable since the NanoSuit® membrane made from Type-III solution constitutes a very effective barrier against gases and solutions. As a consequence, it is possible to obtain good images of surface structures at a much higher resolution. It seems likely that NanoSuit type-III interacts with protein and/or proteoglycan on the surface of cells and tissues to form a stabilizing polymer coat after plasma irradiation or exposure to the electron beam in an SEM [29].

There have been several previous attempts to adapt the SEM for observation of wet samples. Thiberge et al. used polyimide or silicon nitride membranes to protect

samples from the effects of the vacuum [30]. However, this method required the use of high acceleration voltages (15–30 kV) to penetrate the relatively thick membranes. The intense radiation of the electron beam during high magnification imaging was sufficient to cause damage to the specimens. The SSE-based NanoSuit® method (NanoSuit Type-III) can be applied to both living specimens and fixed tissue and requires only low voltage electrons, so imaging occurs in a hydrous/wet state closely approximating the natural condition.

The NanoSuit® method has the advantage of enabling images to be obtained of tissues in, or close to, the living state in a short time at high resolution. However, other observation methods that have been developed so far are also of great value. For example, conventional observation can determine where the surface substance has been washed away with water and an organic solvent during the drying process following fixation. Of course, it is necessary to be aware of the possibility that a change in form has occurred, but it is possible to obtain information different from the NanoSuit® method. Needless to say, it is important to use it in combination with the most appropriate of the various types of microscopes now available.

A novel CLEM approach for examining paraffin sections and the analysis of elements

Histological examination using the LM is currently the gold standard for life science research and diagnostics. However, magnified observations are limited because of the limitations intrinsic to light microscopy. Thus, a dual approach to use both light and electron microscopes, known as correlative light and electron microscopy (CLEM), has emerged and there is currently some competition in its development. Compared to the LM, which was invented in the 18th century, the history of the EM is relatively young, at only 80 years, so the amount of research conducted using the LM is enormous compared to that making use of the EM. Therefore, any new studies should attempt to make use of the massive amount of LM data already available. It is desirable also to be able to observe important parts of the specimen that have been used in the past and to store them again.

We applied the NanoSuit® method to CLEM analysis of paraffin sections. Workflow is very simple. First, the LM is used to determine the location to be observed at high magnification. After LM images are obtained, the cover glass is removed from the slide by immersion in a suitable liquid such as Xylene. NanoSuit Type-II solution is then added, using spin coating, to make a thin layer and the tissue is observed by SEM. The NanoSuit® membrane can be made simply by irradiation with electrons during use of the SEM, and the membrane maintains the fine structure of the section on the slide and provides the electrical conductivity for good-quality SEM observation. Removal of the NanoSuit® membrane after observation is a further advantage. The thin NanoSuit® membrane can be peeled off by running the buffer solution with a pipette not so strong that it damages the tissue, or by shaking the slide in the buffer solution. This allows slides to be re-stained and stored (Fig.7). Thus, the NanoSuit® method represents a novel approach to advancing the field of histology [31].

Figures 8 show examples of pathological specimens observed by the NanoSuit®-CLEM method. This method provides easy observation at the same position by LM and SEM, enabling the advantages of both and avoiding their disadvantages. The LM can be used to observe stained target organelles (Fig.8 red circles), but the image is flat and the magnification is low; while the SEM can be used to produce images at magnifications more than 10,000 times greater and in 3D. The history of this technique is short but now it is possible to combine LM and SEM easily in a non-destructive manner using the NanoSuit®-CLEM method allowing high quality images of any slide material that has been preserved.

In addition to the CLEM histological observation, it is also possible to use energy-dispersive X-ray spectrometry (EDX) analysis of paraffin sections on the same slides. SEM combined with EDX is a spectral technique that provides visual identification of multiple elements simultaneously. To analyze the elemental components of paraffin sections by SEM/EDX, the procedure described in Fig. 7 is applicable to observe identical haematoxylin and eosin (HE) specimens. SEM/EDX analysis can detect the existence of several elements simultaneously, such as Al, Si, Mg, O, and even C [31-33].

The NanoSuit®-CLEM observation method of paraffin sections has various applications. In the immediate future, it is expected that many discoveries will be possible by adding three-dimensional high-resolution information and elemental analysis by SEM/EDX as diagnostic information combined with LM data going back in history more than a century. The increase in CLEM observations by the NanoSuit® method will generate new knowledge and will facilitate the development of new pathological diagnostic and biological sample observation methods.

Immunochromato_NanoSuit® method for disease detection

Human beings are constantly exposed to pathogens such as viruses. As seen with the current SARS-CoV-2 infection, the emergence of a pandemic is a global health hazard with the potential to cause long-term lifestyle changes that cause enormous economic loss and other social disruptions. In order to prevent the spread of infectious diseases, medical test results obtained in real-time are required for early diagnosis and direction of treatment. The widely used RT-PCR method is subject to delay in the arrival of test results and is sometimes affected by false-positive or false-negative results, which are disadvantages particularly in emergencies. Immunochromatography (ICG), or lateral flow assay, is a very convenient testing tool that is easy to operate and can be run by medical staff. Near-patient testing utilizes many commercially produced 'ICG sticks'. The principle is that the antigen-containing sample (e.g., pharyngeal swab, saliva, or urine) is applied to the stick which is then developed by capillary action, with the analyte of interest binding at a zone containing specific antibodies. When the antigen and antibody combine, they develop a visible colour spot or band which confirms the presence of the compound of interest.

Because of its convenience, speed, and low cost, ICG is expected to be a "game-changer", but it has been pointed out that its sensitivity is insufficient compared with

RT-PCR. When macroscopically examining the ICG, a skilled doctor will classify a faint signal as positive. The technology of signal enhancement strategies, such as using colour, electrochemical signals, silver enhancement, magnetic properties, and luminescence, is being developed to enhance the reacted particles on behalf of a skilled doctor, but the sensitivity is increased by at most 10 times (just one degree of magnitude), which still is not comparable to the sensitivity of PCR. Using the surface-enhanced Raman spectroscopy (SERS), the detection sensitivity was significantly improved approximately 37 and 300 fold, but still not popular [34, 35].

Counting reacted particles of ICG using an SEM can increase the sensitivity of ICG tests. The NanoSuit® method can be applied to impart conductivity to the ICG cellulose film made of light elements and enable an increase in sensitivity by direct observation of the target metal particles. When observing a normal ICG sample with an SEM, the image is disturbed due to charging caused by the beam of electrons, and at the same time the substrate thermally expands and moves, and fine particles cannot be observed stably and clearly (Fig. 9A). However, by adding a drop of NanoSuit® solution, conductivity is improved and fine particles can be counted (Fig. 9B). For more than four years, we have been developing the ICG_NanoSuit® method as a highly sensitive method for detecting influenza. When the macroscopic (LM) diagnosis result and the

electron microscopic (SEM) diagnosis results were compared with the diagnosis result of RT-PCR using clinical samples of influenza ICG, the sensitivity (taking RT-PCR as 100%) was 81% with the naked eye and 94% by SEM (Fig. 9C). This was the first ever report to compare ICG visual inspection and SEM, and PCR in patients with possible influenza in actual clinical settings [34].

Current policies regarding the Covid-19 pandemic recommend isolating people with borderline cycle threshold (Ct) values (35<Ct <40), despite some studies reporting that "samples whose signal is detected with a Ct value of 35 or higher are not infectious" [36-38]. Ct refers to the number of cycles needed to amplify viral RNA to reach a detectable level, therefore, if the number of amplifications is increased, there is a high possibility that a misdiagnosis result will be obtained. Recently, Oba et al. reported the concept of setting Ct = 35 as the test threshold, because infectivity is estimated from the amount of virus in saliva [39]. Figure 9C shows that the sensitivity of the ICG_NanoSuit® method is around a Ct value of 35, so it matches the PCR method sensitivity.

With the advantage of being able to detect at a similarly high sensitivity as RT-PCR, but at a test time within a few minutes, the ICG_NanoSuit® method will be useful for automated quantitative measurement, including use of the recently developed SARS-

CoV-2 antigen or IgM/IgG antibody tests for SARS-CoV-2. PCR cannot monitor whether people after vaccination have acquired antibodies, so it will be necessary to use a high-sensitivity antibody test kit. Furthermore, ICG_NanoSuit® has the potential to promptly diagnose emerging infectious diseases and other illnesses, including those affecting livestock, such as avian influenza and classical swine fever. We are currently developing a measuring device dedicated to ICG_NanoSuit® measurement based on SEM and are promoting research and development that will be useful for next-generation health management, including infectious disease control.

Microscopy and Biomimetics – Simple Biomimetics to Near-Future Biomimetics

Many small animals, such as coleopteran and geckos, climb the vertical walls of plants and rocks, and some are able to walk upside down on the ceiling (Fig.10). This indicates that their toes have an adhesive property which can support their body weight and is due to van der Waals force generated by flexible hairs (called setae) whose tip is several hundred nm to several μm in size, accumulated into dense groups (Fig.10A, B, D) [40].

In contrast, anti-cancer drug therapy may cause fingerprints to disappear in some

patients, and since fingerprints have the function of grasping objects, patients who have lost fingerprints often find it difficult to grasp objects in their daily lives. This presents restrictions causing problem which reduce the Quality of Life (QOL).

As one of the biomimetics research activities, an image study meeting was held every week to freely say what the researchers came up with while looking at SEM images observed in different organisms. Among the topics discussed, it was argued that the adhesive strength of setae should not only be dependent on the presence of fine setae at high density, but also the area that comes into contact with the surface of the substrate. Applying biomimetic principles, the researchers considered that it should be possible to improve the QOL of patients without fingerprints, finding or devising an artificial structure with small tip diameter in a deformable material such as cloth. Therefore, the researchers analyzed this problem by computer using a search system [41] called the "Haseyama engine, the interface of the biomimetics image retrieval platform" developed by Professor Miki Haseyama of Hokkaido University and searched for images of artificial objects with the structure and flexibility of seta. As a result, it was found that the structure of fabrics such as NANOFRONT® developed by Teijin Frontier Co., Ltd., is similar to that of setae (Fig.10C).

The researchers have developed gloves that reproduce the adhesive ability of the

legs of small animals in an attempt to improve the QOL of people without fingerprints. They observed how humans grab and flip things, based on which we used cloth that adheres to things easily and constructed special custom gloves (Figure 10E). This is an example of the social implementation of biomimetics research.

As in this example, the beginning of Biomimetics research is to consider whether or not biomimetics can be applied to a technical problem through knowledge of the functions of structures in different organisms. Such knowledge is obtained by observing nature in detail and using them as norms to create human & nature-friendly innovations. The comparison of biological functional principles (Fb) and the technical functional principles (Ft) is the first step of "Simple Biomimetics" and, at this stage, observation with a microscope is indispensable (Fig. 11: Check Fb similar to Ft or not). After this checkpoint, it is necessary to check again whether the biological and technical boundaries match (Fig.11, Bb and Bt). At this stage, it is important to combine the different fields of biology and different technologies as a source of innovation. Next, because organisms can only live within the boundaries in which they can survive, it is necessary to check whether or not the biological quality condition and the technical quality criterion are similar (Fig.11, Qb and Qt). Then, we can begin to move towards social implementation as a biomimetic innovation.

Biomimetics requires interdisciplinary collaboration, but the basis of the idea of biomimetics is to learn from life as we observe it in nature. After millions of years of evolution, creatures have adapted by arriving at structures and mechanisms that are efficient, effective, and enduring. We need to look around us at what life is doing, what it wants, and what kind of interrelationships it has; and above all, the basis of learning life in nature is to know what structures they have. Therefore, microscopy in the indispensable to science. This “Simple Biomimetics” is a solid concept and has the potential to create new research areas, so we should perform “Simple Biomimetics” continuously into the future. In fact, the rate of research publications on biomimetics has been increasing over the past years and now exceeds 3000 papers per year [42].

However, to save the present Anthropocene age and contribute to the SDGs, we must also seek new research and development methods. Biomimetics applies principles and strategies from biological systems in nature to engineering and technological products, processes, and design in general. The potential of biomimetics is considered to be boundless [43], and its significant scientific, social, and economic impacts will have benefits with the potential to save the Anthropocene world. However, nature has a tremendous number of biological systems, and there have already been many investigations of them. Even if research by “Simple Biomimetics” is promoted by one

person or a group of several people, the research may be abandoned, as shown in Fig. 11A; and even if it is successfully implemented in society, the number of successes will be limited. In addition to the speed of “Simple Biomimetics” is relatively slow, because biomimetics itself is a new research field that creates innovation through strong collaboration of different existing research fields. However, interdisciplinary collaboration (i.e., the exchange of deep knowledge between different research fields) is difficult for several reasons, such as differences in technical terms used in different fields. The phenomenon of the current problem is also symbolized by the fact that the number of biomimetic products on the market is still quite small compared to the increase in the number of biomimetic-related treatises [44].

There is an urgent need to accelerate biomimetics research which creates a sustainable society and does not burden the global ecosystem; therefore, it is necessary to solve the current problems mentioned above. To deepen cross-disciplinary collaboration, the need to create a space where researchers in different fields can share information is as indispensable as Simple Biomimetics. The formation of a network of researchers in different fields has been achieved by the Biomimetics Research Group in Japan and is being expanded through the activities of the “Biomimetics Netword Japan” NPO [45]. Based on this network of researchers, it is necessary to form the

"biomimetics of the near future" shown in Fig. 11B. Because Human innovation through biomimetics should take cues from naturally occurring processes, we always have to look at nature, and that data is constantly growing through microscopy. Recently, microscopic data have been digitized, and the accumulation of large amounts of data has begun. Until recently, all the data was lost when a researcher retired or passed away, and data that was not published in academic papers, or was judged of no scientific value by the researcher himself, were abandoned. The movement of "biomimetics of the near future" is good news to solve those things to keep important data for other or future scientists. This wonderful movement should be expanded, but even if only image data is accumulated, it cannot be utilized. It is a must to link the image data with the name of photographers, shooting conditions, research purpose, research memos and notes, and published papers. Being able to trace that information was thinking at the time would be a great hint for successors and it will be a pleasure for the present researchers and continued their research.

Fortunately, a novel biomimetics image retrieval platform based on image retrieval has been proposed by a research team of Japan [41]. Large amounts of image data separately accumulate in different research fields, with each field containing invaluable knowledge. This invaluable knowledge is often difficult to express in words

in the same research field, hindering its ability to be shared with experts outside that field. The "Haseyama engine", image retrieval platform is an implementation of an industrial collaboration platform that supports manufacturing through integrating accumulated knowledge in a cross-field database [41]. In the near future, it will be necessary to develop and improve both the “information science technology” and the Haseyama engine and combine them with big data from the field of microscopy.

Biomimetics and microscopy have always been closely related to each other. In order to fulfil the responsibility of sustainability in the next generation, new Biomimetics and Microscopy should be organically linked through information science technology to support idea-based research. It is necessary to aim for social implementation to solve the problems of the Anthropocene.

Conclusions

Since the invention of the optical microscope in the 17th century, microscopy has

been cultivated and has become an indispensable tool in modern sciences. Recently, because the technique to observe the natural appearance of living things has become indispensable in Biomimetics, the NanoSuit® method was invented to observe living things while they are wet or alive. The photographic data obtained by several kinds of microscopic apparatus have been digitized and have become suitable for processing by information science technology, but the understanding of the photographic data itself has not easily exceeded the qualitative level of individual researchers. Because each researcher is an expert in his/her field, it is difficult to understand the meaning of the microscopic images obtained by other researchers. Biomimetics is achieved by forming multidisciplinary research teams, but researchers are also hard to the microscopic images immediately. It is necessary for the microscopist to give a detailed explanation, and it is also necessary to use computer-based “information science technology” to extract features of each image, extract common terms from a large amount of image data, and calculate variations. It will be necessary to create a server that can store big image data as soon as possible and a data journal to keep the records and priorities of researchers. Further, in order to achieve complex research such as Biomimetics and solve global problems, it is necessary to build a tight connection between idea-supporting information science technology and the huge amount of microscopic data.

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Acknowledgements

We thank the Preeminent Medical Photonics Educations & Research Center of Hamamatsu University School of Medicine for the use of its equipment. This work was supported by Grants-in-Aid for Scientific Research in Innovative Areas ('Innovative Materials Engineering Based on Biological Diversity') [for T.H., M.S.], by JSPS KAKENHI JP18H01869, JP24120001, JP24120004 [for T.H.], JP25292198, 17K08150, 20K06071, JP15H01598 [for Y.T.], Grant-in-Aid for Challenging Exploratory ResearchJP15K14558 [for Y.T.], JP17K08784, JP 20K07423 [to H.K.], by AMED (A508 [to H.K.]), and by Adaptable and Seamless Technology transfer Program through Target-driven R&D (ASTEP) from Japan Science and Technology Agency (JST) JPMJTR20UQ. This work was supported also by grants from the Takeda Science Foundation.

Figure legends

Fig. 1 Schematic drawing of the historical flow of Biomimetics. Beyond the dawn of biomimetics research around 1950," Biomimetic Chemistry", which attempts to imitate enzymes and biological membranes at the molecular level, appeared in the 1970s. Research on "Artificial photosynthesis", which became popular in the 1980s, became

the basis of dye-sensitized solar cells. In the 1990s, nanotechnology was highlighted but the relation with biology diminished, then “Molecular Biomimetics” declined. After that, “Biomimetics Mechanics” and “Biomimetics Materials” have grown continuously. Currently, biomimetics research globally is growing to incorporate large-scale biology such as “Ecosystems Biomimetics” and “BOSSANOVA, Biomimetic Optimization for Symbiotic Sustainable Agriculture inNOVAtion”.

Fig. 2 Each arrow indicate the hook that connects the front and back wings of the carpenter bee, *Xylocopa appendiculata circumvolans* (A, B). It resembles the spiral structure of an industrial spring, although the turns are separated (C). The spirals of the hooks of the front and rear wings turn in opposite directions, and the two wings are firmly combined as a single wing when the bee flies.

Fig. 3. A cherry blossom, *Prumus x yedonensis* with a honey bee (A) and the just scattered petals (B). Observations of cherry blossom petals by LM (C, G, K, O), SEM (D, E, H, I, L, M, P, Q), and LM after SEM observation (F, J, N, R). Rectangles in D, H, L and P indicate position of images magnified in E, I, M and Q, respectively. C-F: An untreated (natural) petal; G-J: A petal following immersion in ethanol solution; K-N: A

different petal following immersion in chloroform solution to remove surface wax. Compared with the specimen treated with ethanol (D, F), that treated with chloroform (H, I) shows that cells in the petal shrank and collapsed, with slight electrostatic charging during SEM observation (I). O-R: Images of a petal subjected to conventional fixation and drying procedures show that (O) it shrank from its size when fresh (white outer frame), as also has the size of each cell (D, E, H, I cf. P, Q). After observation (F, J) it has not changed in size.

Fig. 4 SEM (A, C) and TEM (B, D) observations of plasma-irradiated specimens of mosquito larvae in the absence (A, B) and presence (C, D) of NanoSuit® solution. Without NanoSuit® protection, the specimen was greatly shrunken (A), particularly noticeable in the region marked by outline arrowheads, and there is no NanoSuit® membrane (B) to protect it. In contrast, the specimen treated with NanoSuit® solution (C) shows no apparent shrinkage and the NanoSuit® membrane protecting it (D) is clearly visible at the surface of the cuticle (between the two solid arrowheads).

Fig. 5 Effects of freezing demonstrated by SEM with the use of NanoSuit Type-I solution. A-C: Images of cherry salmon (A) raw, (B) frozen in a -20°C household freezer,

and (C) flash-frozen in liquid nitrogen. A1-A4, B1-B4, C1-C4: Slices of salmon treated as in A-C. Slices from treatments B and C were thawed and all were observed at room temperature after coating with NanoSuit Type-I solution. The three materials look different even when observed at low magnification with an optical microscope (A1-C1), but when observed (in the fresh or thawed state) by SEM at higher magnification, the effects of the different methods of freezing are clearly demonstrated: specimens flash frozen in liquid nitrogen (C2-C4) resemble more closely the original raw tissue (A2-A4) with regular striping of intact sarcomeres in the fish musculature. However, the sample that was frozen more slowly in a household freezer shows no such structure (B1-B4).

Fig. 6 Comparison of normal and cancerous areas of surgically removed stomach tissue observed using the SSE-based method (NanoSuit Type-III) in an FE-SEM. (A), Normal tissue area; (B), cancerous tissue area; These pictures show the comparison of gastric cancer and its adjacent normal sites revealing a marked difference in the structure of the epithelium. (C), cell surface of mouse fibroblasts infected with cytomegalovirus. Cells were treated with NanoSuit Type-III solution and introduced directly to the FE-SEM. Small particles on and around the cell are viruses. The part of

the cell observed at the left side of this figure possesses fine fibers on its right side. In the conventional fixing method, this fine structure often disappears.

Fig 7. Schematic drawing of the workflow for CLEM analysis of paraffin sections with the NanoSuit® method. ①Standard glass slide with coverslip, for LM. ②remove the cover glass. ③ Apply NanoSuit type-II solution to stabilize the specimen for SEM observation. ④Spin coating to remove excess solution ⑤Observe by SEM at the same position observed by LM. ⑥Re-stain and encapsulate with mounting medium and a cover glass. (HE, Haematoxylin and eosin staining method).

Fig. 8 Examples of combined LM and SEM examination of the same material preserved on glass slides. A-B, Slide material from a case of cervical intraepithelial neoplasia (CIN1). A: Immunostaining with anti-HPV L1 antibody was performed on paraffin sections and observed by diaminobenzidine (DAB) staining using the LM. B: The same position, indicated by the red circle in A, was observed by SEM using the SSE-NanoSuit® CLEM method. The distribution of nuclear gold particles (indicated by white arrows) can be observed using a secondary antibody (biotin-labeled anti-mouse antibody) with avidin attached to 40 nm gold particles. C-D, Laryngeal papillomatosis

tested positive for HPV type 6, as shown by staining with 0.5% gold chloride and observed by the NanoSuit®-CLEM method. The LM-observed brown spot in the red circle (C) is a gold-stained region which can be seen as a brighter area (white arrow) by SEM (D).

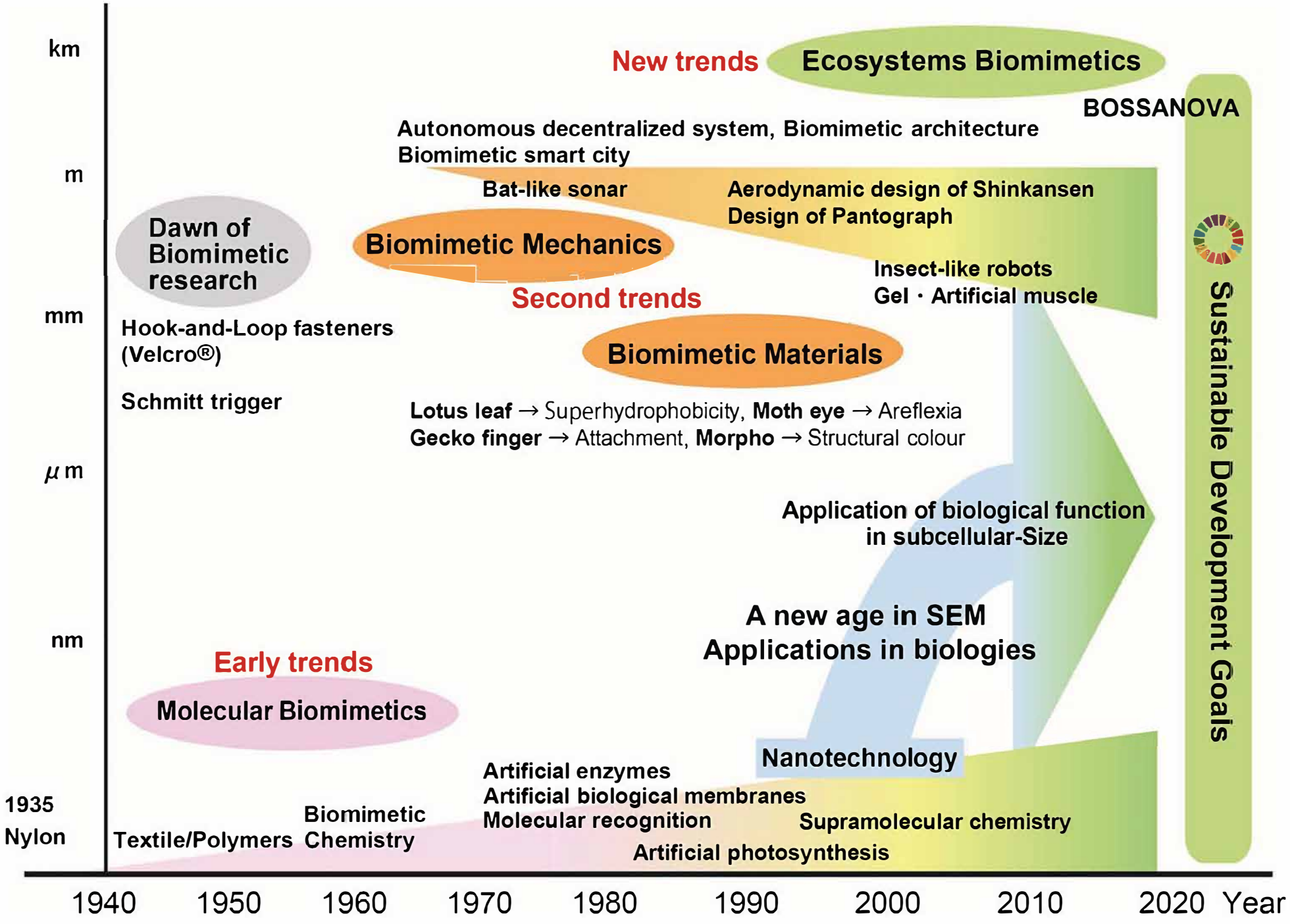
Fig. 9 Visualization of Au/Pt particles on cellulose by SEM (A, B). Representative images of cellulose and Au/Pt-labeled immunocomplex with immobilized antibody without (A) and with (B) NanoSuit® treatment. The insets are magnified images with scale bars of 600 nm. (C) Scatter plot of Cycle threshold (Ct) versus particle counts. Blue dots: Visual + SEM +PCR detection positive. Red triangles: Visual negative, SEM +PCR detection positive. Green diamonds: Only PCR detection positive.

Fig. 10 The "Haseyama engine“, the interface of the biomimetics image retrieval platform, displays part of the entire image database of setae of small animals and the surface of artificial cloth, NanoFront®. Coleoptera (A, B) and geckos can adhere to a ceiling made of glass (D) because of the presence of many fine hairs or setae (A, B, D), each of which is splayed at the distal end. This creates a dense accumulation of very fine fibres which adhere because of the creation of van der Waals forces. Using this

principle as a starting point, biomimetics was considered in constructing gloves (E) for patients with fingertips rendered smooth by the absence of fingerprints, using a type of cloth that improves the ability to grasp objects, and thereby improving the quality of life of such patients.

Fig. 11 Schematic drawing of traditional “Simple Biomimetics” and “Near Future Biomimetics”. The traditional procedure has three checkpoints before biomimetic application, and the results depend on the ability of each researcher. “Near Future Biomimetics” stores various digital data, including microscopic data. Information is extracted using information science technology according to the needs of researchers, using techniques such as data correlation, supporting biomimetic applications with idea-supporting information science technology.

Fig 1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Biomimetic smart city
Bat-like sonar
Aerodynamic design of Shinkansen
Design of Pantograph
Dawn of Biomimetic research
Biomimetic Mechanics
Insect-like robots
Gel・Artificial muscle
Second trends
Hook-and-Loop fasteners (Velcro®)
Biomimetic Materials
Schmitt trigger
Lotus leaf → Superhydrophobicity, Moth eye → Areflexia
Gecko finger → Attachment, Morpho → Structural colour
Application of biological function in subcellular-Size
A new age in SEM
Applications in biologies
Early trends
Molecular Biomimetics
Nanotechnology
Artificial enzymes
Artificial biological membranes
Molecular recognition
Supramolecular chemistry
Artificial photosynthesis
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Fig 2

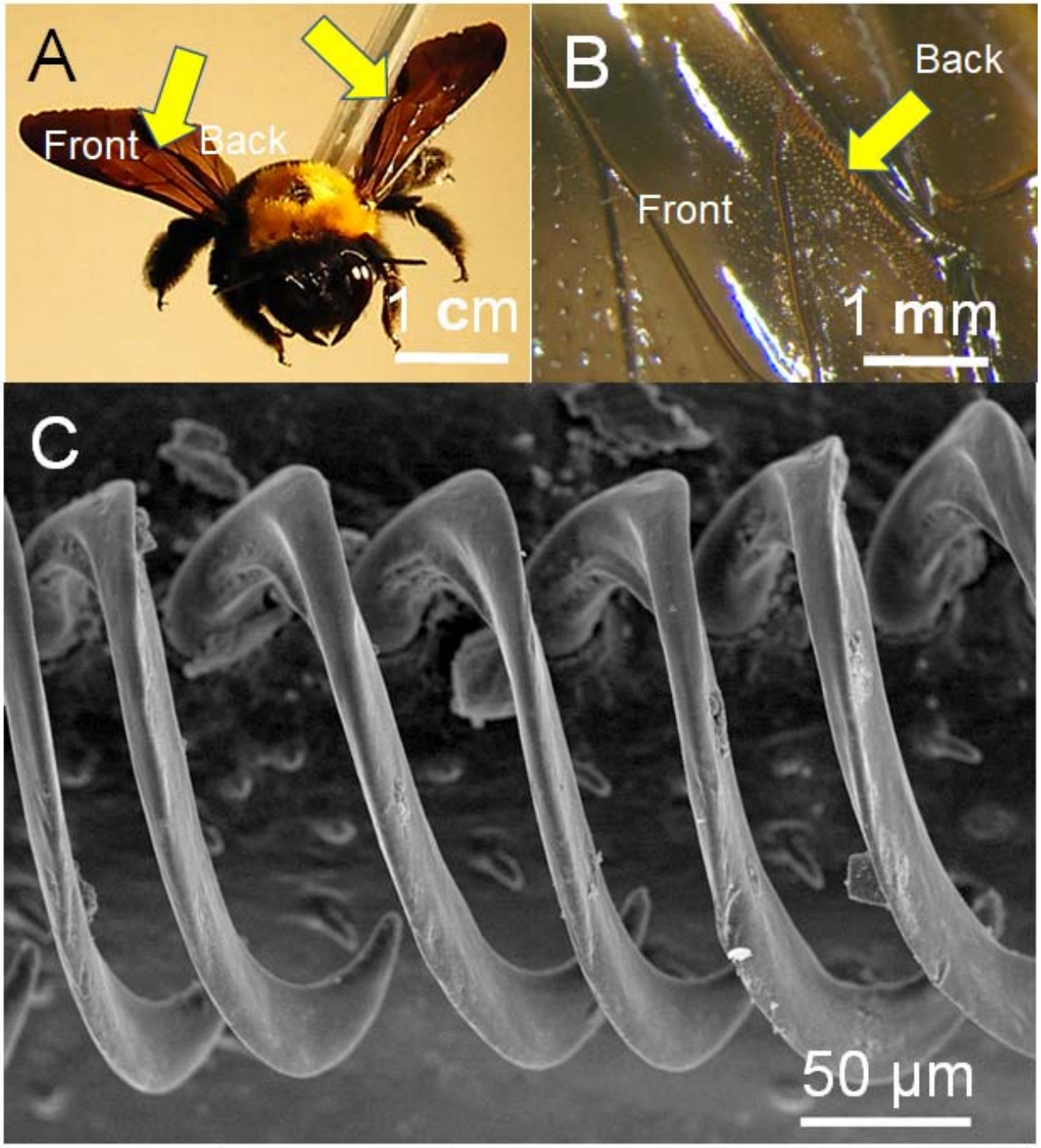

Fig 3

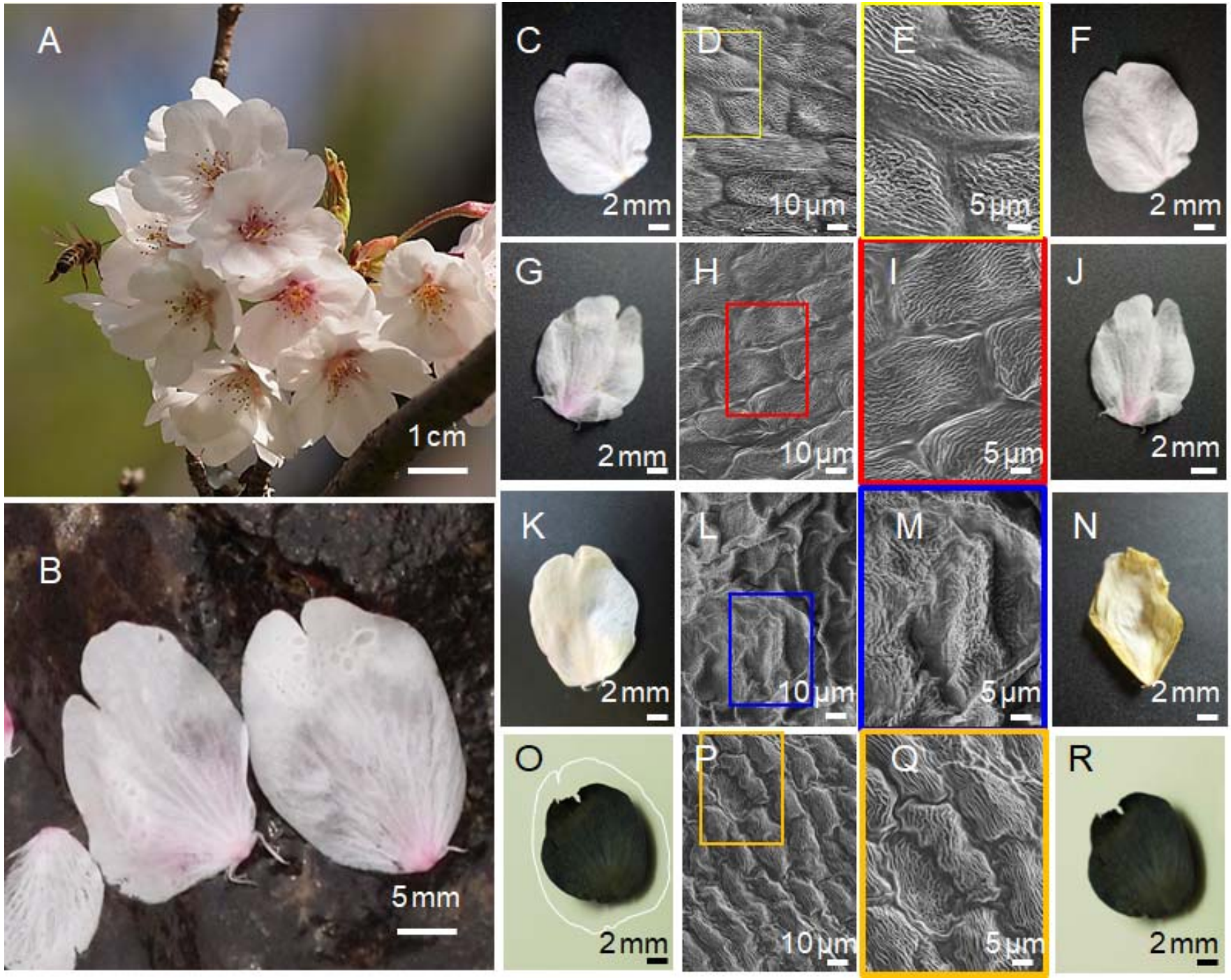
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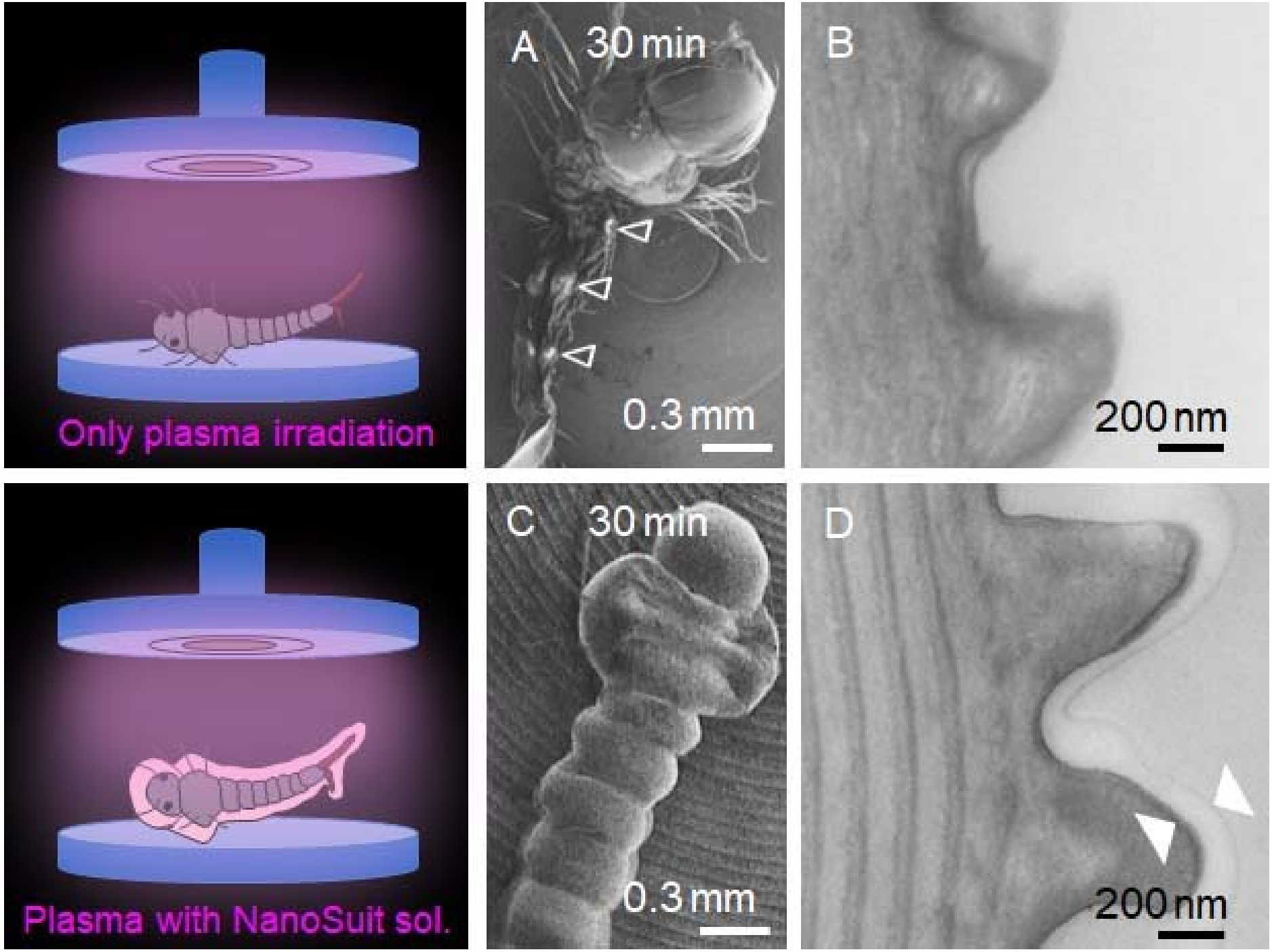
Fig 4


Only plasma irradiation
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C 30 min
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Fig 5

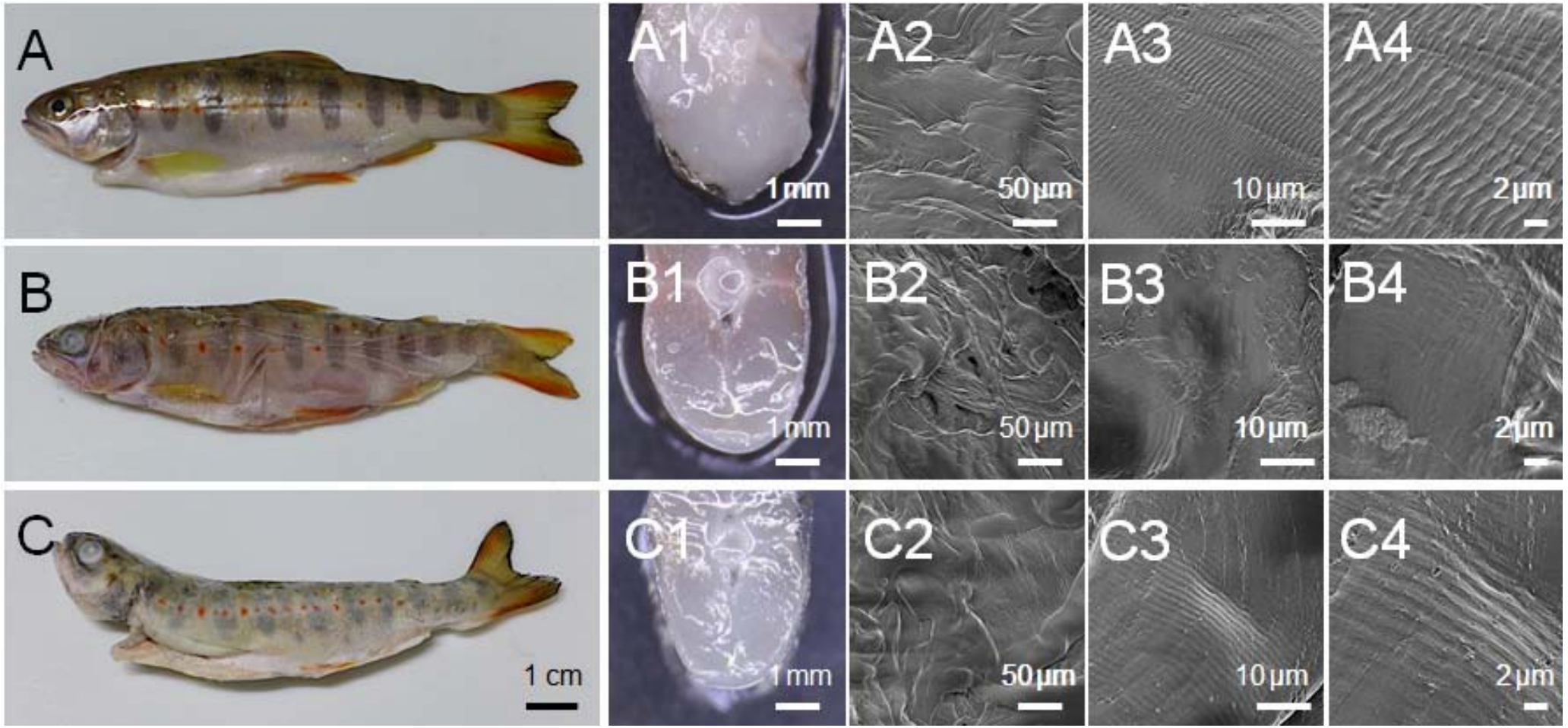
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Fig 6

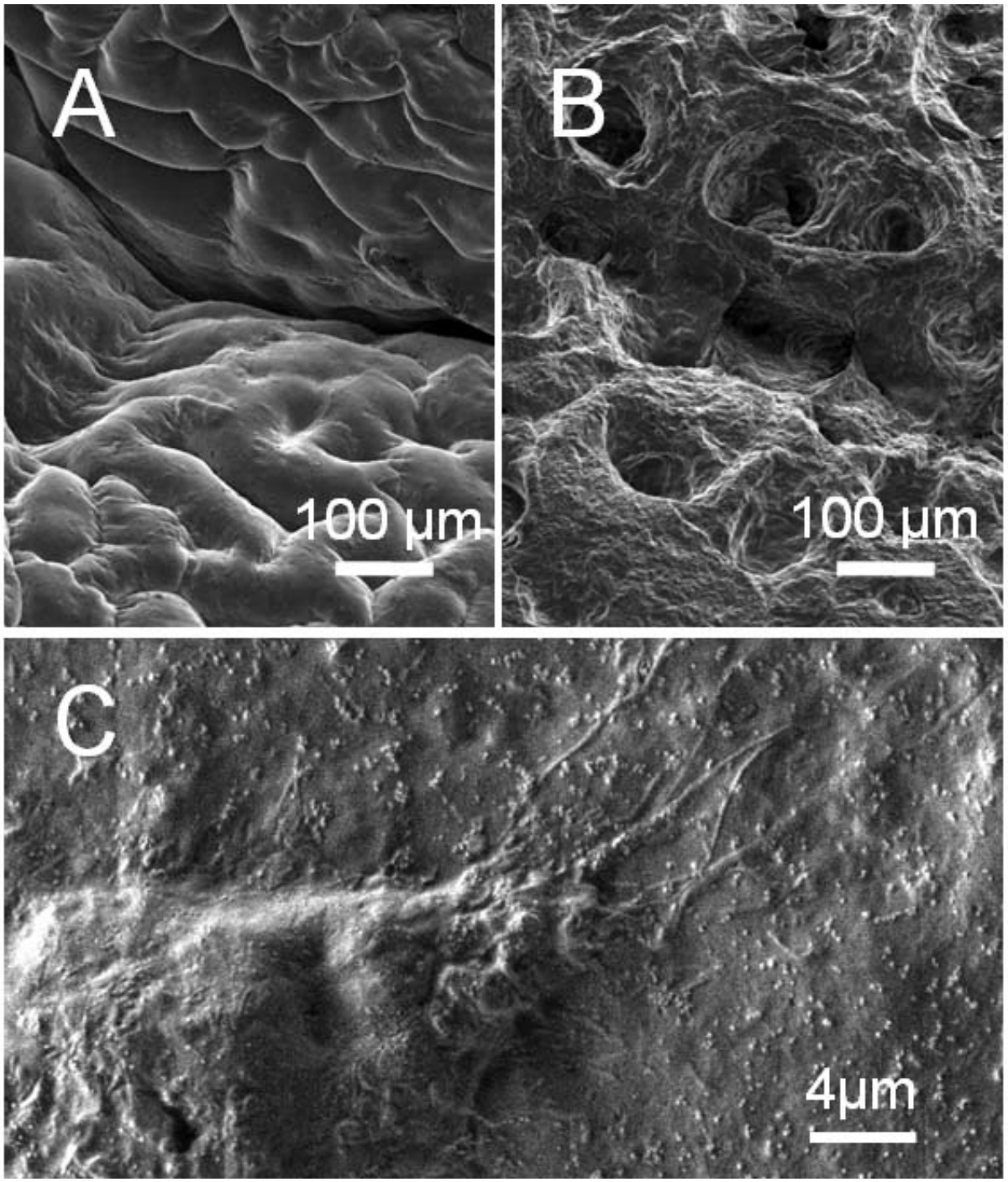


Fig 7

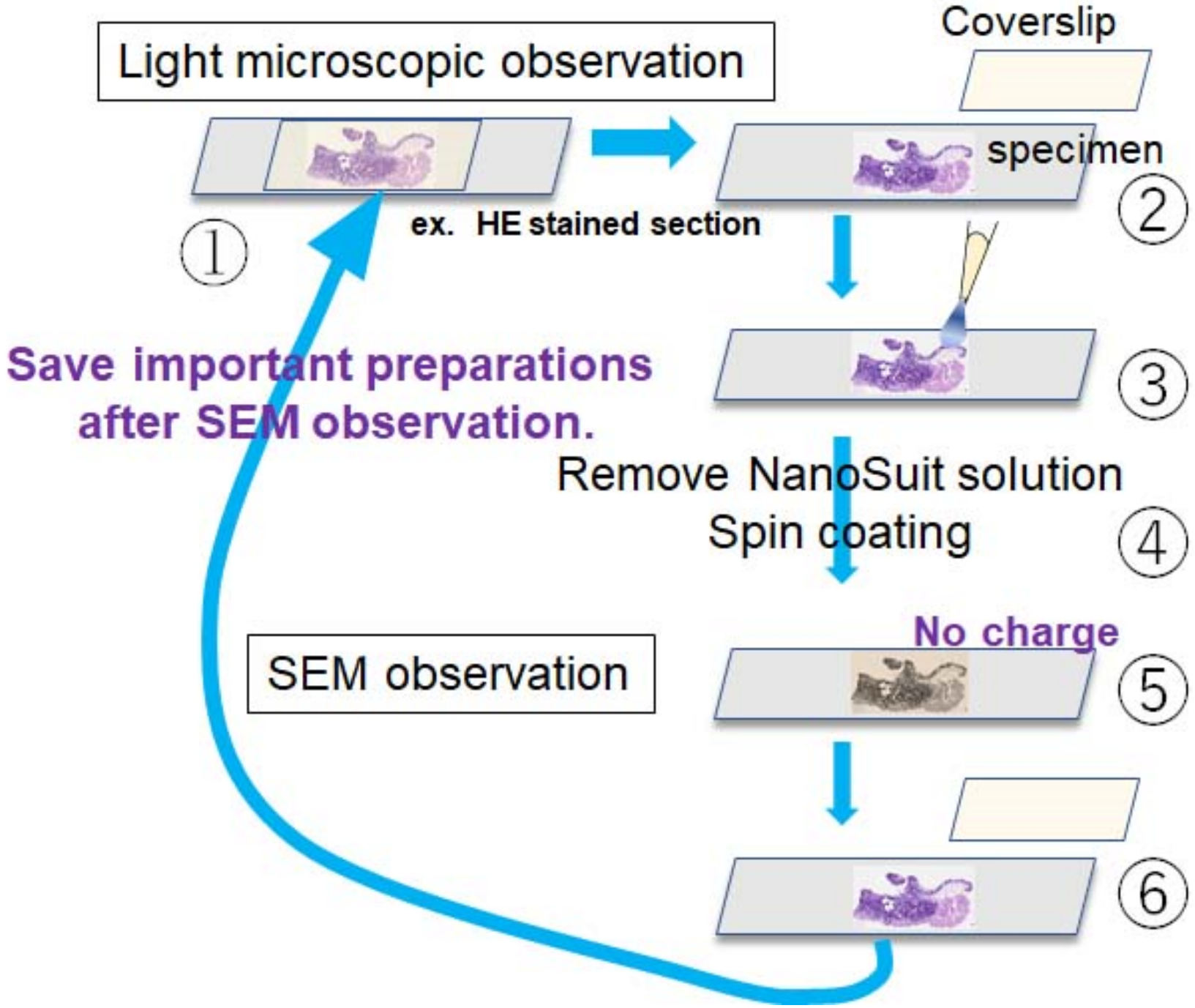

Light microscopic observation
Coverslip
specimen
ex. HE stained section
①
②
③
Save important preparations
after SEM observation.
Remove NanoSuit solution
Spin coating
④
No charge
SEM observation
⑤
⑥

Fig 8

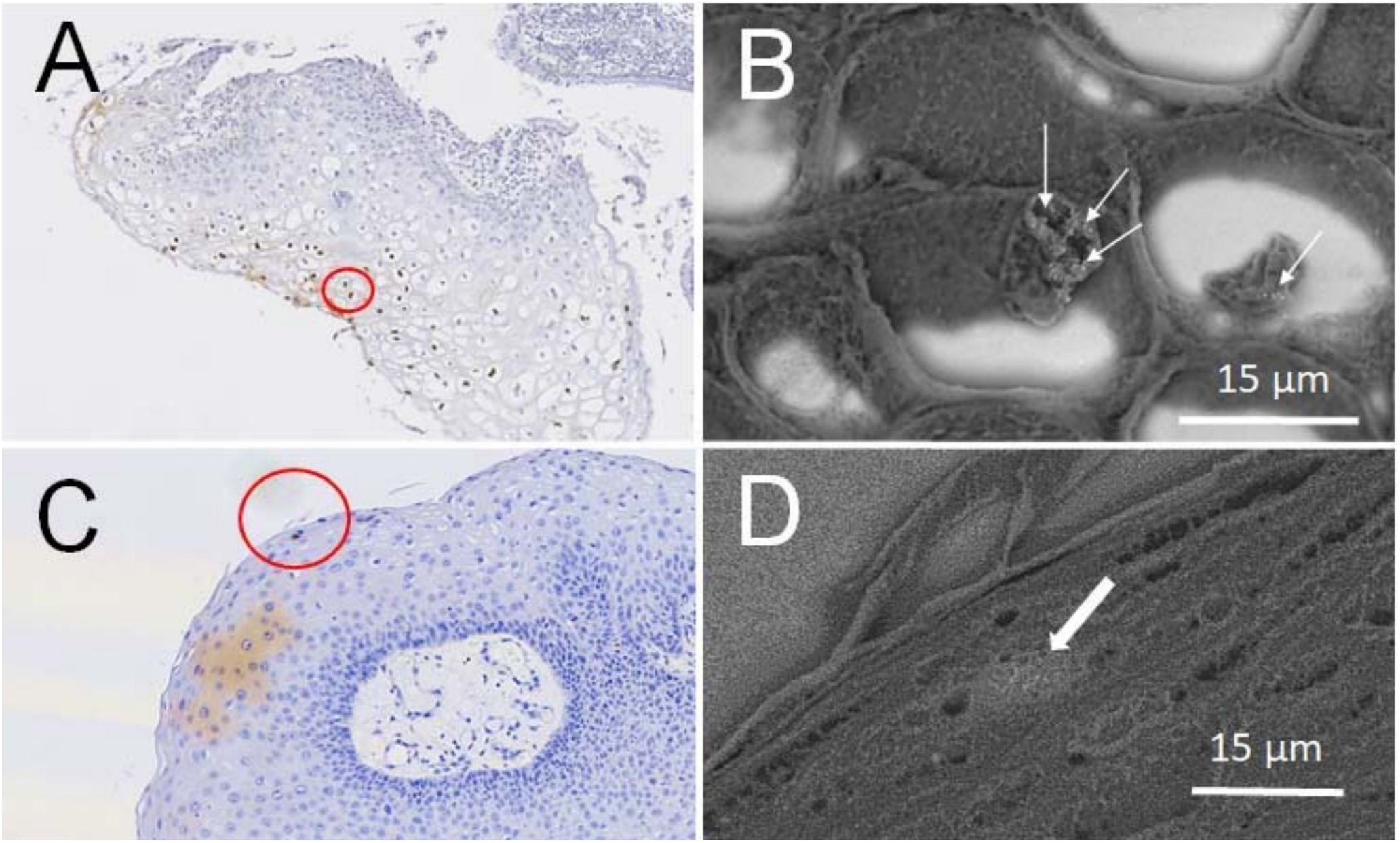


Fig 9

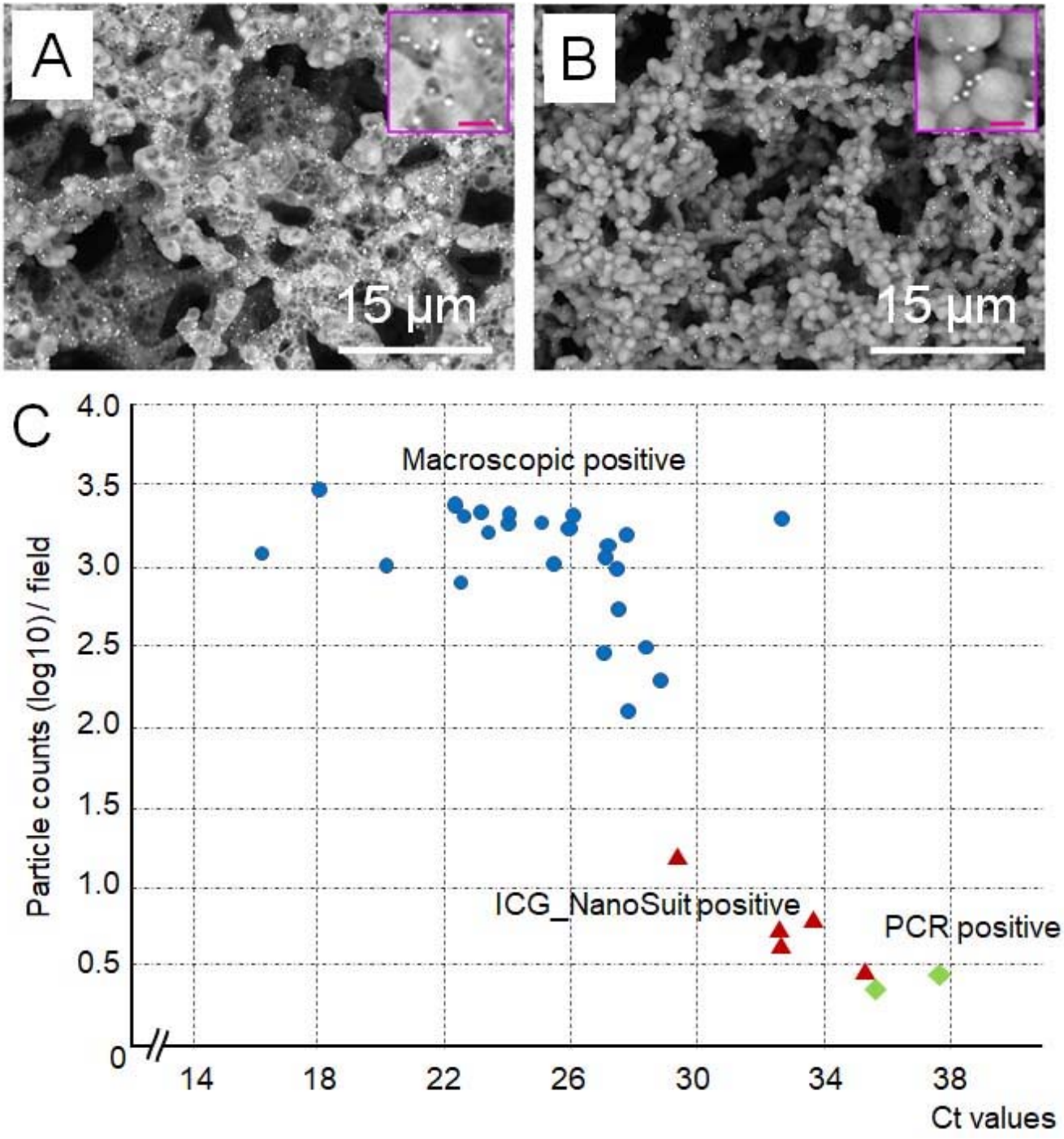
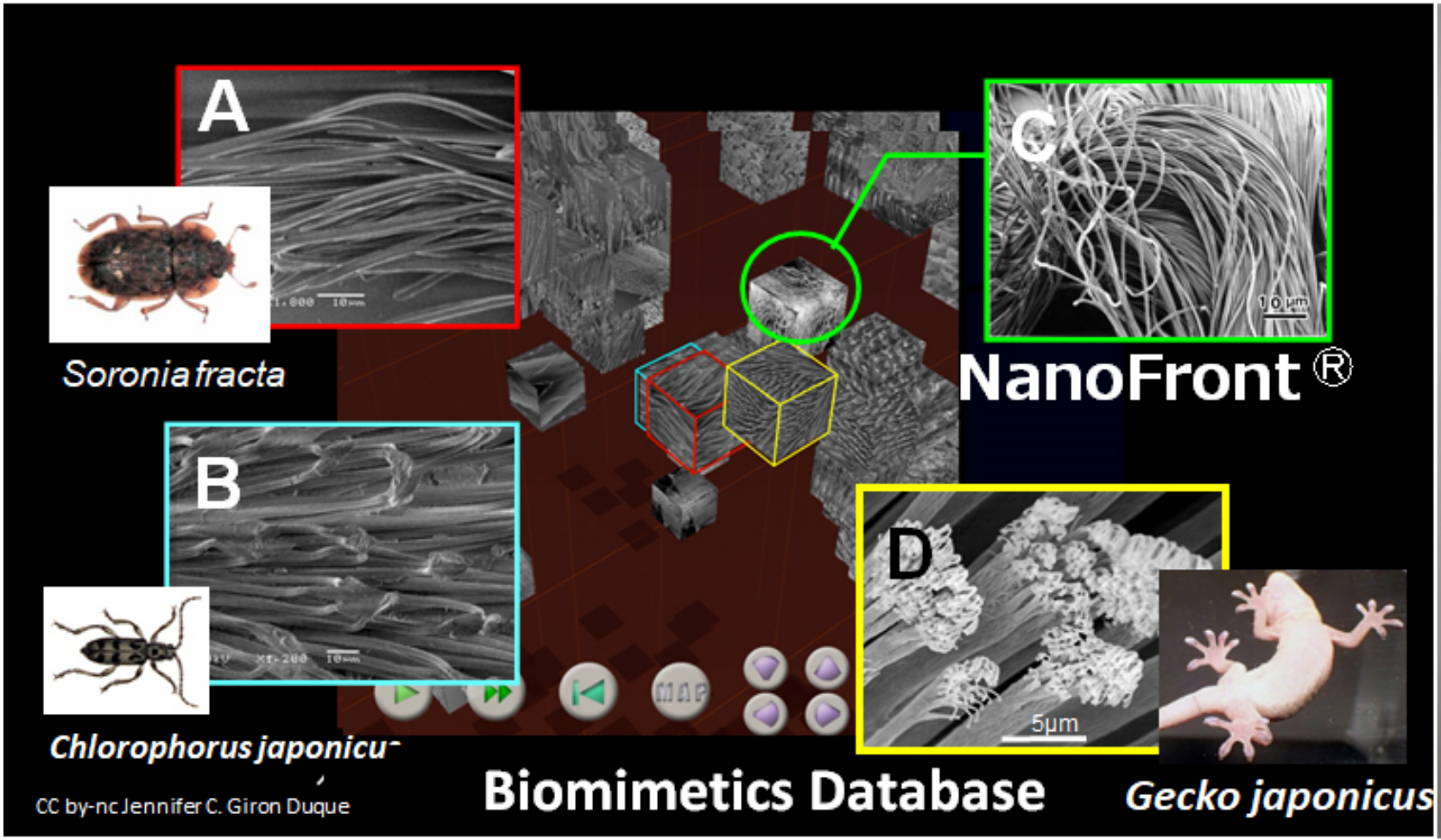
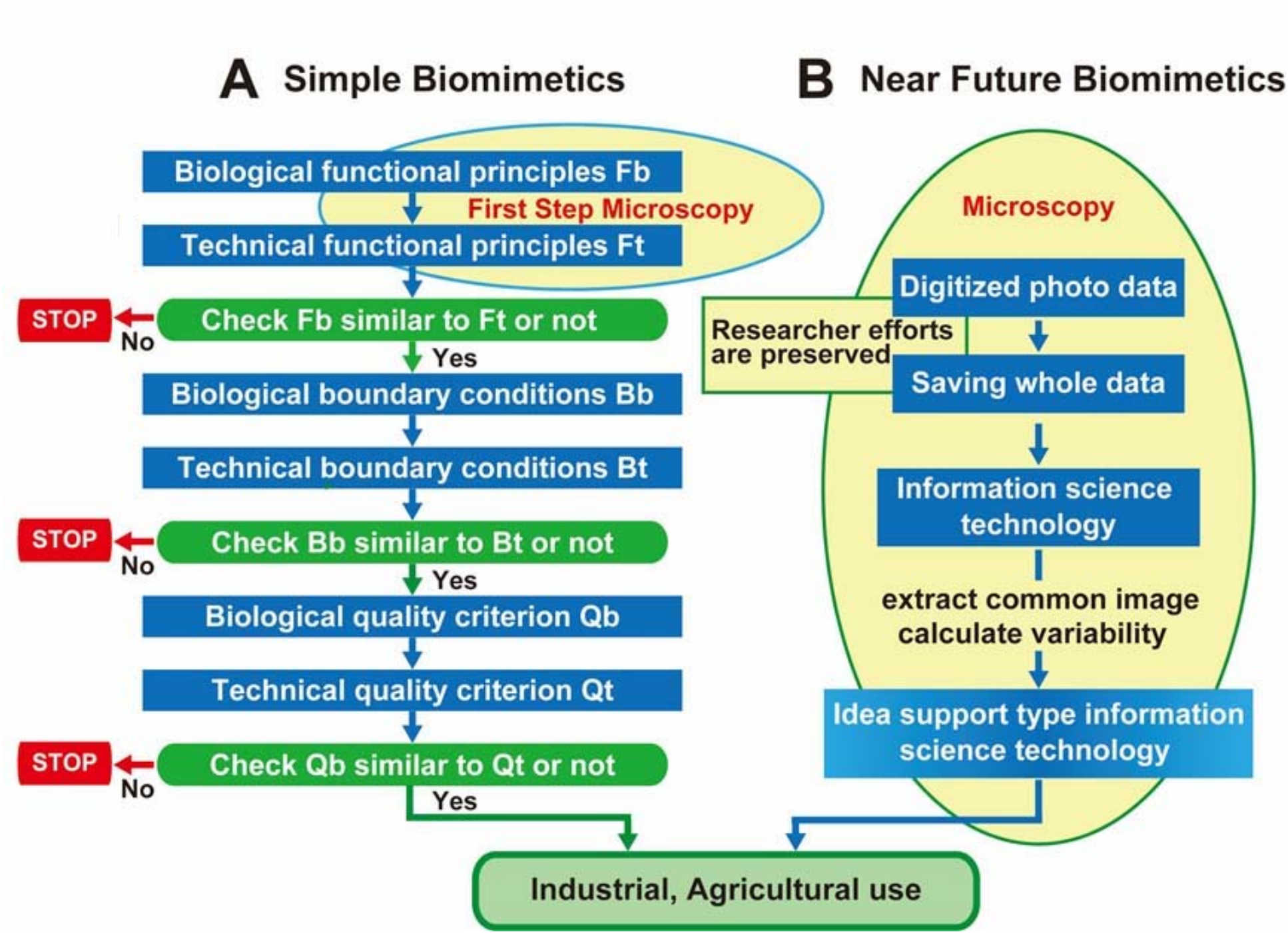

A
15 μm
B
15 μm
C
Macroscopic positive
ICG_NanoSuit positive
PCR positive
Particle counts (log10)/ field
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
14
18
22
26
30
34
38
Ct values

Fig 10


A
Soronia fracta
B
Chlorophorus japonicu
C
NanoFront ®
D
5µm
Gecko japonicus
Biomimetics Database
CC by-nc Jennifer C. Giron Duque


E

Fig 11


A Simple Biomimetics
B Near Future Biomimetics
Biological functional principles Fb
First Step Microscopy
Technical functional principles Ft
STOP
Check Fb similar to Ft or not
No
Yes
Biological boundary conditions Bb
Technical boundary conditions Bt
STOP
Check Bb similar to Bt or not
No
Yes
Biological quality criterion Qb
Technical quality criterion Qt
STOP
Check Qb similar to Qt or not
No
Yes
Microscopy
Digitized photo data
Researcher efforts are preserved
Saving whole data
Information science technology
extract common image calculate variability
Idea support type information science technology
Industrial, Agricultural use