Prof.dr. A. Briegel

Big pictures of small microbes

Universiteit Leiden

Bij ons leer je de wereld kennen

Big pictures of small microbes

Inaugural lecture by

Prof.dr. A. Briegel

on the acceptance of her position as professor of

Ultrastructural Biology

at the Universiteit Leiden

on Friday January 13, 2017.

Universiteit
Leiden

Mijnheer de Rector Magnificus, honored guests, ladies and gentlemen,

Close your eyes and imagine a bacterium…
I cannot read minds, but I assume that many of you will have trouble forming a clear picture in your head. Of course, this probably excludes my microbiology colleagues who most likely think that my request is quite vague since I didn't say which particular species they are supposed to think of. Some of you might imagine little hairy creatures with large teeth and sharp claws. As a child, I imagined microbes like the little ghosts from the Pac-Man computer game, and the white blood cells were the Pac-Man heroes that gobbled up the infections. Instead of a picture, you may just feel negative emotions remembering the last time you or someone in your family fell ill from a bacterial infection.
Bacteria are everywhere. They are the most abundant organisms on earth and impact all aspects of our lives. They determine our health and shape our environment. They are needed to produce yogurt and the lovely Dutch cheeses that have become an essential component of my diet since I moved to the Netherlands. They fertilize our crops and allow livestock to digest plants. They clean our wastewater, and they produce antibiotics. These are just a few examples. We carry about as many microbial cells in us as cells from our own body. Despite this abundance, they are invisible to the naked eye, and we are aware of their presence indirectly during our every day lives. We only notice them when we are sick from battling bacterial infections of pathogenic strains.
Their invisibility prevents most of us from forming a clear picture in our minds. Bacteria often are seen as a nebulous threat that many of us may have trouble grasping.
Until about 350 years ago, we didn't know about microbes at all. The first person to discover them was - of course - a Dutchman.

Dutch Origin of Microbiology
Antoni van Leeuwenhoek started out his career as an apprentice to a cloth merchant and later became a shopkeeper in Delft. Inspired by magnifying glasses that were used to determine the quality of cloth at the time, he began to develop microscopes with unprecedented resolution. Lacking a proper scientific training, but full of unwavering curiosity, he started investigating the microscopic world around him. He examined water from the canals, lakes and the North Sea, swabs from his mouth, blood, semen, plant roots and much more. He documented his observations in painstaking detail and with beautiful illustrations. In the 1670's, he discovered and described little creatures he named 'animalcules', and he could observe them moving around (fig). He carefully calculated the size of these 'very wee animals' to less than 3 μm. He had discovered bacteria. His microscopes were simple, single lens instruments smaller than a modern smartphone. But despite their simplicity, his microscopes were far superior in resolution when compared to all other instruments available at the time. His discovery was met not only with fascination but also with skepticism. Ultimately other scientists could confirm Leeuwenhoek's observations of the animalcules, and he is now generally acknowledged as the father of microbiology.[1]

Today we know a lot more about microbes than Antoni van Leeuwenhoek. But there is still so much we don't know. How do they grow, multiply, communicate with each other and cause infections? In order to understand them we need to take a very close look at them.
Even the best light microscopes available today can not do much better than the microscope that Leeuwenhoek developed. The wavelength of visible light limits the resolution we can get. However, the development of specialized fluorescent labels together with computational analyzing tools enabled the development of super resolution light microscopy. This technique sidesteps the physical limitations of the wavelength of light and its developers were awarded with the Nobel Prize in chemistry in 2014.

This discovery pushes the boundaries of light microscopy to an unprecedented level. It provides the means to localize the fluorescent tags that are attached to your protein of interest at a precision well beyond what can be achieved by traditional light microscopy. The accuracy in the living cell can be determined down to the nanometer level. A nanometer is equal to one billionth of a meter, and for example an *Escherichia coli* cell is about 1 000 nm in diameter. However, the statement often read in press releases that these new methods provide nanometer resolution in the cells is misleading. While it indeed allows the accurate localization of the fluorescent label to this accuracy, these techniques cannot resolve an entire microbial cell with all its machinery at molecular resolution.

The Power of Electrons

However, light is not the only way scientists can see

Another form of microscopy relies on a beam of electrons instead of a beam of light. The wavelength of electrons is about a 100 000 times shorter than that of visible light, and therefore these electron microscopes have a much higher magnifying power. To illustrate this, imagine that I got so frustrated with preparing my speech that I asked my friends at the space agencies to shoot it to the surface of the moon. An electron microscope has enough magnifying power that it would still allow me to read the speech from here.

If I were to change my mind, I would need a space suit to retrieve the speech from the moon to withstand the vacuum in space. Without a suit, the water of my body would begin to evaporate through my skin and orifices, destructively changing my appearance. It would not be a pretty picture. The same actually happens to a biological sample when I insert it unprotected into the high vacuum of an electron microscope. In order to image a biological specimen all the water needs to be removed from the sample, so it will not evaporate nearly instantaneously and completely destroy the material. Obviously, there are no spacesuits for microbes. Instead, the samples can be protected by a plastic embedding. In order to do this, all water has to be removed from the cell. This allows the investigation of cells and tissues, but also introduces a lot of damage and artifacts (fig). Because of this destructive preparation, electron microscopy became less and less popular, and the only reason I am standing here today is my unfailing tendency to ignore good advice.

Don't specialize in Electron microscopy; otherwise you will never get a job in Science

The consensus of my well-meaning advisors was that electron microscopy was an outdated and dying methodology in life sciences. Training in other microbiological techniques such as genome sequencing or bio-informatics would further my career, but by choosing electron microscopy as my specialization I would just end up driving a taxi or serve frietjes at a fast food restaurant. I happily ignored this advice since I believed in the power of electron microscopy: It allows to directly see individual cells, with their complete cellular contents. And at resolutions far beyond what is possible by light microscopy. It allows the direct observation of cellular organelles in eukaryotic cells. To quote the famous Caltech scientist Richard Feynman: "It is very easy to answer many of these fundamental biological questions; you just look at the thing!".

However, my well-meaning advisors did have a point: traditional electron microscopy was inherently limited. But during my PhD training in the research group at the Max Planck Institute in Martinsried under the guidance of professor Wolfgang Baumeister, I learned that we can overcome these limitations. In fact, all the elaborate traditional and destructive sample preparation steps can be completely avoided. A method was developed by a research group around Jacques Dubouchet at the University of Lausanne where the sample is just spread out in a very thin film of liquid and then flash frozen. The freezing has to happen so fast that water molecules don't have time to crystallize. Instead, the arrangement of the water molecules forms a glass-like ice. This results in biological samples, which are essentially free of

artifacts. Additionally, Prof. Baumeister's team was working on the development of procedures to generate three-dimensional images by tilting the sample in the microscope and acquiring images of a sample from many different angles similar to a medical tomogram. These images then can be used to computationally generate three-dimensional models of cells at unprecedented resolution. We can now investigate the detailed cellular content of microbes and unravel the structure and function of molecular machines that the cells need to thrive and multiply. These methods were in development when I joined the group of professor Baumeister as one of the first biology students to apply these methods to microbiology.

The Dawn of Nanobiology

Finally there was a method to look inside of intact cells and in three dimensions, and I got to apply this technique to a wide array of microbes. We opened a new window into the microscopic world - it allows us to glimpse directly into the inside of intact bacterial cells. We learned that bacteria are much more than bags of enzymes - the cells are highly organized and contain molecular machines that allow them to grow, multiply and thrive in ever changing environments.
After my time at the Max Planck Institute in Germany, I moved to Pasadena in California to join the group of professor Grant Jensen at the California Institute of Technology.
Here I continued my studies and made great progress in understanding the secret life of microbes.
Microbes do not have membrane-enclosed organelles, like the much larger Human or eukaryotic cells you have learned about in school. Microbes are still highly organized. Their cells have a defined architecture with tightly regulated localization of the cellular components. Everything has its place. The cells possess a cytoskeleton - a molecular frame - that is involved in the cells' shape, the internal organization and it takes part in the cell's division. Much like Eukaryotes, Microbes use similar proteins to organize their interior. For example, they possess counterparts to the major eukaryotic proteins that organize these cells. Additionally, we discovered yet unknown cytoskeletal elements. We could show that an enzyme essential for the cell metabolism forms such a structural frame, which might indicate how such filaments have evolved in the first place. We have now discovered an amazing diversity of microbial cytoskeletons. We discovered rods, rings, filaments, tubes, sheets, spirals and meshes. This shows, Darwin was right on every scale, when he stated "endless forms most beautiful and most wonderful have been, and are being, evolved".
Microbes possess a variety of structures on their surface, as well. Some are used for the cell's attachment. For example, in a study with the University of Regensburg, we discovered a novel type of cell attachment on the surface of an archaeal species we called the Hamus from the latin word for hook. Each cell is surrounded by a halo of about sixty Hami, each resembling a miniature version of a long barbed wire that terminates in a three-pronged grappling hook. These anchor like ends of the Hami ensure that the cells stay attached to each other, while the barbed wire like 'rope' ensures that the cells don't get too close. These structures are similar to a microbial Velcro that allows the cells to form a highly structured multicellular community.
Other extracellular structures are used for movement. Flagella resemble a molecular whip that is rotated by a microscopic motor that is anchored in the cell envelope. This allows the cell to swim. Another mode of movement is based on string like cell attachment structures called pili. These are filaments that extend, attach to a surface and retract, allowing the cells to crawl along a surface.
Since microbes can actively move through their environment, they also need to determine where they choose to go. Understanding how microbes sense their environment has been a focus of my studies. The cells have a highly sensitive bacterial "nose" that allows them to detect food sources and toxins and actively seek out their preferred environments.
This bacterial nose is composed of thousands of sensors called chemoreceptors. These receptors are highly ordered in a honeycomb like pattern at the cell poles. Molecules indicating a food source or toxins can bind to these sensors. The receptors then activate an enzyme that controls a messenger protein, that

in turn controls the flagella or pili.
Some microbes can even sense the Earths' magnetic field. They contain a series of magnetite crystal spheres that are inside little pockets in the inner membrane of the cells. These magnetic clusters are aligned by cytoskeletal filament to form a microscopic compass needle, which possibly helps their orientation in the environment.
Microbes can also fight for their territory. Some microbes possess a so-called type VI secretion system. These are similar to spring loaded poison daggers. The cells contain tubular structures inside, ready to fire. When an enemy cell comes too close, the tube is triggered, quickly contracts and shoots its poisonous contents into the enemy cell. The cells can fight microscopic battles, like Dutch Golden age merchant galleons, where the secretion tubes fire, disassemble, reassemble and are ready to fire again. (For a recent review on the ultrastructure of prokaryotic cells see[2]).

Microbes in 3D

To illustrate how we can study microbial cells with electron cryotomography, I want to show you a movie of one example of a bacterial cell (supplemental material[2], fig.1). This microbe is called *Bdellovibrio bacteriovorus* and is a predatory bacterium that preys on other microbes. It first attaches to its victim, then slips in between the membranes of its prey and digests the host. It then divides and the daughter cells escape the empty host to seek out new prey. We start with the raw dataset or tilt-series that we collect on the microscope. You can see the microbe while it is being tilted in the microscope. It is embedded in vitrified medium that surrounds the cell. Note the little dark dots all over the images? These are gold particles that we add to the cell cultures before freezing. This allows us to carefully align all the 2D images from the tilt series before we reconstruct the three-dimensional volume.
The final reconstruction reveals a 3D picture of an intact microbial cell. This type of data contains an incredible amount of information - all the structural elements of the cell can be seen at the same time. From this one dataset, you can analyze the cells membranes. In this case, you can see the two membranes characteristic for this type of bacterium called Gram negative. Sandwiched in between the two membranes is the cell wall. This cell wall is a huge polymer consisting of sugar and amino acids that surrounds the whole cell. We can investigate the bacterial DNA - in microbes it is typically one circular chromosome that is twisted like a bread roll. We can study the molecular motors that rotate the flagella and propel the cells forward, or the pili that enable the cells to attach to a surface of the prey cells. We can study the chemoreceptors, which form large honeycomb-like arrays at the cell pole and allow the cells to sense their environment. We can also study the structure and distribution of ribosomes that catalyze the synthesis of proteins.
Taken together, Electron cryotomography allows us to study structure of intact microbes in three dimensions. This answers fundamental questions on how microbes live, how they multiply, move and interact with the environment.

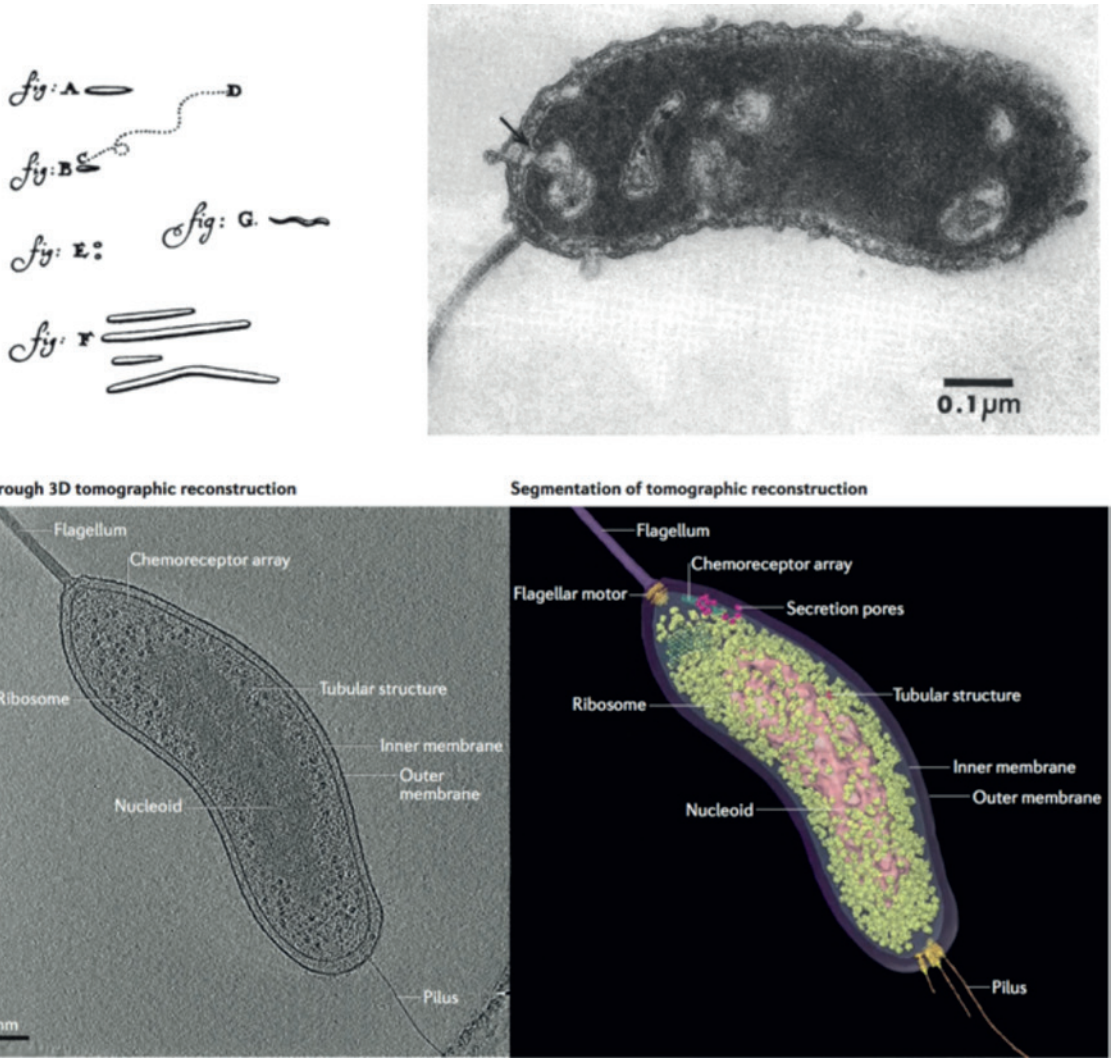

Figure: Microscopy of microbes
Top left shows drawings of bacteria from Leewenhoeck's mouth.[3]Top right: The advent of electron microscopy allowed the study of microbes at much higher resolution as seen here for the bacterium *Bdellovibrio bacteriovorus*.[4] However, the sample preparation destroyed much of the structural details of the cell. Electron cryotomography finally provides a tool to study the delicate structure of intact microbes in three dimensions.[2]

Electron Microscopy - more than pretty pictures

Electron cryotomography is becoming more and more recognized in the field of microbiology. Our research is quite popular for presentations at research conferences. One of the frequent comments we often hear is how pretty our pictures are. On the one hand, such positive feedback is of course flattering, but it also shows we still have work to do. Our work is so much more than mere pretty pictures.

We now have a tool to study microbes in three dimensions at molecular resolution. As I have just shown we can directly observe the molecular machines of the microbes. Much of our work is largely fundamental science: Like in the examples I have mentioned previously, we are seeking answers to basic questions on how microbes grow, divide, or interact with their environment. This type of research is rarely directly applicable for developments of medical cures or industrial applications. There is no short-term profit. But it is an essential component of modern science and it is crucial that this type of research should get financial support from national and international funding agencies. As a society we simply cannot afford to solely invest in research that promises short term rewards in medicine and industry. Truly novel scientific ideas are often risky and a direct benefit for society is difficult if not impossible to predict beforehand.

However, the sad reality is that most funding is only available if a direct beneficial output is convincingly argued before even starting the experiments. In the long run, this approach puts blinders on scientists' eyes and will ultimately come at a high cost for our society. We need to understand the world around us in as much detail as we can and accept that this is a long process with many setbacks. But such fundamental science is essential in order to properly prepare for the challenges that lie ahead in our future.

A recent article in the Boston Globe titled "Lets waste more money on science" (published December 11, 2016) describes several examples how our modern world benefits and depends on fundamental science.

The article describes the story of the young biologist Thomas Brock in 1960. He performed purely curiosity driven research on the microbial communities in the hot springs of the Yellowstone National Park. He found bacteria that could thrive in the near boiling water of the springs, and together with his undergraduate research assistant he managed to grow one of these microbes, the bacterium *Thermus aquaticus*, in their lab. Their work pioneered the extensive study of extremophiles, organisms that can thrive in extreme environments such as in high temperatures, acid or alkaline environments, in salt lakes or at the bottom of the ocean.

Other research groups have continued the research on *Thermus aquaticus* and discovered a molecule they named the taq polymerase. This enzyme is used to synthesize new DNA from an existing strand. Unlike other polymerase molecules of other known organisms, this enzyme can function at much higher temperatures.

Another young biologist named Kary Mullis later developed the polymerase chain reaction or PCR that relies on the special property of the taq polymerase to function at high temperatures. PCR allows the cheap and fast amplification of DNA - billions of copies can be generated in only a few hours. Mullis received the Nobel Prize in chemistry in 1993 for the invention of PCR, and this method it is now used across all disciplines of biotechnology.

The utilization of new knowledge from curiosity driven research is indeed often unpredictable, but the tolerance of uncertainty is an essential component of science that will drive our society forward.

Remember Antoni van Leeuwenhoek? His curiosity alone led him to explore the microscopic world with a tool developed for an altogether different purpose - to count the amount of threads in cloth to determine its quality. He could never have predicted or even imagined the impact his discovery would have on biology, medicine, and even the perspective of humanity's place in the web of life. "The world is not only stranger than we imagine, it is stranger than we can imagine" (adapted from J.B.S. Haldane). When you stay on the safe path

of immediate benefits you will never cross into the astounding undiscovered realms. The microscope's impact didn't stop with the cloth industry or science - his microscope was also later used in art by the famous artist Escher. There is even a replica of the Leeuwenhoek microscope in the Escher museum in The Hague.

Learning Languages

So what brings me here to Leiden? I guess you can say I am here to learn two languages. The first, of course, is Dutch. My progress in the past year is unfortunately still very modest. And only due to the determination of Laura Zondervan, the Institute manager of the Biology Department at the Leiden University, I am now getting the lessons I need to finally make any progress. Thank you Laura for not sending me to the Nuns of Vught. And thank you for being an amazing and much needed friend, even though you refuse to provide me with the puppies I keep asking you for.

The other language I came here to learn is that of microbes. I don't yet know which one is more difficult. How do they decipher the signals from the environment and how are the cells using this information to control their behavior? We have discovered that they can do this, but do not yet know exactly how. This research line is built on my studies that I already started as a PhD student at the Max Planck Institute. I am interested in the structure and function of the molecular machines that enable microbes to find and move to their preferred environmental niches. This behavior is common in nearly all motile bacteria: This molecular 'nose' is composed of thousands of highly sensitive receptors that allows them to "smell" their food sources. Ultimately, I envision that my research will allow us to understand the mechanism underlying this bacterial behavior and might provide the basis for future applications in medicine, wastewater treatments or biosensor technologies.

I will continue this fundamental research line in model organisms such as *Escherichia coli*. But I will also expand this research to microbes that possess multiple chemotaxis sensor arrays. The structure and function of such additional chemotaxis systems are currently not well understood. But understanding these molecular machines is especially relevant for example in pathogens that utilize the chemotaxis as the first step of host invasion. One of the main model organisms that I will be working on here in Leiden is *Vibrio cholerae*, the bacteria that cause cholera.

Vibrio cholerae Infections caused by pathogenic bacteria have been the source of explosive, deadly epidemics throughout history, including pestilence and cholera. Even in the city of Leiden, there were two recorded cholera epidemics in the years of 1832 and 1866 with over a thousand casualties (Historische Canon van Leiden).

During that time, cholera was claiming enormous numbers of human lives all across Europe. The cause for the illness wasn't known. Bad air was thought to be the culprit that caused the suffering and death of so many. The realization that the pathogen was spread through contaminated water was discovered by the English doctor John Snow, who charted cholera deaths in the city of London and overlaid it on a map of the water pumps in the city. He realized that the deaths co-localize with certain wells, and the only group of people spared in the affected areas was the workers of the local brewery that consumed beer instead of contaminated water.[5]

But such epidemics are not just footnotes in history books. Each year, 1.3 billion people are at risk of contracting cholera, and the disease still claims 95,000 lives each year.[6] The urgency to address human diseases caused by Vibrio species is underscored by recent reports in the news: for example, hurricane Matthew not only left destruction behind in Haiti in October 2016, but also resulted in a rise of cholera infections with many casualties. Such natural disasters are not isolated events but are becoming more frequent. The global threat of cholera infections is constantly rising: A new study reports that the warming trend of the sea surface caused by climate change is strongly correlated with the spread of vibrio pathogens.[7]

In order to develop strategic and targeted methods to combat these harmful microbes effectively, it is pivotal to understand the structure and function of these organisms during all stages of the infection. However, most structural studies of pathogenic bacteria have been limited to bacterial cells that are grown in liquid monocultures. This is standard practice in modern microbiology because it facilitates growth of large numbers of cells under efficient and controlled conditions. However, an increasing number of studies are demonstrating that many bacteria undergo radical morphological changes as they adapt to their environmental conditions, many of which cannot be accurately replicated in standard laboratory cultures. We will investigate Vibrio at the molecular level throughout all the environmental and infectious stages. This will provide us with knowledge about the species characteristic makeup and behavior in all stages of its life cycle. Ultimately, I envision that this will allow us to counter serious diseases with a whole new approach.

Currently, one of the most effective weapons against serious diseases is antibiotic treatment. But these come at a cost: when you fight an infection with antibiotics, you also wipe out many of the beneficial microbes in your body which can weaken patients even further. Additionally, the rise of antibiotic resistant strains of bacteria is of growing concern. In the case of cholera, several such strains have already emerged. But what if we truly understand the biology of the pathogen and what triggers the changes in behavior in all stages of infection? Can we use this knowledge to induce a change in the vibrio organism without killing the remaining microbiome and without the risk of resistance development?

I am very excited to share with you that we have just been awarded a research grant called 'building blocks of life' from the Dutch funding agency NWO to study the structure of Vibrio during the infection of zebrafish larvae. This research will be conducted together with my new colleagues Annemarie Meijer from the IBL and the chemist Sander van Kasteren from the Leiden Institute of Chemistry. Besides Annemarie's great insight into all things Zebrafish, she has been an unofficial mentor and friend to me during the past year. She helped me immensely, from teaching me how to put on the traditional necktie, to her patient explanations on the inner workings of the various University committees. Annemarie, I truly don't know what I would have done in the past year without your cheerful advice.

The Netherlands - an ideal environment for high-end microscopy

FEI company

Another partner in the grant is FEI Company, originally a Dutch daughter company of Philips, which is now part of Thermo Fischer. As you can imagine, my work relies heavily on high-end instruments, including the top of the line electron microscopes and accessory equipment. The development and maintenance of such machines is crucial for my research, and I am very glad of the quality and support from FEI. And while FEI is a rapidly growing company, the support I have experienced in the last year was very personal, and I want to especially thank Wim Voorhout, Nico Clemens and Linda van Driel. It is a pleasure working with you and I hope we will continue to do so in the future.

NeCEN

The microscopes I rely on are located inside the NeCEN center, the 'Netherlands Centre for Electron Nanoscopy'. This center is the reason I accepted this position at the Leiden University. NeCEN is the result of a visionary initiative spearheaded by Dutch scientists nine years ago. The team realized that cryo-electron microscopy will play an essential role in the biological sciences, but the required instrumentation is too expensive to be supported by individual research groups. The facility has opened its doors in 2012 under the wing of the chemistry department at Leiden University, but operations didn't run as smoothly as envisioned. Last year, NeCEN has been integrated into the Institute of Biology under the directorship of professor Bram Koster. Under his guidance, the center is now a fully operational national facility with two top-of-the-line microscopes called TITAN Krios machines. Both

machines are equipped with high-end auxiliary equipment and the best detectors currently on the market. In the past year, the necessary computational environment has also been established.
But even the best equipment is useless without highly trained staff to operate it. Bram has assembled an outstanding supporting staff, with Susanne Roodhuyzen as project manager, Ludovic Renault as facility manager, the team of operators Christoph Diebolder, Roman Koning and Julio Ortiz, and the computational scientist Bart Alewijnse. This outstanding team will ensure NeCENs success in the years to come, and I feel very fortunate and confident that I get the best support I possibly can to get the best data with the team and the instrumentation at NeCEN. Bram, you and your research group at LUMC have been incredible supportive to me and my students in the past year. I can't thank you enough for everything you have done for me - including the use of your own lab's facilities, your advice and open ears.

The Institute for Biology
The NeCEN center will have a huge impact for answering many fundamental biological questions in the years to come. I am dependent on its equipment and the support of its personnel. I realize that this center is a significant responsibility for any University and I am truly grateful that NeCEN is under the wing of the Institute for Biology at the Leiden University and supported by the faculty of science. I wouldn't be here if it wasn't for the IBL director Herman Spaink. Herman, I know that without your unparalleled energy and vision I would not be here today. And at the time I decided to accept the position here I didn't even know about the amazing sushi and Mexican food you can cook. Thank you so much for your friendship and support.
My line of work is truly between the disciplines of structural biology and microbiology and I depend on both equally. On one side, I rely on the technical high-end microscopy at the NeCEN facility. On the other side I require facilities to do basic microbiology. I am incredibly fortunate that Gilles van Wezel and his research group have supported me during the past year. He generously shares equipment, lab and office space with my students and me. Gilles, you have been an amazing support and friend to me in the past year, even though I continue to try to steal your students. But the fact that even during your sabbatical in San Diego your students remained faithfully yours despite my attempts to lure them in with chocolate proves that you are simply amazing.
Being embedded in the Microbial Biotechnology and Health cluster allowed me to start collaborations, especially with dr. Dennis Claessen. I am broadening my scientific horizon and we have already embarked on a joint project to study how cells establish polarity, and how some bacteria can live without a cell wall.

Micropia
Now I would like to invite you again to close your eyes and imagine a bacterium…
In contrast to the first time I asked you to do this, you will probably have a very different image in your head. You can now imagine the cells framework, its molecular machines, the cell envelope and some external structures. I hope I have managed to change your view about microbes, but I hope even more that we can share this insight with the broader public as well.
The Netherlands is a marvelous place in terms of outreach and education of microbiology. The only existing museum of microbes in the world is located right here in Amsterdam. This award-winning museum is called Micropia and it is truly worth a visit. You can explore the unseen world of microbes with your whole family, and the many interactive exhibits will ensure no one is bored. I am currently working with the staff at Micropia to add an exhibit showcasing insights from electron cryotomography. So I hope the next time you visit you will see movies that explain the structure of microbes from my lab collected at the NeCEN center at the University of Leiden.

Word of thanks

Before I close, I would like to thank those who have enabled me to come here and accept this appointment. Rector Magnificus and president of the Leiden University Carel Stolker, thank you for the time you took to have lunch with me during my first visit to Leiden. I thoroughly enjoyed our conversation about life and work in California and here. Thank you also to the Dean of the Science faculty Geert de Snoo, for supporting my appointment at the Leiden University and for your continued support during the past year.

Thank you to my PhD students, Eveline Ultee and Wen Yang. Thank you for trusting in the Briegel lab even when we started out with nothing but a pile of equipment in boxes last summer.

I want to say a special thank you to my family.
Thank you to my family in law, who were always there for us, ready with a hammer and a sowing machine, no matter where we decided to move.
Thank you to my parents, who have been my role models and who have supported me throughout my whole life.
And a special thank you to my husband and son, Armin and Kai, who keep me sane and happy even though my career took us from continent to continent and back. Without your unwavering support I would not be here today.

Ik heb gezegd.

References

1 Lane, N. The unseen world: reflections on Leeuwenhoek (1677) 'Concerning little animals'. Philos Trans R Soc Lond B Biol Sci 370, (2015).
2 Oikonomou, C. M. & Jensen, G. J. A new view into prokaryotic cell biology from electron cryotomography. Nature reviews. Microbiology 14, 205-220, (2016).
3 A., L. An Abstract of a Letter from Mr. Anthony Leewenhoeck at Delft, dated Sep. 17. 1683. containing some microscopical observations, about animals in the scurf of the teeth, the substance call'd worms in the nose, the cuticula consisting of scales. Phil. Trans. 14, 568-574. (1684).
4 Burnham, J. C., Hashimoto, T. & Conti, S. F. Ultrastructure and cell division of a facultatively parasitic strain of Bdellovibrio bacteriovorus. J Bacteriol 101, 997-1004 (1970).
5 Johnson, S. How we got to now. (Riverhead books, 2014).
6 Ali, M., Nelson, A. R., Lopez, A. L. & Sack, D. A. Updated global burden of cholera in endemic countries. PLoS Negl Trop Dis 9, e0003832, (2015).
7 Vezzulli, L. et al. Climate influence on Vibrio and associated human diseases during the past half-century in the coastal North Atlantic. Proceedings of the National Academy of Sciences of the United States of America 113, E5062-5071, (2016).

Prof.dr. A. Briegel

2000	Master of Science, Department of Biology, Ludwig-Maximilians- University Munich, Germany
2005	PhD in Natural Sciences, Molecular and Structural Biology Department of the Max-Planck-Institute, Technical University Munich, Germany. PhD Supervisor Prof. Dr. Wolfgang Baumeister
2005-2010	Postdoctoral Scholar, California Institute of Technology (Caltech)/ Howard Hughes Medical Institute (HHMI), USA
2010-2015	Research Scientist, California Institute of Technology (Caltech)/ Howard Hughes Medical Institute (HHMI), USA
2015-present	Full Professor, Institute of Biology, Leiden University, Netherlands

Microbes are everywhere. They are the most abundant organisms on Earth and impact all aspects of our lives. They determine our health and shape our environment. For example, they are needed to produce yogurt and cheese, they fertilize crop, allow livestock to digest plants, they clean our wastewater and they produce antibiotics. Humans carry about as many microbial cells in us as cells from our own body. Yet they are invisible to the naked eye.

Ariane Briegel is professor in Ultrastructural biology and uses the new technology of electron cryotomography to uncover the secret life of microbes. Her research opens a new window into the microscopic world. In particular her work focuses on understanding how microbes sense food and toxins and respond by controlling their motility. To gain insight into the structure and function of the molecular complexes involved in these behaviors electron cryotomography (ECT) allows the direct study microbes in their native state at resolutions capable of visualizing individual proteins.

She will also investigate the changes of microbial organisms through all stages of their lifecycle. Many pathogens, such as *Vibrio cholerae*, change their behavior and structure during infection. She envisions this research will provide a basis for the development of novel treatments to many serious diseases.

Universiteit Leiden