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Natural Superhydrophobic Surfaces

Nanostructures and Wetting States on Collembola Cuticles

Thesis for the Degree of Philosophiae Doctor

Trondheim, December 2016

Norwegian University of Science and Technology Faculty of Engineering Science and Technology Department of Engineering Design and Materials



NTNU

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ISBN 978-82-326-2080-7 (printed ver.) ISBN 978-82-326-2081-4 (electronic ver.) ISSN 1503-8181

Doctoral theses at NTNU, 2016:368

Printed by NTNU Grafisk senter

Preface

This thesis was written at the Department of Engineering Design and Materials (IPM) at the Norwegian University of Science and Technology (NTNU) from autumn 2011 through autumn 2016, under the supervision of Professor Christian Thaulow. Funding was provided by the university (NTNU) as part of their focus program for the strategic research areas.

The work was conducted in close cooperation with Professor Hans Petter Leinaas of the Department of Biosciences (IBV) at the University of Oslo (UiO).

Experiments were performed at the various departments of the Norwegian University of Sciences and Technology (NTNU) except as noted. Sample preparation, machining of equipment, nanoindenter force microscopy and environmental scanning electron microscopy (ESEM) was performed at the Department of Engineering Design and materials. Contact angle goniometry was performed at the Department of Chemical Engineering. Live Collembola were held at the Department of Biology. Live Collembola were provided by Professor Hans Petter Leinaas at the Department of Biosciences at the University of Oslo and by Arne Fjellberg Entomological Research. Scanning electron microscopy, metal- and carbon coating was performed at the EM-lab of the Department of Material Science. Confocal fluorescence microscopy was performed at the Department of Physics. Focused ion beam (FIB) milling and imaging, scanning electron microscopy (SEM), atomic force microscopy (AFM) and freeze drying of samples was performed at NTNU Nanolab.

Acknowledgement

First, I would like to thank my supervisor Professor Christian Thaulow, whose enthusiasm and positivity was a continual source of inspiration. Throughout these five years he has earned my trust and respect many times over. It was Christian who introduced me to his friend, Professor Hans Petter Leinaas, the greatest co-author anyone could hope for. I am deeply thankful for the help; academic, practical and personal, from both of you.

I have shared an office, the "Room for Improvement" with many wonderful people over these five years. Jørn Skogsrud, who I am proud to call a personal friend, and not just a colleague. Inga Ringdalen and Christer Ersland, who made the office a fun and sociable room the first couple of years, and Anette Hagen and Brage Snartland, who did the same the last couple of years. For others who shared our office, Bjørn Rune Rogne, Marie Jørum, and the rest of my colleagues at the department of Engineering Design and Materials, thank you for five great years.

Several people outside of our department have provided great help, advice and company. There's a complete list of labs in the preface and there's at least one person I'd love to thank for each lab on that list. Special thanks to Henriette Vaagland, as well as everyone at NTNU Nanolab. I am also grateful for the contributions of some of the master students in our group: Henrik Olerud, Kristin Fjellvang, Runar Sandnes and Maxime Pujo.

Finally, my warmest thanks to my family and friends. My father, Roy Gundersen, sister Renate Gundersen, brother in law Christian Wangsnes, girlfriend Marianne Dønnessen. And my friends, some from years past, some from my years at university and some more recent. Thank you for making these years enjoyable, thank you for believing in me, and thank you for being there when I needed you.

Abstract

Highly water repellent surfaces, known as superhydrophobic surfaces, have a range of potential applications including self-cleaning, drag reduction and air-retention. The first studies of the superhydrophobic effect was on naturally occurring surfaces, and it was found that superhydrophobicity requires a combination of micro- or nanosized surface structuring and a suitable surface chemistry. Understanding natural superhydrophobic surfaces and their wetting mechanisms can lead to inspiration for the superhydrophobic surfaces of the future. The cuticles of the tiny arthropod Collembola have long been known to be superhydrophobic. The cuticles of Collembola are covered in nanosized surface structures, some of which feature overhang. This compilation thesis combines three articles studying the cuticle structure and wetting of Collembola, they are presented along with a comprehensive introduction to wetting, contact angles, natural and man made superhydrophobic surfaces, providing the necessary context for the three articles. The first article provides a study of twelve different species of Collembola covering a large range of habitats. Ten of these twelve species were found to be superhydrophobic. A detailed comparison of surface structure and wetting properties concludes that there was no direct link between overhanging surface structures and superhydrophobicity on Collembola cuticles. It is further demonstrated that the classic wetting models of Wenzel, Cassie and Baxter are not able to predict the observed contact angles. The second article reports a significant seasonal change in the wetting of the cuticle of a specific species of Collembola. Cryptopyqus *clavatus* is superhydrophobic in winter, but not in summer, and this change in the wetting characteristics is not accompanied by a corresponding change in the cuticular surface structure. The classic wetting models describe a close link between surface structure and wetting characteristics and can not predict this seasonal change. The third article revisits data from the first two articles and succesfully applies a solution presented by Zheng et al. where the three-phase line tension is taken into account. The small size scale of the surface features on Collembola cuticles means that the ratio between the length of the three-phase line and the size of the surface area is very large, which ensures that modest variations of the three-phase line tension can explain large variations in the wetting properties.

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Tension 123

List of Symbols and Abbreviations

A	Area
A_s	Solid surface area
b	Half length, granule top, see Article 1: figure 6
d_0	Edge to edge distance between granules, see Article
	1: figure 6
f	Area fraction, Cassie-Baxter and Zheng models
f_i	Area fraction of surface i
F	Force
g	Gravitational constant
G	Surface free energy
G_0	Free energy in a system where all matter has the properties of the bulk
ΔG	Change in surface free energy
$\frac{1}{h}$	Height
$h(\theta^*)$	Function of the apparent contact angle, equations 31
(*)	and 32
Η	Granule height
l	Length
l_{cr}	Intrinsical chemical length, Zheng model
L	Boundary length
m	Mass
p	Centre to centre distance between granules, see
	Article 1: figure 6
Р	Number of edges in the closest equivalent polygon,
	Article 1
ΔP	Pressure difference
r	Roughness factor, Wenzel model
R	Radius
R_0	Initial radius
R_1, R_2	Radii of curvature
S	Spreading parameter
S	Roughness scale, Zheng model
U	Energy barrier, equation 31
V	Volume
w	Weight
W	Width
W_{AB}	Work of adhesion

α	Tilting angle
γ	Free energy per surface area
γ_{LV}	Surface tension of a liquid-vapour interface
γ_{SL}	Surface tension of a solid-liquid interface
γ_{SV}	Surface tension of a solid-vapour interface
γ_{SV}^0	Surface tension of solid-vapour interface, for a surface
~ .	in equilibrium with its own vapour
θ	Contact angle
θ_0	Inherent contact angle, articles 1 and 3
θ^*	Apparent contact angle
θ_A	Advancing contact angle
$ heta_R$	Receding contact angle
$\theta_{r,\mathrm{Choi}}$	Receding contact angle, Choi model
θ^*_{CB}	Apparent contact angle, Cassie-Baxter model
$ heta_W^*$	Apparent contact angle, Wenzel model
$ heta_Z^*$	Apparent contact angle, Zheng model
$\Delta \theta$	Contact angle hysteresis (CAH)
$\Delta \theta_{\mathrm{Dufour}}$	Contact angle hysteresis, Dufour model
λ	Three-phase line tension
ho	Density
$\Delta \rho_{LV}$	Density difference between the liquid and vapour
	phase
Φ	Apparent contact angle, figure 10
	Applyzic of the expillent profile around a cylinder
ADSA	Avisymmetric drop shape analysis
ADSA	Axisymmetric drop snape analysis
CAH	Contact angle hystoresis
FSFM	Environmental scanning electron microscopy
FIR	Focused ion beam
NI-AFM	Nanoindenter atomic force microscopy
PTFE	Polytetrafluoroethylene
SEM	Scanning electron microscopy
SG	Secondary granules
SLIPS	Slipperv liquid-infused porous surfaces
	Support inquire intraster porous surfaces

1 Overview

1.1 Motivation

Superhydrophobic surfaces are highly water repellent, when water comes into contact with such a surface it forms round droplets that roll off with minimal resistance. This leads to several other functional properties; selfcleaning, drag reduction and air retention. These properties have potential commercial applications ranging from clothing, to solar cells or food processing. Superhydrophobic surfaces have therefore been subjected to increasing interest in recent years. The processes of evolution has led to a wide selection of different surfaces in nature, in species adapted to different environments. Some of these surfaces, such as the lotus leaf, the pitcher plant, and the cuticles of arthropods, have been the inspiration for biomimetic, superhydrophobic surfaces. Biomimetics, the inspiration from and imitation of examples from nature, has been a central method in the study of superhydrophobicity. The exact nature of and mechanism behind natural water repellent surfaces is therefore of great interest beyond the field biology. The cuticles of Collembola have long been known to be highly water repellent, but the mechanism of this water repellency has not been known, nor has quantitative measurements of the wetting of Collembola been made.

1.2 Methods

The experimental work of the thesis included methods normally used in a wide range of scientific fields; from biology through physics, surface chemistry and nanotechnology.

Collembola are tiny arthropods, ranging in size from a few millimeters down to under one millimeter depending on the species. The experiments of this thesis necessitated keeping cultures of live Collembola to provide fresh samples. Work with the Collembola would have been impossible without the close cooperation of Professor Hans Petter Leinaas, of the University of Oslo, who provided initial cultures of Collembola and training in the keeping and handling of live Collembola. Live cultures were kept at the department of Biology at NTNU. Collembola were kept on moist plaster of Paris to ensure a constant high humidity, they were fed with green algae gathered from trees on campus. Hans Petter Leinaas made the selection of which species to study, with an intention to cover different habitats, families and types of surface structures. The Collembola were originally collected in the field by Professor Hans Petter Leinaas and Dr. Arne Fjellberg at various locations in southern Norway, with the exception of one lab stock from the Azores.

Contact angles were measured using the sessile drop method at the Paper and Fibre Research Institute at NTNU. The small size of Collembola caused extra concerns when performing contact angle measurement. Freshly killed samples were used for these experiments. Surface structures were studied with scanning electron microscopes (SEM) at the NTNU EM-lab, NTNU Micromechanical lab, and NTNU Nanolab. Samples were freeze-dried prior to mounting and sputter coated with a thin conductive layer before study in regular SEM. Some imaging done in low vacuum SEM was done on uncoated samples. Cross sections of specific surface features were cut using a focused ion beam on samples with a thick protective coating. The surface structures were also studied using atomic force microscopy at NTNU Nanolab and nanoindenter force microscopy at NTNU Nanomechanical lab. Force microscopy, along with the imaging of cross sections, provided accurate height data of the surface structures. The extent of the wetted area on submerged Collembola was visualized using Nile Red dying and subsequent fluorescence microscopy at the NTNU Department of Physics.

Some of the methods used were presented on national broadcast television on a popular science show called Schrødingers Katt, along with an introduction on where Collembola can be found at the seashore and possible future applications of self-cleaning surfaces.

1.3 Theoretical Background

A comprehensive introduction to wetting and superhydrophobic surfaces is given. The fundamental theories of the wetting of solids, contact angles and contact angle hysteresis are presented, before examples of naturally occurring and synthetic superhydrophobic surfaces are discussed. The intention is to provide a context for the three articles of the thesis.

1.4 Article Summaries

Article 1

"Surface Structure and Wetting Characteristics of Collembola Cuticles", Håkon Gundersen, Hans Petter Leinaas, Christian Thaulow, PLoS ONE, vol 9, no. 2, p. e86783, 2014 [1]

Quantitative measurements of the wetting characteristics of a selection of Collembola cuticles are presented and an effort is made to correlate the wetting performance and surface structuring of the cuticles from a combined technical and evolutionary point of view. It is concluded that superhydrophobicity is a common, but not universal trait among Collembola, that there is no direct link between the presence of overhanging surface structures and superhydrophobicity in Collembola and that the classical Wenzel and Cassie-Baxter models of wetting are not able to predict the observed contact angles on Collembola cuticles.

Article 2

"Seasonal change in the wetting characteristics of the cuticle of the Collembola *Cryptopygus clavatus* (Schött, 1893), Håkon Gundersen, Christian Thaulow, Hans Petter Leinaas, Zoomorphology, vol. 134, no. 2, pp. 211-218, 2015 [2]

A seasonal change is reported in the wetting properties of the Collembola species *Cryptopygus clavatus*, which is superhydrophobic when adapted to winter conditions and wetted when adapted to summer conditions. *C. clavatus* spends the summer submerged, with no visible plastron, grazing on algae in the marine littoral zone. This significant qualitative change in wetting properties was not accompanied with a large change in the surface structure of the cuticle. The change in wetting properties can therefore not be explained by mechanisms that depend on the surface structure.

Article 3

"Collembola Cuticles and the Three-Phase Line Tension", Håkon Gundersen, Hans Petter Leinaas, Christian Thaulow, to be published in the Beilstein Journal of Nanotechnology Data from the first two articles is revisited and a comparison is made to results predicted by a wetting model introduced by Zheng et al. which accounts for the three-phase line tension. Including the three-phase line tension makes the wetting model size scale dependent, which is crucial since Collembola cuticular structures are in the sub-micron scale and therefore have very large three-phase contact lines in comparison to their surface area. The contact angles observed on Collembola cuticles, including the seasonal change of *C. clavatus* can be predicted by assuming a three-phase line tension in the range of 2×10^{-9} N to 2×10^{-8} N, which is within the commonly reported range of magnitude for the three phase line tension.

5

2 Wetting and Superhydrophobic Surfaces

2.1 Introduction

Superhydrophobic surfaces are so water repellent that water droplets stay spherical in contact with these surfaces and easily roll off with minimal resistance [3]. This non-wetting behavior leads to several other interesting phenomena [4,5]: self-cleaning [6–8], drag reduction [9], air-retention [10–12] and plastron respiration [13,14]. These phenomena have a range of potential commercial applications in as diverse fields as solar cells, clothing, oil, gas and maritime industries, and food processing [15,16].

Superhydrophobic surfaces have been known for a long time [17], primarily from natural surfaces [18]. Increasing interest in recent decades has moved the focus from just studying superhydrophobic surfaces found in nature; new interests include replicating natural surfaces, synthesizing entirely new surfaces and formulating models that adequately explain the wetting behavior of these surfaces [4,19,20]. Superhydrophobic surfaces in nature include plant leaves [6,7,11,17,21–24], feathers [4,5,17], insects and other arthropods including Collembola [12, 25–28] and some soils [29]. The hierarchically structured surface of the leaves of the lotus plant (*Nelumbo nucifera*) is perhaps the most well known of these [22]. Man made superhydrophobic surfaces include micro- and nanostructured surfaces [8,30], porous and nanoporous media [31,32] and textiles [18,33,34].

A superhydrophobic surface is frequently defined as a surface that displays a contact angle with water of at least 150° and a contact angle hysteresis of no more than 10° , but a general consensus has not been reached and various definitions have been and are being used in literature [3, 4, 19, 35]. Due to a lack of consensus on the names and definitions for superhydrophobicity, the property is also known by many names: Lotus-Effect[®] [36, 37], ultrahydrophobic [38–40], hydrofuge [25, 27, 41], non-wetting [42] and waterrepellent [26, 43, 44] are among them.

Since the definition of a superhydrophobic surface is based on the quantities of contact angle and contact angle hysteresis, great care is taken in presenting these terms here [3, 4, 19, 20]. Contact angle hysteresis, in particular, is not trivial, and the explanation of contact angle hysteresis phenomena necessitates a discussion of a wide range of wetting phenomena which are further compounded by the often significant topographies of superhydrophobic surfaces. Since the repelling of water seems to be highly dependent on contact angle hysteresis, it is deemed necessary to discuss the phenomenon in detail [37–39, 45]. Furthermore, two different theories have come to dominate the field of superhydrophobics, the Wenzel model [33] and the Cassie-Baxter model [34], despite the fact that they have both have significant shortcomings [46–52]. These theories are nevertheless discussed on the basis that the modern literature on superhydrophobic surfaces can not be understood without knowledge of these theories.

This work contains a detailed introduction into contact angles and various wetting phenomena, including contact angle hysteresis, on smooth and textured surfaces in section 2.3. The various properties and possible applications of superhydrophobic surfaces, as well as a definition of the term itself, and a discussion on why the definition itself is not trivial is presented in section 2.2. Examples of naturally occurring superhydrophobic surfaces are given in section 2.4, while examples of synthetic superhydrophobic surfaces are given in section 2.5. Note that neither of these sections is exhaustive, but should present a selection of some of the more important examples. Finally a discussion of some special cases that resemble superhydrophobic surfaces, but are really something else, is provided in section 2.6. Throughout the work it is assumed that the wetting liquid is water and the surrounding The laws of wetting are the same for other liquids, but the gas is air. nomenclature changes if, for example a liquid hydrocarbon is considered. Surfaces with large contact angles in contact with non-polar liquids are referred to as oleophobic [32, 53] or lipophobic [30, 54], surfaces that repel both polar and non-polar liquids are referred to as omniphobic [55, 56] or amphiphobic [54].

2.2 Superhydrophobicity

2.2.1 What is a superhydrophobic surface?

As the name implies, a superhydrophobic surface is one which repels water, it derives from the words *hydro* (Gr. water), *phobos* (Gr. fear) and *super* (Lat. over). In general a superhydrophobic surface will repel water, staying dry by expelling the water as round droplets which quickly roll or slide away. The word has been used to describe several different types of surfaces and there is no general consensus on the exact meaning of it. To compound matters further, several synonyms have been used to describe the same surfaces: Lotus-Effect^{®1} [36,37], ultrahydrophobic [38–40], hydrofuge [25,27,41], nonwetting [42] and water-repellent [26,43,44] are among them. See [35] for a study of the nomenclature of the field. Here the word "superhydrophobic" is used because it seems to be the most common term in recent publications, and because it is less ambiguous than everyday sounding terms like nonwetting or water-repellent. However, as pointed out by Gao and McCarthy, making light of nomenclature can lead to confusing, or apparently random, usage of poorly defined terms [45]. Care will therefore be made to define exactly what "superhydrophobic" means in the context of this work.

What defines a superhydrophobic surface, what properties does it have and how can this be measured? Water forms round droplets on a superhydrophobic surface (i.e. it displays a high contact angle) and it readily rolls or slides away; i.e. either the contact angle hysteresis $(\Delta \theta)$ is low, or the tilting angle (α) is low, these are related, but the relation relies on several other parameters [3, 4, 20, 45, 57]. Some superhydrophobic surfaces also display the property of air-retention, in which a thin film of air is retained upon submersion, visible as a silvery layer surrounding the superhydrophobic surface (the so-called "Salvinia effect" or "Plastron effect" see sections 2.4.3 and 2.4.4) [11]. Superhydrophobic surfaces also display reduced drag in moving water, this is especially true for air-retaining surfaces [4]. There are also surfaces with very high advancing contact angles, but also fairly high contact angle hysteresis, which results in round, well-defined droplets, that stick to the surface without rolling off, this is sometimes referred to as the "petal effect" [58]. Finally there are surfaces that display near zero contact angle hysteresis and moderate contact angles, which means water readily drains away, but doesn't form round droplets [56]. The term superhydrophobic has been applied to all of these effects. A single term could not, and should not, describe all of these types of wetting behavior, hence a clear definition of the term superhydrophobic is needed.

"Originally any surface with a water-contact angle greater than the maximum observable on a flat surface ($\approx 120^{\circ}$, for PTFE) was considered to be «ultrahydrophobic»" [20]. Today a more common definition seems to be surfaces displaying a contact angle of 150°, or more [3, 4, 19, 20]. Some researchers emphasize that the contact angle hysteresis should also be low, as the contact angle hysteresis seems to be more important for the purpose of how easily a surface sheds water [37–39,45]. The maximum for the contact angle hysteresis is often just stated to be "low", when a value is stated it

¹Registered Trademark, CTM numbers: 001552710 and 001936483

is frequently 10° [4,20]. It should be noted that these values are *arbitrarily chosen*, there is no fundamental difference between a system with a contact angle of 149° and one with a contact angle of 151° , one is simply slightly more hydrophobic than the other. In contrast, the difference between an inherent contact angle below 90° and one above 90° is readily apparent as this is were the cosine of the angle changes sign. Which is crucial in the case of Wenzel's equation (eq 23). For this work a *superhydrophobic* surface is defined as a surface displaying a contact angle of at least 150° and a contact angle hysteresis of no more than 10° .

2.2.2 Properties and Applications of Superhydrophobic Surfaces

Water Shedding

The main property of superhydrophobic surfaces is that of shedding water. Draining away water quickly, leaving a dry surface behind is desirable in several industries. Superhydrophobic clothing would for example allow the wearer to stay dry and warm in rainy weather, without necessitating waterproof fabrics that frequently feature limited breathability. Ice accretion would also likely be reduced for a surface with excellent water shedding abilities, which could lead to great savings for air-lines and offshore operators. However, though some superhydrophobic surfaces also shed ice more easily, there is no general link between water shedding and reduced ice accretion, which is a much more complex problem [19, 59].

The water shedding effect of superhydrophobic surfaces is due to the low contact angle hysteresis, which determines the energy cost associated with a drop moving along the surface [37]. Even completely wetting surfaces can display excellent properties when it comes to draining away water. In windshields a complete wetting is generally more desirable than a superhydrophobic surface. This is because complete wetting (film formation) is not as visually disturbing as the formation of distinct drops, when both drain away quickly [3,4].

Self-Cleaning

The self-cleaning ability, of which the most common example is the Lotus (section 2.4.3) [22], is the ability to wash away impurities with the water

droplets as they roll of the superhydrophobic surface. If a surface contaminated with dirt is subsequently subjected to a light spray of water, the water will form spherical drops which quickly roll of the surface taking dirt with them, see figure 1. Self-cleaning would be desirable in a wide variety of products including optics (glasses and cameralenses), solar cells (where accretion of dirt reduces the energy output), windows, clothing and house paint. Self-cleaning surfaces also have the ability to shed water, but the opposite is not always the case [4, 19].

Self-cleaning generally requires both a high contact angle and a low contact angle hysteresis. It is made possible because the dirt adheres more easily to the drop than the substrate. Even hydrophobic particles can be cleaned away in this way. This is because only weak Van der Waals forces bind the particle to the surface, while much stronger capillary forces bind it to the droplet. Most self-cleaning surfaces are however soiled by non-polar liquids, which also destroys the self-cleaning effect [4, 6, 7, 36, 37].



Figure 1: The self-cleaning surface of a lotus leaf. Reproduced with permission, Copyright 2009 Elsevier, Progress in Materials Science. [7]

Air-Retention

Some surfaces have the ability to retain a thin film of air, which is visible as a silvery film on the submerged solid. This is seen for some plants (e.g. water ferns [11]), insects (e.g. water bugs [12]) and various other animals (e.g. springtails [28]). An example of air-retention is shown in figure 2 where the intertidal midge *Clunio marinus* forms an air film which even extends between extremities. The extremities that penetrate the larger bubble are still covered by a thin film of air [10].



Figure 2: A retained air film on a submerged specimen of the intertidal midge *Clunio marinus*, a) ventral and b) dorsal view. Reproduced with permission, Copyright 2009 Springer, Naturwissenschaften. [10]

A retained air film is interesting primarily in three capacities. Firstly, such an air film can provide respiration under water, this is covered in section 2.4.4. Second such a film may greatly reduce the corrosion of the surface. The surface itself is not really in contact with water, it is in contact with its own air film, effectively negating corrosion. The air film itself must be stabilized by hair-like structures, which are in contact with the water, but the base substrate stays dry under water. The shape and size of these hairs is illustrated in figure 26. Finally, surfaces with retained air films have the possibility to greatly reduce drag [12,19,60], see next section. The intertidal midge (figure 2) is an animal that utilizes a retained air-film primarily for drag reduction, not for respiration [10].

Drag Reduction

A solid moving relative to a fluid experiences a drag force acting in the opposite direction, the result of this is a net energy cost involved in moving an object through a fluid, or a fluid through a pipe or channel. The no-slip boundary condition is a central principle for this field. The condition states that at the boundary between the fluid and the solid, that is at the liquid-solid interface to use the terms of section 2.3, the flow velocity is zero. The

principle was mainly established by the works of George Stokes, Day gives a comprehensive overview of context and literature [61]. The theoretical basis of no-slip and slip flow was covered by McHale [62].

Some superhydrophobic surfaces have shown greatly increased flow rates compared to those that are predicted by the classical models. To accommodate for this the no-slip boundary condition must be revised by introducing a *slip length*. The slip length is defined as the length of the intercept along the axis orthogonal to the interface when a tangent line is drawn along the velocity profile at the interface, shown as b in figure 3 [9].



Figure 3: Velocity profile between two solid surfaces with and without slip. Reproduced under the creative commons 2.0 license, Copyright 2011 Bhushan. [9]

It was shown by McHale et al. [62] that superhydrophobicity alone is not enough to achieve significant drag reduction, a retained air film is also necessary. The limits of drag reduction from a retained air film was calculated to 20 - 30% for a sphere covered by an air film. The drag is reduced because the shear forces induce circulation of air within the air film. There are two competing effects that determine the optimal air film thickness for reducing drag. A thin film will limit the drag reduction by limiting the internal air volume that can be set into circulation. A thick film will increase the cross-section of the system and increase the drag.

2.2.3 Strategies for Achieving Superhydrophobicity

There are three main requirements for making a surface superhydrophobic. The surface chemistry must be such that the inherent contact angle of the system is 90° or more, i.e. the surface must be hydrophobic. There must be a certain roughness that enhances the natural hydrophobicity of the surface. The system must assume the Cassie-Baxter wetting state, to ensure a low enough contact angle hysteresis. Not all of these requirements must be fulfilled to achieve a superhydrophobic state, but a combination of the three results in more stable superhydrophobic surfaces [3,4,19].

A hydrophobic surface chemistry can be achieved in one of two ways. Either, the substrate used can be hydrophobic, or it can be coated in a hydrophobic material. The latter is often preferred in lab scale experiments, coatings with fluorinated polymers or silanes are common. Alternatively, PTFE and similar materials can be used as a substrate, without further coating. Naturally occurring surfaces are often covered with a layer of wax, which provides the necessary hydrophobicity. In some cases that wax self-assembles into crystals, thus improving roughness as well [4,7,63].

Roughness can be achieved in many ways, the various strategies includes fractal materials, selective etching, lithographic methods, self-assembled structures, deformation of polymer films, deposition and copying other rough surfaces through imprint techniques. Lithographic methods remains as one of the most common techniques in the lab. [4] Naturally occurring surfaces often receive their roughness from the epidermal cells, through cuticular folds, concave or convex cells, sometimes forming papillose or hairlike structures. [7] Insects often feature sub-cellular hairs, microtrichia, as a part of the cuticle [63].

The criteria for when the Cassie-Baxter state is favored are described in section 2.3.6. A superhydrophobic surface can either be constructed in such a way that the Cassie-Baxter state is the most energetically favored, or it can be stabilized by maximizing the wetting transition energy barrier. A surface must be very rough and have a high inherent contact angle to ensure that the Cassie-Baxter state is the favored wetting state. This can be achieved with hierarchical roughness, which is common in nature and increasingly common in the field of biomimetic synthetic superhydrophobic surfaces. A hierarchical surface is one that displays roughness on more than one scale, figure 4 gives a schematic overview. [4, 36, 37, 64, 65]

Hierarchical roughness is usually achieved through the combination of several methods. Such as combining microscale lithography with a spray coating with nanotube composites [8], by combining microscale lithography with self-assembled wax crystals [66], or by combining a pattern based on self-assembling patterns of microscale spheres with thermal fracture [30]. Natural surfaces achieve hierarchical roughness through a combination of cellular and subcellular structures, or by combining one scale of structures with self-assembling waxes [7,63].



Figure 4: Schematic overview of the wetting of hierarchical surfaces. Reproduced with permission, Copyright 2011 Elsevier, Progress in Materials Science. [4]

Alternately, the Cassie-Baxter state can be stabilized through different means. This can be done with reentrant geometries, that is, structures with overhang. The overhang represents a large energy barrier against wetting transition because the three-phase line must move quite far past its most stable point before it reaches a new energy minima [25, 28, 53, 55, 67]. Tall, slender pillars are also a way to ensure transition stability [68]. In nature these pillars are hair-like structures that often feature a curve, bend or inclination, such that a surface of parallel fibers is presented against the liquid. Flexible pillars that buckle under load can provide a similar structure. [24, 63, 69]

As previously mentioned, a surface need not fulfill all of these criteria to achieve a superhydrophobic state. Hosono et al. have presented a superhydrophobic surface based with a hydrophilic inherent contact angle [70]. Bico [71] presented a surface with contact angles of $\theta_A/\theta_R = 170^{\circ}/155^{\circ}$ despite having a roughness factor of only $r \approx 1.3$, this was done by ensuring a low area fraction of contact f and a Cassie-Baxter wetting state. Finally, a contact angle of 150° or more is also achievable in the Wenzel wetting state, provided that both the inherent contact angle and the roughness factor are high. For a fluorinated surface with $\theta = 120^{\circ}$, an r > 1.7 is required (from equation 23) though the contact angle hysteresis will likely be high for such a system. Fulfillment of all three criteria do result in more stable superhydrophobicity however.

2.2.4 Perfect Hydrophobicity

The theoretical limit for non-wetting is a contact angle of 180° , as angles larger than this would not make geometric sense. If a system were to exhibit contact angles of $\theta_R = \theta_A = 180^{\circ}$ that would also mean the contact angle hysteresis would be zero; such a system could be referred to as perfectly hydrophobic. A system displaying these properties would have no effective attraction to water.

A contact angle of 180° was predicted by Bartell and Shepard [47] for surfaces consisting of erect fibriles, as can be found in e.g. feathers. Feathers display very high contact angles and low contact angle hystereses, but so far no feather has been observed to display perfect hydrophobicity. Gao and McCarthy [16, 72] have reported surfaces with contact angles of $\theta_R =$ $\theta_A = 180^{\circ}$ on two different occasions. The first with a wafer treated with a toluene solution of CH₃SiCl₃, the second was a compressed sample of a commercially available solid lubricant. The first has been referred to as "Lichao's surface" in later litterature [63].

2.2.5 Omniphobicity

Recent work on non-wetting surfaces have expanded to include the repelling of non-polar liquids. Achieving low contact angles with non-polar liquids is generally more difficult than doing the same with water because water has a remarkably high surface tension as compared to non-polar liquids. This serves to push Young's equation (eq 5) towards low contact angles. Surfaces that repel non-polar liquids are referred to as oleophobic or lipophobic, while surface that repel both polar and non-polar liquids are referred to as omniphobic or amphiphobic. The general approaches to omniphobic surfaces bears semblance to those that attain superhydrophobic surfaces. Tuteja et al. [53, 55], Cao and Gao [32] have focused on reentrant geometry in their work. Yue Li et al. [30] used a surface where pores surrounded by fractal walls provided a combination of high roughness and stable air traps. Huanjun Li et al. [54] achieved an omniphobic surface by coating an array of fluorinated aligned carbon nanotubes, the array of nanotubes provide a combination of extreme roughness, long slender pillars and reentrant geometry. Finally Wong et al. [56] used a porous media to contain a film composed of a liquid that was immiscible with the test liquid.

2.3 Wetting and Contact Angles

When a drop of liquid is placed on a flat solid surface it may either spread out and wet the surface or remain as as a drop with a defined angle of contact with the solid surface [73]. Such drops attains a shape that minimizes the free surface area and forms spherical caps on the solid surface [57,74]. Where these spheres are sectioned is determined by the relative wetability of the solid, which results in a *contact angle* (θ) that can theoretically range from 0° to 180°, representing complete spreading or perfectly spherical drops respectively [75]. Values near the extremes of this range are increasingly difficult to measure and are rarely reported. Extreme values are therefore the subject of some controversy, Adamson and Gast [73] state that "...a contact angle of 180° is fundamentally impossible..." while Gao and Mc-Carthy [16, 72] have reported the synthesis of a material that exhibits a contact angle with water of 180° at two different occasions.

Large drops will be depressed by gravity and therefore have a flattened appearance. A model for calculating the geometry of drops flattened by gravity was presented by Taylor [76]. The flattening of a drop by gravity will not affect the contact angle directly, which can still be observed at the contact line of puddles on smooth solids. Small drops (droplets) retain a spherical shape due to the different scaling of surface tension and weight with respect to size; a small drop radius will render the gravitational force negligible compared to the surface tension [3,74]. Droplets shaped as spherical caps are assumed in this work.

Many different terms have been used for systems exhibiting contact angles of different magnitudes, resulting in a certain amount of confusion [35,74]. Systems that exhibit a contact angle of 0° can be referred to as wetting, or completely wetting. The definition of terms describing larger contact angles is more ambiguous and a general consensus has yet to be reached. A few terms have become common in recent literature and the following terms will be defined for use in this work. Systems exhibiting contact angles below 90° are referred to as "hydrophilic", this includes wetting systems. Systems exhibiting contact angles above 90° are referred to as "hydrophobic". Systems exhibiting very high contact angles ($\theta > 150^\circ$) are referred to as "superhydrophobic". The contact angle (θ) of a droplet is illustrated in figure 5 along with the relative magnitudes of the contact angle for hydrophilic, hydrophobic and superhydrophobic systems.

The term three-phase contact line warrants a short introduction. A drop



Figure 5: The contact angle θ and examples of a "hydrophilic", "hydrophobic" and "superhydrophobic" surface.

shaped like a spherical cap will have a circular interface with the substrate. The Circumference of this interface is known as the three-phase contact line as it is the line where the three different phases of the system are in contact, the vapor, the solid and the liquid. When the substrate is not smooth, but has a certain topography, the contact line will no longer be a perfect circle, but will follow the topography instead. The contact line always refers to the line where the three phases actually meet, however tortuous that line may be, and not to the circumference of the projected circle on the substrate. The importance of this distinction will become clear in the sections concerning rough surfaces.

2.3.1 Surface Energy and Surface Tension

Surfaces are associated with an excess of energy. Surfaces have higher energy relatice to the bulk due to the broken bonds of the atoms or molecules present at the surface. Surface energy is a quantification of these broken bonds. The surface free energy (G) of a system with a surface area A and free energy per unit area γ is given by

$$G = G_0 + A\gamma \tag{1}$$

where G_0 is the free energy of the system assuming that all matter in the system has the properties of the bulk. It is this excess energy associated with surfaces that drives liquids to minimize their surface area by forming spherical droplets.

The surface energy (energy per area) is mathematically equivalent to a surface tension (force per length), and the two terms are often used inter-

changeably [73,77,78]. They are, however, not identical. The surface tension is a tensor acting on the contact line, it is the quantity used to calculate the contact angle. The surface energy is a scalar quantity and a property of the area of a surface, it is the quantity involved in the thermodynamic properties of a surface. In some non-ideal states the surface energy will not be identical to surface tension, in these it is important to recognize the difference between the two. Surface tension and surface energy are numerically identical in ideal systems with flat, uniform surfaces, no surface stresses, constant temperature and equilibrium pressure, this is easier to achieve with liquids than with solids [50].

The equivalence of surface energy and surface tension can be illustrated by a wire frame suspending a liquid film. If one side of the frame is movable it is found that a force F per unit length must be applied to maintain the side in position. If this side moves a small distance so that the total area of the film is increased by ΔA the work done is FdA This work increases the free energy of the system by dG, from equation 1

$$\mathrm{d}G = \gamma \mathrm{d}A + A\mathrm{d}\gamma \tag{2}$$

Equating this with FdA gives

$$F = \gamma + A \frac{\mathrm{d}\gamma}{\mathrm{d}A} \tag{3}$$

For a liquid film the surface energy is independent of the area of the interface $\left(\frac{d\gamma}{dA}=0\right)$. This leads to

$$F = \gamma$$
 (4)

The surface tension is equivalent to the surface energy, q.e.d.. This derivation was taken from [78], but equivalent derivations of variable rigor can be found in numerous introductory textbooks.

The magnitude of both the surface energy and surface tension is dependent on the difference between the bonds of particles in the bulk phase and those present at the surface. This leads to different values for different systems. In the case of liquids, compounds with strong intermolecular interaction (e.g. water) have large values of surface energy and hence also of surface tension. The surface of one phase is in practice an interface between two phases. The interactions of particles at an interface are dependent on the second phase, therefore it is not enough to define a single surface energy of any compound. A separate interfacial energy or tension must be defined for each pair of phases. For a system consisting of a liquid (L), a solid (S) and a vapor V) (e.g. water, glass and air, respectively) the three relevant surface tensions are γ_{SV} , γ_{SL} and γ_{LV} [73].

2.3.2 Fundamental Equations

Young's Equation

Contact angles were first described by Young [79]. Young claimed "... that for each combination of a solid and a fluid, there is an appropriate angle of contact between the surfaces of the fluid, exposed to the air, and to the solid." [79] Young observed that the magnitude of the contact angle was the result of a balance between three forces acting along the interfaces at the three-phase contact line. The three forces acting on the three-phase contact line are the surface tensions on the solid-vapor, solid-liquid and liquid-vapor interfaces (γ_{SV} , γ_{SL} and γ_{LV} respectively). Though Young did not formulate an equation, one can easily be created and has been widely cited as "Young's law" or "Young's Equation", see equation 5 and figure 6. Whyman et al. [80] provide a rigorous derivation of the equation.



Figure 6: Contact angle (θ) and Young's Law illustrated, the magnitude of the contact angle is determined by a balance between the three surface tensions.

An equivalent equation (Eq: 6) was formulated by Dupré [81], who also defined the work of adhesion. The work of adhesion is the change in free

energy (ΔG) upon separation for a system consisting of two parts A and B, $\Delta G = W_{AB} = \gamma_A + \gamma_B - \gamma_{AB}.$

$$W_{SLV} = \gamma_{LV} (1 + \cos \theta) \tag{6}$$

The Spreading Parameter

Some systems will result in complete wetting, instead of forming drops, the spreading parameter (S) is a measure of this.

$$S = \gamma_{SV}^{\circ} - \gamma_{LV} - \gamma_{SL} \tag{7}$$

When S > 0, the wetted surface has a lower energy than the dry surface, so the liquid spreads completely into a thin film. Conversely, if S < 0, then it is energetically favorable for the solid to stay dry and the liquid is limited to a drop with a finite contact angle, i.e. equations 5 and 6 are used. Further discussion of *wetting* systems is beyond the scope of this work, focus will be given to *hydrophobic* and *superhydrophobic* systems.

Capillary Rise and Length

When a tube with a small diameter penetrates the surface of a liquid, the liquid will either climb up along the tube wall or be pushed down by the tube wall to form the appropriate contact angle. A liquid will rise in the tube if the contact angle (θ) is less than 90° and fall if θ is more than 90°. For a small tube the liquid will form a curved surface called a meniscus inside the tube, either convex (for capillary fall) or concave (for capillary rise). Different pressures acting on the meniscus will be in equilibrium at the *capillary height* (h) of the system, see figure 7

The pressure difference across a curved surface was described by Young [79] and Laplace [82] by what is today known as the Young-Laplace equation:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2}\right) \tag{8}$$



Figure 7: Capillary Rise in a Small Tube

where ΔP is the pressure difference, γ is the surface tension of the liquidvapor interface and R_1 and R_2 are the radii of curvature. In a small cylindrical tube $R_1 = R_2 = R$, where R is the radius of the spherical section the meniscus constitutes. Hence equation 8 is simplified to

$$\Delta P = \frac{2\gamma}{R} \tag{9}$$

The following derivation was adapted from Adamson [73]. The pressure difference ΔP is counteracted by gravity acting on the liquid (i.e. hydrostatic pressure), and equation 9 becomes

$$\Delta \rho g h = \frac{2\gamma}{R} \tag{10}$$

where $\Delta \rho$ is the difference in density between the liquid and the surroundings (which may be air, or another fluid), g is the gravitational constant and h is the height of the capillary rise. It is assumed that $R \ll h$. A normalized quantity may be defined from equation 10

$$a^2 = \frac{\gamma}{\Delta \rho g} \tag{11}$$

where a is the capillary length of the liquid. The capillary length is a characteristic quantity for liquids which is sometimes included in different models for wetting, beyond those involving actual capillary tubes (e.g. [55, 83]). The capillary length of water is approximately 2.7 mm [84] Sometimes the capillary length is defined as $a^2 = 2\gamma/\Delta\rho g$, the inclusion or exclusion of the factor 2 derives from the boundary conditions used [73]. For a puddle

forming on a completely non-wetting surface ($\theta = 180^{\circ}$) the thickness of the puddle will be twice the capillary length of the liquid [74].

Equation 10 is correct for systems where the liquid wets the surface of the tube. For systems with a contact angle θ a more general equation is valid.

$$\Delta \rho g h = \frac{2\gamma \cos \theta}{R} \tag{12}$$

The reader is cautioned that the above treatment of capillary rise (equations 10 and 11) is inexact in that it does not take the shape of the meniscus into account. Adamson [73] includes a discussion about the exact solution to the problem of capillary rise.

2.3.3 Contact Angle Hysteresis

Contact angle hysteresis is a complex problem, which is interdependent on several related topics. The reader may wish to review the section on apparent contact angles (section 2.3.4 and the section on rough surfaces 2.3.6 fully or in part while reading this section, depending on previous knowledge. The effect of various complex geometries on the contact angle hysteresis is treated in section 2.3.6 and the measurement contact angle hysteresis is covered in section 2.3.4. The three-phase line tension is discussed in section 2.3.7. Finally, the current models on contact angle hysteresis are reviewed in a separate section (2.3.8 at the end of this chapter (2.3), since knowledge of all the other topics are needed to follow these theories.

Receding and Advancing Contact Angles

Early studies of contact angles showed that numerical values of contact angles were not reproducible for most surfaces. It was observed that a droplet could achieve a range of contact angles on the same substrate [85– 88]. If water is carefully withdrawn from a droplet resting on a solid, e.g. by evaporation or by siphoning with a syringe, the droplet will retain the same contact area but change shape until a certain point when the contact line starts receding. Likewise, a droplet to which more water is added, e.g. through condensation or injection by a syringe, will retain the same contact area but change shape until the contact line starts advancing [37]. Any system therefore displays not one, but two defined contact angles, that of the receding contact line and that of the advancing contact line [42,73,74,80]. These are called the *receding contact angle* (θ_R) and the *advancing contact angle* (θ_A) The difference between these two angles is referred to as the *contact angle hysteresis* ($\Delta \theta$), Eq: 13. Receding and advancing contact angles are illustrated in figure 8.

$$\Delta \theta = \theta_A - \theta_R \tag{13}$$



Figure 8: Receding (θ_R) and advancing (θ_A) contact angles, illustrated by sessile droplets with water siphoned or injected by a syringe.

A metastable state can be achieved with a droplet displaying the receding contact angle, the advancing contact angle or any angle in between [37]. Therefore the contact angle of a system is not a single value, and any reports of a single value are of limited value as it is impossible to know where in the range of possible contact angles the reported angle belongs. The importance of the contact angle hysteresis was realized quite early and various methods were developed to measure the advancing and receding contact angles accurately [47–49,85,86,89]. Studies presenting only a single value as *the* contact angle of a system are nevertheless still being published [12, 26, 28, 70]. This should be avoided as it is not always easy to deduce which of the characteristic contact angles it is that is being reported, or if it is one of any number of metastable contact angles [90].

Drops on Tilted Planes

If a droplet is placed on a smooth solid surface which is tilted the droplet will start moving down the tilted plane (there are some exceptions to this, the so-called "Petal Effect" which will be discussed in section 2.4.3). The
tilting angle (α) is defined as the angle at which the drop moves with a constant speed. When the drop is moving in such a way it displays both a minimum and a maximum contact angle (θ_{Min} and θ_{Max}), and all the angles between these extremes [57,91]. The front of the contact line will be *advancing* and the back of the contact line will be *receding*, see figure 9.



Figure 9: Tilting angle (α) illustrated, along with receding (θ_R) and advancing (θ_A) contact angles, by a droplet running down a tilted plane with constant speed.

The 2D-representation (figure 9) trivializes the problem, as the contact angle values will vary around the entire perimeter of the drop, but events at the downhill-most and uphill-most points on the contact line are very similar to those that occur during advancing and receding contact angle measurements, respectively [92]. The two contact angles are generally assumed to equal the advancing and receding contact angles (θ_A and θ_R), but there is no a priori theoretical justification for this. The assumption that the angles formed by a sliding drop equal θ_A and θ_R is correct for the 2D-model shown in figure 9, but for an actual 3D drop it is an approximation [93]. This distinction is rarely cited, and further treatment will use the approximations of $\theta_{Min} = \theta_R$ and $\theta_{Max} = \theta_A$.

The fact that deformed drops can be observed clinging to non-horizontal surfaces without running down, such as rain on a car window, disproves Young's statement (section 2.3.2) and proves the existence of contact angle hysteresis. If there was a unique contact angle defined by the nature of the solid and liquid, as Young claimed, then "...a drop would necessarily slide when tilting the substrate: displacing the liquid along the plane does not modify any surface energy, yet lowers gravitational energy." [57] The clinging drop corresponds to a metastable state, stabilized by the contact angle hysteresis.

The mathematical relation between the tilting angle α and the receding and advancing contact angles (θ_R and θ_A) was derived by Furmidge [91], citing previous similar results by MacDougall and Ockrent [86]. In equation 14 m and g are the mass and gravitational constant, and W is the width of the droplet (measured perpendicular do the direction of tilt/movement). Furmidge's equation balances the force of gravity acting on a drop on a tilted surface against the resistance against movement generated by the contact angle hysteresis:

$$\frac{mg(\sin\alpha)}{W} = \gamma_{LV}(\cos\theta_R - \cos\theta_A) \tag{14}$$

As can easily be seen from equation 14 the tilting angle and the contact angle hysteresis are closely related, and often display similar orders of magnitude, but they are not identical. The close relation between contact angle hysteresis and the tilting angle means that the contact angle hysteresis, and not the absolute size of the contact angles, is the relevant parameter for water shedding. A perfectly clean piece of glass with $\theta_R = \theta_A = 0^\circ$ is an extreme example, where droplets will not form but any water will form a film and drain away easily [3]. A contact angle hysteresis of 0° will correspond to a situation where no force is required to move a droplet [92]. An infinitesimal tilting angle will then be observed, with droplets moving down the tilted surface without distortion of the drop shape.

Early Work on the Contact Angle Hysteresis

Early theories attributed the contact angle hysteresis to various causes including inaccuracy of measurement, polluted test surfaces and failure to provide smooth test surfaces [51, 86]. "Explanations of hysteresis were in general not explanations but short lists of things that can affect the contact angle; roughness, chemical heterogenity, and interactions between the probe fluid and the surface." [74] Bartell's and Hatch's [85] study of Galena provides an early mention of contact angle hysteresis as an inherent attribute of the system, as opposed to a result of inaccuracy. Serious treatment of the subject was first performed by MacDougall [86], who concluded that "a liquid drop when $\theta_A \neq 0^\circ$ exhibits two characteristic and reproducible contact angles $\theta_A(\text{const.})$ and $\theta_R(\text{min.})$ ".

The two causes of contact angle hysteresis most frequently encountered in literature are surface topography and chemical heterogenity [46,50,86,90,92, 94,95]. Both have received serious scrutiny from early studies until today. Pease [89] provided an early discussion of chemical heterogenity, comparing hydrophobic surfaces to "mosaics of polar and non-polar groups, arranged with regularity on a crystal face, and with statistical distribution on an amorphous solid surface". Such a surface would provide numerous possible metastable states with different positions of the contact line. Pease correlated the contact angles to work of adhesion and work of wetting stating that the receding contact angle is related directly to the line of greatest possible mean work of adhesion and that the advancing contact angle is dependent upon the greatest possible amount of work necessary to wet the solid surface. Later works citing Pease have emphasized his focus on the contact line, according to Pease the contact angle hysteresis can be regarded as a contact line phenomenon (not a surface phenomenon) [90].

Bartell and Shepard [47–49] conducted a study of a number of wax surfaces with carefully prepared roughness. Paraffin wax was prepared with pyramidal asperities of known height and angle of inclination. They identified contact angle hysteresis as an effect of the physical structure of the surfaces and proposed a model in which the system exhibits an equilibrium contact angle (microscopically) along the entire contact line, with sample topography resulting in a different apparent contact angle (macroscopically).

Shuttleworth [51] introduced the concept of energy minima and energy barriers to the field of contact angle hysteresis. According to Shuttleworth's model the surface roughness alone is sufficient to explain contact angle hysteresis. A drop of liquid will only assume the state of absolute minimum energy, the equilibrium contact angle, on an ideally smooth surface. On a rough surface there will be asperities which act as energy barriers for the recession or advance of contact lines. Thus, the contact line may come to rest in a metastable state, where asperities pin the contact line (acting as energy barriers) in a non-equilibrium position.

Johnson and Dettre [87, 88] performed a computer simulation and consequent experimental validation of the wetting of solids with a wide range of roughness. Like Shuttleworth [51] they considered contact angle hysteresis to be caused by the pinning of the contact line in metastable states, local energy minima stabilized by energy barriers. Unlike Shuttleworth who identified the maximum possible apparent contact angle(see section 2.3.4) as the advancing contact angle and similarly the minimum possible apparent contact angle as the receding contact angle, Johnson and Dettre considered these to be unstable states representing the energy barriers between observable states. The model was an idealized rough surface of concentric ridges presented in figure 10 found in section 2.3.4. A finite number of states fulfilling the boundary conditions of constant drop volume and constant inherent contact angle were identified and considered in terms of free energy. This was done for several degrees of roughness (height and spacing of ridges).

2.3.4 Measuring the Contact Angle

The contact angle is a very visible quantity, which seems familiar to most without the need for a deep understanding of the underlying mechanisms. It is quite easy to understand that a small amount of water will form a discrete drop (with a finite contact angle) on a wax paper, while a drop of oil will spread out (contact angle of 0°) on the same wax paper; concepts such as surface tension seem abstract in comparison. The contact angle is visible to the naked eye, so measuring it should be trivial. The vast breadth of different methods for measuring contact angles may then come as a surprise. Textbooks and encyclopedias on surface chemistry almost invariably include a chapter discussing the different available methods, and new methods are still being developed. It turns out that constructing a tangent of the liquid surface where it contacts the solid and measuring the enclosed angle is considerably easier to describe than to perform [73,75,77]. A selection of classical and modern methods will be briefly described in this section.

Apparent Contact Angle

A distinction must be made between the *inherent* and the *apparent contact* angle. The inherent contact angle (θ) is that which is actually formed at the three-phase contact line, this is often found to be in agreement with Young's law. The apparent contact angle (θ^*) is the angle that is macroscopically observable and readily measurable. For a rough surface the two can be extremely different. The observed contact angle is, in general, the sum of the inherent contact angle and a topography-dependent contact angle with local variation.

In figure 10 the distinction is illustrated with an idealized rough surface. The idealized surface is circularly symmetrical around the z-axis, with an equation $z = z_0(1 + \cos \frac{2\pi x}{x_0})$ in cylindrical coordinates (x, z, ϕ) where $2z_0$ is the height of a ridge and x_0 is the period of the surface. It is assumed

that the volume of the drop is constant and that it always meets the surface with a constant, inherent angle θ . It can be seen from the figure that the apparent contact angle ϕ is the sum of the inherent angle θ and the slope angle α of the surface at the point of contact [87].



Figure 10: A Drop on an Idealized Rough Surface. Reproduced with permission, Copyright 1964 American Chemical Society, Advances in Chemistry. [87]

2.3.5 Classic Methods of Measuring the Contact Angle

The *tilting plate method* proved facile in early experiments. In this method a smooth plate is partially submerged and rotated until a position is found at which the liquid makes horizontal contact with the solid. The method utilizes the fact that it is easier to distinguish the horizontal than a tangent [77]. Huntington [96] provided an early description of the technique (see figure 11) thanking a Dr. H. A. Wilson(no related references) for the suggestion. By performing two measurements the contact angle θ can be found by the following equation

$$\theta = \frac{180^\circ - \alpha}{2} \tag{15}$$

where α is the angle through which the sample must be rotated to go from one horizontal state to the other. Various difficulties with this method have been cited. Aligning the plate such that the contact with the liquid is perfectly horizontal can be challenging, the method requires a large sample of both the liquid and the solid (which must be a smooth plate) and the method is quite vulnerable to contamination [75]. Another concern is that the mechanism of rotating the plate introduces vibrations that may cause the contact line to slip across asperities (the vibrational energy overcomes the energy barriers), resulting in the measurement of an ill-determined angle somewhere between the receding and advancing contact angle(see section 2.3.3) [50].



Figure 11: The Tilting Plate Method. Reproduced with permission, Copyright 1906 Royal Society of Chemistry, Transactions of the Faraday Society. [96]

The Wilhelmy Plate is a technique which, despite its age, simplicity and inaccuracy, remains relatively common [75]. In this method a thin plate is suspended at a liquid surface from the arm of a balance such that the lower edge of the plate is level with the liquid surface, removed from the plate. This results in a mensicus around the perimeter of the plate. The weight of this meniscus (w) can be measured, which can be analyzed to give a value for $\gamma_{LV} \cos \theta$ through the following equation

$$w = L\gamma_{LV}\cos\theta \tag{16}$$

where L is the perimeter of the plate. Note that this method yields a measure of $\gamma_{LV} \cos \theta$, such that unless γ_{LV} is previously known, another experiment must be performed to determine an independent value for θ [77]. Alternatively $\sin \theta$ can be determined directly by measuring the height of the meniscus h and using the Laplace equation of capillarity (equation 17), where $\Delta \rho_{LV}$ is the difference in density between the liquid and vapor phase and g is the gravitational constant; this will also requires a known γ_{LV} if an independent value of θ is to be found [75]. The method is illustrated in figure 12.



Figure 12: The Wilhelmy Plate Method

One of the strengths of the Wilhelmy plate method is that the two measurements (weight and height of the meniscus) can be combined, and so can the equations (16 and 17), so that both γ_{LV} and θ can be determined from a single experiment [77].

$$\gamma_{LV} = \frac{\Delta \rho_{LV} gh^2}{4} + \frac{w^2}{\Delta \rho_{LV} gh^2 L^2} \tag{18}$$

$$\cos\theta = \frac{4\Delta\rho_{LV}gh^2Lw}{(\Delta\rho_{LV})^2g^2h^4L^2 + 4w^2}$$
(19)

Like the tilting plate method, the wilhelmy plate method has the disadvantage of requiring a plate shaped sample. A similar method has been developed for any shape with a known perimeter and constant cross section, the Wilhelmy gravitational method. In this method the sample, which ideally is a cylindrical fiber, is partially immersed in the liquid and the force acting on the sample is measured by a balance. Conceptually, simply replace the plate in figure 12 with a fiber and lower its bottom edge a known distance below the bulk surface of the liquid. $\gamma_{LV} \cos \theta$ can be found from the following equation

$$F = L_w \gamma_{LV} \cos \theta + V \Delta \rho_{SL} g \tag{20}$$

where F is the force required to balance the system, L_w is the wetted perimeter, V is the displaced liquid volume and $\Delta \rho_{SL}$ is the difference in density between the liquid and the solid. This method requires a welldefined sample and is more useful for e.g. carefully manufactured glass fibers than for natural fibers [75].

The entire discussion of the Wilhelmy methods has revolved around hydrophilic solids, the methods can be used for hydrophobic solids ($\theta > 90^{\circ}$) as well, but the capillary rise will then be replaced by an analogous capillary depression. The equations used will be the same, but the sign of several quantities will change.

Direct measurement of the observed angle is a viable method which has seen a large variety of implementations since early investigations [86,97,98]. Sessile drops can be placed on a horizontal test surface and observed with microscope, camera or a projection system and the angle can be measured with a protractor, dedicated software (in the case of digital cameras) or simple geometric relations (e.g. $\tan \frac{\theta}{2} = \frac{h}{R}$ for a spherical cap of height h and radius R [85]). Sessile drops, and their direct analogy sessile bubbles, are illustrated in figure 13. Note that sessile bubbles are analogous, but opposite of sessile drops, in that highly wetting surfaces will form round bubbles. Solid, liquid and air systems are often used, but the air phase can be replaced with a second liquid. A related method uses a tilted test surface instead of a horizontal one, the setup then becomes equivalent to the situation shown in figure 9 in section 2.3.3. The horizontal plate method will reveal the advancing contact angle, if liquid is added to the droplet such as via a syringe. Likewise the receding contact angle will be revealed if liquid is removed from the droplet. Simply placing a droplet on the plate will result in any angle in between the two extremes, but usually the advancing angle will be observed as the droplet *advances* onto the test surface when it is placed there. The droplet on the tilted plate will display the advancing contact angle on the lower edge and the receding contact angle on the higher edge, or an approximation thereof as noted in reference [93] and section 2.3.3. [73, 75, 86, 97–99]

The direct measurement method is an easily understood method that requires only a small sample of the solid and the liquid. A disadvantage of the method is its sensitivity to vibration, which makes especially the receding



Figure 13: Use of sessile drops and bubbles for contact angle determination, adapted from [73]

contact angle difficult to measure. Extreme values (θ close to 0° or 180°) are also difficult to measure. Very high contact angles in particular are difficult to observed due to a depression of the drop by gravity [50,72,75].

Please note that *this is not an exhaustive list* of the classical methods. A more extensive review of the classical methods was provided by Neumann [100]. Bartell [85] provided a comparison of some of the earliest methods.

Modern Methods for Measuring the Contact Angle

A variant of the direct measurement method described in the previous section still sees frequent use. The method is usually referred to as the sessile drop method and uses a syringe to deliver the liquid drop on a horizontal test surface. A microscope fitted with a digital camera is placed on one side of the test surface, while a light source is placed on the opposite, with the test drop positioned directly between the light source and the microscope. The entire setup can be placed on a vibration-dampening table. Water can be added or withdrawn with the syringe to achieve the advancing or receding contact angle. A computer is used to analyze the image, a common method is to use dedicated software to fit the actual drop profile to a theoretical one. The well known axisymmetric drop shape analysis (ADSA) technique fits the drop profile to a Laplacian curve, this will yield both θ and γ_{LV} provided $\Delta \rho_{LV}$ is known. Figure 14 shows two drops as observed in a typical setup. [75, 77, 101, 102]

If a flat surface is not available, the same general setup (light source, microscope and camera) can be used to provide an image of the capillary rise or deptression around a cylindrical sample. The *analysis of the capillary profile around a cylinder* (ACPAC) method utilizes a digital camera and image analysis software to determine the wetting properties of any cylindrical



Figure 14: A drop showing $\theta \approx 90^{\circ}$ and a drop with $\theta >> 90^{\circ}$ as observed in a setup for the sessile drop method with a digital camera.

sample [75].

Early implementations of the ADSA technique used a capillary fitted within the horizontal test plate to create a drop from below, this was done to ensure a gentle deposition of the drop, while providing a free apex. The first image fitting software systems required a free apex to determine contact angles and surface tensions accurately. Advances in image analysis strategies have made it possible to determine these values without a free apex, thus allowing a syringe or capillary to stay in the drop throughout the test. This allows a great increase in the flexibility of the technique as liquid can be added or siphoned directly to or from the drop, and liquid lenses floating on a second liquid can be held stationary by the capillary [101].

Very Large Contact Angles

As the apparent contact angle approaches 180° , it becomes increasingly difficult to measure with the sessile drop method. Gravity can cause drops to sag near the contact lines. Additionally diffraction and scattering near the triple-phase contact line can cause it to appear blurred. This combination of sagging and blurring can lead inconsistent determination of the contact angle [102], or consistent underestimation [103]. This effect is size dependent, where larger drops display a larger error, but even relatively small droplets (sub-microliter) showed some error.

A new technique that was recently proposed utilizes the Bond number ($Bo = \frac{\rho g l^2}{\gamma_{LV}}$ where l is the maximum width of the droplet) in a perturbation solution to the sagging problem. Using only the measured width of the drop and the capillary length as additional input. The method allows the accurate

determination of contact angles close to 180° from the imaging of sessile drops [102].

Gao and McCarthy [16,37,72], in connection with their synthesis of surfaces with reported contact angles of $\theta_R = \theta_A > 176^\circ$, saw the need to establish a technique to qualitatively distinguish surfaces with "slight affinity for water ($\theta_A/\theta_R = 175-179^\circ/175-179^\circ$) and those with no affinity ($\theta_A/\theta_R = 180^\circ/180^\circ$). The method involves affixing the test surface up-side down and slowly lowering it onto the top of a sessile drop resting on a horizontal plate. Contact between the test surface and the drop is repeated, with some compression of the drop. "Surfaces with contact angles <180° exhibit affinity for the droplet during attachment and release." By utilizing this method, contact angles of $\theta_R = \theta_A = 180^\circ$ have been reported.

Special Considerations

In the beginning of this section it was pointed out that in general, it is the apparent contact angle (θ) that is observed, but that a different inherent contact angle (θ^*) is present on the microscopic scale. The wetting of a surface at a microscopic scale is interesting in relation to understanding the mechanisms of wetting and non-wetting, espescially with regards to contact angle hysteresis on rough surfaces. With this in mind a technique was recently developed that utilizes the diffraction of coherent light to evaluate the "microscopic contact angle" and the shape of the menisci at the microscopic scale. The observed values were comparable to the macroscopic inherent contact angle θ , confirming that the contact angles follow Young's Equation at the microscopic scale. [104]

The testing methods described in the preceding sections all require relatively large, flat or cylindrical, solid samples. Testing the wetability of powders falls outside the scope of this thesis, but several methods have been developed; a review of the different available techniques was provided by Lazghab [83].

2.3.6 Contact Angles and Rough Surfaces

Contact angles are dependent, not only on the surface chemistry, but also on surface topography. There are two main models of drop interaction with a rough surface, that of wetting along the entire solid liquid interface, and that of a composite wetting state with only the top of surface peaks wetted leaving intermittent air pockets. Both of these models will be discussed in this section, as will the transition between these two states.

The Limits of Smooth Surfaces

Contact angles can theoretically range from 0° to 180°, beyond this the angle approach no longer makes geometric sense. Smooth surfaces display a range of contact angles from complete wetting (0°) to hydrophobic ($\theta > 90^{\circ}$), but there is a general consensus that the highest extremes of contact angles can not be achieved without considerable roughness [3,4,19,20,38,74]. A study of a wide selection of smooth surfaces revealed no contact angles for water droplets higher than $\approx 120^{\circ}$, observed on fluorinated polymers [105]. The same study concludes that the highest theoretical contact angle for water on a real smooth solid would be 156°, given by an energy balance based on a surface with no surface energy. Any contact angle observed to be higher than 156° therefore cannot be explained by the energy balance of a smooth system.

Wenzel Model and Cassie-Baxter Model

When a drop of liquid is in contact with a surface with some topography there are two general states it can assume. The drop can wet the entire surface, penetrating the "valleys" between any surface features. Or it can stay suspended on top of the surface features, allowing pockets of air to remain between "peaks". The drop can also assume a combination of the two, in which the liquid penetrates a part of the valleys between the surface features, but not all the way to the bottom [3]. The wetted state is commonly referred to as the Wenzel state after R.N. Wenzel who was the first to describe it [33]. The suspended state is referred to as the Cassie-Baxter State after A.B.D. Cassie and S. Baxter [34], it has also been referred to as the "Fakir State" owing to its resemblance of a fakir resting on a bed of nails. The states are illustrated in figure 15.

Wenzel introduced a roughness factor to describe a non-smooth sample. The roughness factor equals the ratio between the flat projected area of a surface and the actual surface area.



Figure 15: The Cassie-Baxter state, transitional state, Wenzel state and Impregnated state.

$$r = \frac{\text{Actual surface}}{\text{Geometric surface}}$$
(21)

Since a rough surface essentially has "more" surface available, any surfacedependent phenomena are enhanced on a rough surface. If this is applied to the Young equation (equation 5), the terms involving the solid should be multiplied by r. If this is done we get

$$r\gamma_{SV} = r\gamma_{SL} + \gamma_{LV}\cos\theta_W^* \tag{22}$$

This leads to the Wenzel equation which gives the apparent contact angle of a rough surface (θ^*) as a function of the inherent contact angle (θ) according to Young's equation.

$$\cos\theta_W^* = r\cos\theta \tag{23}$$

Equations 22 and 23 can be expanded to include smooth surfaces if r = 1. The net effect of the Wenzel state is an enhancement of the surface's intrinsic tendency towards wetting or non-wetting. Large roughness values (r >1) make hydrophobic surfaces more hydrophobic and hydrophilic surfaces more hydrophilic. The effect of surface roughness is to amplify the effect of the inherent contact angle θ on the apparent contact angle θ^* , so that relatively small changes in the inherent contact angle can give large changes in the apparent contact angle for systems with a large roughness factor r [3,4,33,57].

Wenzel assumed that the liquid would be in contact with the solid on the

entirety of the actual surface area. Cassie and Baxter [34] considered the contact between liquid drops and a porous solid, where the drops rest atop asperities on the solid surface such that only a fraction of the area beneath the drop is in contact with the solid. Cassie and Baxter reasoned that the fraction of a drop that rests on the solid asperities f_1 would follow the law for contact with the solid, while the fraction that rests on the air pockets between the asperities f_2 would act as if in contact with air. Assuming that $\cos \theta_{Air} = -1$, this yields equation 24.

$$\cos\theta_{CB}^* = f_1 \cos\theta - f_2 \tag{24}$$

From this it can be seen that a small fraction of contact (f_1) between the drop and the solid will result in a large contact angle, and as f_1 approaches zero, the contact angle will approach 180°. Certain geometries, such as spikes on a flat field, can achieve small fractions of contact (small f_1) with a relatively low roughness [71].

This model was later expanded by Cassie to include chemically heterogeneous surfaces, i.e. surfaces where the "valleys" are replaced with solid areas with a different surface energy and thus a different contact angle than the base surface.

$$\cos\theta_{CB}^* = f_1 \cos\theta_1 + f_2 \cos\theta_2 + \dots + f_n \cos\theta_n \tag{25}$$

Here f_i and θ_i correspond to the area fraction and inherent contact angle of each chemically homogeneous area. Cassie [106] commented that the original system where n = 2 and the two areas where solid and air respectively was the most important specific case.

The Wenzel and Cassie-Baxter equations (equation 23 and 24) can be combined for surfaces where the tops of the asperities are not perfectly smooth (i.e. surfaces with hierarchical structures) [4].

$$\cos\theta^* = r_1 f_1 \cos\theta - f_2 \tag{26}$$

Here r_1 is the roughness factor of the asperity tops corresponding to area fraction f_1 . The general trend of this equation is that a small fraction of contact $(f_1 < f_2)$ and a large roughness factor (r) makes the surface more

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hydrophobic, provided $\theta > 90^{\circ}$. For systems where $f_1 = 1$ and $f_2 = 0$, the equation reduces to the Wenzel equation (eq. 23).

The area fractions of Cassie and Baxter are often assumed to equal $f_1 + f_2 = 1$. The fractions are therefore sometimes simplified to $f_1 = f$ and $f_2 = 1 - f$. This is incorrect for two reasons. First, in Cassie and Baxter's article [34] this unity is not presented. Second, and more importantly, this identity is only valid for the case of perfectly flat-topped asperities with no penetration of liquid between the asperities. The errors involved in using the incorrect forms of the equation has been estimated to 3° to 13° for superhydrophibic surfaces [107].

The Impregnated State

A third state of wetting can be observed for drops on rough surfaces. That of the impregnated state, in which liquid fills the room between the asperities also outside of the drop, see figure 15. The apparent contact angle for such a system can be found by assuming that the inherent contact angle of a water-filled pore is 0° , i.e. the cosine is 1, so that by the same approach as for equation 24 the following can be found:

$$\cos\theta^* = f_1 \cos\theta + f_2 \tag{27}$$

where f_1 is the fraction of the drop that is in contact with the solid islands and f_2 is the fraction of the drop that is in contact with water-filled pores. This can also be viewed as a special case of equation 25 with only two phases, one of which is n = 2 and $\theta_2 = 180^{\circ}$ [108,109]. This state of wetting is sometimes referred to as the Cassie impregnated state, or just the Cassie state, a handle that can be confusing because of its similarity with the wellknown Cassie-Baxter state. The two states are therefore referred to as the *Cassie-Baxter state* and the *impregnated state* in this work.

Criticism Against Cassie, Baxter and Wenzel

Both the Cassie-Baxter [34] and Wenzel [33] models received criticism from an early point [47–51], but both are still among the most widely cited in the field [52]. Their use has however been the subject of some controversy, which can be colorfully illustrated by the titles of two articles from 2007 "How Wenzel and Cassie Were Wrong" [52] and "Cassie and Wenzel: were they really so wrong?" [110].

The two main objections to the Cassie-Baxter and Wenzel models are that they fail to describe contact angle hysteresis, and that they treat the contact angle problem from an area perspective rather than a contact line perspective [3, 42, 46–50, 52, 74, 89, 110, 111].

Pease [89], although he did not quote either Wenzel or Cassie-Baxter, presented a contemporary theory that described the contact angle as a contact line dependent problem. "The junction is really a line of tension acting on the fluid surface. It is affected by the properties of the different groups of the solid surface which the line happens to cross."

Bartell and Shepard [47–49], in their series of papers in 1953, provided an alternative theory for the wetting of a surface with some topography. They presented experimental results that disproved Wenzel's theory, which failed to predict the apparent contact angle, and contact angle hysteresis, of systems where the roughness of the solid-liquid interface differed from that of the three-phase contact line [48].

Extrand [46] prepared surfaces with carefully characterized chemically heterogeneous zones. On a surface consisting of material 1, circular patches of material 2 was introduced. The interfacial tensions (γ_{SV} and γ_{SL}) and thus also the inherent contact angles (θ) of the two materials were different. Drops of water with diameters larger than the diameters of the circular patches were placed in such a manner that the circular patches of material 2 were concentric with the drops. Since $r_{drop} > r_{patch}$ the patches were therefore entirely covered by the drops, while the three phase contact line was in contact only with material 1. It was found that the inherent contact angles of material 2 was irrelevant for the apparent contact angle of any of the drops.

The experiments of Bartell, Shepard [47–49] and Extrand [46] were repeated by Gao and McCarthy [52]. They found the need to carefully and systematically present the lackings of Wenzel's and Cassie and Baxter's theories and present experimental results to back their claims. The results of the experiments were the same as the previous studies: the contact angle was only dependent on the topography and chemistry at the contact line and unaffected by any changes in the drop interior and the study concluded: "Wenzel's and Cassie's equations are valid only to the extent that the structure of the contact area reflects the ground-state energies of contact lines and the transition states between them." [52]

The experiments of Bartell [48], Extrand [46] and Gao [52] are summarized in figure 16. In column **a** a smooth, slightly hydrophilic surface in shown; column **b** shows surfaces with topography or chemical heterogenity; column \mathbf{c} shows surfaces where the topography or chemical heterogenity is limited to a patch in the middle of the drop and shows contact angles as predicted by equations 23 24 and 25; column \mathbf{d} shows the experimentally observed results of the same surfaces. Rows I, II and III correspond to Wenzel's equation (eq. 23), Cassie-Baxter's equation (eq. 24) and Cassie's equation (eq. 25) respectively. For the Wenzel state (row \mathbf{I}) the introduction of surface roughness across the whole surface enhances the wetting properties of the surface and reduced the contact angle $(\mathbf{I} \mathbf{b})$. The inclusion of a small rough area in the middle of the drop is predicted to reduce the contact angle a bit, according to how much the average roughness has increased $(\mathbf{I} \mathbf{c})$, the observed result was that the patch of roughness had no effect $(\mathbf{I} \mathbf{d})$. For the Cassie-Baxter state (row II) the roughness would increase the contact angle (II b); limiting the roughness to a patch within the confines of the drop would increase the contact angle to a lesser degree according to the Cassie-Baxter equation $(\mathbf{II} \mathbf{c})$; the observed result was that roughness within the confines of the drop did not affect the contact angle (**III d**). For the Cassie equation (row III) the situation is the same as for the Cassie-Baxter equation (row II), but the roughness has been exchanged with a surface of a different chemical composition (higher inherent contact angle), the effect is the same. The trend for all of these is that the equations predict \mathbf{c} a result somewhere between the angles of **a** and **b**, but the observed angles **d** were identical to **a**.

A less harsh treatment of the theories of Wenzel, Cassie and Baxter was presented by McHale [110] who pointed out that the original equations are correct for surfaces with such a random distribution of roughness that it does not matter where the drop is placed. McHale further suggested the modification of r and f to r(x) and f(x), i.e. local values, for non-random heterogeneous surfaces. He also concluded that the contact angles will be determined by the properties at the contact line.

Wenzel, Cassie and Baxter presented the view that the contact angle was dependent on surface energy, when in reality it is dependent on surface tension along the contact line. The mathematical equivalency of a surface



Figure 16: An overview of the predictions of Wenzel [33], Cassie and Baxter [34] and the results of Bartell and Shepard [48], Extrand [46]and Gao and McCarthy [52]. Column **a** shows a smooth, homogeneous surface. Column **b** shows a surface with roughness or chemical heterogenity. Column **c** shows the predictions of Wenzel, Cassie and Baxter's equations for surfaces where the modifications of column **b** are limited to a patch within the confines of the test drop. Column **d** shows the experimental results of Bartell, Shepard, Extrand, Gao and McCarthy. Row **I** corresponds to Wenzel's equation (eq. 23), row **II** corresponds to the Cassie-Baxter equation (eq. 24) and row **III** corresponds to the Cassie equation (eq. 25). [33, 34, 46, 48, 52]

tension (force/line) and a surface energy (energy/area) can lead to the false conclusion that they are the same, an incorrect view that is still occasionally printed in textbooks. Surface energy is a more immediately useful concept for calculating the thermodynamics of a wetting system, but it does not determine the contact angle. Wenzel, Cassie and Baxter's equations are still both useful and correct so long as the properties at the contact line are used to determine the roughness factor r and wetted fraction f [3,50,52,74, 110]. Cheng et al. [111] presented a series of experiments that illustrate the contact line dependence of wetting in an impressive, visual manner.

Wetting Transition

It was initially noted in section 2.3.6 that a drop on a rough surface can either wet the entire surface, rest atop the asperities or assume a mixed state. All of these states have been experimentally observed. Which of these states has the lowest overall energy (i.e. which is thermodynamically stable) is dependent on several factors. For any system a set of different metastable states will exist, these are states with local energy minima, but not the global energy minimum. Transition from one metastable state to another, or to the stable state is hindered by energy barriers. This has practical value since, in general, the Cassie-Baxter state is preferred due to its lower contact angle hysteresis. A simplified energy map of the different wetting states is included in figure 17. [3, 57, 65, 84, 109, 112, 113]



Figure 17: A simplified energy map of different wetting states for a rough hydrophilic solid, adapted from [109]

If we review the Wenzel equation (eq. 23 we see that for very large values of r the equation can predict an apparent contact angle of more than 180° . This does not make physical sense, as the basis of the state described by Wenzel's equation is that of wetting of the solid, which would not happen if the contact angle was 180° . The Cassie-Baxter state on the other hand, readily describes a system with little to no contact between the solid and the liquid, but the state of air pockets are unlikely to be favored for surfaces with only moderate hydrophobicity (θ just above 90°). If the two equations (eq. 23 and 24) are combined and solved for $\cos \theta_t$ we get:

$$\cos\theta_t = \frac{-f_2}{r - f_1} \tag{28}$$

Which yields a value between -1 and 0 for $\cos \theta_t$, the cosine of the contact angle where the two states are equivalent. [84]. Here θ_t is the critical contact angle of transition. For a surface of a given structure (i.e. a given r for the Wenzel state and given f_1 and f_2 for the Cassie-Baxter state) this equation thus predicts whether the Wenzel state or the Cassie-Baxter state is favorable based on the inherent contact angle (θ) of the material compared to the critical contact angle of transition (θ_t). If $\theta > \theta_t$, the Cassie-Baxter state will be energetically favored.

Observation have been made, of the same system achieving both wetting modes in turn, this means that the state representing the global energy minimum is not always achieved and thus that energy barriers that block this transition must exist. Furthermore it is generally observed that drops tend to stay in the wetting mode in which they are placed, i.e drops carefully deposited on a surface tend to be in the Cassie-Baxter state while drops originating at the surface, such as from condensation, tend to be in the Wenzel state. However, once an external force is applied (pressure, vibration etc.) the system switches to the energetically favorable state, which is frequently the Wenzel state [65, 112, 113].

The transition from one wetting state to the other has been described with pressures. An external pressure applied to a drop in the Cassie-Baxter state can push it down into the structure forming a Wenzel contact. This is relevant for such situations as rain, since raindrops can have very high impact pressures (≈ 14000 kPa) on flat surfaces [114]. An experiment that can simulate this involves placing a drop between two identical surfaces and compressing them with a micrometric screw. Based on this approach, Lafuma et al. [84] observed a transition from receding contact angles of about 160°, to receding contact angles of about 40° following a treatment were a pressure was applied and then relaxed. These results clearly showed a permanent change in the wetting properties of a system as a result of applied pressure.

Wetting transitions can be induced in many different ways in addition to pressure: gravity, bouncing of droplets, evaporation of droplets, electric fields and vibration of droplets. The various methods give estimates of the pressure needed to initiate the wetting transition in the range of 100-300 Pa (lower than the impact pressure of rain by several orders of magnitude) for 10 μ l droplets on micrometrically scaled rough surfaces, with higher stability for naturally occuring surfaces [109].

When the geometry of a system is well defined the transition energy barriers can be calculated. The magnitude of the energy barriers are lower than the energy of evaporation and higher than the energy of thermal fluctuation in the droplests. If the energy barriers were not much larger than the thermal fluctuations, the drops would easily assume the global energy minimum. If the energy barriers were not much smaller than the energy of evaporation, then droplets would evaporate before they transitioned from one wetting state to another [109].

One of the most frequently studied systems is that of a surface covered with an array of identical pillars produced by lithographic techniques. The surfaces are preferred since the geometry is both highly predictable and relatively easy to describe mathematically. By using pillars of different sizes, different density distributions on the surface, different cross sections, different coatings and different tops a large range of parameters have been assessed. A scanning electron micrograph of a typical system is shown in figure 18. Describing the effect of all of these parameters on the wetting transition barrier is beyond the scope of this article and the reader is recommended to see the individual articles [65, 68, 84, 112, 113] or Bormashenko's review [109] from Green Tribology. Note that the study of this type of systems is common beyond the sub-field of wetting transition, such pillar arrays are frequently seen in all parts of wetting theory.



Figure 18: A typical system of pillars created by lithography used for the study of wetting phenomena. Reproduced with permission, Copyright 2010 Springer, Science China Physics, Mechanics and Astronomy. [68]

Luo et al. [113] were able to observe the transition from the Wenzel state to

the Cassie-Baxter state directly, using a setup similar to that of Lafuma et al. [84]. Their work considered surfaces with features of varying angles with the substrate, including reentrant geometries (e.g. pillars with overhang), which makes the calculations much more complex, but also has a profound effect on the transition energy barrier. For systems with significant overhang, the Cassie-Baxter state can be stabilized even for systems with $\theta < 90^{\circ}$ [53, 55]. Hensel et al. [67] provided a model and simulation results indicating a stabilization of the Cassie-Baxter state for a system with any inherent contact angle, based on a complex "serif T" surface structure.

Roughness and Hysteresis

Roughness and wetting states have an effect, not only on the apparent contact angle, but also on the contact angle hysteresis. Generally it has been observed that the Wenzel state increases the advancing contact angle, but lowers the receding contact angle as compared to a smooth surface; thus leading to an overall increase in contact angle hysteresis. In the case of the Cassie-Baxter state both the advancing contact angle and the receding contact angle are increased, their relative change is such that the contact angle hysteresis is slightly decreased as compared to a smooth surface [3, 4, 109]. This means that the a transition from the Cassie-Baxter state to the Wenzel state involves a large change in the value of the receding contact angle, while the change in the value of the advancing contact angle is more moderate [84].

Early solutions to the problem of contact angle hysteresis on a rough surface assumed that the inherent contact angle of the smooth solid would be observed along the entire contact line, but that the apparent contact angle would adapt to surface topography. When a contact line advancing along a solid substrate encounters an incline, the drop will stop advancing until an apparent contact angle approximately equal to the sum of the contact angle of the smooth surface and the angle of inclination is attained [49]. The prerequisite that the microscopic contact angle always equals the inherent contact angle necessitates a certain raggedness to the contact line on a microscopic scale. Any roughness would thus make an advancing or receding droplet deviate from its ideal form of a spherical cap, but only at the size scale of the roughness features [51]. This has been observed directly, see figure 19, for systems of cured polydimethylsiloxane (PDMS) on an array of pillars with reentrant geometry [42]. The details of this will be treated more closely in section 2.3.8.



Figure 19: A receding droplet of PDMS on an array of pillars. Reproduced with permission, Copyright 2009 Elsevier, Journal of Colloid and Interface Science. [42]

2.3.7 Three-Phase Line Tension

As was pointed out in the introduction to this section, the three-phase contact line is the line where the three phases meet, in the case of a droplet of liquid on a solid substrate surrounded by air it corresponds to the circumference of the droplet. The three-phase line tension is a tension, or energy, associated with this line. Due to differences in the intermolecular interaction near the three-phase line as compared to those of the interfaces or bulk there is also a difference in the energy of molecules located at or near this line, see figure20a. The complexity of the phenomenon can be compounded by assuming that the interactions are not limited to a simple mathematical line, but rather a confluence zone, see figure 20b [115, 116].

The three-phase line tension is easy to describe conceptually, an excess energy is associated with the contact line, but difficult to measure experimentally or calculate rigorously. The tension is difficult to calculate because of the number of phases and forces involved, it is difficult to measure because of its limited magnitude. For this reason a general consensus regarding the value for the three-phase line tension has yet to be reached. In fact the





(a) Interactions at the three-phase contact line compared with those at a two-phase interface. The length of the arrows indicate the strength of interaction, the dashed arrow indicates the interaction between two interfaces caused by their proximity. [115]

(b) The three-phase confluence zone [115]

Figure 20: The contact of three phases. Reproduced with permission, Copyright 2004 Elsevier, Advances in Colloid and Interface Science. [115]

reported values vary over several degrees of magnitude, and even the sign is disputed! The best estimates seems to be somewhere between 10^{-9} and 10^{-6} J/m, or between 1 nN and 1 μ N, with a clear majority reporting positive values [115].

The three-phase line tension can be included in equations to calculate contact angles, normally the effect of including this miniscule quantity would be negligible, but some systems relevant for superhydrophobic surfaces exhibit extremely large contact lines and thus a strong dependency on the line tension [38]. An example of such a modified contact angle equation (based on equation 24) was proposed by Zheng et al. [68].

$$\cos \theta^* = -1 + (1 + \cos \theta)(1 - \frac{l_{cr}}{S})f$$
 (29)

where $S = \frac{A}{L}$ is a shape-dependent roughness scale determined by the boundary length L and area A of the surface features that is in contact with the liquid if the system is in a Cassie-Baxter state (section 2.3.6) and l_{cr} is defined as

$$l_{cr} = \frac{\lambda}{\gamma_{LV} + \gamma_{SV} - \gamma_{SL}} \tag{30}$$

where λ is the three-phase line tension. In this specific study, λ was treated as a fitting parameter due to the lack of a well documented value, it was determined to be 1.57 10⁻⁸ J/m for the system in question [68].

2.3.8 Current Models for the Contact Angle Hysteresis

Contact angle hysteresis, the difference between the receding and advancing contact angles of a system, is a complex problem which involves surface chemistry, topography, the three-phase line tension and the wetting state of the system. It is generally agreed that the effect is caused by energy barriers separating the various energy minima, in essence trapping the drops contact line in a metastable position. Therefore the drop's contact line will not move immediately upon altercation, but require a minimum energy level which is built up as the contact angle changes until the point where it advances or recedes. However a general theory of the contact angle hysteresis is still lacking [109].

A relation between the energy barrier U of the movement of the three-phase line along a substrate and the contact angle hysteresis is given by:

$$\Delta \theta = \left(\frac{8U}{\gamma R_0}\right)^{\frac{1}{2}} h(\theta^*) \tag{31}$$

where $h(\theta^*)$ is a function of the apparent contact angle given by

$$h(\theta^*) = \frac{(1 - \cos\theta^*)^{1/12} (2 + \cos\theta^*)^{2/3}}{2^{1/3} (1 + \cos\theta^*)^{1/4}}$$
(32)

and R_0 is the initial radius of the spherical droplet before deposition on the substrate [80]. Some important results can be gained from this relation. The difference between the contact angle hysteresis of systems in the Wenzel wetting state and the Cassie-Baxter suspended state is explained by a difference in U, which has been shown to be bigger for the Wenzel state. The reasoning behind that is that advancement onto the top of asperities should not impose energetic barriers, whereas advancement along an increasing incline should [37]. Furthermore the dependence on the apparent contact angle, given by $h(\theta^*)$ (equation 32) is only significant for very high or very low contact angles. Low contact angles lead to a low value of h and thus a low contact angle hysteresis (highly hydrophilic surfaces should not exhibit large contact angle hystereses). On the other hand, h becomes significant for large values of θ^* , such that systems with very high contact angles and low contact angle hystereses must be explained by low values of U [80]. Note that the model does not include precursor films or any form of surface heterogenity.

If the energy barrier of the movement of the three-phase line U is important for the determination of the contact angle hysteresis, then it is equally important to determine U. It has been assumed that drops moving along a surface in the suspended Cassie Baxter state (resting on top of asperities) do so in a rolling manner. If this is the case, then the energy barrier against advancing onto a new asperity should be negligible. Meanwhile the energy barrier against dewetting an asperity top upon leaving it could be significant [37]. Thus, it is the dewetting of asperities that is the major contributor to Ufor a rolling droplet. I.e. the dewetting of asperities is the rate limiting factor of droplet motion. If each asperity is small, then the energy barrier presented against dewetting a single asperity would be small. If the advancement of the droplet is assumed to require only a single (or a few) asperity to be dewetted at a time, the overal energy barrier against droplet motion would be determined by the energy barrier against dewetting a single asperity, and should be decreased by decreasing the size scale of the asperities [117].

As the size scale of asperities decreases, the absolute length of the contact line between the drop and the asperities will increase. Bevelling the tops of asperities would also increase the length of the contact line. When the length of the contact line is long, a term like the three-phase line tension can become important, since the amount of total line tension is dependent on the length of the contact line. An effect of this is the stabilization of the suspended Cassie Baxter wetting state [68]. As pointed out previously the Cassie-Baxter state is expected to give lower contact angle hysteresis. Another effect is that ground state energies of the different metastable states is increased, effectively lowering the energy barriers, thus leading to lower contact angle hysteresis [90].

When we introduce curvature to the top of the asperities the sign of the curvature must also be considered. A negative curvature (concave asperity tops) could trap microdroplets when the drop dewets them. The result would be that the rupture of multiple microscopic capillary bridges between the main drop and the microdroplets would greatly increase the energy barrier U for moving the receding contact line [92].

Choi et al. [42] connected several of the points raised to present an alternate model based on the Cassie-Baxter relation, which accounts for directionality by using differential area fractions instead of assuming isotropic area fractions. Choi et al. used this model to predict the receding (equation 33) and advancing (equation 34 contact lines of a system in a suspended wetting state.

$$\cos\theta_R^* = r_f f_{d,R} \cos\theta_1 + (1 - f_{d,R}) \cos\theta_2 \tag{33}$$

$$\cos\theta_A^* = r_f f_{d,A} \cos\theta_1 + (1 - f_{d,A}) \cos\theta_2 \tag{34}$$

Where r_f is the roughness fraction within the wetted area fraction along the contact line. $f_{d,R}$ and $f_{d,A}$ are the differential area fractions of the system, which are both highly geometry dependent and anisotropic.

To summarize, contact angle hysteresis is caused by energy barriers preventing the movement of the contact line which must be overcome to move the contact line from one metastable state (energy minimum) to another [109]. This can be minimized by, minimizing the apparent contact angle (i.e. for wetting systems) [80] or by assuming the Cassie-Baxter wetting state instead of the Wenzel wetting state [37]. Decreasing the size scale of the asperities will further decrease the contact angle, by reducing the energy barrier of each dewetting event [117], stabilizing the Cassie-Baxter state [68] and increasing the ground state energies [90]. The contact angle hysteresis can be predicted for the suspended wetting state, but this is not trivial and will require detailed knowledge of the system and the direction of contact line movement [42].

2.4 Naturally Occuring Superhydrophobic Surfaces

Nature has provided an abundance of different superhydrophobic surfaces ranging from plant leaves through arthropod cuticles, birds feathers and soils. Naturally occurring superhydrophobic surfaces have been known and studied for longer than synthetic superhydrophobic surfaces. The nonwetting properties of these surfaces are increasingly being used as the inspiration for *biomimetic* synthetic surfaces [4, 5, 19, 60].

2.4.1 Hierarchical Structures

Natural materials often have features that span or repeat over several length scales. The resultant *hierarchical structures* often display properties that are not replicable with structurally less complex materials. Roughness on several length scales has been proved to enhance and stabilize superhydrophobic properties. Some have gone as far as proclaiming hierarchical structures a prerequisite for superhydrophobicity [4, 37, 65].

The tendency for hierarchical structures is seen in all of the following subsections. Plants often display a certain roughness on the sub-cellular level (e.g. with papillose epidermal cells) which is on the micrometer scale, while the same structures are covered in epicuticular waxes which self-assemble into crystals with sub-micron lengths. The same is seen in insects where subcellular hairs (microtrichia) are covered in grooves, scales or epicuticular waxes. Feathers have a structure that repeats from the visible, similar to that of a fractal, with rachis carrying barbs carrying barbules carrying barbicles. In short, roughness on multiple scales, wich was recognized as important to create superhydrophobic surfaces in section 2.2, is very common for natural superhydrophobic surfaces [7, 17, 63, 118].

2.4.2 Multifunctional Surfaces

Natural surfaces rarely have only a single purpose. The many natural superhydrophobic surfaces frequently also allow respiration, are thermally insulating, are transparent or have a specific color. In some cases the superhydrophobicity is a prerequisite for respiration (see section 2.4.4 about plastron respiration). This inherent multifunctionality makes the natural superhydrophobic surfaces even more interesting as it is often desireable to combine sever surface properties in a single product. Self-cleaning solarcells is an example of a product that would require a multifunctional surface (transparency and superhydrophobicity), others include anti-fouling ship hull coatings (anti-adhesion and drag reduction) and "breathing" rain-

garments (air-permeability and superhydrophobicity).

The following sections focus on specific properties of selected natural surfaces, but it can be fruitful to keep the whole of the animal in mind when studying these surfaces. The superhydrophobic hair-cover of aquatic insects is of course also permeable to air, while the superhydrophobic structure on the eyes of some insects is also transparent.

2.4.3 Plant Leaves

The water repellent properties of plant leaves has been known for a long time. The first systematic studies of contact angles on leaves were performed by Fogg [21] and Cassie [17] in the 1940's. In addition to observing high contact angles, the mirror-like sheen which is a characteristic of air trapped beneath the water was observed on leaves. Today a wide selection of leaves have been studied and it has been shown that several display superhydrophobic and/or self-cleaning properties. Notable examples include the lotus [4, 6, 7, 22, 24, 118], the salvinia water ferm [7, 11], rice [7, 118], "elephant ear" (Colocasia esculenta [4] and pitcher plants (Nepenthes) [7, 23, 56, 118]. A readily available example for the curious is the leaf of the broccoli plant, on which Cassie [17] observed the silvery sheen of a superhydrophobic surface under water in 1945. The surface structure of plant leaves is determined by the epidermal cells, which may be papillose (form bumps), display epicuticular folding (giving a wrinkled appearance) or even concave (forming pits); these attributes are usually on the micrometer scale [7, 118]. Epicuticular waxes (long chain hydrocarbons or alcohols on the leaf surface) can further modify both the structure and chemistry of leaf surfaces, these frequently form complex three-dimensional crystalline microstructures with features down to the sub-micro and nanometer level [4, 118].

Lotus Leaves

No discussion of superhydrophobic surfaces would be complete without mention of the sacred lotus (*Nelumbo nucifera*). This aquatic plant has long been known for its remarkable ability of self-cleaning, which enables it to emerge pristine from the muddy waters in which it grows. The lotus has been held as a symbold of purity for this reason. Today the "Lotus-Effect"[®] is both sometimes used as an expression for self-cleaning or superhydrophobicity in general, and it is a registered trademark² of Wilhelm Barthlott (one of the early researchers [22]) which has been commercialized in the Lotusan[®] product range of self-cleaning paints. The lotus leaf has been one of the prime inspirations for biomimetic superhydrophobic surfaces in the past decade [3, 4, 7, 11, 18, 22, 23, 36, 37, 119, 120].

Contact angles in excess of 160° were measured on the adaxial leaf surfaces of lotus leaves, the contact angle hysteresis has been estimated to anywhere between a few degrees an evanescence. The result is a remarkably efficient self-cleaning effect that many researchers have tried to understand and copy. Lotus leaves are hierarchical surfaces, where papillose epidermal cells form the first level of roughness in the form of rounded bumps and tubular selfassembled epicuticular wax crystals form the second level of roughness. The structure was studied in detail by Bhushan and Jung. The papillae had a height of 13 μ m and a midway width of 10 μ m, corresponding to a width to height ratio of approximately 3/4. The papillae lacked a sharp apex, but neither were they flat-topped, instead displaying a peak radius of 3 μ . The self-assembled tubular wax crystals were found to consist of large hydrocarbon molecules. The height of the wax crystals was 0.78 μ m, the midway width was 0.40 μ m and the peak radius was 0.15 μ m. [121] A scanning electon micrograph of a typical papilla covered in wax crystals is shown in figure 21. The hierarchical structure is characteristic for the lotus leaf, but it is not the only example of such structures [4, 7, 22].



Nelumbo nucifera

Figure 21: Several papillae covered in tubular wax crystals from the adaxial surface of the leaf of the Lotus (*Nelumbo nucifera*, the insert shows a close-up of the wax crystals.). Reproduced with permission, Copyright 2009 Elsevier, Progress in Materials Science. [7]

 $^{^{2}\}mathrm{CTM}$ numbers: 001552710 and 001936483

The Salvinia Effect

The floating water fern *Salvinia molesta* displays a combination of superhydrophobic and hydrophilic sites on a complex surface structure that results in a very robust capacity for air-retention under water. Upon submersion the water fern brings a thin film of air covering the leaves down under the water surface, which causes a silvery appearance when the plant is submerged. This mechanism has been coined the "Salvinia effect". The plant can retain this air film for several weeks, making it one of the most durable air films studied [11].

"The upper side of the floating leaves of S. molesta is densely covered with complex multicellular hairs. Four hairs are grouped together and elevated on a large emergence, leading to structures with a total height of about 2 mm. The terminal ends of the four hairs are connected, forming an eggbeater-shaped structure." [11] Different members of the genus have between one and four multicellular hairs (trichomes) that may or may not be connected at the terminal ends. The trichomes are known to be covered in crystalline rodlets of epicuticular wax. The combination of trichomes and wax creates a superhydrophobic surface, a typical example of a hiearchical plant structure with hydrophobic wax. Careful analysis of the area where the four trichomes of S. molesta are connected revealed that this spot lacked the wax that created a nanoscale roughness on the rest of the trichomes. A low temperature SEM investigation revealed that the tip of the eggbeater-shaped structures were hydrophilic. It is this combination of a superhydrophobic hair structure with hydrophilic tips that creates the highly stable air film. The hydrophilic tips serve to "pin" the air film at a hair's length from the leaf surface. The surface structure and its interaction with water is shown in figure 22 [6, 7, 11].

Pitcher Plants

The carnivorous pitcher plants (*Nepenthes*) display a remarkable surface structure which may prove important for further research into biomimetic surfaces. The epidermal cells of the pitcher plants have extensive cuticular folding, resulting in a micro textured surface that can hold a liquid film. This liquid film (a polymeric aldehyde) presents an extremely slick surface to any insects that would walk on the plant. The result is that insects that step on the rim of the plant slide down into the middle of the plant



Figure 22: The eggbeater structure of *Salvinia molesta* and its interaction with water at the end of the trichomes and a schematic overview of the leaf, hairs and air-film. Reproduced with permission, Copyright 2010 Wiley, Advanced Materials. [11]

were they are digested. This happens because the lubricating liquid film trapped by the micro textured surface repels the oils on the insect's feet, the normally excellent adhesion of insect feet is thus negated. The plant displays very low friction and almost no contact angle hysteresis for a wide range of compounds [7,118]. This effect has recently been replicated with the intention of creating a self-repairing omniphobic surface, so called slippery liquid-infused porous surfaces (SLIPS) [23, 56], the details of which are discussed in section 2.5.

The Petal Effect

The petals of roses (subgenus Rosa) frequently feature convex epidermal cells with extensive cuticular folding, but no epicuticular waxes [7]. These surfaces therefore display a wetting behavior that is distinctly different from that of the lotus. Feng et al. [58] were the first to report the "close array of micropapillae on the surfaces of the petal of red rose (rosea Rehd)" [sic] with contact angles measured to 152.4° , which resulted in spherical looking droplets which still adhered to the petals upon being turned upside down (i.e. a tilting angle α of over 180°). This effect is also apparent on the petals (flower leaves) of other flowers. Petals are transient organs the purpose of which is to attract pollinators. As such, petals are not required to be self-cleaning, which is a defense against pathogens. Furthermore three-dimensional wax structures on the petals would lead to unfavorable conditions for pollinators [7]. The combination of a high contact angle with strong adhesion is likely caused by the impregnated wetting state (see section 2.3.6) [58].

2.4.4 Insects

Wetting phenomena are surface (or contact line) dependent, and as such are more dominant than bulk dependent phenomena, such as gravity, for small animals. The small size scale of insects means that surface phenomena play a much bigger role in their life than for creatures the size of a human. Insects could literally get stuck in a drop of rain if they did not have any non-wetting adaptations. As a response to this insects have evolved a range of special wetting properties and also exploit surface and contact forces for other purposes, such as adhesion. [3,60,68,122–124]

Insects achieve the superhydrophobic effect through a combination of surface structuring and modification of surface chemistry. Insects are often covered in tiny "hairs" (microtrichia and setae, subcellular filiform protuberances, see figure 25b) that provide hierarchical roughness. The cuticle (including trichia and setae) composed of a chitin-protein complex which is covered in a layer of epicuticular wax. The combination of hierarchical roughness with the high inherent contact angle of the wax results in surfaces that can display very high contact angles and very low contact angle hysteresis. The wetting properties are exploited for such purposes as staying dry, walking on water and breathing under water. The rest of this section is divided according to the purpose of the special wetting properties. [5, 10, 12, 63, 125–127]

Non-Wetting

Early studies on the wetting of insects focused on specially adapted species, such as waterbugs and insects living on the water surface. This obscures a vital point, which is that a large range of insects display remarkable non-wetting properties. Wagner provided a study of the wings of a large variation of insects. [125]. A correlation between the wing-surface to bodymass ratio and the wetting properties of the wings was found. Large-winged insects were found to have "very unwettable" wings, which also showed a self-cleaning effect. Small-winged insects were, in contrast, found to have hydrophilic wings.

The special hydrophobic properties of insects are caused by a variety of different surface structure. In addition to the "hairs" mentioned earlier [25], scale like structures, ridges, granules and folds have also been observed [125]. The combination of different structural elements forming hierarchical rough-

ness and reentrant geometry (overhangs) constitute two different strategies employed by insects. Springtails (*Collembola*, insect-related arthropods) have been found to have striking geometrical patterns of granules and ridges measuring between a few hundred nm to a few μ m. It has been theoretized that the pattern and structure of these granules are responsible for the robust water-repellency of collembola cuticle, the overhang on individual granules has been emphasized [5, 28, 41, 127]. Overhang on microscopic features may serve to stabilize a suspended wetting state by greatly increasing the energy barrier for wetting the base cuticle. The general effect of overhang on wetting was discussed in section 2.3. Various surface structures from a selection of arthropods are shown in figure 23.

Water Walking

One of the most striking abilites of certain insects is the ability to walk on water. The legs of the water strider (*Gerris remigis*) has been found to have remarkably water reppelent legs. The nature of the water striders legs was briefly reported in 2004 [26] and later explained in further detail [129]. From recoded force-displacement curves the supporting force of a single leg was found to be 1,52 mN (or about 15 times the body-weight of the water strider). SEM imaging revealed that each leg is covered in microtrichia (tiny hairs) which are adorned with nanoscale grooves. The grooved hairs provides a hierarchical roughness which is able to displace about 300 times its own volume in water before the leg penetrates the surface (see figure 24).

The water strider is far from the only arthropod to display the ability of water walking. An excellent review of water walking arthropods in general is available [63]. A group which includes, in addition to insects, springtails (*collembola*) and fisher spiders (*Dolomedes triton*). Podura aquatica is a springtail that lives its entire life on the water surface, its superhydrophobic cuticle received early study by Noble-Nesbitt [41]. In contrast with the water strider, *P. aquatica* is superhydrophobic on most of its surface, not just the legs. In fact, the unguis (claws) of *P. aquatica* are wetted by water, in contrast with those of the water strider. The combination of wetting unguis and superhydrophobic tibiotarsus enables walking on the water surface with some traction, while a hydrophilic ventral tube provides adhesion to the water surface to prevent the animal from being blown away.

The water bug *Notonecta glauca*, which will be more closely discussed with regards to its plastron respiration, exploits the wetting effects in the opposite



(a) Plate-like scales from the forewing of the butterfly *Lysandra bellargus* [125]



(c) Striking geometric pattern of epicuticular granules on the springtail Xenilla maritima



(b) Detail from the elytron of the grasshopper *Oedipoda caerulescens* [125]



(d) Setae-covered cuticle of the springtail *Folsomia quadrioculata*



(e) Specialized scales from the butterfly *Parantica sita* [128]



(f) Dense cover of microtrichia from the underside of the elytra of the water bug *Notonecta glauca* [12]

Figure 23: Scanning electron micrographs of various insect and springtail cuticles. Parts a, b and e reproduced with permission, a and b Copyright 1996 Wiley, Acta Zoologica [125], e Copyright 2009 Springer, Naturwissenschaften [128]. Part f reproduced under the creative commons 2.0 license, Copyright 2011 Ditsche-Kuru et al. [12].



Figure 24: The hierarchical structure of water strider legs, with setae (hairs) and grooves on the setae. Reproduced with permission, Copyright 2007 American Chemical Society, Langmuir. [129]

way by walking submerged, up-side down on the underside of the water-air surface (figure 25a). *N. glauca* has a solution similar to that of *P. aquatica* where most of its surface is superhydrophobic, while the tips of each leg can be wetted [12].



Figure 25: (a) The waterbug *Notonecta glauca* walking on the underside of the water surface. Reproduced under the creative commons 2.0 license, Copyright 2011 Ditsche-Kuru et al. [12] (b) Microtrichia and larger setae providing two levels of roughness on the tibia and femur, stabilizing the plastron of *Clunio marinus*. Reproduced with permission, Copyright 2009 Springer, Naturwissenschaften. [10]
Plastron Respiration

Streams, lake shores and the tidal zones are areas that can be alternately dry and flooded. Plastron respiration is a powerful adaption to this type of environment since it enables respiration in well-aerated water as well as air [13]. First a short note on the respiratory systems of insects is in order. Insects do not possess lungs like humans do, but respire by direct gas exchange. This gas exchange normally takes place through tracheae, specialized air-channels, but some species do not have a tracheal system and instead respire by diffusion through parts of the cuticle [130]. As a result of this insects can respire as long as they are in contact with air, inhalation is not necessary.

The plastron is a thin layer of air on the surface of the insect, stabilized by superhydrophobic hairs. The gas composition of the air layer will be subject to changes due to the metabolism of the insect and the concentrations in the surrounding water. The insect will consume oxygen and produce carbon dioxide. Oxygen will diffuse from the surrounding water into the air layer, once the oxygen activity in the air layer drops lower than that of the surrounding water. Likewise carbon dioxide will diffuse from the air layer to the surrounding water, once the carbon dioxide activity in the air layer exceeds that of the surrounding water. The result is that, not only is oxygen replenished, but the build-up of carbon dioxide is prevented. This is made possible by the relatively high solubility of carbon dioxide in water, as compared to the solubility of oxygen [10, 13, 25, 131].

In addition to the exchange of gases necessary for the insects metabolism, the exchange of nitrogen is inevitable. An air layer controlled by a constant external pressure (such as a bubble) has a variable volume, and will eventually collapse as nitrogen continuously diffuses from the bubble to the surrounding water. Such temporary air layers are not true plastrons, but physical gills and require frequent resurfacing of the animal to replenish the air. By contrast an air layer with a constant volume can exist indefinitely, but a finite pressure difference across the air-water interface will have to be supported [10, 12, 25]. In the case of plastron respirating insects this pressure difference is supported by superhydrophobic hairs, which keeps the air-water interface from making contact with the base cuticle of the insect [69]. Crisp [25] provided a sketch of different hair types that fulfill this function (figure 26, while Ditsche-Kuru has performed a detailed structural study of the different parts of the waterbug *Notonecta glauca* (figure 25a [12].



The two different size scales of microtrichia and setae are clearly illustrated in figure 25b.

Figure 26: Different hair possessed by plastron respirating insects, drawn to scale. Reproduced with permission, Copyright 1948 Royal Society of Chemistry, Discussions of the Faraday Society. [25]

Plastron respiration is not limited to insects, but has also been observed for other arthropods such as certain species of springtails and spiders [14,27,63]. Artifical plastron respiration has also been achieved by encasing a spider in a ball of a porous, superhydrophobic material and submerging the ball. Plastron respiration is still limited to animals with a relatively high surface area to oxygen consumption ratio; a human would for example require a plastron with a surface area of 90 m² [14]. The details of plastron respiration is beyond the scope of this work, but the extensive study by Flynn and Bush is recommended as further reading [132].

2.4.5 Feathers

Early investigation into the feathers of aquatic birds were performed to determine what "water-proofing agent" the birds employed to stay dry while swimming. Following Cassie and Baxters theory regarding the wetting properties of porous surfaces it was realized that the superhydrophobic properties of feathers are due to their structure as opposed to their surface chemistry. Feathers are built up of a central rachis (shaft) to which numerous parallel barbs are attached. One size scale smaller, the barbs serve as the shaft to which numerous parallel barbules are attached, which are fitted with hooked barbicles. Thus the surface consists of numerous parallel fibres similar to the ideal structures in Cassie and Baxter's model and this structure is present at two length scales making the overall structure supremely suited for water-repellency. [4, 5, 17, 25, 34]

The parallel barbules of a feather provides a reentrant geometry (overhang) that can be used as a base for oleophobicity, provided some surface modification is applied. This is because a surface composed of parallel fibers can obtain a composite wetting state even if the inherent contact angle is under 90°. Biomimetic replication of feathers therefore show promise beyond mere copying [55,87].

2.4.6 Superhydrophobic Soils

Soils can be composed of very fine particles. When the size of soil particles is smaller than the capillary length of water, a suspended state (as described by Cassie and Baxters equation, section 2.3.6) can be achieved. This typically happens if the soil particles are covered with a hydrophobic compound, which would give the system and inherent contact angle above 90° [3,29,57]. The phenomenon of superhydrophobic soils is imitated in the toy "Magic Sand" [15].

Superhydrophic soils are very vulnerable to drought as water-uptake is effectively hindered by the superhydrophobic effect. Erosion can easily occur, e.g. during rain, when water runs off the hydrophobic soil, washing the top layers with it. Such soils may also form liquid marbles (see section 2.6.3), which subsequently roll off showing very high mobility on all solid surfaces [29, 133].

2.5 Synthetic Superhydrophobic Surfaces

There are numerous approaches to creating synthetic superhydrophobic surfaces. The general requirements that must be fulfilled were discussed in section 2.2.3. The various synthesis routes usually involves the creation of a micro-, nano- or hiearchical roughness, and a subsequent coating of this surface with a hydrophobic compound. Creating microscale or nanoscale roughness can be done in an almost limitless number of ways. Bhushan divided the various methods for doing so into five categories in his review: lithography, etching, deformation, deposition and transfer. [4] The same division will be used here, in addition to a discussion of Biomimetics in general and a note on some early results in the field of synthetic superhydrophobic surfaces.

2.5.1 Biomimetics

Biomimetics comes from *bios* (Gr. life) and *mimetikos* (Gr. imitative) and has come to mean the process of studying, understanding and copying nature. It bears much resemblance to terms like bionics and biomimicry. The fundamental principle of biomimetics is that nature has had millions of years of evolution to perfect its designs. The solutions to various problems found in nature are therefore often both sophisticated and robust. Scientists in the field of biomimetics seek to understand nature's solutions, and copy them. This is in no way unique for the field of wetting theory, the connection between bird's wings and fixed wing aircraft is perhaps the most striking example. Biomimetic methods have however been important for the field of superhydrophobic surfaces because of the plethora of natural superhydrophobic surfaces which have served as inspiration since the beginning. The Lotus-Effect® paint was one of the first commercial successes in the field, which resulted from the biomimetic study of the Lotus. [4, 5, 7, 9, 19, 22, 66]

Biomimetics can also copy the *mechanisms* that occur in nature, without copying the structure. An example of this is synthetic plastron of Shirtcliffe et al. [14] Plastron respiration was demonstrated with a synthetic superhydrophobic porous sphere based on a sol-gel foam. The plastron functioned as its natural counterparts, and was able to provide an encased spider with air. Plastrons found in nature are not based on foam-like structures, they are generally stabilized by sub-cellular hairs. [10, 12, 25, 69]

2.5.2 Early Results

Gao and McCarthy have on several occasions pointed out that what we refer to as superhydrophobic surfaces today were known in the 1940s and that much of the old literature seems to have been forgotten. [38, 74] In one of their publications they used a patent from 1945 [134] to convert a commercially available textile into a superhydrophobic surface. [18]

Herzberg et al. reported the synthesis of a surface with a hierarchical surface roughness in 1970 [135], using the same method based on a solution of CH_3SiCl_3 as Gao and McCarthy later used to create a surface with "perfect hydrophobicity. [72] Herzberg et al. also reported a surface with zero contact angle hysteresis, which would have been both "superhydrophobic" and "self-cleaning" in the modern terminology.

The modern revitalization of the field was spearheaded by a 1994 study by Kawai et al. which were the first to use lithographic methods to synthesize highly precise geometries to test the various wetting theories. They found that Wenzel's theory could not predict their results, indicating the neeed for further research. [136] Onda et al. reported "Super-Water-Repellent Fractal Surfaces" [sic] in 1996, but also super wetting fractal surfaces, illuminating the point that surfaces with extreme roughness could be used to achieve novel wetting properties. [44] Finally, Barthlott and Neinhuis' much cited report on the self-cleaning ability of the lotus was published in 1997. [22].

Since these early reports the field has grown remarkably. Guo et al. included statistics on the number of articles published related to "superhydrophobic" in the five years leading up to their review, a fivefold increase in papers per year in as many years. [19]

2.5.3 Lithography

There are several related methods called lithography, one of the most common for use in the study of superhydrophobic surfaces in photolithography. In photolithography a substrate is illuminated through a patterned mask such that only certain areas of the substrate is activated. The substrate is usually a silicon wafer coated with a photoresist. Related methods utilize electron beams or x-rays in a similar manner. A method referred to as "soft lithography" will be covered in section 2.5.7.

Lithography is a widely used method, both to create superhydrophobic surfaces and to study the mechanisms of wetting. Figures 18 and 19 featured structures created with lithographical methods. Figure 27 shows a few further examples. The structures of figure 19 [42] were created primarily to

study wetting mechanisms.



Figure 27: Examples of geometries created with lithographic methods. *Top* row: pillars with various cross sections by Öner and McCarthy [39], *Second* row, from left: "microhoodoos", pillars with overhang by Tuteja et al. [55], early result by Kawai et al. [136], *Bottom row*: network of ridges with overhang, by Choi et al. [42] Reproduced with permission, top row Copyright 2000 American Chemical Society, Langmuir, second row left Copyright 2008 National Academy of Sciences, PNAS, second row right Copyright 1994 The Japan Society of Applied Physics, bottom row Copyright 2009 Elsevier, Journal of Colloid and Interface Science.

Several researchers have used lithographic methods to copy the structure of the lotus leaf (see section 2.4.3). Lithography is then used to create the microscale roughness (that consisting of the papillose cells), while a second length scale of roughness is added through other means. Bhushan et al. coated the top of lithographically etched cylinders with self-assembled wax nano-crystals similar to those which naturally occur on the Lotus. [66] Jung and Bhushan used a spray coating with a carbon nanotube composite to provide the second degree of roughness to the structure. [8]

2.5.4 Etching

Etching, the removal of material from a substrate can be performed in several manners. The process can be isotropic (etches in every direction) or anisotropic (gives straight walls), it can be selective (etching only certain materials) or have low selectivity and it can be wet (involving immersion in a bath of chemicals) or dry (usually involving a plasma chamber). Of the different available methods chemical etching offers isotropic and selective wet etching. Electrochemical etching is slightly less isotropic than chemical etching, but offers different selectivity. Sputter etching is a highly anisotropic etching with very low selectivity, while reactive ion etching has relatively high anisotropy with moderate selectivity. [137]

Etching is usually combined with some other method to achieve a patterned surface, commonly lithography. Shirtcliffe et al. used a combination of lithography and etching to create copper-based superhydrophobic surfaces. [138] Etching can also be done without prior lithography, such as by Yang et al. [139] By combining several methods of etching quite complex structures can be achieved, such as the "microhoodoos" of Tuteja et al. (shown in figure 27) which were produced by a combination of lithography with a reactive ion etching and an isotropic etching procedure. [55]

2.5.5 Deformation

Roughness can also be created by deforming a base material. Zhang et al. reported that stretching a tape of PTFE consisting of fibrillar PTFE crystals in a direction perpendicular to the fibriles resulted in a superhydrophobic surface. Their reasoning was that by stretching the tape the distance between the fibriles extending out from the surface increased, effectively lowering f_1 in Cassie and Baxter's equation (eq 24). [140]

Li et al. used the thermal stress fracture of a ceramic material to increase the roughness of their superhydrophobic material. The material was originally synthesized through the self-assembly of polystyrene spheres into a closep-acked pattern between which an In_2O_3 pattern was precipitated. When the polystyrene spheres were removed with a subsequent heat treatment it was

discovered that the greater thermal stresses induced in the patterns created by bigger polystyrene spheres led to extensive thermal stress fracture in the oxide, creating a surface with much enhanced roughness. [30]

2.5.6 Deposition

Various deposition techniques have been used, not only to create roughness, but also to coat rough surfaces created through different methods with a hydrophobic coating. Fluorination [44, 54, 139, 141] and silanization [31, 52, 55, 72, 135] are common approaches to the latter, both have become staples of the field. Such treatments can be applied with methods such as dip coating, spin coating and spray coating, but also through more complex methods such as chemical vapor deposition (cvd) or evaporation.

An even wider range of deposition methods is used to create roughness. Techniques based on self-assembly are interesting, in that they can be flexible and cheap, if a suitable precursor is found [4]. The self-assembly of suspended spheres into tightly packed layers has been utilized both as a method of templating [30] and as a way of creating a structure with hierarchical roughness by combining polystyrene and silica spheres of different size [32]. Both of these also used silanization to provide a hydrophobic inherent contact angle. While Hosono [70] achieved superhydrophobicity with a hydrophilic inherent contact angle by using a chemical bath deposition method where brucite cobalt hydroxide crystals self-assembled into pinshaped crystals. Some substances self-assemble into very rough surfaces through fractal growth [44] or formation of nanocrystals which were used as the second scale of roughness by Bhushan et al. [66]. Liu et al. [142] used a self-assembly method to structure an array of aligned carbon nanotubes into a hydrophobic honeycomb-like pattern. Innovative use of self-assembly techniques can also result in self-repairing superhydrophobic surfaces. This was done by Puretskiy et al. [40] when they combined a perfluorinated wax with colloidal particles that spontaneously segregated at the wax-air interface.

Deposition techniques allow several layers to be applied in succession. Different layer by layer techniques can be used to build up porous structures or create hierarchical roughness. Silica spheres and silica nanoparticles have been used in layer by layer methods to create transparent and antireflective superhydrophobic films [31, 32, 141].

So-called "sol-gel" methods have also been succesfully used to create superhydrophobic surfaces. Here the solution is applied through e.g. dip coating [43] or spin coating [143, 144], before the phase-separation creates enhanced roughness in the deposited film.

2.5.7 Transfer

Topography can be transfered from one surface to another, e.g. from a template to product, through various means. One of the benefits of such methods is that a slow and costly process can be used to create a precise master surface, which will later be duplicated in a cheap and efficient manner. Soft lithography or nanoimprint lithography are popular methods. Soft lithography involves creating a master template (or using a natural surface for this purpose) and subsequently creating a mold (negative) of the template, e.g. with polyvinylsiloxane dental wax. The negative can then be used as a mold, e.g. for casting with epoxy resin. The specific method presented here as an example was used by Bhushan et al. [9].

Often a large number of the presented methods can be combined. An example is that of Gao et al. [145] whose synthesis of a biomimetic mosquito eye analog involved a complex process with lithography, deposition, transfer and self-assembly methods. It was published as a "soft-lithography" method.

2.5.8 Other Methods

Several synthesis routes are not easily categorized into one of the five categories of Bhushan et al. [4] One common route which has not been touched upon is that of textiles and fibers. The fibrous surface of a textile, especially "microfiber" textiles, display a large degree of roughness, often on multiple size scales. The waterproofing of textiles by modification of the fibers by a hydrophobic compound was mentioned in the initial publications by both Wenzel [33] and Cassie and Baxter [34]. As mentioned previously, Gao and McCarthy used a commercially available textile to produce a superhydrophobic surface [18]. Furthermore, the water- and stain-proofing of textiles is frequently mentioned as one of the possible applications of superhydrophobic coatings [3, 7, 14, 19, 129, 146]. Textiles are an example of a type of surface we see daily that displays the necessary roughness for attaining superhydrophobicity. The benefits of textiles can be taken one step further by using electrospinning to produce highly uniform and superfine fibers. The method has already seen use in producing superhydrophobic surfaces [55], but more importantly continues to be a topic for research [147, 148].

Nanotubes, of which carbon nanotubes are the most common, show promise as a base for superhydrophobic coatings. Their fine size creates extremely rough surfaces when they are grown aligned in arrays, but they are inherently hydrophilic, which means they have most commonly been coated [54] or manipulated in some other manner to create superhydrophobic surfaces [142]. In addition to arrays of aligned nanotubes, they have also been used in a spray coated composite to apply a second size scale of roughness to a microstructured surface [8].

2.5.9 SLIPS

Recently a superomniphobic, self-repairing and transparent surface called SLIPS (slippery liquid infused porous surface) was reported [56]. Its significance is not solely due to the combination of several highly attractive properties in one surface, but also in the fact that its approach to the problem of achieving superhydrophobicity was completely novel (for synthetic surfaces). The various strategies presented in section 2.2.3 were exchanged with the principle that immiscible liquids can move relative to each other with vanishing contact angle hysteresis. Thus, if a liquid film is retained on a surface, any liquid immiscible with the film will readily slide off it. The pitcher plant presented in section 2.4.3 uses the same strategy.

The focus is moved from the interaction between the solid and the liquid that is to be repelled, to the simpler relation between the solid and the film that is to be retained. This does not require as precise a surface topography, as long as the film liquid wicks into and preferentially adheres to the solid. It can be achieved by infiltrating a low surface energy porous solid with a low surface tension liquid. This was achieved by using a porous network of PTFE nanofibers as the substrate and various perfluorinated liquids as the film liquid. The results were contact angle hystereses below 2.5° for alkanes, glycols, glycerol and water [56]. The limits of this approach is the stability of the liquid film, how long it stays in the pores before it evaporates or leaks out and the chemical properties of the liquid, which must be immiscible with any impinging liquid, but should also penetrate the substrate. Still, SLIPS is likely to introduce two important themes to the field of superhydrophobics. That of integrating non-wetting with selfhealing and self-cleaning capabilities. And that of moving the field from the two- or three-phase models of Wenzel and Cassie-Baxter to more complex views [23].

2.6 Non-Wetting without Superhydrophobic Surfaces

There are a few occurrences where very high contact angles of liquid drops can be observed without superhydrophobic surfaces. This is of general interest to the field of superhydrophobicity since it may provide alternate approaches to the study of some common properties. It is also included for the sake of completion, to highlight the fact that spherical droplets can form without a superhydrophobic substrate.

2.6.1 Liquids with Hight Surface Tension

The most mundane case of high contact angles without a highly hydrophobic (or olephobic/omniphobic) surface is that of liquids with very high surface tensions. Water is regarded as a high surface tension liquid compared to many organic liquids, but there are other liquids with still higher surface tensions. Mercury is an example of such a liquid, with a surface tension of $\gamma_{LV}^{Hg} = 485 \text{ mN m}^{-1}$, compared to that of water $\gamma_{LV}^{H_2O} \approx 72 \text{ mN m}^{-1}$. It is obvious that a large value of γ_{LV} will skew the Young equation towards high contact angles θ given more moderate values for γ_{SL} and γ_{SV} . A very high surface tension will tend to force the liquid into a shape where the surface area is minimized, i.e. a sphere, which displays a high contact angle. The theoretical maximum contact angle of a mercury droplet on a smooth, lowenergy surface is $\theta^{Hg} > 160^{\circ}$. For this reason, mercury displays obvious non-wetting properties that have been known for a long time and used as a simile to water on superhydrophobic surfaces [3, 22, 34, 50, 76, 79, 86, 105].

2.6.2 Leidenfrost Droplets

The phenomenon of Leidenfrost droplets can be observed when a drop liquid is deposited on a solid surface with a temperature much higher than the boiling point of the liquid. The drop assumes a state of complete nonwetting where it displays contact angles of $\theta_R = \theta_A = 180^\circ$, a state with zero contact angle hysteresis and hence no barrier towards movement. This is readily observed when e.g. water is spilled on a hot plate in the kitchen or liquid nitrogen is spilled on a lab bench of ambient temperature. The phenomenon was first described by Leidenfrost in his 1756 work "De Aquae Communis Nonnullis Qualitatibus Tractatus", a translation of the section regarding Leidenfrost droplets is available [149]. The drop never comes into contact with the solid because a film of vapor forms between the drop and the solid so that the drop i levitating on a cushion of its own vapor. The phenomenon therefore has nothing to do, conceptually, with the field of superhydrophobicity, since there is no solid-liquid contact, but the macroscale properties are identical [3, 19, 57, 62].

2.6.3 Liquid Marbles

When particles adhere to the fluid-fluid interface of an emulsion they can stabilize the emulsion. If the encapsulated fluid is a liquid and the surrounding fluid is air, the resulting system is known as a liquid marble. The creation, properties and potential uses of liquid marbles was the subject of a recent review by McHale and Newton [133]. Liquid marbles are droplets, with small particles covering the air-liquid interface. The marbles therefore show a mixture of the properties of the liquid and the solid. Since the bulk of the marble is a liquid it can assume any shape, largely dependent on surface tension and gravity, much like a liquid when not in contact with a solid. Additionally, since the surface of the marble is covered in particles it displays no wetting phenomena with solid substrates and a reduced rate of evaporation.

Liquid marbles are created when water droplets come into contact with small (up to tens of μ m) hydrophobic particles, the particles will self-attach and self-assemble into a shell around the droplet. A short study of the surface free energy of a liquid marble reveals that even hydrophobic particles energetically favor attachment to the liquid-air interface. Liquid marbles can be supported by any solid substrate without interaction between the encapsulated liquid and the substrate, since there is no wetting the shape of the liquid marble is dependent on gravitational effects, and not contact angles. Small liquid marbles will be approximately spherical, larger liquid marbles will be puddles, this will be determined by the size of the marble in relation to the capillary length (a, see equation 11 in section 2.3.2). Liquid marbles can even be supported by a liquid substrate, provided the liquid

substrate does not wet the covering particles all the way to the interface of the encapsulated liquid. Liquid marbles display some of the same effects as droplets on superhydrophobic surfaces, extreme mobility and spherical forms. The principles of contact angles and wetting can be used to described the attachment of particles to the air-liquid interface; but the general result is quite distinct from that of superhydrophobic surfaces [3, 19, 133].

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3 Article 1

"Surface Structure and Wetting Characteristics of Collembola Cuticles", Håkon Gundersen, Hans Petter Leinaas, Christian Thaulow, PLoS ONE, vol 9, no. 2, p. e86783, 2014

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The article, as published contained an error that was corrected 11 July 2014: "The images for Figures 3 and 5 are incorrectly switched. The images for Figures 4 and 6 are also incorrectly switched. The figure legends appear in the correct order."

Surface Structure and Wetting Characteristics of Collembola Cuticles

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Abstract

The cuticles of the arthropods Collembola (springtails) are known to be superhydrophobic, displaying such properties as water-repellence and plastron formation; overhanging surface structures have been suggested as the source of these properties. Superhydrophobicity is closely related to surface structuring and other surfaces with overhanging structures have been shown to possess robust superhydrophobic properties. In effort to correlate the wetting performance and surface structuring of the cuticles, from both a technical and evolutionary point of view, we investigated a selection of Collembola species including species from several families and covering habitats ranging from aquatic to very dry. The observed contact angles of wetting was in general larger than those predicted by the conventional models. Not all the studied Collembola were found to have superhydrophobic properties, indicating that superhydrophobicity is common, but not a universal trait in Collembola. Overhanging structures were found in some, but not all Collembola species with superhydrophobic ticts; which leads to the conclusion that there is no direct link between overhanging surface structures and superhydrophobicity in Collembola.

Citation: Gundersen H, Leinaas HP, Thaulow C (2014) Surface Structure and Wetting Characteristics of Collembola Cuticles. PLoS ONE 9(2): e86783. doi:10.1371/ journal.pone.0086783

Editor: Markus J. Buehler, Massachusetts Institute of Technology, United States of America

Received July 31, 2013; Accepted December 14, 2013; Published February 3, 2014

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Funding: The funding was provided by the Norwegian University of Science and Technology, NTNU, through a program for strategic funding of materials-related research projects. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Superhydrophobic surfaces, showing functional properties like self-cleaning, air-retention and drag reduction, have enjoyed increasing interest in recent years [1-4]. Evolution has led to a wide selection of different surfaces in nature, fitted for species in different environments. Some of these surfaces, such as the lotus leaf, the pitcher plant, and the cuticle and hairs of aquatic insects [5-11], have been the inspiration for biomimetic, superhydrophobic surfaces. Although excellent water shedding properties have been achieved the methods are often vulnerable to small imperfections or contamination, yield surfaces of low durability or are vulnerable to weathering [1,12–14]. The investigation of natural surfaces that have yet to receive attention may provide inspiration for novel solutions to these challenges. The cuticles of Collembola (springtails) have long been known to be highly waterrepellent [15-17]. Surface topography is know to be of vital importance to the wetting behavior of a surface, several studies have concluded that significant surface roughness is a prerequisite for superhydrophobic behavior [1,2,4,8,9]. Two recent studies have highlighted the cuticles of Collembola from a biomimetics point of view [18,19]. One of these emphasized the importance of comparing species relatedness and habitat types in order to better understand the evolutionary aspects of the surface structure modifications [18]. The other study documented robust water shedding and air retention properties with water, as well as several organic liquids, on Collembola cuticles. Microscopic structures with overhang were suggested as a possible explanation for the superhydrophobic and omniphobic properties [19].

Superhydrophobicity is an effect that causes water to roll off a surface with very little resistance. This can result in a self-cleaning effect when contaminations adhere to the water droplets and roll off with the droplets. Upon submersion such surfaces can retain a thin layer of air on the surface; this greatly reduces flow resistance in water and is also the basis for the plastron respiration of arthropods [1,8,20,21]. Generally a combination of a hydrophobic surface (displaying inherent contact angles (θ_0) of 90° or more) and surface structuring is required to achieve superhydrophobic effects. Arthropod cuticles achieve this by combining cuticular structuring with a cover of tiny hairs and hydrophobic cuticular waxes [22].

Collembola are small six-legged arthropods that represent one of the oldest and most abundant (in numbers of individuals) terrestrial animal groups on earth. Thus they have a long and diversified evolutionary history (nearly 400 million years) of adaptation to life on land. The cuticles of Collembola show complex surface structures, including both respiring surfaces and thicker parts that block gas exchange [15]. Figure 1 shows several Collembola and an SEM image of its cuticle. The cuticles are known to have strong anti-wetting properties, with the ability of plastron formation around submerged animals [17]. The variations in the patterns of surface structures, in relation to habitat conditions and species relatednes, is of both evolutionary and biomimetics interest. Collembola live on or below the ground and are thus highly affected by soil water conditions. Respiring through the body surface they also run the risk of dessication. In dryer habitats adaptation appears mainly to involve a reduction in the respiring surface and thereby an improved protection against

water loss. Under wet conditions hydrophobic properties and improved plastron formation facilitating gas exchange may be more important [17]. Self-cleaning is also an important aspect of the superhydrophobic cuticle, as soil dwelling animals may come in intimate contact with harmful substances and pathogens. The appearance of the Collembola cuticles is often very clean, with no visible contamination even when studied in scanning electron microscopes (SEM).

The arthropod cuticle in general consists of a chitin-protein complex with a cover of epicuticular wax [22]. In Collembola, we find that thinner sections, without wax, and thicker parts covered by wax form recognizable geometric patterns. These thicker parts, or protuberances, are commonly referred to as granules. The basic pattern appears to consist of triangular granules connected by ridges in hexagonal rings [23]. The size of these basal units may vary, but are usually in the order of a few hundred nanometers. These granules have been shown to have overhanging structures in some species, where parts of the granule extend beyond the base of the granule, like the eaves of a roof. Helbig et al. suggested that this overhang was an important, but neglected, characteristic of non wetting Collembola cuticles[19]. Several triangular granules may fuse to quadrangular granules arranged in rectangular patterns. These structures, including both hexagonal and rectangular configurations, represent general patterns found in most Collembola at different parts of the body, and in all major taxonomic groups.

However, there are also some systematic differences between these groups, such as the tendency to form secondary granules (involving several primary granules) in several families of the superfamily (section) Poduromorpha. Figure 1 shows both larger, secondary granules and smaller, primary granules connected by ridges. In the superfamily Entomobryomorpha some genera of the family Isotomidae tend to modify their cuticle mainly by changing the connecting ridges and thus individual areas of thin cuticle between the granules, while in other genera the size of the individual granules may change greatly without affecting the size of the thin cuticle units. Lastly, other families of the Entomobryomorpha show little cuticle modification at all, possessing a more or less uniform cover of the hexagonal configuration, possibly with wax cover also on the thinner parts [16,24]. Thus, in the latter group, it is possible that gas exchange occurs through the pores of the granules [16], rather than across the thinner parts of the cuticle, as argued for other Collembola groups [15,25]. Figure 2 shows one species (X. maritima) with a typical hexagonal

configuration and one species (*A. laricis*) with enlarged granules. Such systematic characteristics represent differences in the structural patterns on which evolution will act, and may lead to very different solutions to the same environmental challenges; i.e. between unrelated species living in the same habitat. This emphasizes that in order to improve our understanding of cuticular wetting properties from an evolutionary perspective, one should compare related species from different habitats as well as species from different families living in similar habitats.

In the present work we investigated the cuticles of twelves species of Collembola from four different Collembola families in an effort to explain the wetting properties of Collembola cuticles based on wetting principles and evolution. The selected species represent a wide range of habitats from high mountains to the coast, including both extreme drought as well as littoral and aquatic climes. Analyses of the structural arrangement of thinner and thicker parts of the cuticle, including the presence of overhang, were performed to quantify basic parameters in models of wetting behavior (e.g. roughness and solid surface fraction). The resulting, theoretical estimates of contact angles were compared with experimental contact angle measurements of water droplets on cuticles.

Results

The advancing and receding contact angles of water droplets on the cuticles of a selection of Collembola was measured with the sessile drop technique, the contact angle hysteresis was calculated as the difference of the advancing and receding angles. The measured contact angles are presented in table 1; the uncertainty in the presented values correspond to one standard deviation as calculated from the population of measured values for each species.

When the numbers in table 1 were compared to the standard criteria for superhydrophobicity (θ_A and θ_R exceeding 150° and $\Delta \theta$ under 10° [3]) ten out of twelve tested species were found to be superhydrophobic. This includes species from all the tested families (Hypogasturidae, Onychiuridae, Isotomidae and Entomobydiae) and a variety of habitats (intertidal zone, terrestrial, litter layer, watter-logged soil, forest floor, grassland and marsh) displaying the full range of moisture (very dry to aquatic) and flood danger (no danger to intertidal zone) [26]. Only species 8 and 12 (*C. clavatus* and *X. maritima*) were not superhydrophobic; water droplets were also observed to stick to these two species, where on all other tested species they would slide off. These two



Figure 1. Live Collembola and their cuticle structure. (a) Several specimens of Onychiurus sp., the scale bar is 1 mm. (b) SEM image showing the cuticle structure of Onychiurus sp.. A pattern of large, secondary granules (solid arrow) are shown and in between these a pattern of small, primary granules (dashed arrow), the primary granules are connected by ridges. doi:10.1371/journal.pone.0086783.g001

Surface Structure and Wetting of Collembola



Figure 2. Scanning Electron Microscopy (SEM) images of species 10 and 12. Left: species 10 A. laricis Right: species 12 X. maritima. The images, at 10 000X magnification, show structures typical for the dorsal metasoma. Species 12 has a typical structure of triangular granules, connected by ridges, organized in a hexagonal pattern. Species 10 has markedly enlarged granules, in a variation of forms, organized in a varied pattern closer to square than hexagonal. doi:10.1371/journal.pone.0086783.g002

species represent two very different habitats; *C. clavatus* is active submerged in rock pools, and as such is always wet, while *X. maritima* lives on the crusts of lichens on boulders and standing tree trunks, which may become very dry for long periods [26].

Structural parameters were measured with scanning electron micrographs (SEM). The results are presented in table 2 where values for species marked with a * are based on secondary granules while the rest are based on primary granules. Figure 2 presents micrographs of two example species (species 10, *A. laricis* and species 12, *X. maritima*). Height data and the presence of overhang was determined from cross-sections created with focused ion beam (FIB) milling and subsequent SEM imaging. The FIB cross sections of *A. laricis* and *X. maritima* are shown in figure 3, note the presence of overhang on *X. maritima*. SEM images of the other species are included as supporting information, figures S1, S2, S3, and S4.

Ladie I. Contact Andle Measuremen	Table 1	. Contact	Angle	Measureme	nt
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#	Species	θ_A	θ_R	$\Delta \theta$	SG
1	Hypogastura viatica	$167.9\pm1.5^{\circ}$	$163.2\pm1.6^\circ$	$4.7\pm2.2^{\circ}$	yes
2	lsotomurus prasis	$168.2\pm3.3^\circ$	$164.1\pm3.7^{\circ}$	$4.1\pm4.9^{\circ}$	no
3	Onychiurus sp.	$170.2\pm2.4^\circ$	$166.2\pm2.9^{\circ}$	$4.0\pm3.8^{\circ}$	yes
4	Folsomia quadrioculata	$170.5\pm2.6^\circ$	$166.5\pm2.0^\circ$	$4.5\pm2.6^\circ$	no
5	Anurophorus septentrionalis	168.3±2.3°	$164.8\pm2.4^{\circ}$	$3.5\pm3.4^{\circ}$	no
6	Desoria olivacea	$164.4\pm3.7^{\circ}$	$162.5\pm2.1^\circ$	$1.9 \pm 4.8^{\circ}$	no
7	Archisotoma besselsi	$169.6\pm2.2^{\circ}$	$164.5\pm3.1^\circ$	$5.0\pm3.8^{\circ}$	yes*
8	Cryptopygus clavatus	$140.6\pm3.1^\circ$	$118.4\pm10.9^{\circ}$	$22.3\pm11.4^{\circ}$	no
9	Orchesella flavescens	$150.2\pm6.0^{\circ}$	$152.9\pm4.4^{\circ}$	$-2.7\pm7.5^\circ$	no
10	Anurophorus laricis	$157.4\pm2.5^\circ$	$161.2\pm8.3^{\circ}$	$-3.8\pm8.7^{\circ}$	no
11	lsotoma anglicana	$154.3\pm4.0^{\circ}$	$158.6 \pm 1.9^{\circ}$	$-4.3\pm4.5^{\circ}$	no
12	Xenylla maritima	$156.5 \pm 9.8^{\circ}$	$132.4 \pm 11.9^{\circ}$	$24.1 \pm 15.4^{\circ}$	no

Results of the contact angle measurement for each of twelve species of Collembola. The measured advancing (θ_A) and receding (θ_R) contact angles, whith standard deviation, the calculated contact angle hysteresis $(\Delta \theta)$ and the absence or presence of secondary granules (SG). * The secondary granules of A. besselsi are enlarged primary granules. doi:10.1371/journal.pone.0086783.1001

Figure 4 illustrates how the different values were measured from cuticle micrographs. Based on these measurements (table 2) we estimated some important parameters (roughness factor (r), solid area fraction (f), differential solid area fraction in the receding direction (f_r) , estimated contact angle from the Wenzel equation (θ_W) [27], estimated contact angle from the Cassie-Baxter equation (θ_{CB}) [29], estimated contact angle hysteresis from Dufour's method $(\Delta \theta_D)$ [30] and the estimated receding contact angle from Choi's method $(\theta_{r,Choi}^*)$ [30]) as presented in table 3. Neither the Wenzel equation, Dufour's method nor Choi's method predicted superhydrophobic behavior for any of the species. The Cassie-Baxter equation only predicted superhydrophobic behavior for species 1 and 7, and then only when the secondary granules were considered. The following assumptions were made: $f_a = 0$, i.e. that the differential area fraction in the advancing direction is zero, which means that the advancing edge of a droplet is not in contact with the substrate. This assumption is reasonable for a system where the droplet is resting on the top of discrete asperities, since any incremental advancement of the contact line from a set of asperities will be into the empty space between asperities, where the solid area fraction is zero [30]. $r_f = 1$, i.e. that the tops of the granules are assumed to be smooth. The inherent contact angle for the cuticle substrate was assumed to be $\theta_0 = 105^\circ$. The predicted values are compared to the measured values in figure 5 for contact angles and in figure 6 for contact angle hysteresis; the figures are based on data from tables 1 and 3.

Wenzel's roughness parameter r is the ratio between the nominal contact area and the actual contact area assuming complete wetting (i.e. Wenzel state); the different cuticular structures were approximated to repeating geometric patterns in order to estimate a value for r. The solid fraction of the surface, f, is used in the Cassie-Baxter relation, it can be estimated as $f = \frac{A_s}{A_s}$ from the nominal area of the section of the cuticle containing a single granule (A) and the nominal surface area of the top of a granule (A_s) . f_r is the differential area fraction, as used by Choi et al. [30], found as $f = \frac{2b}{2b+p}$ where the parameters *b* and *p* were measured from SEM images as illustrated in figure 4. r, f, and f_r can, when combined with parameters from table 2 be used to calculate the contact angle for the surface as predicted by Wenzel's equation $(\cos \theta_W = \cos \theta_0 r)$ [27], the Cassie-Baxter equation $(\cos \theta_{CB} = \cos \theta_0 f)$ [28], Dufour's model for contact angle hysteresis $(\Delta \theta_D)$ [29] and the receding contact angle according to Choi's model $(\theta_{r,Choi}^* = \arccos[f_r(r_f \cos \theta_0 + 1) - 1])$ [30].

#	Species	P [nm]	d ₀ [nm]	<i>H</i> [nm]	<i>L</i> [nm]	Α [μm ²]	$A_s \ [\mu m^2]$	Overhang
1	H. viatica	6	370	210	970	0.134	0.068	yes
1*	H. viatica	3	1950	1150	2790	3.66	0.54	yes
2	I. prasis	5	970	155	1570	0.77	0.15	no
3	Onychiurus sp.	6	380	175	750	0.108	0.041	yes
3*	Onychiurus sp.	3	1000	846	3180	2.55	0.77	yes
4	F. quadrioculata	4	860	290	2670	1.37	0.51	no
5	A. septentrionalis	3	570	332	4430	2.31	1.40	yes
6	D. olivacea	6	430	100	910	0.181	0.054	yes
7	A. besselsi	6	320	110	560	0.059	0.022	yes
7*	A. besselsi	4	620	355	1150	0.509	0.070	yes
8	C. clavatus	4	420	120	2240	0.655	0.39	no
9	O. flavescens	6	520	233	1230	0.278	0.098	no
10	A. laricis	4	490	165	5370	2.85	1.98	no
11	I. anglicana	4	630	193	1825	0.635	0.22	yes
12	X. maritima	6	1040	440	2350	1.06	0.35	yes

Surface structure characteristics, as measured on scanning electron micrographs. P: number of edges in the closest equivalent polygon; do: longest regular distance between primary granules; H: height of granules; L: length of the three-phase contact line for the wetting system of one granule; A: nominal area of a section of cuticle containing a single granule; As nominal surface area of a granule; the final column denotes the presence of overhanging structures. Rows marked * present values based on secondary granules.

doi:10.1371/iournal.pone.0086783.t002

The structural parameters of the granules can also be estimated with nanoindenter atomic force microscopy (NI-AFM). This method proved more challenging than using SEM and FIB cross sections on Collembola cuticles and the primary granules of all species could not be imaged. Corresponding values based on NI-AFM are available for most of the species as supporting information, figures S5 and S6, tables S1 and S2.

Discussion

The results presented in table 1 indicate that superhydrophobicity is a quite general characteristic of the Collembola cuticle, independent of habitat and phylogeny (relatedness). However, the two species Xenylla maritima and Cryptopygus clavatus showed some clear exceptions to this general trend.

As contact angles approach 180° they get increasingly difficult to measure accurately [31]. The small size of the Collembola compared to the droplets used also makes accurate measurement challenging. The negative contact angle hystereses measured for several species are therefore likely the result of experimental error for samples with very small, but positive, contact angle hystereses. The qualitative observation of droplets sticking to two of the species (Xenylla maritima and Cryptopygus clavatus), however indicates significant contact angle hysteresis for these two species. Interestingly these two species occupy habitats at the two extreme ends of the humidity range. C. clavatus is active on the bottom of small rock pools [32]. A wettable cuticle seems to be a presupposition for the species to walk freely in a submerged state, as can be seen under stereo-microscope when they easily penetrate the water surface and become completely wetted (personal observation). X. maritima, in contrast, lives in drought exposed lichen crusts on rocks and tree trunks[26], where the risk of submersion is minimal. X. maritima shares this habitat with A. laricis which has a very different cuticle structure with distinct superhydrophobic properties. A possible explanation for these fundamentally different traits of two lichen living species is that they appear to have very different strategies to

survive in their habitat. A. laricis is heavily protected against water loss by a heavy armor of large, wax-covered granules. While X. maritima is very tolerant to water loss (personal observations). X. maritima is quite inactive when dehydrated, and in order to utilize the improved conditions when humidity increases, it must recover the water balance quickly. A wettable cuticle may facilitate such recovery.

The contact angle experiments were only performed on the dorsal metasoma of the Collembola. This was because the droplets that could be produced in the experimental setup was relatively large compared to the size of the Collembola, such that placing a droplet on and examining the contact angle on smaller parts of the animal(e.g. antennas, limbs or head) was challenging and would not yield accurate or reproducible results. In short, the dorsal metasoma was the only area large enough and uniform enough to accommodate the measurement of contact angles. Only SEM and AFM images from the dorsal metasoma were used in the numerical analyses, as such the results of the contact angle measurements and the results of the mathematical predictions should be commensurable as they are both based solely on data from the dorsal metasoma.

There are normally three criteria that should be fulfilled for a surface to achieve a stable superhydrophobic effect: The intrinsic contact angle of the surface should be 90° or more (i.e. hydrophobic), surface structuring should create considerable roughness and the system should assume a Cassie-Baxter wetting state. Not all of the three criteria need necessarily be met, but each contribute to the stability of a superhydrophobic state [1,8]. The epicuticular waxes of Collembola and other arthropods are known to be inherently hydrophobic, such that any wax-covered surface would contribute to water repellency [15,16,22]. Further, recent research into the effect of overhanging structures on wetting suggests that these structures may stabilize the Cassie-Baxter wetting state by providing a formidable energy barrier against wetting state transition [33-36]. The cuticles of several species of

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Surface Structure and Wetting of Collembola



Figure 3. SEM images of FIB cross sections of species 10 and 12. Left: species 10 *A. laricis* magnification 8 000X, Right: species 12 *X. maritima* 15 000X magnification. The images show sections of the cuticle where a prism shaped part has been removed by FIB milling, while the structuring around it was protected by a layer of platinum, to reveal cross sections of the granules. A single granule is highlighted by a white circle in each image. In species 10 there is no evidence of overhang, in species 12 overhang is present. doi:10.1371/journal.pone.0086783.g003

Collembola display overhang, which has been proposed as an explanation for the commonly observed excellent water repellency of Collembola cuticles by Helbig et al [19]. We must therefore consider the different surface structures, including overhang, of the studied Collembola to determine what creates the differences in wetting behavior.

In this study, we primarily emphasize the differences between the two non-superhydrophobic species (X. mariima and C. clavatus) and all other tested species. Though all tested species were hydrophobic ($\theta > 90^\circ$) a considerable contact angle hysteresis was observed for these two species. A contact angle hysteresis in this range will hinder droplet movement on the cuticle surface, the result is that water droplets can stick to X. maritima and C. clavatus, albeit with a large contact angle, while they simply slide off the other tested species as the curvature of the cuticle itself is larger than the sliding angle of a droplet resting on the cuticle. This constitutes a clear, qualitative difference in the wetting behavior of these two species.

It is difficult to see any clear trends when we compare the structural parameters (table 2) between superhydrophobic and non-superhydrophobic species. *X. maritima* possesses the largest

intergranular distance (d_0) for primary granules, but this is not much larger than those of e.g. *L* prasis and *F. quadrioculata. C. clavatus* has a rather small d_0 . Another important parameter is the height of the granules (H). *X. maritima* had the highest primary granules of all investigated species, which should help to stabilize the Cassie-Baxter wetting state and enhance hydrophobic properties [37]. *C. clavatus* had the second smallest primary granule height. Thus, neither separate nor in combination did the two parameters discriminate between the species that did and did not show superhydrophobic properties. E.g. two of the superhydrophobic species *I. prasis* and *D. olivacea* had about the same d_0 and smaller *H* than *X. maritima* and *C. clavatus* respectively. Also, the contact line length (*L*) as well as the two area parameters (*A* and *A_s*) of both *X. maritima* and *C. clavatus* are midrange, neither exceptionally large nor small.

The roughness (r) and solid area fraction (f) were calculated in order to evaluate the surfaces according to the theories of Wenzel, Cassie and Baxter [27,28]. The results, shown in table 3 and compared with the measured values in figure 5, clearly underestimate the real contact angle of all studied species. The Collembola cuticles considered in this work are certainly more complex than

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Figure 4. Measurement of structural parameters. The schematic shows how the structural parameters A, A_{st} b, d₀, L and p were measured from SEM images doi:10.1371/journal.pone.0086783.g004

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the regular, geometric patterns considered by Wenzel, Cassie and Baxter. When describing such complex, natural surfaces with a single value for roughness r or area fraction f, care was taken to make conservative translations. The values for r were calculated based on surface features smaller than those studied by Wenzel, while the values for f were calculated based on only the tops of the granules, thus neglecting the ridges; both of these considerations should lead to higher predicted contact angles for these models. The contact angle hysteresis was estimated based on Dufour's formula [29], but this overestimated the contact angle hystereses as compared to the measured values for all studied species, as shown in figure 6. Finally Choi's model [30] was used to estimate the receding contact angle, this underestimated the receding contact angle for all the superhydrophobic species, but interestingly overestimated it for X. maritima and C. clavatus.

Some key assumptions were made in the use of these models, namely that the intrinsic contact angle for the cuticle surface was $\theta_0 = 105^\circ$, the tops of the asperities were smooth $(r_f = 1)$ and the differential area fraction in the advancing direction was $f_a = 0$. The intrinsic contact angle of waxes consisting mainly of hydrocarbon chains are expected to be in the range of 90° to $110^\circ,$ while the intrinsic contact angles of flat samples of the waxes of insects, as well as that of chitin are approximately 105° [22,38]. If the intrinsic contact angles were larger than the assumed values, the predicted values of the Wenzel, Cassie-Baxter and Choi models would increase. Though even if the value was assumed to be as high as $\theta_0 = 120^\circ$ (a value typical for smooth polytetrafluoroethylene/Teflon) these models would only predict superhydrophobic behaviour for half of the investigated species. The models are still unable to differentiate between the superhydrophobic and nonsuperhydrophobic species, even with this unreasonably high estimated intrinsic contact angle. Smooth asperity tops is a less than likely assumption in that the granules display a slight curvature instead of completely flat plateaus, the assumption was made to simplify the Choi equation. The actual roughness parameter r_f is likely between 1 and 2, as $r_f = 2$ corresponds to hemispherical granule tops. In order to predict contact angles above 150° a roughness parameter of over 3 is needed, which is clearly higher than what the SEM images indicate. The Choi model is not able to differentiate between the superhydrophobic and non-superhydrophobic species regardless of what value is assumed for r_f . Finally, the assumption that the differential area
¥	Species	r	f	f_r	$\boldsymbol{\theta}_W$	θ_{CB}	$\Delta \theta_D$	$\theta^*_{r,choi}$
	H. viatica	2.49	0.34	0.29	115.6°	136.3°	12.2°	139.7°
*	H. viatica	2.74	0.13	0.24	118.4°	153.4°	10.8°	143.1°
	l prasis	1.32	0.16	0.24	103.3°	150.0°	10.3°	143.0°
	Onychiurus sp.	2.02	0.28	0.27	110.6°	140.5°	12.4°	141.3°
*	Onychiurus sp.	2.89	0.23	0.33	120.1°	143.9°	14.9°	136.5°
	F. quadrioculata	1.39	0.27	0.35	104.0°	140.9°	15.2°	135.6°
	A. septentrionalis	1.66	0.38	0.40	106.7°	133.6°	12.7°	132.1°
	D. olivacea	1.44	0.23	0.27	104.5°	144.2°	9.8°	141.1°
	A. besselsi	1.87	0.27	0.25	108.9°	140.9°	11.5°	142.2°
*	A. besselsi	1.99	0.12	0.22	110.2°	154.3°	9.9°	144.6°
	C. clavatus	1.32	0.37	0.39	103.3°	133.9°	12.3°	132.9°
	O. flavescens	1.86	0.26	0.28	108.9°	141.6°	6.8°	140.1°
0	A. laricis	1.26	0.41	0.41	102.6°	131.3°	8.1°	131.5°
1	I. anglicana	1.48	0.28	0.29	104.9°	140.5°	7.8°	139.4°
2	X. maritima	1.73	0.25	0.26	107.4°	142.5°	7.4°	141.4°

Calculated parameters, r: roughness factor, f: solid area fraction, f_j : differential solid area fraction in the receding direction, θ_{W} : estimated contact angle from the Wenzel equation, θ_{CB} : estimated contact angle from the Cassie-Baxter equation, $\Delta \theta_D$: estimated contact angle hysteresis based on Dufour's method and $\theta^*_{r,choi}$: estimated receding contact angle based on Choi's method. Rows marked * present values based on secondary granules.

doi:10.1371/journal.pone.0086783.t003

fraction in the advancing direction $f_a = 0$ is a result of the Cassie-Baxter (i.e. suspended) wetting state, which is the state modelled by the Choi model. Assuming a non-zero fraction will reduce the predicted advancing contact angles from perfect non-wetting, resulting in two values for the model $(\theta^*_{a,Choi} \text{ and } \theta^*_{r,Choi})$, but will not affect the values of $\theta^*_{r,Choi}$ which are the results discussed in this paper. We conclude that the established models can not be used to estimate the wetting properties of Collembola cuticles as the calculated values diverge too much from the measured values. This discrepancy between measured and estimated contact angles might indicate that either the models need to be modified, or that important parameters are not taken into account.

Overhang (reentrant granule geometries) has been suggested as such a parameter [19]. Overhang is not readily included in the classical models as only surface features are taken into account, what is going on beneath the surface in contact with the droplet is not considered at all by the Cassie-Baxter model for example. The presence of overhang would lead to a slightly larger roughness coefficient (r) in the Wenzel model, but the increase would be largely insignificant compared to the discrepancy between the predictions of the Wenzel model and the measured contact angles. The role of overhang is largely that of increasing the energy barrier between the Cassie-Baxter state and the Wenzel state, such that it prevents drops from spreading down between the granules on the cuticle surface [34,35]. This means overhang can readily explain why a drop would stay in the Cassie-Baxter state instead of the Wenzel state, but it cannot explain why the measured contact angles are larger than those predicted by the Cassie-Baxter or Choi models.



Figure 5. Measured contact angles compared to predicted contact angles. Measured advancing (θ_A) and receding (θ_R) contact angles with one standard deviation error bars as compared to the values predicted by the Wenzel (θ_{W}), Cassie-Baxter (θ_{CB}) and the Choi ($\theta_{r,Choil}$) equations. The minimum limit for contact angles considered superhydrophobic is denoted by a dotted line at 150°. Rows marked with an asterisk (*) denote predicted values based on secondary granules. doi:10.1371/journal.pone.0086783.g005

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Figure 6. Measured contact angles hysteresis compared to contact angle hysteresis predicted by the Dufour method. The maximum limit for contact angle hysteresis considered superhydrophobic is denoted by a dotted line at 10°. Rows marked with an asterisk (*) denote predicted values based on secondary granules. doi:10.1371/journal.pone.0086783.g006

Overhang is present on some, but not all of the studied Collembola. Of the two non-superhydrophobic species, X. maritima has overhang on the granules, while C. clavatus does not. Of the superhydrophobic group several species have overhang (e.g. A. septentrionalis and D. oliviaca) while several others lack overhang (e.g. F. quadrioculata and A. laricis). Thus, in contrast to the suggestion of Helbig et al. [19], our results do not indicate a direct link between the presence of overhanging structures and superhydrophobicity. In fact, there is no single parameter that explains why X. maritima and C. clavatus do not display the same superhydrophobic effect as all the other tested species.

The secondary granules present on some Collembola are significantly larger than the primary granules. This results in larger values for the intergranular distance (d_0) and granule height (H)when secondary granules are considered. The effect of secondary granules seem uncertain. In a purely Cassie-Baxter wetting state the presence of secondary granules would completely mask the effect of primary granules as any water would be suspended on the tops of the larger, secondary granules. This results in a significantly lower solid area fraction (f) when the secondary granules are considered, and consequently higher estimated contact angles (θ_{CB}) . In a Wenzel wetting state the secondary granules would slightly increase the roughness (r) and thus the estimated contact angle (θ_W) . However both models still severely underestimate the contact angle. The presence of secondary granules did not influence the estimated or measured values for the contact angles significantly. Examples of closely related Collembola species from very different humidity conditions showing almost identical secondary granule configurations are included as supporting information, figure S7. The lack of variation in the secondary granules for a wide variation in humidity indicate that the secondary granules are not a key part in the adaptation to humidity conditions.

Collembola normally possess a cover of microscopic hairs. The number of hairs and their arrangement are usually sufficiently conservative to be used as a taxonomic tool [26], but the length of the hairs may vary greatly even between closely related species. Wetting properties are also affected by the number and length of the hairs covering the body surface. [22,39] If the hair-cover, rather than the cuticle structure, is quantified and used with existing wetting models different values for the contact angles would be predicted. A cover of curved hairs may act in much the same way as structures with overhang and provide robust superhydrophobicity [40]. However, for Collembola the hairs are of microns to tens of microns in scale while the granules are on a scale of hundreds of nanometers, models that incorporate the contact line energy will therefore differ between the two and would be more likely to predict high contact angles for the granules.

Superhydrophobic cuticles were observed for a variety of Collembola species from different families and several different habitats. This was not a universal trait however as two of the tested species did not display the superhydrophobic effect, including a Collembola adapted to extreme drought (X. maritima) and one adapted to aquatic habitats (C. clavatus). No single structural parameter was observed that could explain the lack of superhydrophobicity in only two of the species. No direct link was found between structural overhang and superhydrophobicity as both structural overhang, and the lack thereof, was observed on both superhydrophobic and non-superhydrophobic cuticles. The most widely used equations underestimated the contact angles of the cuticles. This indicates that more sophisticated models are needed to explain the observed wetting behavior of Collembola cuticles. Closer study of the reasons behind this underestimation may yield interesting results from a biomimetics point of view, as a novel solution for achieving robust superhydrophobicity.

Materials and Methods

For droplets in contact with a substrate the contact angle θ is defined as the angle between the droplet, and the substrate at the contact line (i.e. at the droplet circumference). The receding contact angle θ_R is the contact angle for a droplet with a receding contact angle θ_A is the contact angle for a droplet with a vacuum contact line, e.g. for a shrinking droplet, while the advancing contact angle θ_A is the contact angle for an advancing contact line, e.g. a growing droplet. The difference between the advancing and receding contact angles is deemed contact angle hysteresis $\Delta \theta$. The quantities θ , θ_A , θ_R and $\Delta \theta$ are illustrated in figure 7.

Contact angles were measured with the sessile drop method, using a KSV CAM 200 contact angle goniometer and KSV CAM Optical Contact Angle and Pendant Drop Surface Tension Software v.4.04. The test liquid, de-ionized micropore water, was deposided with a syringe on the dorsal metasoma of Collembola that were fixed to microscope slides with double-sided adhesive tape. Advancing and receding contact angles were attained by leaving the syringe tip in the droplet and adding or siphoning liquid. An example of a droplet in contact with a Collembola is shown in the inset of figure 7. Uncertainties were estimated based on the sample standard deviation of the data set. The samples were classified as superhydrophobic or not superhydrophobic based on the following criteria: both the advancing and receding contact angles (θ_A and θ_R) should exceed 150° and the contact angle hysteresis ($\Delta\theta$) should not exceed 10°.

There are two general models for the interaction between liquids and rough surfaces. The liquid can be in complete contact with the surfaces, filling any openings between asperities. This model is referred to as the Wenzel state [27]. The second model assumes the liquid will not penetrate between the asperities on a

rough surface, keeping the liquid suspended in a composite contact partially touching the tops of the asperities and partially hanging suspended between the asperities. This is referred to as the Cassie-Baxter or composite state [28]. Considerable research has been conducted since the initial works of Cassie, Baxter and Wenzel. Modern theories of wetting, such as those of Choi [30] and Dufour [29], consider more complex partial wetting states and differential approaches where simple area fractions are no longer used; surfaces with submicron structures also makes it important to consider the three-phase contact line [29,30,41,42].

The approach of Choi et al.[30] is a variation of the Cassie-Baxter model; suspended wetting is assumed, but instead of using a single value for the solid area fraction (f) a differential approach is used with different values for the solid area fraction in the advancing and receding directions. Dufour's model [29], is a purely mechanical approach to describing contact angle hysteresis. The deformation energy needed to "stretch" drops before they detach from a surface is considered. For a system where the drop rests on the top of asperities, such as the granules of Collembola, the deformed volumes from each asperity corresponds to the solids of revolution of catenary curves. These deformation volumes can be estimated from the size and shape of the asperities. The contact angle hysteresis $(\Delta \theta)$ can then be estimated, either from an assumed value for the apparent advancing contact angle, or a posteriori from measured values of the apparent advancing contact angle.

The sizes of the the thinner and thicker parts of the cuticle structures were measured, as well as the height of and distances between these structures. Theses sizes were measured from Scanning Electron Microscopy (SEM) images taken with a Hitachi Su-6600 or with a FEI Helios NanoLab DualBeam FIB (using the electron beam). The samples were freeze dried and fixed to stubs using silver glue or carbon tape. The samples were coated with a thin layer of carbon and sputter coated with Pt.

Focused Ion Beam (FIB) milling was performed with a FEI Helios NanoLab DualBeam FIB to obtain cross-sectional SEM images of the surface structures. The samples were prepared as for general SEM imaging. Subsequently a thick (μm scale) protective layer of Pt was applied using the deposition mode of the instrument. Then a cubic section of the cuticle was removed with the ion beam, the region of removal was placed such that it intersected one or more granules. Afterwards the sample was tilted such that the cross-section of the granules could be imaged.

Collembola are non-regulated invertebrates which are not subject to any special laws or regulations related to animal experiments in Norway. The species studied are not endangered or protected. The samples were identified according to the key of Arme Fjellberg [32], species 3 was only identified to the family of Onychiuridae and not to a specific species within this family. Samples were collected in the field at various locations in southern Norway, except species 3 which was from a lab stock of Azorean origin (held by Leinaas). All field locations were in public areas with no special restrictions on the gathering of invertebrates. The samples were kept at high relative humidity on moist plaster of paris and fed with bark covered in green algae. The samples were killed with chloroform vapor immediately preceding the experiments to ensure freshness.

Twelve species were selected for this investigation. The selection was made to present the different surface structure modifications in Collembola, as well as presenting species from different families and from habitats ranging from extremely dry to very wet. The species included of the order Poduromorpha: *Hypogastura viatica* and *Xenylla maritima* from the family Hypogasturidae and an unidentified species from the Onychiuridae family; and the order Entomobryomorpha: *Anurophorus laricis, Anurophorus septentrionalis, Folsomia quadrioculata, Archisotoma besselsi, Cryptopygus clavatus, Desoria olivacea, Isotomurus prasis* and *Isotoma anglicana* from the family Isotomidae and *Orchesella flavescens* from the family Entomobryidae.

The following information on the species is based on [26,32] and own observations: *H. viatica* is a surface active species living at, or near, the intertidal zone, it moves away from the water at high tide. *X. maritima*, though of the same family as *H. viatica*, inhabits a

Figure 7. Contact Angles: The contact angle θ is defined as the angle between the droplet, and the substrate at the contact line. The receding contact angle θ_R is the contact angle for a droplet with a receding contact line, e.g. for a shrinking droplet, while the advancing contact angle θ_A is the contact angle for an advancing contact line, e.g. a growing droplet. Surfaces that when in contact with water display a contact angle of less than 90° are hydrophilic, surfaces with contact angles of more than 90° are hydrophobic and surfaces with both advancing and receding contact angles of more than 150° as well as contact angle hysteresis less than 10° are superhydrophobic. The inset shows a sessile droplet in contact with a springtail. doi:10.1371/journal.pone.0086783.g007



very different habitat, it lives on the crusts of lichens on boulders and standing tree trunks, which may beome very dry for prolonged periods; the species is highly drought resistant. A. laricis is from the same habitat as and often co-occurs with X. maritima. A. septentrionalis occurs in moderately dry forest floor, it is closely related to A. laricis. F. quadrioculata is a species typical for the lower litter layer, a habitat that is rarely flooded but may sometimes become quite wet, it is not surface active and is sensitive to dessication. A. besselsi is an intertidal species, which unlike H. viatica may become submerged during high tide. C. clavatus is found in association with rock pools and other small bodies of water near the shore. It is active under water, where it feeds on algea while submerged without showing signs of plastron formation. D. olivacea is a surface dwelling speces from wet habitats with water logged soil. I. prasis is a big, surface dwelling species found in wet and humid habitats, though usually not in the same habitats as D. olivacea. I. anglicana is a big surface dwelling species found in moderately humid habitats both on the forest floor and on open land. O. flavescens is another big, surface dwelling speceis that may be found together with I. anglicana as well as in wet, marshy habitats.

Supporting Information

Figure S1 SEM images of species 1 through 6. Top left: species 1 *H. viatica* 10 000X magnification, Top right: species 2 *I. prasis* 10 000X magnification, Mid left: species 3 *Onychiurus sp.* 20 000X magnification, Mid right: species 4 *F. quadrioculata* 10 000X magnification, Bottom left: species 5 *A. septentrionalis* 10 000X magnification, Bottom right: species 6 *D. olivacea* 10 000X magnification. The structures shown are typical for the dorsal metasoma. (TIF)

Figure S2 SEM images of species 7 through 12. Top left: species 7 *A. besselsi* 20 000X magnification, Top right: species 8 *C. clavatus* 15 000X magnification, Mid left: species 9 *O. flavescens* 10 000X magnification, Mid right: species 10 *A. laricis* 10 000X magnification, Bottom left: species 11 *I. anglicana* 10 000X magnification, Bottom right: species 12 *X. maritima* 10 000X magnification. The structures shown are typical for the dorsal metasoma.

(TIF)

Figure S3 Cross section SEM image of species 1 through 6. Top left: species 1 *H. viatica* 20 004X magnification, overhang is present, Top right: species 2 *I. prasis* 20 000X magnification, no overhang, Mid left: species 3 *Onychiurus sp.* 50 000X magnification, overhang is present Mid right: species 4 *F. quadrioculata* 25 000X magnification, no overhang, Bottom left: species 5 *A. septentrionalis* 20 004X magnification, overhang is present, Bottom right: species 6 *D. olivacea* 50 000X magnification, overhang is present. The structures shown are typical for the dorsal metasoma. (TIF)

Figure S4 Cross section SEM image of species 7 through 12. Top left: species 7 *A. besselsi* 50 000X magnification, overhang is present, Top right: species 8 *C. clavatus* 20 000X magnification, no overhang, Mid left: species 9 *O. flavescens* 35 005X magnification, no overhang, Mid right: species 10 *A. laricis* 8

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000X magnification, no overhang, Bottom left: species 11 *I. angliana* 120 000X magnification, overhang is present, Bottom right: species 12 *X. maritima* 15 000X magnification, overhang is present. The structures shown are typical for the dorsal metasoma. (TIF)

Figure S5 Nanoindenter AFM (Ni-AFM) image of species 1 through 6. Top left: species 1 *H. viatica* Top right: species 2 *I. prasis* Mid left: species 3 *Onychiurus sp.* Mid right: species 4 *F. quadrioculata* Bottom left: species 5 *A. septentrionalis* Bottom right: species 6 *D. olivacea.* The magnifications are indicated by the scale bars. The structures shown are typical for the dorsal metasoma. (TIF)

Figure S6 Nanoindenter AFM (Ni-AFM) image of species 7 through 12. Top left: species 7 *A. besselsi* Top right: species 8 *C. clavatus* Mid left: species 9 *O. flavescens* Mid right: species 11 *I. anglicana* Bottom left: species 12 *X. maritima.* The structures shown are typical for the dorsal metasoma, with the exception of species 9 where the structure of the head is shown, due to challenges in imaging the metasoma. (TIF)

Figure S7 Scanning Electron Microscope (SEM) images showing closely related species with secondary granules. Top left: *H. tullbergi*, Top right: *H. viatica*, Bottom: *C. longispina*. The images have 5000X magnification and show both secondary and primary cuticle granules. (TIF)

Table S1 Surface Structure Characteristics Based on NI-AFM. Surface structure characteristics, as measured on nanoindenter atomic force micrographs. *P*: number of edges in the closest equivalent polygon; d_0 : longest regular distance between primary granules; *H*: height of granules; L: length of the three-phase contact line for the wetting system of one granule; *A*: nominal area of a section of cuticle containing a single granule; A_s nominal surface area of a granule. Rows marked * present values based on secondary granules. (PDF)

(PDF

Table S2 Calculated Parameters Based on NI-AFM. Calculated parameters, based on nanoindenter atomic force micrographs; r: roughness factor, f: solid area fraction, f_r : differential solid area fraction, receding direction, θ_W : Estimated contact angle from the Wenzel equation, θ_{CB} : Estimated contact angle from the Cassie-Baxter equation, $\Delta \theta_D$: estimated contact angle hysteresis based on Dufour's method and $\theta_{r,chol}^*$: estimated receding contact angle based on Choi's method. Rows marked * present values based on secondary granules. (PDF)

Acknowledgments

The authors would like to thank the reviewers appointed by PLoS One.

Author Contributions

Conceived and designed the experiments: HG. Performed the experiments: HG. Analyzed the data: HG HPL CT. Contributed reagents/materials/ analysis tools: HG HPL. Wrote the paper: HG HPL CT.

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3.1 Article 1, Supporting Information

Additional images, tables and equations for "Surface Structure and Wetting Characteristics of Collembola Cuticles" were included as supporting information.

Supporting Information

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This is supporting information for "Surface Structure and Wetting Characteristics of Collembola Cuticles".

SEM Images

Scanning Electron Microscope (SEM) images of the cuticle structures of all studied collembola are presented in figures 1 and 2. SEM images of the cross-section of granules are also included (figures 3 and 4), legends indicate the presence of overhanging structures. Species 10 and 12, although included in the main text, are also included here for the sake of completion.

NI-AFM

Nanoindenter Atomic Force Microscopy (Ni-AFM) images were taken with a Ti950 Nanoindenter from Hysitron using a 90° cube corner tip. Both freshly killed and freeze dried samples were studied, without any applied coating. The samples were fixed to stubs using silver glue. Species 10 and the primary granules of species 3 were not imaged, this is because the cuticles of some collembola are very challenging to image with this method. It is assumed that the hairs contribute to this challenge. The NI-AFM images are presented in figures 5 and 6, the height of the granules, and other structural parameters can be estimated from the NI-AFM data, these are presented in table 1. These may be used to calculate relevant parameters and be used to predict contact angles, see table 2. The procedures and models are identical with the ones discussed for SEM images in the main text.

Secondary Granules

Figure 7 shows the cuticle structures of three related species of collembola from habitats of different humidity. *Hypogastura tullbergi* are found in dry tussocks, *Hypogastura viatica* are found in a selection of more moist habitats while *Ceratophysella longispina* are found in wet habitats. The structure, size and distribution of secondary granules appear to be very similar in the three species.

Models of Wetting

When a drop of liquid is in contact with a surface with some topography there are two general states it can assume. The drop can wet the entire surface, penetrating any "valleys" between the asperities. Or it can stay suspended on top of the asperities, allowing pockets of air to reamin between the asperities. The wetted state is commonly referred to as the Wenzel state, while the suspended state is referred to as the Cassie-Baxter state. The two states are illustrated in figure 8.

Wenzel intorduced a roughness factor to describe a non-smooth surface. For any nominal section of the sample there is an actual surface area, and there is a geometric surface area equal to the flat projected area, the roughness factor equals the ratio of these.

$$r = \frac{\text{Actual surface area}}{\text{Flat projected surface area}} \tag{1}$$

This roughness factor is used in Wenzel's equation, which predicts the "apparent" or observed contact angle θ_W^* for a rough surface based on the intrinsic contact angle θ_0 (that wich would apply to a completely flat surface) and the roughness factor r.

$$\cos\theta_W^* = r\cos\theta_0\tag{2}$$

Cassie and Baxter considered the contact between liquid drops and a porous solid, where the drops rest atop asperities on the solid surface such that only a fraction of the area beneath the drop is in contact with the solid. Cassie and Baxter reasoned that the fraction of a drop that rests on the solid asperities f_1 would follow the law for contact with the solid, while the fraction that rests on the air pockets between the asperities f_2 would act as if in contact with air. Assuming that $\cos_{Air} = -1$, this yields the Cassie-Baxter equation.

$$\cos^*_{CB} = f_1 \cos \theta_0 - f_2 \tag{3}$$

Figure Legends



Figure 1. SEM images of species 1 through 6. Top left: species 1 *H. viatica* 10 000X magnification, Top right: species 2 *I. prasis* 10 000X magnification, Mid left: species 3 *Onychivrus sp.* 20 000X magnification, Mid right: species 4 *F. quadrioculata* 10 000X magnification, Bottom left: species 5 *A. septentrionalis* 10 000X magnification, Bottom right: species 6 *D. olivacea* 10 000X magnification. The structures shown are typical for the dorsal metasoma.



Figure 2. SEM images of species 7 through 12. Top left: species 7 A. besselsi 20 000X magnification, Top right: species 8 C. clavatus 15 000X magnification, Mid left: species 9 O. flavescens 10 000X magnification, Mid right: species 10 A. laricis 10 000X magnification, Bottom left: species 11 I. anglicana 10 000X magnification, Bottom right: species 12 X. maritima 10 000X magnification. The structures shown are typical for the dorsal metasoma.



Figure 3. Cross section SEM image of species 1 through 6. Top left: species 1 *H. viatica* 20 004X magnification, overhang is present, Top right: species 2 *I. prasis* 20 000X magnification, no overhang, Mid left: species 3 *Onychiurus sp.* 50 000X magnification, pverhang is present Mid right: species 4 *F. quadrioculata* 25 000X magnification, no overhang, Bottom left: species 5 *A. septentrionalis* 20 004X magnification, overhang is present, Bottom right: species 6 *D. olivacea* 50 000X magnification, overhang is present. The structures shown are typical for the dorsal metasoma.



Figure 4. Cross section SEM image of species 7 through 12. Top left: species 7 A. besselsi 50 000X magnification, overhang is present, Top right: species 8 C. clavatus 20 000X magnification, no overhang, Mid left: species 9 O. flavescens 35 005X magnification, no overhang, Mid right: species 10 A. laricis 8 000X magnification, no overhang, Bottom left: species 11 I. anglicana 120 000X magnification, overhang is present, Bottom right: species 12 X. maritima 15 000X magnification, overhang is present. The structures shown are typical for the dorsal metasoma.



Figure 5. Nanoindenter AFM (Ni-AFM) image of species 1 through 6. Top left: species 1 *H. viatica* Top right: species 2 *I. prasis* Mid left: species 3 *Onychiurus sp.* Mid right: species 4 *F. quadrioculata* Bottom left: species 5 *A. septentrionalis* Bottom right: species 6 *D. olivacea.* The magnifications are indicated by the scale bars. The structures shown are typical for the dorsal metasoma.



Figure 6. Nanoindenter AFM (Ni-AFM) image of species 7 through 12. Top left: species 7 *A. besselsi* Top right: species 8 *C. clavatus* Mid left: species 9 *O. flavescens* Mid right: species 11 *I. anglicana* Bottom left: species 12 *X. maritima.* The structures shown are typical for the dorsal metasoma, with the exception of species 9 where the structure of the head is shown, due to challenges in imaging the metasoma.



Figure 7. Scanning Electron Microscope (SEM) images showing closely related species with secondary granules. Top left: *H. tullbergi*, Top right: *H. viatica*, Bottom: *C. longispina*. The images have 5000X magnification and show both secondary and primary cuticle granules.



Figure 8. The Wenzel and Cassie-Baxter wetting states. The Wenzel state assumes complete contact between the droplet and the substrate, with liquid filling any valleys. The Cassie-Baxter state assumes a droplet resting on top of asperities, with no liquid penetrating inbetween the asperities.

Tables

#	Species	P	d_0 [nm]	H [nm]	L [nm]	$A \ [\mu m^2]$	$A_s \ [\mu m^2]$
1	H. viatica	6	310	310	1210	0.15	0.10
1*	H. viatica	3	2110	1500	3640	8.00	0.99
2	I. prasis	5	790	150	1300	0.60	0.13
3*	Onychiurus	3	1490	950	4560	8.85	1.51
4	F. quadrioculata	4	730	120	2400	0.93	0.40
5	A. septentrionalis	3	540	150	1990	0.73	0.29
6	D. oliviaca	6	360	180	950	0.14	0.058
7	A. besselsi	6	230	40	480	0.35	0.017
7*	A. besselsi	4	520	140	1420	0.57	0.14
8	C. clavatus	4	300	130	1750	0.42	0.22
9	O. flavescens	6	230	120	1410	0.19	0.14
11	I. anglicana	4	590	180	2000	0.64	0.30
12	X. maritima	6	560	220	2800	0.66	0.55

Table 1. Surface Structure Characteristics

Surface structure characteristics, as measured on nanoindenter atomic force micrographs. P: number of edges in the closest equivalent polygon; d_0 : longest regular distance between primary granules; H: height of granules; L: length of the three-phase contact line for the wetting system of one granule; A: nominal area of a section of cuticle containing a single granule; A_s nominal surface area of a granule. Rows marked * present values based on secondary granules.

#	Species	r	f	f_r	$ heta_W$	θ_{CB}	$\Delta \theta_D$	$\theta^*_{r,Choi}$
1	H. viatica	2.64	0.66	0.33	117.3°	116.8°	13.6°	136.4°
1*	H. viatica	3.07	0.12	0.23	122.2°	153.9°	9.6°	143.9°
2	I. prasis	1.28	0.22	0.21	102.9°	144.9°	8.8°	145.4°
3*	Onychiurus	2.13	0.17	0.31	111.7°	149.2°	14.1°	138.3°
4	F. quadrioculata	1.24	0.44	0.33	102.5°	129.7°	14.9°	136.8°
5	A. septentrionalis	1.45	0.40	0.34	104.6°	131.8°	14.0°	135.8°
6	D. oliviaca	1.62	0.43	0.27	106.4°	130.4°	9.7°	141.3°
7	A. besselsi	1.54	0.049	0.27	105.6°	163.7°	12.3°	141.0°
7*	A. besselsi	1.82	0.24	0.28	108.4°	143.0°	12.5°	140.5°
8	C. clavatus	1.46	0.53	0.36	104.7°	124.5°	6.6°	134.7°
9	O. flavescens	1.28	0.71	0.38	102.3°	114.3°	7.9°	133.6°
11	I. anglicana	1.53	0.47	0.33	105.5°	127.6°	8.6°	136.9°
12	X. maritima	1.54	0.83	0.35	105.5°	108.1°	9.5°	135.1°

Table 2. Calculated Parameters

Calculated parameters, based on nanoindenter atomic force micrographs; r: roughness factor, f: solid area fraction, f_r : differential solid area fraction, receding direction, θ_W : Estimated contact angle from the Wenzel equation, θ_{CB} : Estimated contact angle from the Cassie-Baxter equation, $\Delta\theta_D$: estimated contact angle hysteresis based on Dufour's method and $\theta^*_{r,choi}$: estimated receding contact angle based on Choi's method. Rows marked * present values based on secondary granules.

4 Article 2

"Seasonal change in the wetting characteristics of the cuticle of the Collembola *Cryptopygus clavatus* (Schött, 1893), Håkon Gundersen, Christian Thaulow, Hans Petter Leinaas, Zoomorphology, vol. 134, no. 2, pp. 211-218, 2015 Reproduced under the creative commons 4.0 license, Copyright 2015 Gundersen et al.

Available with open access online.

http://link.springer.com/article/10.1007/s00435-015-0254-y

Zoomorphology DOI 10.1007/s00435-015-0254-y

ORIGINAL PAPER

Seasonal change in the wetting characteristics of the cuticle of the Collembola *Cryptopygus clavatus* (Schött, 1893)

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Received: 6 August 2014/Revised: 14 January 2015/Accepted: 16 January 2015 © The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract The littoral Collembola Cryptopygus clavatus spends the summer submerged, grazing on algae under water, and the winter on dry land. The cuticles of Collembola are, in general, highly water repellent, often superhydrophobic; the cuticle of C. clavatus has, in contrast, been described as not water repellent. Wetting properties are closely tied to surface structuring, and previous studies of Collembola cuticles have used the pattern of cuticular granules to explain the superhydrophobic properties of these cuticles. The wetting properties of the cuticles of C. clavatus were measured on animals acclimated to summer and winter. A significant difference in wetting performance was observed. Animals acclimated to winter conditions showed superhydrophobic non-wetting properties. Animals acclimated to summer conditions were not superhydrophobic, water droplets readily adhered to their cuticles. This large change in wetting behavior of the cuticle could not be explained by changes in the cuticular surface structure, which were very limited. Instead, we suggest a change in the epicuticular wax layer could explain the differences.

Communicated by Andreas Schmidt-Rhaesa.

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Department of Biosciences, University of Oslo, P.O. Box 1066, Blindern, 0316 Oslo, Norway e-mail: h.p.leinaas@ibv.uio.no **Keywords** Collembola · Cuticle · Seasonal adaptation · Superhydrophobic · Wetting

Introduction

The cuticles of Collembola (springtails) are in general highly water repellent, and often superhydrophobic (King et al. 1990; Helbig et al. 2011; Ghiradella and Radigan 1974; Noble-nesbitt 1963; Gundersen et al. 2014). As a result, most Collembola will form a protective plastron and float to the surface upon submersion, and consequently very few Collembola are able to remain active under water (King et al. 1990; Nickerl et al. 2012). In contrast, the cuticle of the littoral Collembola Cryptopygus clavatus (Schött, 1893), which spends extensive periods submerged with no visible plastron, has been described as "not water repellent" (Fjellberg 2007). C. clavatus grazes on algae in the rocky marine littoral zone. It may be observed creeping along the bottom of brackish rock pools and under the water film on rocks after rain. During winter, C. clavatus remains in shelter and does not venture out to graze either on land or in rock pools (Fjellberg 1998, 2011).

The wettability of a surface by water is quantified by the contact angle. When a drop of water is resting on a solid surface, it forms a spherical cap (Quéré and Reyssat 2008; Gao and Mccarthy 2009). The relative wettability of the solid results in a contact angle (θ) which can range from 0° (perfectly wetting) to 180° (perfect non-wetting), see Fig. 1. Surfaces with contact angles of 90° and up are referred to as hydrophobic (Gao and Mccarthy 2009; Gu 2002).

If water is carefully siphoned from a drop resting on a solid, the drop will retain the same contact area but change shape until a certain point when the contact line starts receding. Likewise, a drop to which more water is added will



Fig. 1 a Contact angle θ , the angle between the substrate and the drop. Contact angles of 90° and up are considered hydrophobic. b Receding contact angle θ_R for a shrinking droplet. c Advancing contact angle θ_A for a growing droplet

retain the same contact area but change shape until the contact line starts advancing (Gao and McCarthy 2006). Any system therefore displays not one, but two defined contact angles, the *receding contact angle* (θ_R) and the *advancing contact angle* (θ_A), see Fig. 1. The difference between these two angles is referred to as the *contact angle hysteresis* ($\Delta\theta$). The mobility of a drop on a surface is determined by the contact angle hysteresis, a low contact angle hysteresis allows drops to move freely on a surface (Gao and Mccarthy 2009; Shirtcliffe et al. 2010). For a surface to be considered superhydrophobic, an advancing contact angle (θ_A) of at least 150° and a contact angle hysteresis ($\Delta\theta$) of no more than 10° is required (Bhushan and Jung 2011).

The cuticles of Collembola are, with few exceptions, superhydrophobic (King et al. 1990; Helbig et al. 2011; Gundersen et al. 2014; Hobæk et al. 2011). Superhydrophobic cuticles in Collembola and other arthropods protect against drowning and pathogens, this occurs through the formation of a plastron upon submersion and through the self-cleaning effect, respectively (King et al. 1990; Gundersen et al. 2014). An animal that respires through the body surface, as Collembola do, or through a tracheal system, as most insects do, can survive indefinitely in aerated water as long as a thin layer of air is retained around the cuticle or the opening of the trachea. This air retention is enabled by superhydrophobic structures on the cuticle, setae, microtrichiae or a combination thereof (Crisp and Thorpe 1948; Bush et al. 2007; Neumann and Woermann 2009). Some insects, notably their larvae, and apparently also the Collembola C. clavatus respire under water through direct gas exchange with water.

Studies on the wetting behavior of Collembola cuticles have so far focused on the surface structures present on the cuticles (Helbig et al. 2011; Gundersen et al. 2014; Nickerl et al. 2012), which include a network of granules and ridges on a submicron scale (Lawrence and Massoud 1973), see Fig. 2. The presence of overhanging structures (Helbig et al. 2011) and their exact size, distance and height (Gundersen et al. 2014) are likely important factors in determining the wetting behavior of the cuticles. Microsized structures with overhang, where the top of the structures are wider than the base, resulting in a reentrant cross section similar to that of a mushroom or one-legged table, are known to enable robust superhydrophobic behavior. Studies of ordered arrays of micropillars with overhanging features (Choi et al. 2009; Cao et al. 2007; Tuteja et al. 2008) and different cross sections (Zheng et al. 2010) have been performed in an effort to create more robust, synthetic superhydrophobic surfaces.

Superhydrophobic effects are known to be a combination of surface chemistry and surface structures, mostly inherently hydrophobic surfaces with a nano-scale or hierarchic surface roughness (Shirtcliffe et al. 2010; Bhushan and Jung 2011). The surface chemistry of Collembola cuticles is modified by epicuticular waxes, which likely play a dual role of protecting against desiccation and improving non-wetting properties (Ghiradella and Radigan 1974; Noble-nesbitt 1963; Gundersen et al. 2014).

The purpose of this work was to study and explain the fundamental change in wetting properties of the cuticle of *C. clavatus* from summer to winter conditions. The cuticle changes from readily wettable in summer conditions to superhydrophobic in winter conditions. Conventional models of wetting and the current understanding of why the cuticles of most Collembola are superhydrophobic would indicate a structural change in the cuticle from season to season, whereas the current understanding of Collembola cuticular structures is that there is no mechanism that can change these during the life span of individual Collembola.

Experimental method

Live *C. clavatus* (Collembola are non-regulated invertebrates which are not subjected to any special laws or regulations related to animal experiments in Norway.) were collected by



Fig. 2 Scanning electron microscopy (SEM) images showing the cuticle of *C.clavatus*. The dorsal metasoma is shown at the joint between the third and fourth segment of summer-acclimated (\mathbf{a} , \mathbf{b}) and winter-acclimated animals (\mathbf{c} , \mathbf{d}); at 1,000× and 15,000×

magnification. Atomic force microscopy (AFM) images are also shown of summer- (e) and winter-acclimated animals (f), color coding in AFM images are based on height difference

Arne Fjellberg at Tjøme, Norway, in October 2011. One set of animals was acclimated to winter conditions by being kept at 3 °C in dry conditions, while another set of animals was acclimated to summer conditions by being kept submerged at 10 °C. The animals were killed with chloroform vapor immediately preceding the experiments.

The wetting properties of the cuticle were determined through the sessile drop method. Drops were applied with a syringe (31 gauge stainless steel, Hamilton Company) and observed with a KSV CAM 200 contact angle goniometer. The data were analyzed with KSV CAM Optical Contact Angle and Pendant Drop Surface Tension Software v.4.04. The test liquid used was de-ionized micropore water. The tests were performed at room temperature at ambient humidity and pressure. Advancing and receding contact angles were attained by leaving the tip of the needle in the drop and adding or siphoning liquid. Drops were deposited on the dorsal metasoma. Some animals were also completely submerged by depositing drops that were larger than the animals. Submerged animals were investigated for visible signs of a retained air layer.

Atomic force microscopy (AFM) was performed in ambient conditions. A TI-750 UBI nanomechanical testing system from Hysitron was used with a 90° cube corner tip to scan the surface in contact mode. AFM brings a piezocontrolled probe with a very fine diamond tip into contact with the sample and allows the scanning of surface images with height data; this method allowed a direct measurement of granule height.

Scanning electron microscopy (SEM) was performed with a Hitachi SU 6600 using the secondary electron detector. Animals were killed and immediately freezedried. Dried animals were coated with carbon in an SEM Turbo Carbon Coater from Agar Scientific (typical settings: $t = 2 \times 8$ s, E = 4.8 kV), prior to the SEM study.

Animals studied in Cryo-SEM were frozen in nitrogen slush and transferred to a Gatan Alto cryo box set to -95 °C; the animals were sputter-coated with approximately 5 nm gold palladium alloy and subsequently transferred to the chamber of a Hitachi S-4800 field emission SEM which was kept at -150 °C. SEM and Cryo-SEM allowed imaging and subsequent measurement of the size and shape of cuticular granules.

Cross sections were milled with a FEI Helios Nanolab dual-beam Focused Ion Beam (FIB) and imaged with the SEM beam of the same tool. Animals were prepared as for SEM imaging with an additional coat of platinum applied with an Edwards Sputter Coater S150B (typical settings: t = 60 s, I = 20 mA). A thicker protective field of platinum was applied with the FIB system on the area where the cross section was performed. FIB enables site-specific

milling with nanometer resolution, which allowed cross sections of granules to be obtained.

Results

Contact angles are presented in Table 1: the results of the summer-acclimated animals are the same as those previously reported in a wider study of the wetting of Collembola cuticles in a large selection of species (Gundersen et al. 2014). When subjected to the commonly used criteria for superhydrophobic surfaces (Guo et al. 2011), an advancing contact angle (θA) of at least 150° and a contact angle hysteresis ($\Delta \theta$) of no more than 10°, the cuticles of specimens acclimated to winter conditions were found to be superhydrophobic, while specimens acclimated to summer conditions were not superhydrophobic. Test droplets were observed to cling to summer-acclimated animals, while they were deflected by the winter-acclimated animals, see Fig. 3.

Figure 2a, c shows the cuticle at $1,000 \times$ magnification with the joint between the third and fourth segment of the dorsal metasoma visible. Figure 2b, d shows the cuticle of the same region at $15,000 \times$ magnification. Figure 2e, f shows AFM images of the cuticular structure. The SEM

Table 1 Contact angles: advancing contact angles (θ_A), receding contact angles (θ_R), an contact angle hysteresis ($\Delta\theta$) of winter- and summeracclimated animals; all values are given with a statistically attained standard deviation value from a set of measurements

	$\theta_{\rm A}$	θ_{R}	$\Delta \theta$	Superhydrophobic
Winter-acclimated	$166^{\circ} \pm 2^{\circ}$	$166^{\circ} \pm 2^{\circ}$	$pprox 0^\circ \pm 3^\circ$	Yes
Summer-acclimated	$140^{\circ} \pm 3^{\circ}$	$118^{\circ} \pm 11^{\circ}$	$22^{\circ} \pm 11^{\circ}$	No



Fig. 3 Images from the contact angle goniometer illustrating highly contrasting behavior with water forming a round droplet on a winteracclimated animal and adhering to a summer-acclimated animal

Table 2 Surface structure characteristics: distance between granules (d_0) , height of granules (H), length of the three-phase contact line for the wetting system of one granule (L), the nominal area of the air pocket between granules (A), the nominal surface area of a granule (A_s)

	d_0	<i>H</i> (µm)	<i>L</i> (μm)	A (μm)	As
Winter-acclimated	0.42 ± 0.06	0.12 ± 0.01	2.2 ± 0.8	0.65 ± 0.5	0.39 ± 0.3
Summer-acclimated	0.38 ± 0.06	0.12 ± 0.01	2.4 ± 0.4	0.73 ± 0.2	0.42 ± 0.1



Fig. 4 Differences in the cuticle granule curvature for animals acclimated to winter (*left*) and to summer (*right*). Winter-acclimated animals tend toward convex curvature, as compared to straight-edged granules. Summer-acclimated animals tend toward concave curvature,

and AFM images were used to determine the surface structure characteristics used in the common wetting models, i.e., the pitch, size, and shape of granules, and these are presented in Table 2, see (Gundersen et al. 2014) for a close discussion on the determination of such characteristics from a SEM image. The sole significant difference found was a change in the curvature in the edges of the granules, which in winter-acclimated animals were straight to convex and in summer-acclimated animals tended to be straight to concave (see Fig. 4). This difference can be quantified by comparing the area contained within a traced circumference around a granule with the area contained within an equivalent straight-edged granule. The ratio between these areas (traced in red and blue in Fig. 4) was $\approx 1.02 \pm 0.02$ for summer-acclimated animals and $\approx 1.14 \pm 0.02$ for winter-acclimated animals.

Cross sections of the cuticle obtained with dual-beam Focused Ion Beam (FIB) milling and SEM imaging are presented in Fig. 5a, b. The purpose of imaging cuticle

as compared to straight-edged granules. **a**, **b** Granules traced in *red*, with straight-edged equivalent granules indicated in *blue*. **c**, **d** The general cuticle structure. *Scale bars* are 500 nm (**a**, **b**) and 10 μ m (**c**, **d**)

cross sections was to observe the presence or lack of overhanging structures, which fundamentally alters wetting behavior when present. No overhanging geometry was observed in any cross sections.

Discussion

A significant, qualitative difference in wetting properties was observed for summer-acclimated animals of *C. clavatus* as compared to winter-acclimated animals. The properties for summer-acclimated animals are in stark contrast to the properties observed on most Collembolan cuticles, which are almost universally superhydrophobic (Gundersen et al. 2014; Helbig et al. 2011). A superhydrophobic cuticle would, however, make the usual summer behavior of *C. clavatus* impossible (Fjellberg 1998, 2011). According to the prevailing knowledge of wetting and superhydrophobic surfaces, this change in



Fig. 5 Scanning electron microscope (SEM) images of cross sections of cuticles milled with a Focused Ion Beam (FIB). Images with 12,000X and 20,000X show a cross section of the granular structure

wetting must be caused by either a change in surface structuring, a change in surface chemistry or a combination of the two (Shirtcliffe et al. 2010; Bhushan and Jung 2011).

The most important surface features on the cuticle of C. clavatus, with regards to wetting performance, are the cuticular granules and setae. The cover of setae, while important for the wetting performance for many arthropods (Bush et al. 2007), remained unchanged for summer- and winter-acclimated animals of C. clavatus. The granules can be quantified by their size, shape and the distance between them. The size of and distance between the granules remained the same. In terms of shape, the presence or lack of an overhang, or undercut, beneath the granules is an important feature in wetting systems (Tuteja et al. 2008), and has been suggested as the main contributor to the superhydrophobicity of Collembola cuticles (Helbig et al. 2011). However, as no overhang was observed on either summeror winter-acclimated animals, this cannot explain the change in wetting behavior either. The only observable change in surface structure was a slight shift in the shape of the cuticle granule edges from convex curvature on winteracclimated animals to concave curvature on summer-acclimated animals. Adjusting for this small change in contact line length and granule area in classical (Cassie and Baxter 1944) and modified wetting models (Zheng et al. 2010; Choi et al. 2009) does not yield qualitatively different results. While the physical characteristics of the cuticle surface has been assumed to be the main contributor to the non-wetting properties of Collembola cuticles (Helbig et al. 2011; Gundersen et al. 2014), for C. clavatus this is clearly not the case, as evident by the drastic difference in wetting behavior for a very similar surface structure. However, the minor change in granule shape could reflect changes in the wax layer.

covering the dorsal metasoma of a winter-acclimated animal (**a**) and a summer-acclimated animal (**b**). There are no indications of overhanging structures on the granules, which appear quite flat

The thicker parts of the cuticle surface are covered by an epicuticular wax layer that blocks gas exchange (Noble-nesbitt 1963). Covering the cuticle in a wax layer is a trade-off between efficient respiration and protection against desiccation, as Collembola respire, but also lose water by evaporation, through the gas permeable sections of the cuticle that lack a wax layer (King et al. 1990; Noble-nesbitt 1963; Leinaas and Fjellberg 1985). The distribution of permeable surface area and gas blocking areas form the well-known cuticular patterns of Collembola (Lawrence and Massoud 1973; Leinaas and Fjellberg 1985). This has the added effect of creating the superhydrophobic effect which is present in many Collembola (Helbig et al. 2011; Noble-nesbitt 1963; Gundersen et al. 2014) as the wax layer provides the hydrophobic surface chemistry which, in combination with micro- or sub-micro-scale roughness, is required for superhydrophobic non-wetting (Gao and McCarthy 2006; Shirtcliffe et al. 2010; Bhushan and Jung 2011). A reduction in the epicuticular wax layer as part of the summer acclimation can therefore explain the change in wetting behavior.

The cover of epicuticular waxes is subjected to two different selections: one which balances respiration and desiccation and one in which a superhydrophobic cuticle is the result. Modification of the cuticle structure as adaptation to specific drought exposure of the habitat has been well documented (Leinaas and Fjellberg 1985). By comparison, evolution of superhydrophobicity is less obvious, as it seems to be a general characteristic of Collembola from different habitat types, and not limited to habitats that are especially exposed to flooding (Gundersen et al. 2014). However, the seasonal change in the wetting properties of *C. clavatus* clearly shows the result of a direct selective change. While superhydrophobic cuticles protect Collembola from drowning during submersion (King et al. 1990; Ghiradella and Radigan 1974), it would also prevent

C. clavatus from its characteristic underwater grazing behavior. Plastrons (a local bubble of retained air) around superhydrophobic cuticles provide enough buoyancy to prevent the submersion of animals during normal conditions, or return the animal to the surface after forced submersion. *C. clavatus* would not be able to overcome the buoyancy of a plastron nor would it be able to graze and walk underwater with a plastron. During winter, there is no need for the ability to graze under water, as *C. clavatus* spends the season in inactivity. However, the need to prevent water loss is ever-present, as is the ability to survive occasional events of submersion during flooding.

It is therefore likely that the change in wetting behavior is an adaption to a twofold challenge. The non-wetting effect needs to be reduced in summer to allow grazing, while the water vapor permittivity needs to be reduced in winter. Since both of these qualities may be controlled by the extent and chemical characteristics of the epicuticular wax layer, a seasonal change in the wax layer can explain both how *C. clavatus* is able to adapt, as well as why it shows such excellent water repellence in winter. A change in the epicuticular wax layer could also explain the small change in granule shape that was observed. A more extensive wax cover on the top of the granules in animals acclimated for winter could result in the more convex shape.

The changes in the wetting properties of the cuticle of summer- and winter-acclimated animals of C. clavatus cannot be explained by structural changes of the cuticle. There is no known mechanism that would allow individual Collembola to change the shape and size of its granules; and no change was observed. The changes in wetting properties can, however, be explained by changes in the epicuticular wax layer, and such changes are already a known mechanism of adaptation to drought exposure in other species of Collembola. We therefore suggest changes in the epicuticular wax layer as a model of explanation for the seasonal changes in wetting properties. We stress that more detailed studies of the cuticular waxes of C. clavatus and other Collembola are needed if a complete understanding of their wetting, and changes in wetting performance is to be reached. This would contribute to the understanding of natural superhydrophobic surfaces, where surfaces with switchable wetting properties are especially interesting, and would also be of great interest to the evolutionary understanding of how these animals adapt to their environments.

Acknowledgments The authors thank Arne Fjellberg for collecting the animals, and Antje Hofgaard at the EM-Lab for biological sciences at UiO, and for equipment used at the NanoLab, Nanomechanical Lab, and EM-Lab at the departments of Chemical Engineering, Engineering Design and Materials, and Biology at NTNU. **Conflict of interest** The authors declare that they have no competing interests.

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5 Article 3

"Collembola Cuticles and the Three-Phase Line Tension", Håkon Gundersen, Hans Petter Leinaas, Christian Thaulow, to be published in the Beilstein Journal of Nanotechnology

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Collembola Cuticles and the Three-Phase Line Tension

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Abstract

The cuticles of most springtails (Collembola) are superhydrophobic, but the mechanism has not been described in detail. Previous studies have suggested that overhanging surface structures play an important role, but such structures are not a universal trait among springtails with superhydrophobic cuticles. A wetting experiment with a fluorescent dye revealed the extent of wetting on exposed surface structures. Using simple wetting models to describe the composite wetting of the cuticular surface structures result in under predicting the contact angles of water, including the three-phase line tension allows prediction of contact angles in the observed range. The discrepancy between the contact angle predicted by simple models and those observed is especially large in the springtail *Cryptopygus clavatus* which changes, seasonally, from superhydrophobic to wetting without a large change in surface structure; *C. clavatus* does not have overhanging surface structures. This large change in observed contact angles can be explained with a modest change of the three-phase line tension.

Introduction

Collembola, a group of small, terrestrial hexapods, have been known to possess remarkable water repellent properties.¹⁻⁷ Robust water repellence has been the subject of extensive research, with naturally occurring surfaces providing the most well known examples of this effect.^{8,9} This effect has great potential for use in functional surfaces with effects like self-cleaning, drag reduction and air-retention.¹⁰⁻¹² The field of superhydrophobic surfaces has made extensive use of biomimetic methods, where imitation of natural surfaces provide the basis for man made surfaces.^{9,13,14} The exact nature of and mechanism behind natural water repellent surfaces is therefore of great interest beyond the field biology.

While the water repellency of Collembola has long since been described in general, macroscopic terms, a specific mechanical explanation has been lacking. Cassie and Baxter described a composite wetting state, where water wets only the tops of surface features, without wetting the substrate in between.¹⁵ The composite wetting state assumed by Cassie and Baxter is well known in a range of other natural superhydrophobic surfaces, ⁹ and the stability of the composite state on Collembola cuticles in particular has been the subject of recent study.^{4,7} The apparent contact angle of a composite wetting state is predicted by the Cassie-Baxter equation, which underestimates the contact angle of Collembola cuticles⁵ and sub-micron sized surface structures in general.¹⁶ The Cassie-Baxter equation also fails to predict changes in contact angle without an accompanying change in surface structure, such as the seasonal change in wetting characteristics for the collembolan species *Cryptopyqus clavatus*.⁶

The three-phase line tension is an energy term associated with the line of contact between three phases (most commonly solid, water and air) in partially wetted systems.¹⁷ By including a three-phase contact line term in the equation for the apparent contact angle, Zheng et al.¹⁶ were able to predict the size scale dependency of the apparent contact angle for sub-micron surface structures. The effect of the three-phase line tension on the apparent contact angle is significant for systems with a large three-phase line length in relation to the wetted surface area. The micron and sub-micron sized cuticular granules of Collembola have such a relation. We propose that using the equation of Zheng et al. can predict the high contact angles observed in Collembola in general, as well as provide a possible mechanism for the seasonal change of *Cryptopygus clavatus* in specific.

The three phase line tension (λ) , or *line tension* for short, is an energy term related to the parts of a system where three phases meet. In the case of a droplet of liquid resting on a solid surface, the three phase line is simply the perimeter of the drop. In the case of a drop resting on the top of surface roughness features (i.e. a Cassie Baxter model state), the three phase line is the sum of the perimeters of each wetted roughness top.

The molecules near three phase contact lines are subject to different intermolecular forces, compared to molecules in bulk phases, which results in a line tension. This is analogous to how the balance of intermolecular forces acting on a molecule near a two phase interface result in an interfacial tension, see figure 1. Reported magnitudes of the line tension for different systems and experimental methods range from $\lambda = 10^{-10}$ N to $\lambda = 10^{-6}$ N, with a majority of studies on solid-liquid-vapor systems falling in the higher end of this range.¹⁷



Figure 1: Imbalance of intermolecular forces for molecules near the three phase contact line. The length of the arrows indicate the relative strength of the intermolecular interactions with the different phases. Adapted from Amirfazli and Neumann.¹⁷

Partial wetting is a state where a liquid rests on the top of surface roughness features such that the roughness tops are wetted, while the substrate between tops is not wetted. Partial wetting is often referred to as a composite wetting state or a Cassie state after the early proposed equation of Cassie and Baxter (eq:1) based on a surface energy argument for the case of a droplet resting on a composite surface.¹⁵

$$Cos(\theta_{CB}^*) = f_1 Cos(\theta_1) + f_2 Cos(\theta_2) + \dots$$
(1)

Where θ^* is the apparent contact angle, f_i is the fraction of the surface *i* in contact with the water drop and θ_i is the inherent contact angle of a smooth surface *i*. For the special case of a water drop on the roughness tops of a chemically homogeneous rough surface (i = 1) in air (i = 2), equation 1 is reduced to equation 2 through the assumptions that $f_1 + f_2 = 1$ and $Cos(\theta_2) = -1$.

$$Cos(\theta_{CB}^*) = -1 + f(Cos(\theta_0) + 1)$$
⁽²⁾

Where f is the solid area fraction, θ_0 is the inherent contact angle of the smooth solid.

Zheng et al. included a the three phase line tension in the Cassie Baxter equation, see equation $3.^{16}$

$$Cos(\theta_{\rm Z}^*) = -1 + (1 + Cos(\theta_0))(1 - \frac{l_{\rm cr}}{S})f$$
(3)

Where S is the "Roughness Factor" $S = \frac{A_s}{L}$, the ratio between the cross-sectional area (A_s) and perimeter (L) of a surface roughness top. And l_{cr} is an "intrinsical chemical length" given by equation 4.

$$l_{\rm cr} = \frac{\lambda}{(1 + Cos(\theta_0))\gamma_{\rm lg}} = \frac{\lambda}{\gamma_{\rm lg} + \gamma_{\rm sg} - \gamma_{\rm sl}} \tag{4}$$

Where λ is the three phase line tension and γ_{lg} , γ_{sg} and γ_{sl} are the interfacial energies of the liquid-gas, solid-gas and solid-liquid interfaces.

Equation 3 can be written in an equivalent form without the novel parameters (S and l_{cr}), see equation 5.

$$Cos(\theta_{\rm Z}^*) = -1 + f(Cos(\theta_0) + 1 - \frac{\lambda L}{A_{\rm s}\gamma_{\rm lg}})$$
(5)

Zheng et al.¹⁶ developed the equation to describe the case of droplets resting on the top of small surface roughness features, treating the three phase line tension as a parameter to describe different contact angles observed for surfaces with feature tops with different size scales but identical solid area fraction. Zheng et al. reported that a line tension magnitude of 1.57×10^{-8} N was found to provide a good agreement between results and theory for roughness scales down to $S \approx 0.3 \ \mu m$, below which the equation predicts contact angles of 180°.

Certain assumptions or simplifications must be used in order to apply these equations to Collembola cuticles. Thicker and thinner parts of the Collembola cuticles form recognizable patterns. The thicker parts are referred to as granules, and are connected by sections of intermediate thickness referred to as ridges. These granules are of sub-micron size, usually in the range of a few hundred nanometers, and typically form a hexagonal pattern of triangular granules connected by straight ridges.¹⁸ A rhombic pattern of rhombic granules is also common,^{5,18,19} this is the type of pattern on *Cryptopygus clavatus*.⁶ The partial wetting state where only granule tops are wetted can be approximated by simple tesselating patterns. The repeating unit is three-sided prism, surrounded by a triangular open space, for approximately hexagonal cuticle patterns. For approximately rhombic cuticle patterns, the repeating unit is a four-sided prism, surrounded by a square open space. See figure 2, for a comparison of the hexagonal and rhombic approximations. The two characteristic lengths (l_1 and l_2) can be used to determined the relevant parameters; $f_{\text{Hexagonal}} = l_1^2/l_2^2$, $S_{\text{Hexagonal}} = l_1/4 \sqrt{3}$, $f_{\text{Rhombic}} = l_1^2/l_2^2$, $S_{\text{Rhombic}} = l_1/4$.

Both the Cassie-Baxter model and Zheng's models of wetting, include the inherent contact angle of the substrate (θ_0) as a parameter. Substrates with $\theta_0 < 90^\circ$ are not expected to form stable composite wetting states, which is a prerequisite of both models. Surface structures with reentrant geometry (overhang) can support composite wetting states with any value of θ_0 .²⁰ Such geometry occurs in several collembolan species, ⁴ but is not a universal trait in these animals.^{5,6} The upper limit of θ_0 is about 120° for real surfaces, observed on perfluorinated polymers, or 156° for a theoretical surface with no surface tension.²¹ Insect waxes fall in the range of 90° to 110°, typically around 105°, which is also the inherent contact angle of water on chitin.^{22,23} The range of reasonable values for θ_0 is therefore limited to 90° – 120°; where



Figure 2: A simple, tessellating pattern that can approximate Collembola cuticles for the partial wetting state where only granule tops are wetted. The two characteristic lengths $(l_1 \text{ and } l_2)$ can be used to determined the relevant parameters; $f_{\text{Hexagonal}} = l_1^2/l_2^2$, $S_{\text{Hexagonal}} = l_1/4\sqrt{3}$, $f_{\text{Rhombic}} = l_1^2/l_2^2$, $S_{\text{Rhombic}} = l_1/4$. The geometric patterns are overlaid in SEM images of Xenylla maritima (hexagonal) and Cryptopygus clavatus (rhombic) for comparison.
the lower bound is a requisite of the composite wetting state and the upper bound is the highest known value for real surfaces.

The model for predicting the apparent contact angles becomes size scale dependent when the area to perimeter ratio (S) of surface features is included. The magnitude of the size scale dependency is determined by the three-phase line tension (λ) . The exact magnitude of λ is not known for the Collembola cuticle, water, air three-phase system. Zheng's model can either be used with measured contact angles to estimate λ or with estimated values of λ to predict the apparent contact angle θ^* of systems with known geometry. For low values of λ the contact angles predicted by equation 5 approach that of the Cassie-Baxter model (equation 2), which means that f is the dominant factor, high values of λ give $\theta^*_Z = 180^\circ$. Exactly what constitutes "low" and "high" values of λ is determined by Zheng's "Roughness Factor" S, as an example f = 0.25 and $S = 0.1 \mu m$ predicts a contact angle within 0.5° of that of the Cassie-Baxter model for $\lambda < 10^{-10}$ N, while $\theta^*_Z = 180^\circ$ is predicted for $\lambda > 10^{-8.27}$ N.

Figure 3 shows the effect of varying the inherent contact angle (θ_0) , the roughness factor (S) and the solid area fraction (f) in the Zheng model $\theta_Z^*(\lambda)$. Small values of λ yield a result that approaches that of the regular Cassie-Baxter model (a horizontal line), while high values of λ results in a prediction of perfect non-wetting (shown as $\theta_Z^* = 180^\circ$). Between the extremes of pure Cassie-Baxter behavior and pure non-wetting a critical range of λ is found, where the exact magnitude of λ determines the contact angle. Changing the roughness factor shifts this critical range of λ , but does not qualitatively change the behavior. Changing the solid area fraction changes the value predicted by the Cassie-Baxter model, and thus shifts the minimum value of θ_Z^* for low values of λ . Changing the inherent contact angle shifts both the minimum value of θ_Z^* and the critical range of λ .

The composite wetting state assumed by the Cassie-Baxter model, as well as the derivative Zheng model, can be demonstrated by a wetting experiment with a dye. Nickerl et al demonstrated a lipid layer (epicuticular wax) covering all parts of the Collembola cuticle,



Figure 3: The effect of θ_0 , S and f on the model of Zheng et al.:¹⁶ $\theta_Z^*(\lambda)$. A system with $\theta_0 = 105^\circ$, $S = 0.1 \mu m$ and f = 0.25 is shown as a red line in each graph.

using time of flight secondary ion mass spectrometry.²⁴ A lipophilic dye, such as Nile Red, will bind to any part of such a layer it came into contact with. The parts of the cuticle that were wetted by the dye can then be visualized with fluorescence microscopy.

Results and Discussion

Collembola cuticles were dyed with a water acetone solution of Nile Red and imaged with fluorescence microscopty, a selection of cuticles are shown in figure 4. The tops of primary and secondary granules are clearly visualized, the base between granules was not visualized on any samples. This indicates that the tops of the granules were wetted by the dye solution, while the base cuticle was not wetted. This is in accordance with the assumption of a composite wetting state, with a wetted area fraction (f) corresponding to the area fraction of cuticular granules.

The area fraction covered by granules (f) is the main parameter used to estimate apparent contact angles by the Cassie-Baxter equation (equation 2). Nickerl et al.¹⁹ studied the cuticle structure of a larger selection of Collembola. The geometric measurements (granule size and distance) can be used to estimate the area fraction covered by granules (f) for species with regular granule patterns, figure 2 shows such an approximation for rhombic and hexagonal granule patterns. We applied this estimation method to the measurements of Nickerl et al. which yielded a range of granule area fractions from 0.111 to 0.709, compared to a range of



Figure 4: Stained samples imaged with fluorescence microscopy, showing the tops of primary and (present in d and g) secondary granules. The light areas are those where the lipophilic dye has bonded with the surface, indicating wetting contact between the dye solution and a lipid layer. The images were obtained with confocal fluorescence microscopy using incidental light of 488 nm wavelength and a bandpass filter (565-615 nm). **a**) *C.clavatus* (winteracclimated), **b**) *C. clavatus* (summer-acclimated), **c**) *F. quadrioculata*, **d**) *H. viatica*, **e**) *I. prasis*, **f**) *O. flavescens*, **g**) *Onychiurus sp.*, **h**) *P. flavescens*.

0.137 to 0.697 from a reassessment of our measured values published in⁵ by the same method of estimation. The selection of species by Nickerl et al. covered all orders of Collembola (Entomobryomorpha, Poduromorpha, Symphypleona, Neelipleona), the selection of species by Gundersen et al was intended to cover a diverse range of Collembola surface structures and habitats ranging from extremely dry to very wet. Since all orders of Collembola and a wide range of surface structures and habitat types are considered in these two studies, it seems likely that the granule area fraction of most Collembola will fall within the two extremes of 0.111 to 0.709. If equation 2 is used to estimate the contact angle of this range of values of f, the resulting range is 118° to 157°.

Direct measurement of the contact angles of Collembola cuticles are scarce, but their wetting behavior is variously described as "non-wetting"^{24,25} "anti-wetting"^{2,25} and "unwettable".¹ The common classification of "superhydrophobic" surfaces requires an apparent contact angle exceeding 150°, and a contact angle hysteresis no larger than 10°. The predicted contact angles for all but the lowest values of f (and consequently hightest values of θ_{CB} *) yielded by the Cassie-Baxter equation do not reflect the observed, apparent contact angles of most Collembola cuticles. The authors⁵ previously found that the Cassie-Baxter equation systematically underestimated the contact angle, compared to measured values.

The Zheng model (eq 3) includes the roughness parameter S and the three-phase line tension λ . S, the ratio of three-phase contact line length and wetted surface area, can be calculated from surface structure data. A reassessment of measured values published in⁵ yielded a range of 0.039 μ m to 0.37 μ m in the Collembola species studied. λ , the threephase line tension, of water on Collembola cuticles, or similar systems, is not known. There are two possible approaches, assume a single value for λ for all Collembola species studied, and use it to predict apparent contact angles. Alternatively, assume that λ can vary from one species to another and use measured values of the contact angle to estimate reasonable values of λ . Estimated values of λ for each species is shown in figure 5 where f and Sare based on the reassessment of data from⁵ and $\theta_0 = 105^{\circ}$ was assumed. All intersections between the observed contact angle θ^* and predicted apparent contact angles by the Zheng model θ_Z^* are marked, while the sets of f and S that mark the upper and lower bounds for θ_Z^* for the studied species are shown as solid lines. All estimates of λ where found in the range from $\lambda = 2 * 10^{-9}$ N to $\lambda = 2 * 10^{-8}$ N; the values are summarized in table 1. This is within the range of published values for three-phase line tension in vapor-liquid-solid systems.

The Collembola Cryptopygus clavatus changes between superhydrophic water repellance with plastron formation upon submersion in winter conditions; and active grazing underwater with no visible plastron in summer conditions.⁶ This change in wetting behavior is not accompanied by any structural changes in the cuticle. Gundersen et al concluded that changes in the epicuticular wax layer was a possible explanation. Assuming $\theta_0 = 120^\circ$ in equation 2 yields a predicted contact angle of $\theta_{CB}^* \approx 135^\circ$, below the contact angle observed in both summer- and winter acclimated animals, see figure 5. The coverage of epicuticular wax was previously assumed to be either the top of the cuticular granules, leaving the areas between the granules exposed, or the entirety of the cuticle, recent studies conclude that the entire cuticle is covered.^{1,2,24} These two give the same result in a wetting model that assumes contact only at the top of the cuticular granules. Study with a lipophilic dye is not suited to differentiate between the two, but would have revealed any loss of wax coverage on the top of granules upon summer-acclimation. Figure 4 shows stained samples of winter- and summer-acclimated *C.clavatus* under fluorescing conditions. The immediate conclusion is that the top of the granules are covered in epicuticular wax in both the winter- and summeracclimated state, and that changes in the extent of the wax layer can not explain the seasonal change in wetting behavior.

Figure 5 shows the discrepancy between measured values of the apparent contact angle (dashed red and blue lines) and the predictions of the Cassie-Baxter model (solid, red and blue lines) along with the Zheng model (solid black line). Values for f and S were based on assessment of SEM images from Gundersen et al.⁶ The intersect between the Zheng model (described by equation 3) and the measured values give estimates for the value of λ on C.

clavatus, $\lambda = 8.82 \times 10^{-9}$ N for winter-acclimated animals and $\lambda = 4.21 \times 10^{-9}$ N for summeracclimated animals. This modest change in the magnitude of the three-phase line tension can explain the seasonal change in wetting characteristics found in *C. clavatus* from summerto winter-adaption, without large structural changes in the cuticle.



Figure 5: Left: Measured and predicted apparent contact angles (θ^*) as a function of $log(\lambda)$ for *C.clavatus* adapted to winter and summer conditions. Assumptions: $\theta_0 = 105^\circ$, f = 0.588, $S = 0.176 \ \mu m$, based on results described in.⁶ Right: θ_Z^* as a function of $log(\lambda)$ for the two combinations of f and S that yield the highest and lowest estimated values of λ found among species in Gundersen et al.⁵ with estimated values of λ noted for each species.

Conclusion

The very large apparent contact angle of water on Collembola cuticles can not be predicted by the conventional wetting models.⁵ The parameters in these models, wetted area fraction (f)and inherent contact angle (θ_0) , can vary within a certain range, but not enough to explain the observed contact angles. The wetted area fraction can be demonstrated by experimental methods, as shown here, as well as through mathematical modelling of the energy needed to transition from composite wetting to non-composite wetting.⁴ This yields a wetted area that is determined by the area covered by cuticular granules, which for Collembola constitutes a range from 0.111 to 0.709 in the work of Nickerl et al.,¹⁹ compared to a range of 0.137 to 0.697 in a reassessment of data from our previous work.⁵ The Cassie-Baxter model¹⁵ greatly underestimates the apparent contact angles of these cuticles for most of this range of

Species	$S \ [\mu m]$	f	$\lambda[\mathrm{N}]$	Notes	
Anurophorus laricis	0.37	0.70	$1.68 \mathrm{x} 10^{-8}$		5
$A nurophorus \ septentrional is$	0.32	0.60	$1.60 \mathrm{x} 10^{-8}$		5
Archisotoma besselsi	0.039	0.37	$1.97 \mathrm{x} 10^{-9}$		5
Archisotoma besselsi	0.061	0.14	$2.71 \mathrm{x} 10^{-9}$	Based on secondary granules	5
Cryptopygus clavatus	0.17	0.59	$4.40 \mathrm{x} 10^{-9}$	Summer-acclimated	5
Cryptopygus clavatus	0.18	0.58	$4.21 \mathrm{x} 10^{-9}$	${\it Summer-acclimated}$	6
Cryptopygus clavatus	0.18	0.60	$8.82 \text{x} 10^{-9}$	Winter-acclimated	6
Desoria oliviaca	0.059	0.30	$2.62 \mathrm{x} 10^{-9}$		5
Folsomia quadrioculata	0.19	0.37	$9.67 \mathrm{x} 10^{-9}$		5
Hypogasture viatica	0.070	0.50	$3.50 \mathrm{x} 10^{-9}$		5
Hypogasture viatica	0.19	0.15	8.16×10^{-9}	Based on secondary granules	5
Isotoma anglicana	0.12	0.33	$3.82 \mathrm{x} 10^{-9}$		5
Isotomurus prasis	0.095	0.19	$4.33 \mathrm{x} 10^{-9}$		5
Onychiurus sp.	0.054	0.38	$2.78 \mathrm{x} 10^{-9}$		5
Onychiurus sp.	0.24	0.30	$1.21 \mathrm{x} 10^{-8}$	Based on secondary granules	5
Orchesella flavescens	0.080	0.35	$2.11 \text{x} 10^{-9}$		5
Xenylla maritima	0.15	0.33	$5.32 \mathrm{x} 10^{-9}$		5

Table 1: Estimated values of the three phase line tension (λ). Estimates are based on primary granules, unless otherwise noted.

f compared with the measured values of water on Collembola cuticles. The inherent contact angle, while theoretically ranging from 0° to 156°,²¹ is limited to a reasonable range of 90° (the minimum for a stable composite wetting state) to 120° (the highest known for a smooth solid). This range of inherent contact angles is not sufficient to explain the range of observed apparent contact angles. The assumption for Collembola cuticles in this work was $\theta_0 = 105^\circ$, which corresponds to that of many insect waxes and chitin.^{22,23} The model for predicting apparent contact angles becomes scale dependent when the three phase line tension (λ) is considered.¹⁶ In the specific case of Collembola, the size scale is of a magnitude where the three phase line tension can explain the discrepancy between the observed apparent contact angles and those predicted by classical models. A three phase line tension in the range of $2x10^-9$ N to $2x10^-8$ N can account for this difference, this is within the range of previously reported values of the three phase line tension.¹⁷

Materials and Methods

Springtails are non-regulated invertebrates and not subject to animal experiment laws in Norway. The species studied are not endangered or protected. The animals were collected in the wild in Norway in public areas with no restrictions on the gathering of invertebrates. The animals were killed with chloroform vapor immediately before experiments.

Nile Red dye was dissolved in acetone to form a stock solution at 1 mg per ml. This was further diluted 1:100 with acetone, and 1:100 with 10 %Vol Acetone (aq) to form one pure acetone dye and one ageous acetone dye. Samples were soaked in a dye solution for 5 minutes and subsequently rinsed with acetone and air-dryed.

Samples were studied with a Zeiss 510 Confocal Laser Scanning Microscope. Fluorescense microscopy was performed with incidental light of 488 nm wavelength and a bandpass filter (565-615 nm). Reflected light microscopy used a bandpass filter (480-520 nm). All imaging was done with a water immersion objective, with the samples immersed in purified water. The cuticle (including granules and ridges) of the dorsal metasoma was studied.

Samples were mounted on SEM stubs with silver glue and imaged with no applied conductive layer (i.e. no metalization or carbon coating). An FEI Quanta FEG 450 ESEM was used, utizling the large field-detector, which detects a combination of secondary and backscattered electrons, in the low-vacuum mode. Typical imaging settings were 0.50 mBar (water vapor) chamber pressure, $E_{Acc}=10$ kV.

Some unstained arthropod cuticles will autofluoresce, this was observed for several of the studied species: *C. clavatus*, *F*, *quadrioculata*, *H. viatica*, *I. prasis* and *Onychiurus sp.*. Setae, rings in the cuticle around the base of setae, primary and secondary granules were imaged in fluorescent lighting on unstained samples (not all features were equally autofluorescent on all studied species). This autofluorescence effect can easily be distinguished by its weaker luminescence. On average one order of magnitude higher light intensity was required to visualize features based on autofluorescence alone, as compared to stained samples.

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Graphical TOC Entry

