(Univ. Prof. Dr. Friedrich Aumayr)

BACHELORARBEIT

Nanostructures of plantwaxes: Biomimetic usage against *Dictyna civita*

Ausgeführt am Institut für
Angewandte Physik
der Technischen Universität Wien
Wiedner Hauptstraße 8-10 / 134
1040 Wien

unter der Anleitung von

Associate Prof. Dipl.Ing. Dr.techn. Ilse-Christine GEBESHUBER

durch

Gabriel SZABO
Matrikelnummer 01329434

Wien, August 6, 2017

____
Gabriel Szabo

Abstract

Nanotechnology is one of the latest growing fields of research in nearly every part of it, starting with biology, medicine, physics and other technological fields of research and ending with art, where structural colors have a very high potential to create art that has never been produced before.

This thesis results out of a problematic found in façade building, where the pollution of the wall spider cannot be prevented yet. One attempt could be the usage of nanotechnology. Since nanotechnology is one of the "younger" and developing areas of research, little is known about the affects on the human body, this is why the first chapter of this thesis is about the toxicity of nanotechnology for humans. Based on the so-called Lotus-Effect, some thoughts are presented regarding properties can be applied on façades, which are non-toxic for humans. Different approaches are discussed, along to the presentation of various methods of measuring surfaces and determining their properties, using the bird of paradise blossom and the banana leaf as examples representing the huge variate of nano-structured plant-surfaces.

Kurzfassung

Nanotechnologie ist eines der zur Zeit am stärksten wachsenden Forschungsgebiete und das in nahezu jedem Fachbereich unterschiedlichster Studien- und Wissenschaftsrichtungen, angefangen bei Biologie und in der Medizin, über die Physik und sonstige Anwendungen in der Technik, bis hin zur Kunst, wo mit den Strukturfarben ein sehr hohes Potential, zur Erschaffung von Kunst noch nahezu ungenutzt ist.

Grundlage für diese Bachelorarbeit war die Problematik im Fassadenbau, die speziell auf die Verschmutzung von Fassaden durch die Mauerspinne eingeht. Dieses Problem konnte bis jetzt noch nicht behoben werden und es dürfte auch noch einige Zeit dauern, bis es gelöst wird. Ein Lösungsansatz wäre die Verwendung von Nanotechnologie. Da diese allerdings, im Vergleich zu anderen Forschungsgebieten noch sehr jung ist, ist noch nicht ausreichend geklärt, wie sich spezielle Nanopartikel auf den menschlichen Körper auswirken können. Aus diesem Grund handelt das erste Kapitel von der Nanotechnologie und ihrer möglichen Toxizität. In weiterer Folge wurde besonderes Augenmerk auf den sogenannten Lotus-Effekt gelegt, welcher womöglich mit nicht giftigen Pflanzenwachsen auf Fassaden realisiert werden könnte. Hierfür werden verschiedene Ansätze und Methoden behandelt, mit denen man Rückschlüsse auf die Oberflächenstruktur und deren nanontechnologische Beschaffenheit ziehen kann. Dafür wurden, stellvertretend für eine Vielzahl von Pflanzen mit speziell strukturierten Wachsen, die Strelizien und die Bananenblätter untersucht.

Contents

1 Introduction to Nanotechnology

1.1 Introduction to Nanotechnology

Nanotechnology is one of the largest growing fields in science, and the numbers of attempts of using nanotechnology in every section of our lives are rapidly increasing.

The manipulation of matter on an atomic and molecular scale is often associated with this apprehension, so it is more a hypernym to a variety of fields of research. There are also different ways to realize effects caused by nanostructure or even nanoparticles, which are bottom up and top down methods. There are also attempts to reproduce structures seen on different plants and animals, for example the so-called lotus-effect, structural colours in wings of butterflies, adhesives from diatoms and attachment of flies caused by nano-structured hairs.

It also has to be differentiated, if it is desired to use single nanoparticles or whole nano-structures, because here the processes also differ. Silver nanoparticles for example are examined for their usage in drug development, because some experiments have shown that those particles have got antimicrobial effects, whereas nanostructures in plant waxes and on some animals are examined for technical usage, for example reducing friction, reflecting a certain wavelength of light and in this case, preventing insects from walking on different surfaces.

Right now it is even possible to "reprint" nano-structures of different butterflies; more on that can be found in chapter 2.3 (on page 18).

Since 1959, when nanotechnology was first discussed by the physicist Richard Feynman, it changed a lot of different innovations and has led to new ways of thinking and experimenting. With the invention of the Scanning Tunneling Microscope in 1981, scientists were able to manipulate individual single atoms for the first time, which was the initial start of the huge field of research called nanotechnology [1].

1.2 Toxicity of nanoparticles

Although Nanotechnology offers enthusiastic and promising applications, several dangerous and harmful effects appeared, which resulted in a discredit of Nanotechnology.

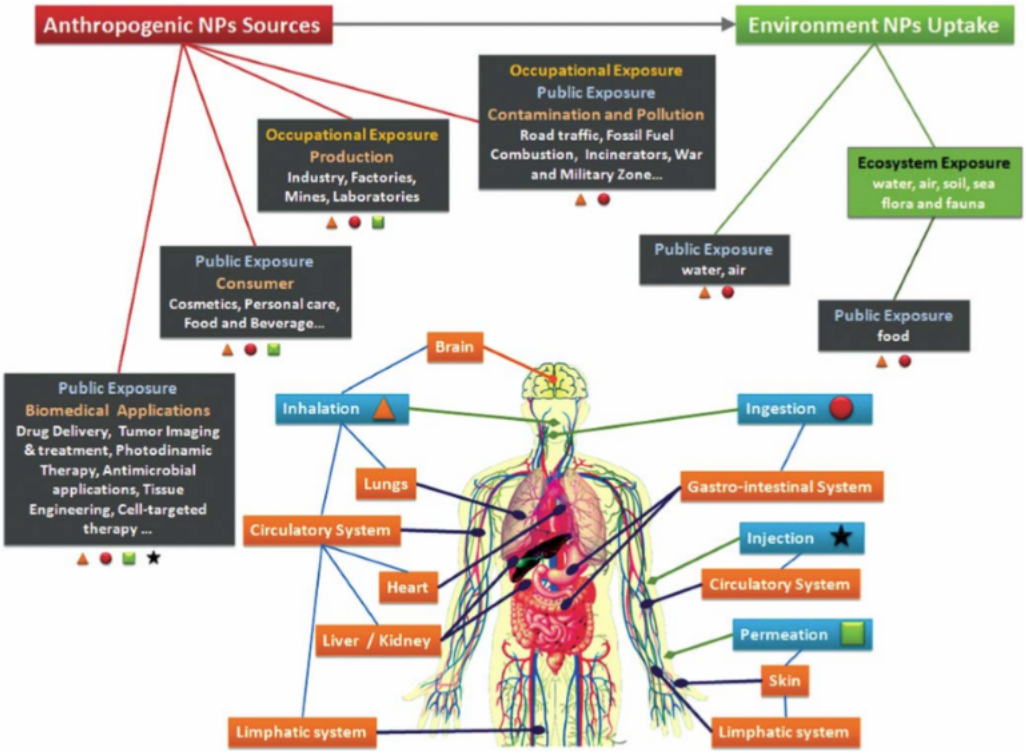


Figure 1.1: Human exposure to nanoparticles and several ways of entry into the human body[2]. © 2014, Current Medicinal Chemistry

Figure 1.1 shows that the human body is capable to uptake nanoparticles in different ways, which dramatically increases the toxicity of some of them. Scientists have shown that the mechanisms underlying nanoparticle uptake, following their biological and toxicological properties, strongly vary depending on their size, shape and surface charge. There have been many experiments analysing the tissue distribution depending on their size, for example Gold nanoparticles with a size of 10 nm were widespread in different organs, while larger nano-particles from 50 to 250 nm were only found in the liver, spleen and blood. Objects of research in this case were rats [2].

Using the example of crystalline Nickel sulphide particles, it was shown that Nickel sulphides were selectively phagocytized (a cell engulfs a solid particle to form an internal compartment) by the cells, which leads to dissolving in the cytoplasm, whilst Nickel ions get released and damages the heterochromatin. This effect explains why certain nanoparticles are able to damage the DNA what subsequently can lead to cancer.

Another hazardous effect is the inability of alveolar macrophages to remove inhaled, ingested or ingressed nanoparticles, which can induce inflammatory response and release of inflammatory cytokines with harmful consequences [2].

1.3 Incubation of nanoparticles

As it was shown before, there are many ways to incubate nanoparticles, which are described in more detail in the following chapters.

1.3.1 Inhalation

Inhalation is believed to be the most common way to uptake nanoparticles. As already mentioned in chapter 1.2, the depositioning and entering depends on the size of those particles. That means the main particles are deposited in the nose, mouth and larynx, the smaller granules can reach the bronchial tree, where they can agglomerate and start damaging surrounding tissue.

A direct relation between inhaling and incidence of lung cancer was demonstrated in epidemiological studies on refinery workers who were exposed to metal particles.

Another well-known case of cancer caused by particles is the asbestos inducted lung-cancer. If the particles are smaller than a specific size, they can be transported further on to different organs and tissues, which means inhalation might cause different types of cancer.

1.3.2 Permeation

This way of uptake is the most controversial way discussed and scientists are still not sure, how high the related toxicity really is. The risk seems to be minimal, because the skin normally behaves as a protecting barrier, but nevertheless it is porous to nanoparticles and holes are offered by the hair follicles which can act as entry for fine particles.

Some experiments showed that several nanoparticles reached the lymph nodes and further on the vascular system through the skin, which can lead to damage and modifying of the DNA.

This might me be a concern regarding the fact that we use products containing nanoparticles every day, like toothpaste, deodorant, sunscreen, foundation and many more. Still it has to be kept in mind, that permeation through skin is quite unlikely compared to inhalation, so as already mentioned before the risks are at a very low level [2].

1.3.3 Ingestion

The uptake through ingestion has to be handled carefully, because on the one hand it is not that well researched compared to the other two ways mentioned before and on the other hand, because a growing field of research is investigating on drugs containing certain kinds of nanoparticles, which might have a positive effect on the human body.Several studies showed that the human body can handle those small particles the best through ingestion, although there are still some materials, sizes and shapes which are toxic [2].

Altogether blaming nanoparticles to be toxic for humans is not quite right and one should consider and be aware of the fact that there are, like in every situation in life, nanoparticles that are useful and some that are toxic. Regarding this fact, there are several studies that deal with classification of toxic and non-toxic materials, sizes and shapes [2].

2 Probable field of usage for Nanotechnology

Nanotechnology is afflicted with a stigma regarding its possible toxicity. As already mentioned in 1.2, not all materials, sizes and shapes are toxic for the human body. This chapter will give some examples generally and especially solutions regarding the façade pollution from so-called wall spiders (*Dictyna civica*).

2.1 Wall spider and façade pollution

2.1.1 Attachment mechanisms of wall spiders (*Dictyna civica*)

Killing spiders with chemicals doesn't only harm them but also in most cases the insecticides are toxic for the environment too. So the better way to get rid of pollution caused by wall spiders is to focus on the mechanisms with which spiders can climb on the wall and to prevent them to be able to do so. In figure 2.1 different types of those mechanisms are shown.

Regarding the *Dictyna civica*, only mechanical attachment mechanism plays a role, because the family of *Dictynidae* only uses rare so-called claw tufts for attachment. Most mechanisms for attachment use the principle of mechanical interlocking with substrate structures, which is associated with the low effort hence there is no material discharge necessary, which would be necessary with adhesive secretions. The structures are relatively simple and because of their universality and efficiency they tend to work great on rough substrate.

The most emerging attachment devices are claws. Their structure is more or less similar,

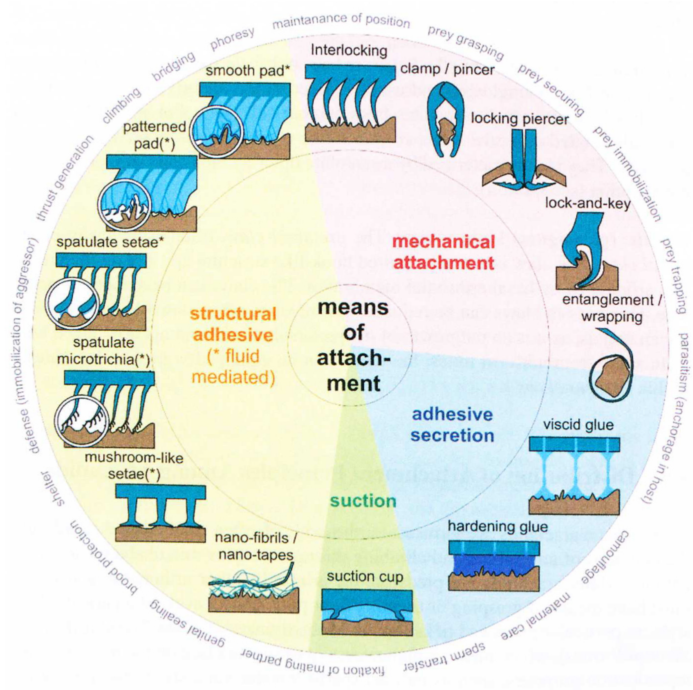


Figure 2.1: Schematic overview on different attachment mechanisms [3]. ©2016, Springer

whereas their material and shape may vary. The base is broad and flexibly hinged and absorbs stress and provide adaptability and resistance of the interlocking while moving. In contrast to that the shaft is curved ventrally and enhances the attachment ability. Due to different substrates and its resistance, the material of it must be very stiff and the sharper the tip is, the better it works. Here the risk of breaking the claw increases the thinner it gets, so the claw tip is normally enhanced with incorporated metal ions or special structuring to strengthen it up.

Figure 2.2 shows how sharp and fine those claws are. With this in view it seems to be obvious that spiders are able to attach on very small structured substrate or even on dust and dirt particles. Regarding the wall spider, there might be one way of attachment, which could be more or less easily prevented. Their movement on for examples façades can take place through attaching on dust or dirt particles with a very small-sized diameter. They could be washed away, if the structure of the façade would be superhydrophobic as it is already known from the Lotus-Leaf or the Banana-Leaf. With every shower of rain the possibility of finding something to attach on decreases rapidly, because the rain drops would intake the particles while running off the façade.

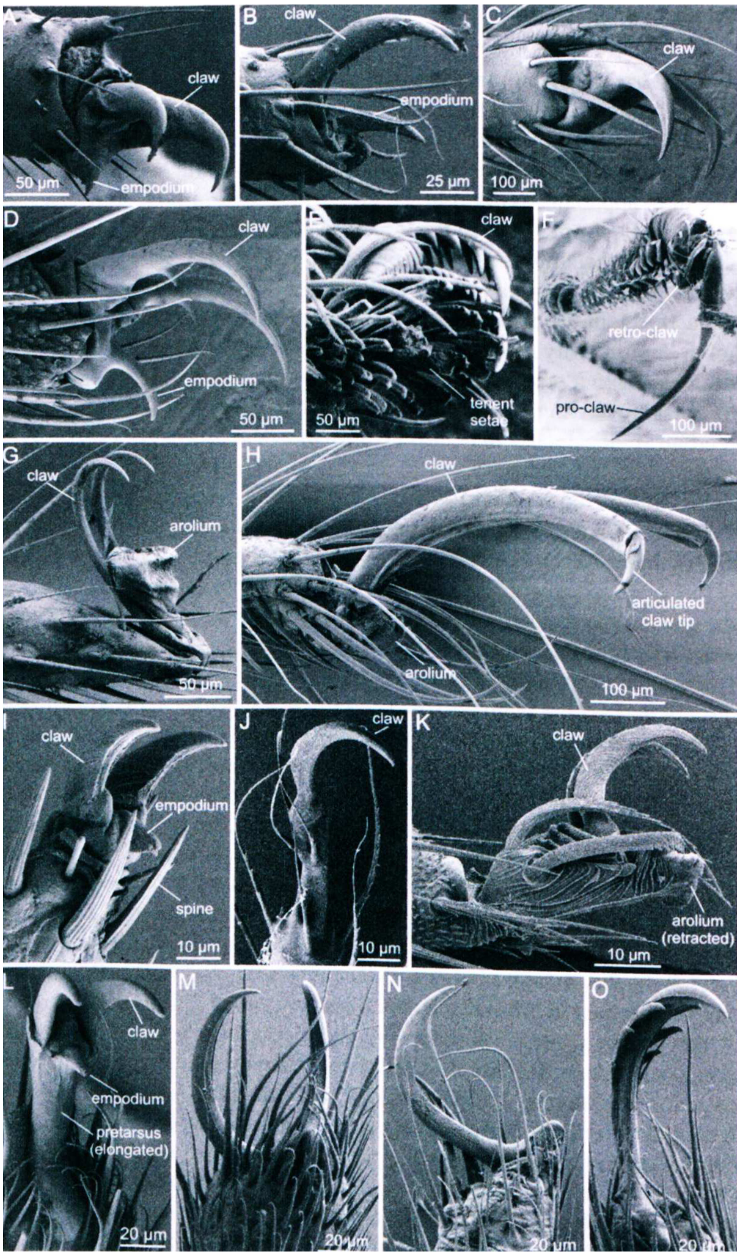


Figure 2.2: Claws of walking legs in arachnids [3]. © 2016, Springer

It could also appear that a certain kind of structuring prevents arachnids from walking on surfaces because they might "taste" bad, regarding the fact that they are rigid and do not bear any sensory organs, but they often got chemosensors for checking the surface they are on. However, this aspect is not so well researched, so this is only an assumption and can be tested by applying a certain structure to an infested area of wall spiders.

In chapter 2.1.2 there is also another factor listed from where the pollution gets so heavy, namely the silk and the resulting spider webs [3][4].

Silk of those spiders is attached to so called attachment discs consisting of a mixture of glue and nanofibres. To understand the function and construction of those silk discs, I. Grawe et al. [4] investigated discs of different arachnids on various surfaces. The substrates used were glass, Teflon and the upper surface of leaves of Sycamore maple (*Acer pseudoplatanus*) to represent a widespread generic plant surface. Glass was chosen because of its hydrophilic properties and because it provides a high surface free energy, whereas Teflon has a very low surface free energy and is hence highly anti-adhesive.

The base plate consists of numerous parallel and crossing loops of pyriform glue-coated fibres that are denser in the central part of the disc, as it is shown in figure 2.3.

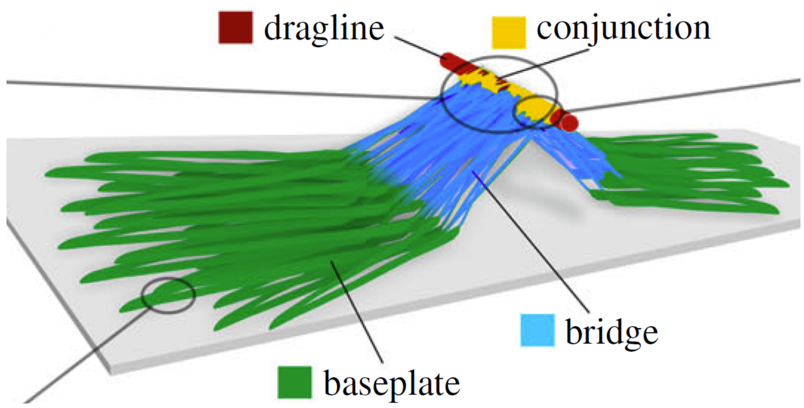


Figure 2.3: Schematic of attachment discs [4]. © 2014, Journal of Royal Society

Between the conjunction and the base plate, there is also a web-work of glue-coated fibres spread, which are building branches between the so called conjunction and the substrate. The conjunction, as its name already says, is the connection between the disc and the dragline, which later builds up the web itself. Further on the different parts of the disk were examined with fracture mechanics. This means that the force was measured over time until the parts broke. Here it was recognized that the highest pull-off-forces of the base plate were found to be at glass, whereas Teflon had significantly lower pull-off-forces.

In figure 2.4 those effects, regarding the base plate, were shown by the area of the coated surface, which is proportional to the pull-of forces [4].

As it has been shown, the lower the surface free energy and the higher the anti-adhesive the substrate is, the harder it gets for spiders to build a web which is capable of carrying the weight it should under normal circumstances. It also could be that because of the lower adhesive forces, the spider webs can get washed away by normal rain or get so heavy that they drop off the surface.

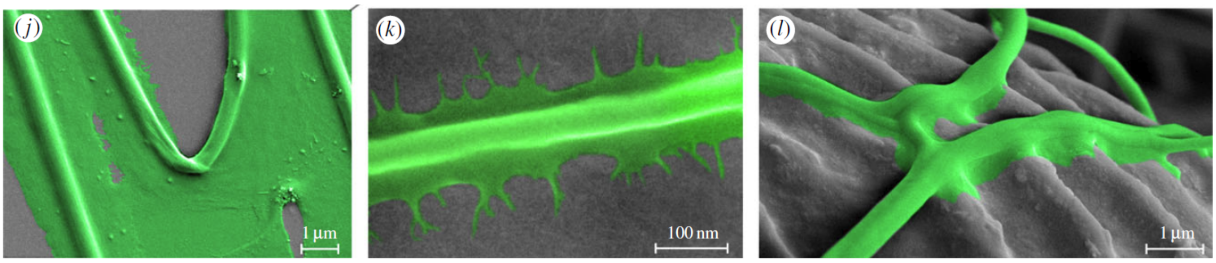


Figure 2.4: Single pyriform (glue silk protein found in attachment discs) glue-coated fibres of the base plate on different substrates. (j) glass, (k) Teflon and (l) upper surface of Sycamore maple [4]. © 2014, Journal of Royal Society

2.1.2 Façade Pollution

Spiders can leave dark stains on the façade, which is not only unattractive, but also a nutrition medium for different bacteria and fungi, which leads to damage of the façade. Those dark spots are not spiders at all (the spiders only measure 1 to 3 mm in diameter), they are spider-nets full with insects, dirt and other visible particles like dust.

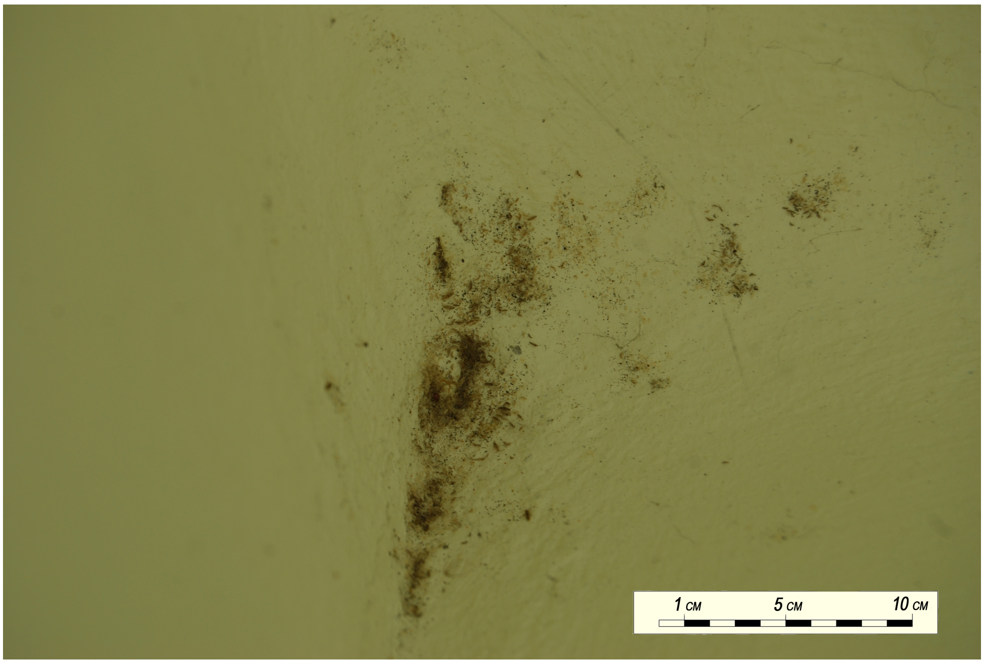


Figure 2.5: Pollution caused by wall spiders.

Wall spiders use slots and cracks to hide themselves, but they are not able to increase them, which is a positive effect regarding the further investigation, because it means that it is not necessary to kill them to save the façade, but further on it is essential to prevent them from entering the wall. Interestingly, wall spiders prefer lightened up areas and freshly painted façades, which allows some further investigation on whether they react on focused UV-radiation, but more on that in section 2.3.

Another problem concerning spiders is the weak effectiveness of insecticides, because their effect decreases rapidly under exposure of UV-radiation and weather in common, not to mention the environmental damage accompanied by those insecticides. Worth mentioning is the fact, that bees and butterflies suffer under those harmful conditions, which directly affects our environment and further on our lives. Insecticides are also damaging organisms living in water. Those few examples describe quite well that to prevent our buildings from pollution, investigations on less harmful materials and on solutions, which might not be harmful at all, need to be done [5].

2.2 Nanotechnology as solution

In the last decades, scientists from different fields of research investigated on functional surface structures of plants. Interestingly, plants created various protective outer coverages over the last 460 million years of evolution, called cuticle. One of the most important attributes, not only for the problem mentioned in section 2.1.2, but also commonly regarding the ambient environment, is the hydrophobicity.

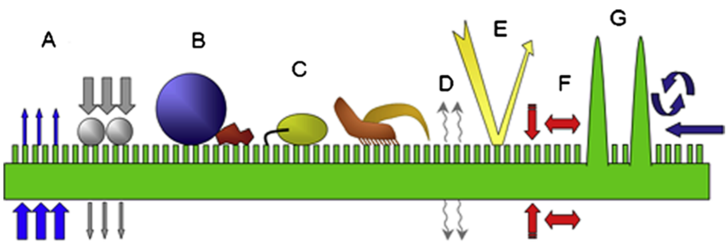


Figure 2.6: Schematic of structured plant-surface [6]. © 2009, Elsevier

As you can see in the schematic in figure 2.6, nano- and microstructures also control the water loss/ leaching from the interior, prevent potential harmful particles from entering, minimize adhesion to different sorts of insects, reflect certain kinds of wavelengths and regulate surface and body temperature.

2.2.1 Waxes in plants

The epidermis cells, which are the outermost cells of plants, show different settings of morphology, but are principally built based on the same principle, shown in figure 2.7. Here, the top layer, called cuticle, can be seen. This part of the epidermis cell is, as already mentioned and shown in figure 2.6, the most important and most functional part and is

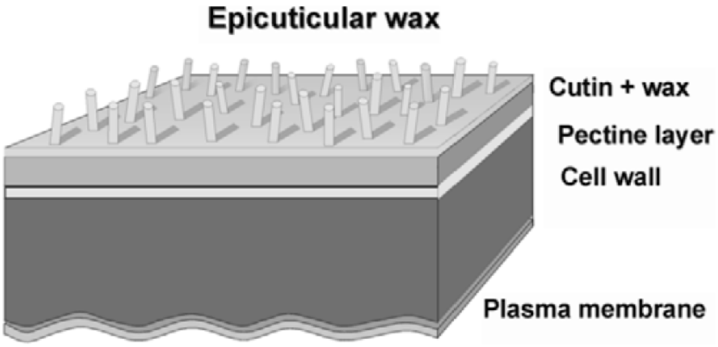


Figure 2.7: Schematic of the outermost layers of the plant epidermis cells [6]. © 2009, Elsevier

found on most of the land-living plants. The cuticle is basically built up from cutin and hydrophobic waxes, a polyester-like biopolymer, composed of hydroxyl hydroxyepoxy fatty acids, and sometimes also by cutan, which consists of polymethylene chains. Scientists have shown large differences between the micro- and nanostructural build-up of several plants in different development stages [6][7].

This factor also plays a big role in whether the plants are hydrophobic or superhydrophobic. Wax films mostly get combined with three-dimensional waxes, which have different sizes and shapes, e.g. tubules or platelets [6].

The pectin-layer connects the cuticle to the much thicker cell-wall underneath. Those layers are not always formed as flat underground, but also can be structured like the cutin above. Via analyzing the cross-sections of different plants, it was shown that the sometimes varying thickness of this layer leads to different effects and formation of wax-crystals at the surface [7][8].

The last layer separates the living part from the outer nonliving part and is called the plasma membrane.

For further investigations only some kinds of plants can be used, especially those with wax structures, because some of the plants, for example those plants in dry habitats often got hairs attached on their surfaces, which prevent them from uncontrolled water loss and also from reflection of certain kinds of wavelengths [6][7][8].

2.2.2 Lotus leaves

Thinking of superhydrophobic plant-surfaces, the first plant coming to mind is the Lotus leaf (*Nelumbo nucifera*) and its so-called Lotus-effect [13]. The selfcleaning effect has been known in Asia for more than 2000 years, but investigations first started in the 1970s with invention of the Scanning Electron Microscope.

Hierarchical surface structure was found to be responsible for the superhydrophobicity and the selfcleaning effect. These structures are built by randomly orientated small wax tubules on top of what looks like round pyramids, as you can see in figure 2.8. Caused by this effect, adhesion for particles and wetability from the plant's surface are reduced so much, that the tension of water droplets leads to contact angles above 150°, which means that the droplet is barely in contact with the Lotus leaf.

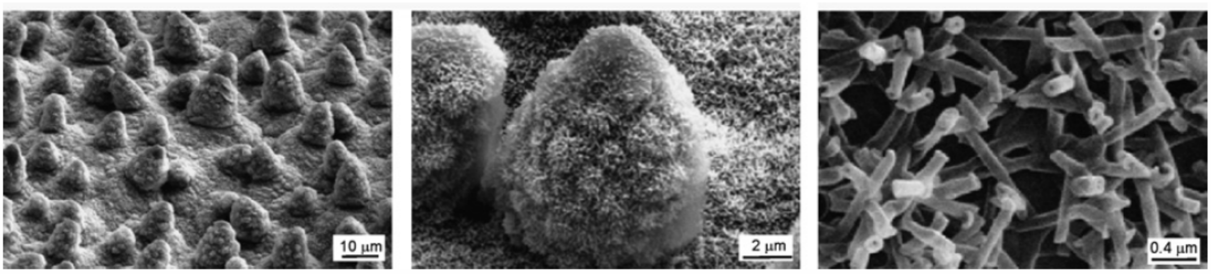


Figure 2.8: SEM micrographs of the Lotus *(Nelumbo nucifera)* leave surface [6]. © 2009, Elsevier

In figure 2.9 you can see a schematic describing the hierarchical structure and why this structure one has the smallest contact surface and hence nearly no adhesion. Flat microstructured surfaces show the most adhesion, which is why we barely find them alone on most of the plants, but more often in combination with nano- and hierarchical structures. There were many more plants found to exhibit hydrophobicity, so the so-called Lotus-effect occurs not only on Lotus, but also on cabbage turnip (*Brassica oleracea*), on different genera of violet and nasturtium (at the surface of the blossom), on the blossom leaves of the winter rose and on countless more plants [8][9].

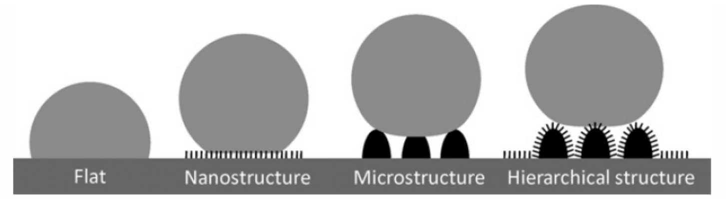


Figure 2.9: Schematic of hierarchical structures causing superhydrophobicity [9].© 2009, Soft Matter

2.3 Thoughts

Regarding the problems caused by the wall spider, there are different possible ways to solve them and one way is nanotechnology.

Chapter 1.2 was about toxicity of Nanotechnology and its negative impact on our health, so suggesting nanotechnology as solution might appeal to be the wrong way. In fact, every human being gets in contact with nanostructures every day and sometimes even ingests some of them. Grapes for example have a nanostructured wax layer, which is not toxic at all. So one thought of using non toxic materials to apply nanostructures to façades is the usage of plant waxes or if possible synthetic waxes based on natural plant waxes.

The next problem is the deposition of those materials on larger surfaces. One way might be to add nanotubules out of plant waxes to the façade painting, which might lead to a similar or the same superhydrophobic effect seen on different plants described in section 2.2.2. Another thought is to heat up the wax, apply a thin layer on an existing bottom layer of common façade paint and let it cool down at a certain temperature. Koch et al. [9] have shown in some experiments, that it worked under certain conditions on small areas, so maybe this process can be applied to larger areas.

The most important thought, which might not be found in production or research, was to use stamps with a nanostructured pattern and plant waxes as stamp material.

Zobel et al. [10] have shown, on the sample of the *Morpho peleides*, that it is possible to create a master stamp with a compound used in dentistry. In cooperation with a qualified supplier for stamps with nano-scaled imprints, it could be possible in the near future, that the façades can be "colored" with such stamp roll.

A further step to investigate the problem caused by wall spiders would be the consequence of increased UV-radiation on those spiders, which might be "produced" by a certain nano-scaled pattern reflecting more UV-radiation.

Further on, some plant surfaces will be investigated in this thesis, whether they might be capable of being stamped and if they bring along the needed structure.

The last thought is that the wax crystals could be "ironed" on different surfaces or could be depositioned through vapour on certain surfaces.

3 Experimental methods

3.1 Possible methods

To measure the surface of certain plants, which might have a micro- or nano-structure fit the mentioned problem best, there are different types of measurement possible, like STM, SEM, AFM and even optical microscopy. For the first two mentioned types, the samples would have to be prepared first, like coating them with a thin layer of gold atoms to make the surface conductible. The preferred method is the AFM method, which was performed and hence will be described at first. It measures the force between the tip and the atoms on the surface. The methods described after that will be SEM and optical microscopy.

3.2 Theory of AFM measurements

The AFM measures forces between the cone end of the tip and atoms structured on the surface of the investigated material. By moving the tip in x- and y-direction, the AFM records a 2D-model of the surface. Adding the z-axis, the microscope is able to record 3 dimensional visualizations. There are different modes of operation, in which the AFM can run and they will be described in the next section.

The picture gets generated through reflection from a laser focusing on the top of the tip to a four quadrat photo diode (deflection sensor), from which an external PC can calculate the resulting image. In figure 3.1 the main parts are graphically shown. The laser is not in the picture, but the beam is more or less represented by the dotted line between the deflection sensor and the top of the tip [11].

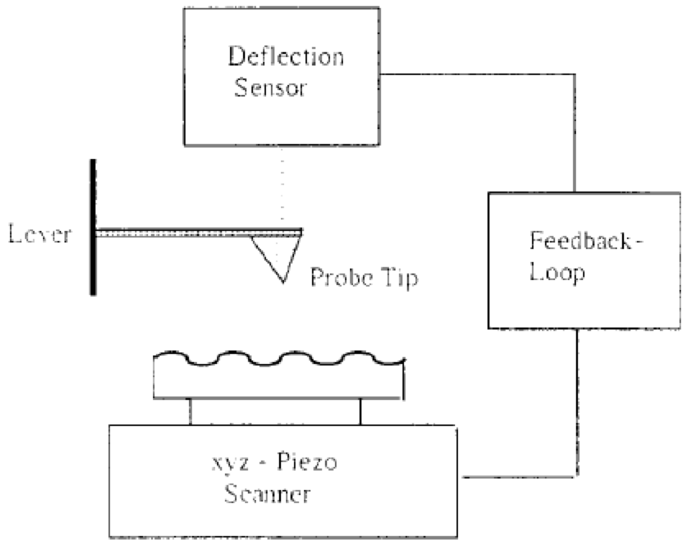


Figure 3.1: Principle of the AFM microscope, describing combined work of the (canti)-lever, deflection sensor and the feedback-loop as connection to the PC [11].

3.2.1 Modes of operation

There are two main operating modes, which are the static-modes, also called dc-modes, and dynamic modes called ac-modes.

In the static mode, the tip and its body bend because of the force acting between the tip and outer atoms of the surface, until it reaches a steady state. It is known from the Hook's law, that the deflection z_t of the beam focusing on the cantilever is proportional to the force $F = c_B * z_t$, where c_B is the spring constant of the cantilever.

$$c_B = \frac{3 * E * I}{l^3} \tag{3.1}$$

$$I = \frac{b * d^3}{12} \tag{3.2}$$

As it is shown in equation 3.1 and further on in equation 3.2, the moment of inertia I is directly proportional to the dimensions, and the Young's modulus E depends on the material of the cantilever. Both parameters are important for the accuracy of the measurement.

There are two different methods of static modes, which are the equiforce mode and the variable deflection mode. While measuring the surface, one can keep the deflection constant by constantly changing the height of the sample (or the tip) in relation to the probing tip. This is the most common mode of the static modes, called equiforce mode. The alternative is to keep the already mentioned height constant and which is the second mentioned mode, which allows high scanning speeds and is commonly used for atomic scale imaging.

In contrast to the static mode, the cantilever is oscillating close to its resonance frequency when operated in dynamic mode, because it is more stable than working with the resonance frequency itself. There are also different "sub-modes", which vary in measuring the change of amplitude, the phase-shift or the deflection.

Compared to the above mentioned modes, the recording of force vs. distance curves does not involve a change in lateral position but it shows a correlation between force and distance from the tip to the sample. The deflection z_t is monitored as a function of the sample position z_s. In contact mode long-range attractive and short-range repulsive forces are in equilibrium due to the elastic deformation of the lever. For accurate measurement of soft surfaces, the elastic deformation mode is neglected and one of the dynamic modes has to be chosen, from which the distance between probing tip and sample is derived. Thus the mentioned forces can be determined.

It is also possible to measure force gradients in the ac-mode, which is more accurate, especially in non contact mode [11].

3.2.2 Deflection sensors

The resolution of deflection sensors was found to be sufficient at atomic resolution. The first sensors were based on electron tunneling, but tunneling is rather sensitive to contaminants and other interactions with the surrounding. Due to that, optical interferometry, laser beam deflection and capacitance methods have been developed.

The optical methods mentioned use the characteristics of visible light to measure a change in distance, as is is known for example in the Michelson-Interferometer.

Regarding the capacitance method, the change of electric capacity due to a change of length is used to to get information on the surface measured [11].

3.3 Theory of SEM measurements

The Scanning Electron Microscope (SEM) is one of the most versatile instruments available for analyzing different surfaces including the morphology and chemical composition. Despite of high importance in scientific research, optical microscopy has limitations due to its resolution compared to the SEM.

The resolution limit is defined as the minimum distance by which two structures can be separated and still appear as two different objects. It is known that the limit of resolution depends on the wavelength of the emitting source. Due to electrons having a much lower wavelength than light, the resolution of the SEM is therefore much better [12].

3.3.1 Principle of the Scanning Electron Microscope

Figure 3.2 shows the principle of the SEM. Electrons are accelerated from a field-emission cathode through a voltage difference between cathode and anode, which has a range from 100 eV up to 50 keV. Those electrons are afterwards focused by a system of magnetic and electrostatic lenses to the surface of the sample. There they either generate light emission through exciting the atoms of the sample, or they generate so-called secondary electrons, which can be imaged via video amplifier. There would be a third possibility of measurable emission, the backscattered electrons, but those are not important for the measurements done in this thesis [12].

3.3.2 Secondary electrons

The SE mode is the most important one, because these electrons can easily be collected by a positively charged collector grid, based at a certain angle to the sample holder. Behind this grid, the secondary electrons are accelerated onto a scintillator, from where the electrons get multiplied by a photomultiplier and are then visualized, as already mentioned in chapter 3.3.1. The typical energy of secondary electrons is very low at about 5 eV and only electrons from a very minor depth beneath the sample surface (about 2 nm) can leave the sample and are therefore detectable.

The resulting resolution of SEM using secondary electron emission is between 5 and 20 nm and it depends on the diameter of the electron beam, on lens aberrations and diffraction errors, regarding the SEM. Errors like pollution of the sample, mechanical vibrations, electromagnetic stray fields, scattering of electrons in the sample and damage of the sample through generation of heat are also probable to downgrade the resolution [12].

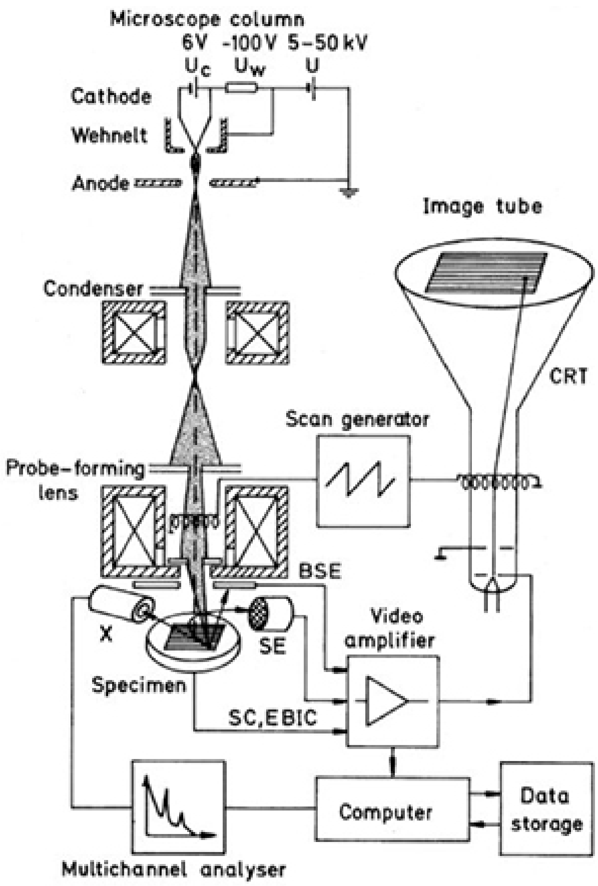


Figure 3.2: Principle of the SEM microscope [12]. © 1998, Springer

4 Measurement and interpretation

4.1 AFM measurements

The first attempts to measure the surface texture of the banana leaf (*Musa paradisiaca*) (top and bottom, but only the bottom has superhydrophobic characteristics) and the blossom of the bird of paradise (*Strelizia reginae*) were done with the AFM (MFP, 3D, Asylum Research, Santa Barbara, USA). Tips with a lower resonant frequency were used (about 75 kHz), because they are better to measure larger scaled structures.

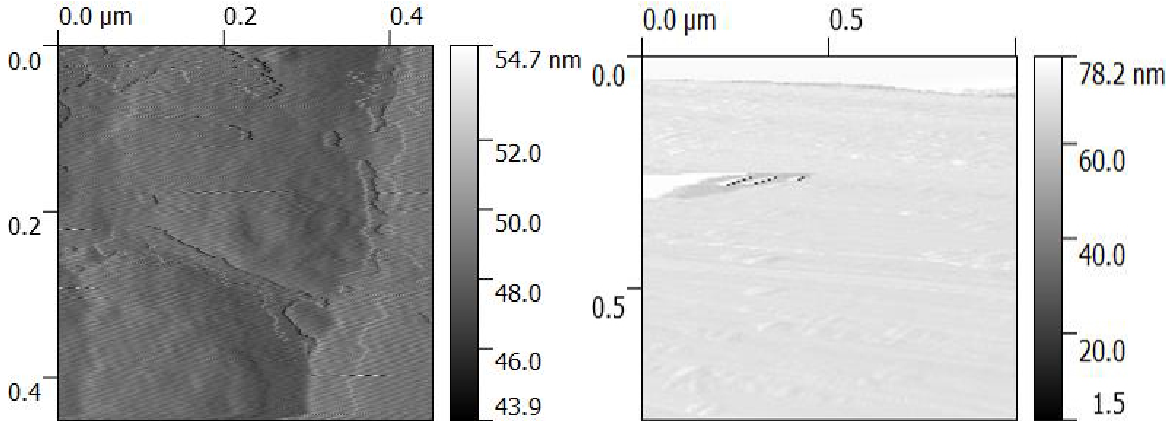
4.1.1 Preparation

For the measurements, the leaf and the blossom were cut in to small pieces of 5 mm x 5 mm small and were placed on double-sided adhesive tape out of copper, which was previously attached to a glass slide. After that, the samples were left to dry under atmospheric conditions for two days. Before placing them under the AFM, the rippled edges were cut off to prevent damage for the tip.

4.1.2 Results

After three weeks of effort to get applicable results without any success, the thoughts were that the structures of the wax crystals are too complex and the depth of them might exceed the resolution of the AFM. Another factor might be the flexibility of the wax crystals, which makes it complicated to tell which and whether a signal is correct. In addition to the mentioned complications the wax is pretty sticky which probably leads to a contamination of the tip and therefore to unusable measurements.

Figure 4.1 shows, that the image of the banana leaf seems to be usable, whereas the measurement of the bird of paradise did not bring up information about the surface. In chapter 4.2 and chapter 4.3 it will be shown, that both measurements are useless.



(a) Attempt to measure banana leaf

(b) Attempt to measure bird of paradise AFM,

Figure 4.1: Attempts to measure banana leaf (*Musa paradisiaca*) and bird of paradise (*Strelizia reginae*) using the AFM

4.2 Optical Microscope Measurements

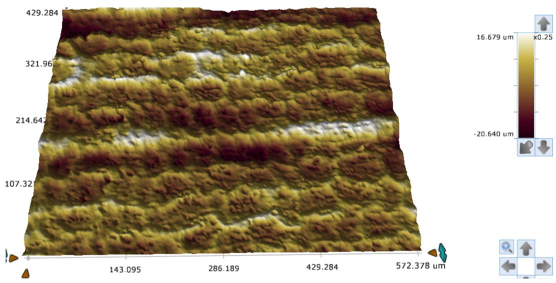
The second attempt was the measurement with an optical microscope. Therefore the samples were examined with a device, provided by Bruker, called Contour Elite and with the kind help of one of their technicians.

4.2.1 Preparation

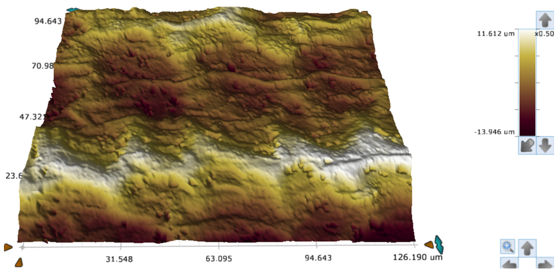
The samples were the ones used for the measurements described in chapter 4.1 and did not need further preparation for this measurement.

4.2.2 Results

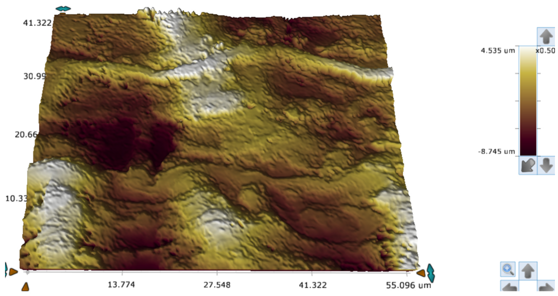
Using Contour Elite pictures of the banana leaf and the bird of paradise blossom were made for better knowledge of the structure on the surface. First the images of the banana leaf are shown, then those of the bird of paradise blossom.



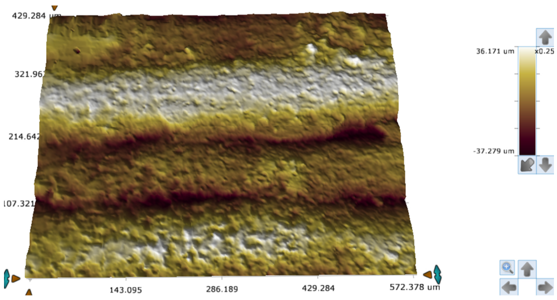
(a) banana leaf, 570 μm x 570 μm



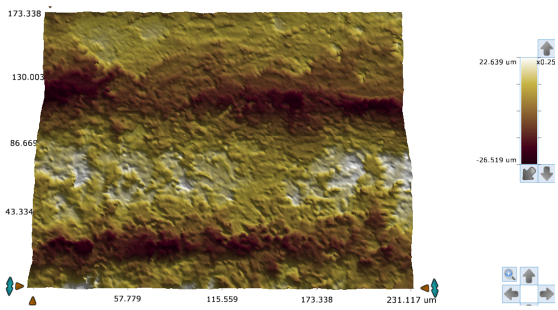
(b) banana leaf, 100 μm x 100 μm



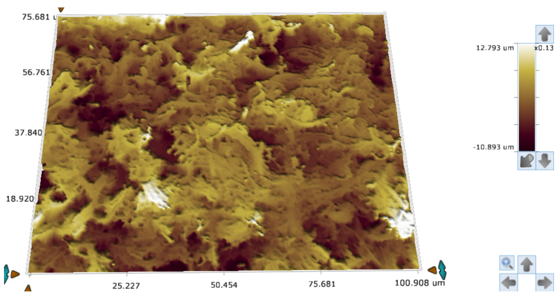
(c) banana leaf, 55 μm x 55 μm



(d) bird of paradise, 570 μm x 570 μm



(e) bird of paradise, 300 μm x 300 μm



(f) bird of paradise, 100 μm x 100 μm

Figure 4.2: Measurement of banana leaf (*Musa paradisiaca*) and bird of paradise blossom (*Strelizia reginae*) using optical microscopy

Based on these measurements it became clear, that the structure is not measurable with the AFM, because the depth exceeds the resolution of it, as it was already predicted in chapter 4.1.2, because the maximum z-distance for the piezo of the AFM is below 10 μm.

4.3 SEM Measurements

The last microscope used in this thesis is the SEM, which should provide the resolution needed for the investigated nanostructures. Therefore the SEM of the Institute of Solid State Physics of TU Wien, with the support of Karin Withmore, was used to achieve the best results.

4.3.1 Preparation

The probes were still the same as used for the measurements in chapter 4.1 and 4.2. To get a conductive surface (wax crystals are non-conductive), the samples were sputtered with a layer of 4ňm thick combination of gold and palladium (ratio: 60-40), which took about 5 minutes. The samples were attached to a double-sided adhesive carbon tape, which is conductive. The target-holder was made of stainless steel. The samples had to be dried to not pollute the SEM. Figure 4.3 shows the samples as placed on the target-holder.

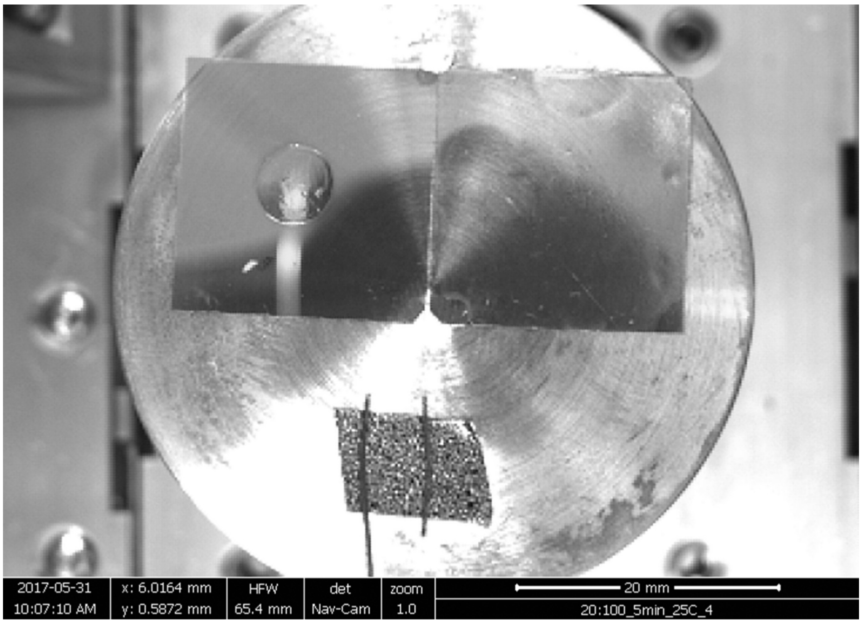


Figure 4.3: Target-holder of the SEM.

4.3.2 Results

In figure 4.4 an overview comparable to the structure in figure 4.2 is shown.

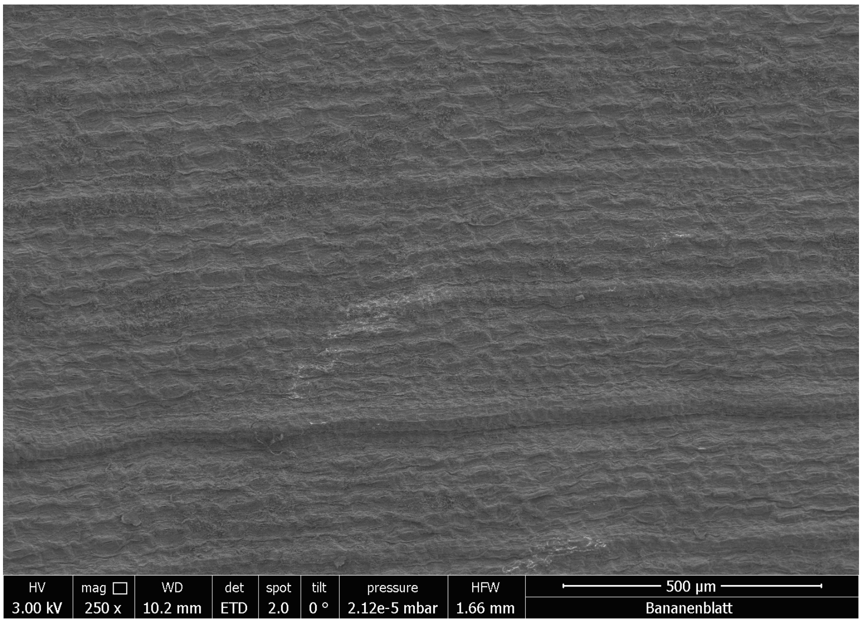


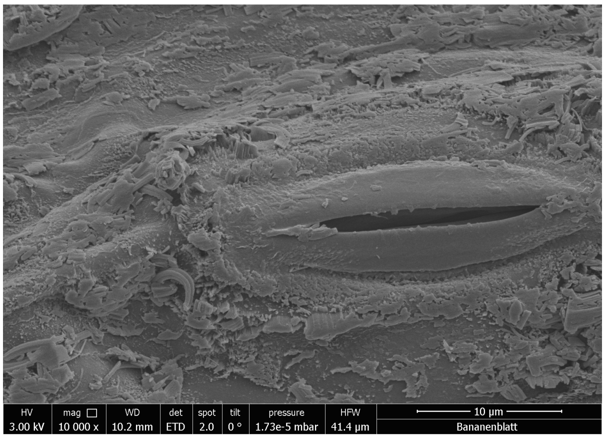
Figure 4.4: SEM Measurement of banana leaf (*Musa paradisiaca*)

Figure 4.5 was needed to see, if wax crystals could be ironed and if the structure is capable of being printed to certain surfaces. Therefore, different images of different resolutions and places on the surface were made. With these images it is clear, that the wax crystals are not suitable to be reprinted with the technique used by Zobel et al. Therefore the structure should have been quite flat in a certain direction, so that the material for the stamp-negative could be easily removed to imprint it on for example epoxy resin.

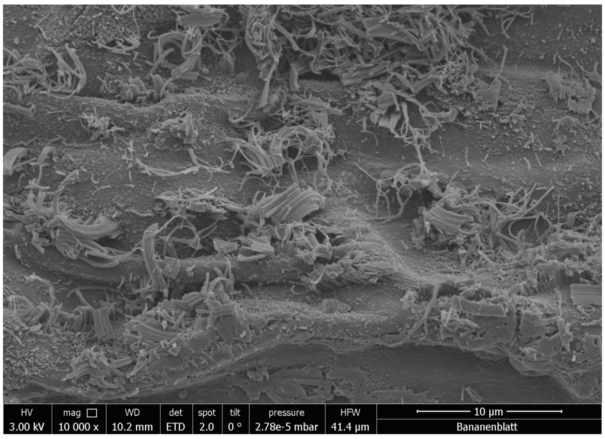
Figure 4.6 shows quite the same structure as it was measured with the optical microscope in as it can be seen in figure 4.2.

Here the same "problem" is obvious as that has already occurred with the banana leaf wax crystals. They are too complex to be printed, as it is clearly seen figure 4.7 (a-d).

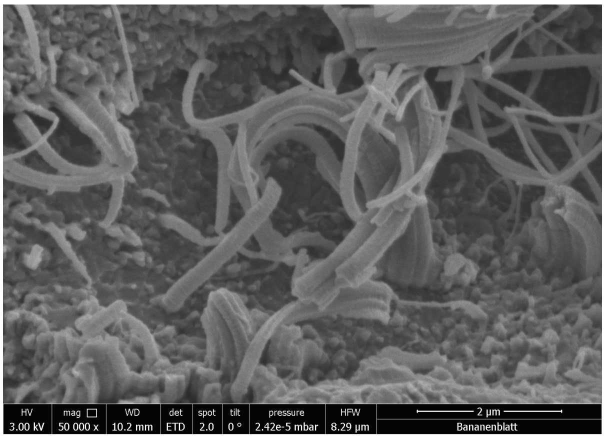
The last surfaces that were measured were two metal plates, one smooth and one rasped. Both were heated up until they reached temperatures of about 170°C, then the banana leaves were "rubbed" on them to simulate the ironing process.



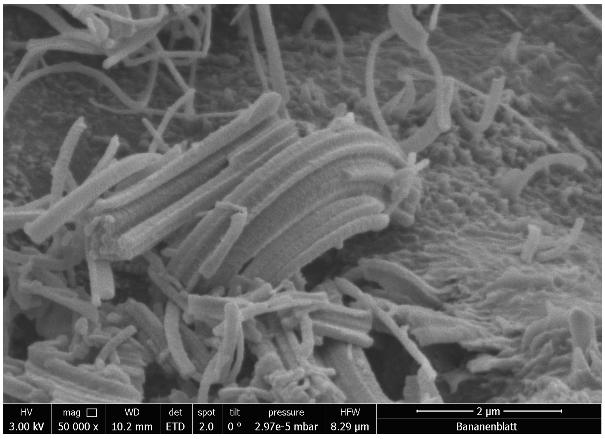
(a) banana leaf, 40 μm x 30 μm



(b) banana leaf, 40 μm x 30 μm



(c) banana leaf, 8 μm x 6 μm



(d) banana leaf, 8 μm x 6 μm

Figure 4.5: Measurement of banana leaf (*Musa paradisiaca*) using the SEM with different resolutions

In figure 4.8 it can be seen, that not much of the wax has been transferred to the surface. This might have a few reasons, which are now mentioned but not further investigated due to a limit of time for this thesis.

The first possible reason might be the temperature, which maybe should have been higher to ablate more of the wax. The second changeable factor is the time of cool down. Kerstin Koch et al. [9] have successfully tried to apply wax crystals through vapour depositioning, where they cooled down the sample very slow. As it is seen in figure 4.8

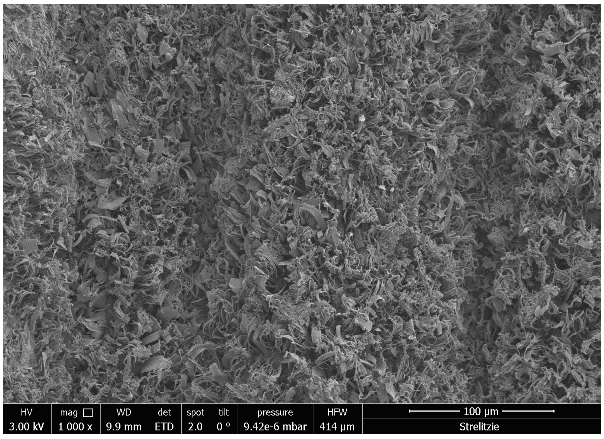
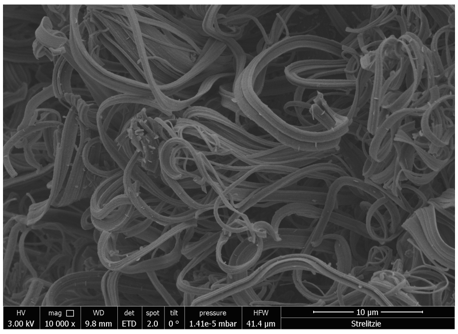
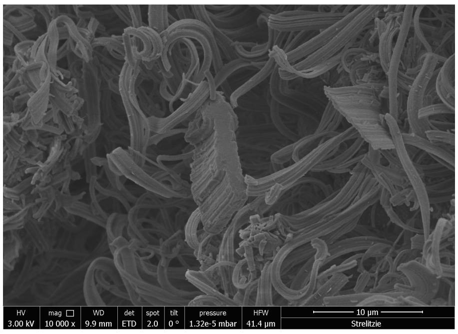


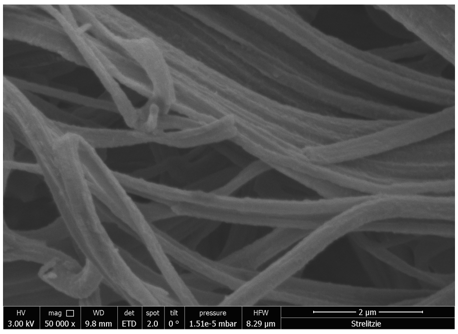
Figure 4.6: SEM measurements of bird of paradise blossom (*Strelizia reginae*)



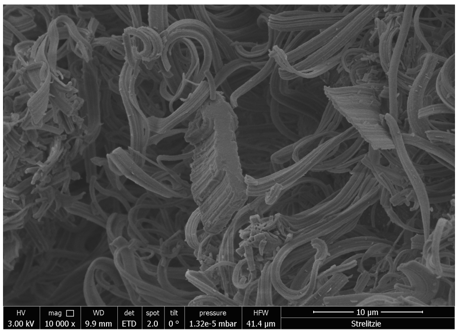
(a) bird of paradise, 40 μm x 30 μm



(b) bird of paradise, 40 μm x 30 μm

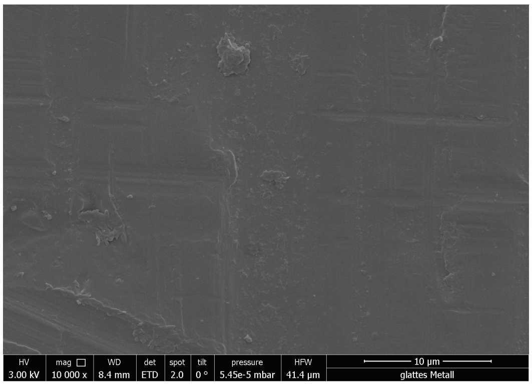


(c) bird of paradise, 8 μm x 6 μm

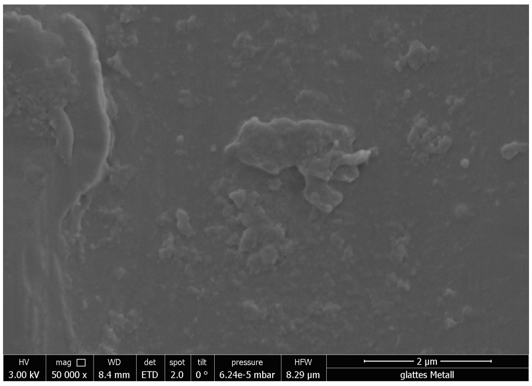


(d) bird of paradise, 8 μm x 6 μm

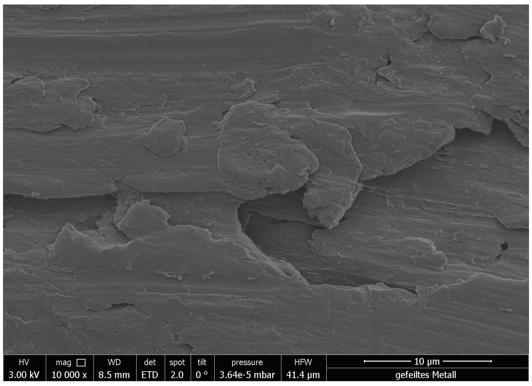
Figure 4.7: Measurement of bird of paradise blossom (*Strelizia reginae*) using the SEM with different resolutions



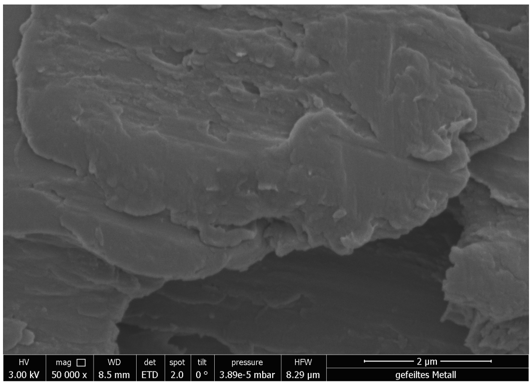
(a) smooth metal surface, 40 μm x 30 μm



(b) smooth metal surface, 8 μm x 6 μm



(c) rasped metal surface, 40 μm x 30 μm



(d) rasped metal surface, 8 μm x 6 μm

Figure 4.8: SEM measurements ironed metal surfaces

(c-d), the rasped metal could have ablated more of the wax than the smooth one has, so maybe by diversifying the roughness it could be investigated, which grade of roughness would fit best.

4.4 Wetability studies

In chapter 2.1.1 it is mentioned, that spiders use dust particles and other polluting particles to achieve attachment on certain surfaces, which could be prohibited by a superhydrophobic surface, where those particles can be easily washed away. Therefore the surfaces of banana leaf and the bird of paradise blossom were investigated on whether they have those properties.

This can be achieved by applying small droplets of water on the surface, taking a picture orthogonal to the object and measuring the contact angle. Surfaces are superhydrophobic when this angle exceeds 150°. In figure 4.9 and 4.10, where the complementary angles were measured, it can be seen that both surfaces have superhydrophobic properties, which makes them a matter of interest for further investigations of their wax crystals.

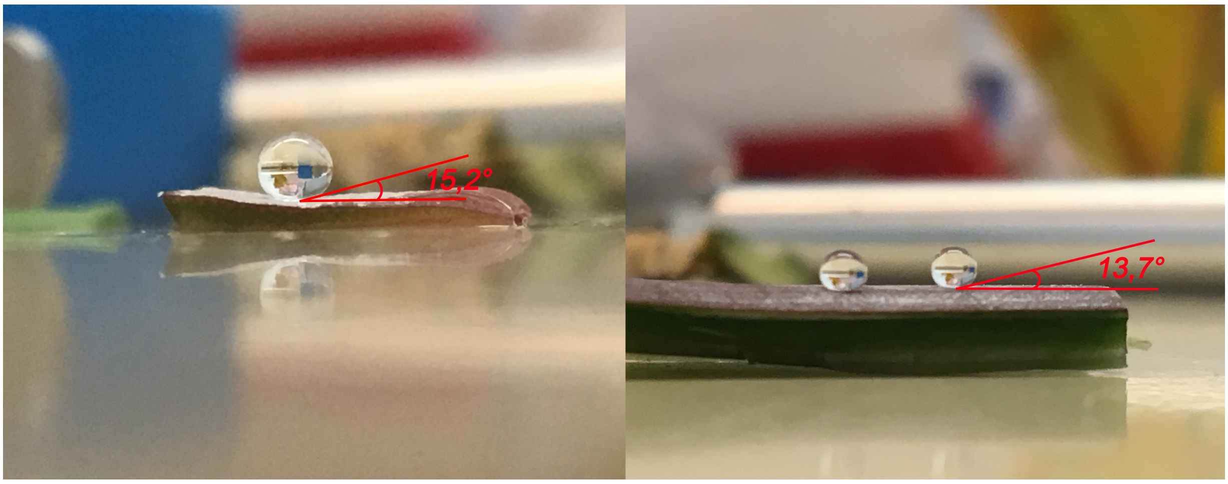


Figure 4.9: Wetability studies of the surface of bird of paradise blossom (*Strelizia reginae*).

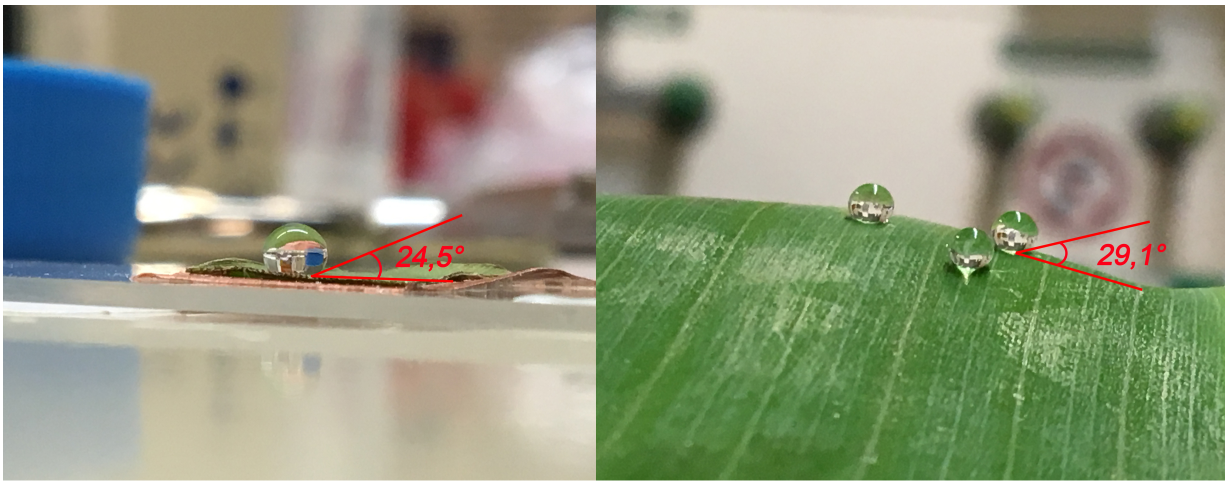


Figure 4.10: Wetability studies banana leaf (*Musa paradisiaca*).

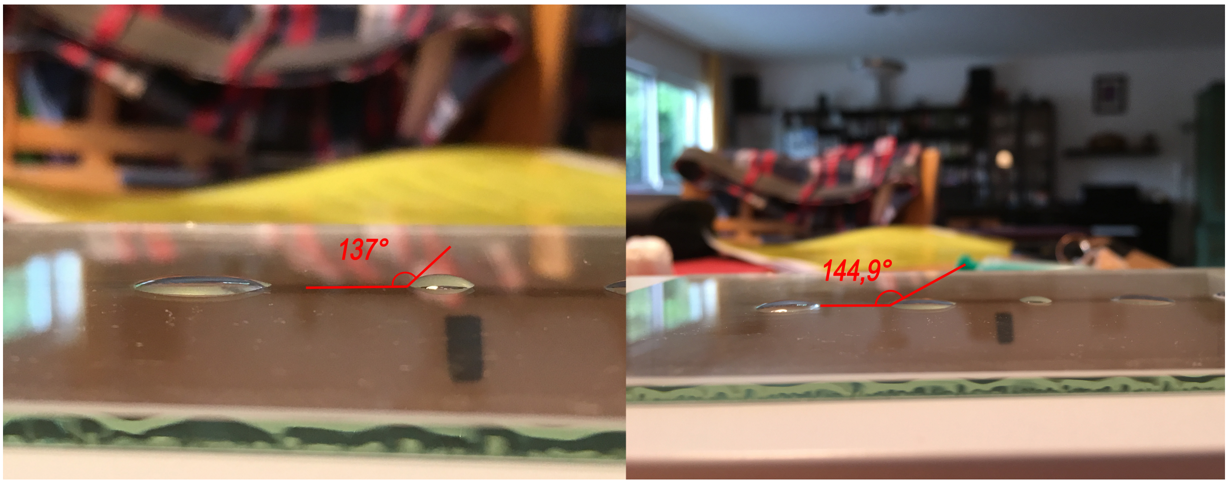


Figure 4.11: Wetability studies glass plate.

In figure 4.11, where the complementary angles were measured, it can be seen, that glass has hydrophilic properties, because the contact angle is lower than 50°. Compared to 4.10 and 4.9 the difference is clearly visible between a hydrophilic and a hydrophobic surface.

5 Conclusion and Outlook

Nanotechnology used in a responsible way is probably not as dangerous as some people might think, considering the fact that there are lots of structures with which humans get in contact everyday. Thinking of the problems this thesis is dealing with, there are some possibilities mentioned which could be investigated with a positive outcome and further on solve those problems.

The highest potential might be in the use of heating up synthesized wax crystals and letting them cool down on the destined area in a certain time, such as ironing or vaporising.

To use the imprinting way there has to be found a structure that is capable of being imprinted on the one hand, and it must not be toxic for humans and the environment on the other hand. Though it is currently the option with the lowest potential due to the unknown structures, it will have the highest potential once those missing "ingredients" are found, because then "nanocolors" could probably be painted like normal watercolors.

Not only would nano-structured colors find their field of usage as a useful insecticide, but also could they be applied on nearly every surface to give them additional and useful functions, such as reflecting certain wavelengths of light, which could be useful to cope with some of consequences the climate change, but this acquires more research to be done.

List of Figures

Bibliography

[1] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K Kim, Y.S. Lee, D.H. Jeong, M.H. Cho Antimicrobial effects of silver nanoparticles. *Biology and Medicine*, 95-101, 2007.

[2] M. A. Zoroddu, S. Medici, Alessia Ledda, V. Nurchi, J. I. Lachowicz and M. Peana Toxicity of Nanoparticles. *Current Medicinal Chemistry*, 3837-3853, 2014.

[3] S.N. Gorb, J.O. Wolff. Attachment Structures and Adhesive Secretions in Arachnids. *Springer*, 25-62, 117-140, 153-162, 2016.

[4] I. Grabe, S.N. Gorb, J.O. Wolff. Composition and substrate-dependent strength of the silken attachment discs in spiders. *Journal of Royal Society*, 1-9, 2014.

[5] Umweltschutz Zürich. Spinnennetze an Fassaden. www.umweltschutz.zh.ch, last visited: 06.08.2017, 2014.

[6] K. Koch, B. Bhushan, W. Barthlott. Multifunctional surface structures of plants: An inspiration for biomimetics. *Elsevier*, 137-178, 2009.

[7] A. Eichler-Volf, A. Kovalev, T. Wedeking, E.V. Gorb, L. Xue, C. You, J. Piehler, S.N. Gorb, M. Steinhart. Bioinspired monolithic polymer microsphere arrays as gerically anti-adhesive surfaces. *IOPscience*, 1748-3190, 2016.

[8] W. Barthlott, M. Mail, B. Bhushan, K. Koch Plant Surfaces: Structures and Functions for Biomimetic Innovations. *Springerlink*, 1-40, 2016.

[9] K. Koch, B. Bhushan, Y.C. Jung, W. Barthlott. Fabrication of artificial Lotus leaves and significance of hierarchical structure for superhydrophobicity and low adhesion. *Soft Matter*, 1386-1393, 2009.

[10] S. Zobel, W. õSalvenmoser, T. Schwerte, I.C. Gebeshuber, M. Schreiner. *Morpho peleides* butterfly wing imprints as structural colour stamp. *IOPscience*, 1748-3190, 2016.

[11] E. Meyer. Atomic Force Microscopy. *Progress in Surface Sience*, 1-17, 1992.

[12] Ludwig Reimer. Scanning Electron Microscopy: Physics of Image Formation and Microanalysis. *Springer*, 1-20, 1998.

[13] W. Barthlott, C. Neinhuis Purity of the sacred lotus, or escape from contamination in biological surfaces. *Springer*, 1-8, 1998.