*“Mijnheer de Rector Magnificus, leden van het College van Bestuur,*

*Collegae hoogleraren en andere leden van de universitaire gemeenschap.*

*Zeer gewaardeerde toehoorders.*

*Dames en heren”.*

Thank you all for having come from close and afar to this academic ceremony in which I want to give you an introduction to my academic field, share the excitement for it and show glimmers of what the future might brings us. I want to share with you a bit of my scientific and personal road to this day and I want to convince you that concerning the university administration I am in fact a woman.

[S2] But first things first. Let me tell you about my field of research, *computational microscopy*.

Today’s images are no longer just recordings of photons or electrons, but results of computerized processing, reconstruction and analysis - and that is exactly where my research enters. Where the instrument development encounters physical barriers, the combination with clever computational approaches allows overcoming these barriers.

In everyday terms you can think about it the following way. You replace the microscope with your smart phone and take a picture. [S3] But then do not like the result and start to manipulate it. These types of image manipulation kept scientists busy in the 80s. To make the little boxes appear around the faces before taking a picture kept scientist busy for another 10 years.

[S4] It becomes a bit more interesting if I want to make a landscape picture to capture the whole applied physics building. [S5] The landscape picture in fact consists out of many individual pictures that must be stitched together as you see here.

[S6] Now *computational microscopy* is a step further. It is not only processing of the obtained images. First it is about designing a strategy to obtain the desired information from the object. That involves all aspects starting from the scientific question to the illumination, the instrument, detection and processing. It is not optimization of each component alone, but the combination of them all. You can also view this as a system engineering’s problem. Potentially we use a feedback loop such that the acquisition of the images changes in real-time depending on the analysis.

For example in fluorescence microscopy such an integrated approach was at the basis for the Noble prize in Chemistry 2014. After this rather dry introduction I want to show you 3 minutes of an animation on microscopy in Delft [S7,8] [movie]

[S9] In the movie you have seen Antonie van Leeuwenhoek, who is a famous Delft citizen for his many discoveries in biology using his home-build microscopes. As you might have noticed the university has appointed me Antonie van Leeuwenhoek professor. I am also working on microscope technology to improve imaging for cell biology, but this naming is solely a coincident.

Now a word on the title of my talk: [S12] Room at the bottom. It is inspired by the title of a now famous talk by Richard Feyman in 1959 for the American Physical Society. There he made a detailed argument on the possibilities for what is now called nanotechnology. He formulated a future vision on what could be possible in principle for the miniaturization of machines. For example miniaturization of transistors into integrated circuits - the core of todays computers. He also advocated the need of much better microscopes to see all of the tiny machines. [movie][S13]

His speech did not have much impact initially as the vision was too far away from what could be technically realized for more than 20 years. Feynman’s main point, however, was that physicists should start making tools to study biology at the nanometer scale – that there is plenty of room at the bottom for exciting new research where others at the time thought that space – is the final frontier. Enrico Fermi is quoted to have asked “*Where is everybody*?” Voicing his astonishment that earth apparently has not seen signs of alien visitors.

[S14] Before I tell you where there is still room at the bottom, let’s have a look at what can we do right now. Right now we can manipulate single atoms, build some of his tiny machines but we can do much better than Feynman envisioned for imaging with light as you have seen in the animation. We shift what we can resolve to the nanometer scale with localization microscopy; from seeing red blood cells now nearly seeing DNA. And we put our technical developments and methods in the use of cell biology to visualize the machinery of life.

[S16] Here you see me in front of a painting in the Kroeller-Mueller museum in the Hoge Veluwe that is very much related to localization microscopy as you have seen in the animation. [S17] The images formed by localization microscopy consist out of many, many individual points, the localizations. That is very similar to the technique in this canvas by the French painter Seurat [Aussprache: Soera]. He painted in a technique later termed “pointillism”. If you zoom in very closely you see that he painted the image not by brushes but by putting small dots very close to each other, like in localization microscopy.

[S18] The best we have done as a community up to now for putting localization microscopy to use is this example from Xiaowei Zhuang and her group at Harvard. They discovered the spatial organization of structure proteins along an axon, which are the transmission highways of neuronal cells for signaling. The distance of the actin rings matched tretamers of spectrin. This discovery would not have been possible with electron microscopy, as it cannot identify specific proteins although the resolution would be more than sufficient.

[S20] Today we can also image structures fabricated with nanotechnology. Here you see an example of a DNA Origami design. Just as in paper origami, you can fold DNA the way you want by clever designing a base pair sequence to let it self-assemble structures with fluorescent molecules attached as this TU Delft logo. [S21] Here you see some of these logos imaged with localization microscopy. Note the relative poor signal to noise ratio of the logos. That is due to the limited density of fluorophores on a single structure that is already much smaller than the wavelength of light. As we now image many of these structures - why not combine the information of all of them into one new super-super resolved structure? [S22] We did that for 500 structures and obtained an image where you can see the individual sites on the DNA origami at a distance of less than 5 nm! That is better than wavelength over 140. Where Feynman thought that only wavelength over 2 is possible due to the structure of light. The underlying physics he used for his reasoning is still valid, we just sidestepped it in a clever way.

[S23] Another thing he could also not imagined is the immense power of computers nowadays. They allow us for example to detect signal just above the noise, which is the crucial first step in the image processing pipeline for localization microscopy. Here we computed a complicated statistical test for the presence of single molecules at each pixel of this image and find 7 of 8 signals that are hardly visible at all. Using massive parallel computations on graphic cards this evaluation only took 1 second.

[S24] I also want to share with you some of my clever, but just not clever enough moments in hindsight. Here you see a publication from 2005, one year before the seminal papers on localization microscopy came out, which led eventually to the Nobel Prize in 2014. We did a lot of things quite right (the ones in green), but not all – the ones in red. That meant our results were not the breakthroughs demonstrated a year later. The really annoying thing is that we had the same technology in the lab that they used. Once we saw their results our reaction is be best described by this quote from John Cruijff. On the bright side WE Moener and Eric Betzig shortly acknowledged our work in their Nobel Lectures, but of course mainly because they did the red parts better.

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Now you are eager to hear where is the room at the bottom, but first I want to switch gears and go back in time and tell you about my way into science and how I come to stand here in Delft today.

Why an interest for physics? [S25]

Obviously if you want to understand why things are the way they are, physics is the way to go. And “things” means, not people and their doings, but for instance to wonder why is it dark at night.

The first time I experienced the beauty of doing an experiment was in high school where we had to choose an experiment and show it in front of class. Showing an experiment to class also directly let me appreciate being a teacher. It turned out that it is much harder to be on the other side. But I also directly experienced teaching as a lot of fun.

This experiment was initially done around 1910 by Milikan who found that charge did only occur in quantized multiples of a unit charge - the charge of the electron. I also found that in high school and that really thrilled me. A simple hands-on experiment, revealing fundamental physics that can be conducted in high school.

Just much later did I learn that this experiment is a foremost example of conformation bias in science that is still present today. It took a long time before step by step other scientists reported always little bigger values in small steps until they converged to the current value, which is 7 standard deviations outside the reported measurement uncertainty [this means that Milikan thought that the correct value was likely to be wrong with a probability of 400 billion to 1]. So why did the other scientists not report the correct value directly but only step by step? Very likely they thought a too high number was wrong and so they discarded it even before making it public or the reviewers did it for them.

Why Holland and Delft? [S26]

I grew up and later studied physics in the southern part of Germany, but decided that an Erasmus year in Stockholm would be a good idea. There I met a very nice girl from Holland and we lived happily ever after. So Holland it was for a PhD. And a Russian guest professor at that time in Munich told me that Delft is the place to be in the Netherlands. After the PhD I went to the Max-Planck Institute for Biophysical Chemistry in Göttingen on a NWO scholarship to go abroad. From there I took an excursion to industry in Eindhoven and worked for FEI on image analysis for electron microscopy before coming back to Delft.

 In short, my path has been a mix of professional opportunities and the discovery that Dutch women are the best.

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Once in the Netherlands it turned out to be wise to be aware of the cultural and sociological setting for being professionally successful. [S31] For the Dutch context the following article in the Volkskrant [from Keyan Shahbazi] from June this year, summaries for me one really puzzling aspect about the Dutch value system.

*“It boils down to that we are autonomous individuals in the Netherlands who have the right to an opinion and to bluntly voice it, such that unreserved solidarity, harmony and courtesy are not leading principles but only obstacles for honesty. However, all perception of inequality is undesirable at the same time. And anybody who deviates from this happiness of the mean, is as a matter of principle suspect. ”*

 For all our foreign scientists it is good to be aware of both these aspects of Dutch culture. The expectation to conform to the happiness of the mean might for example not be present at all in the US. In academia that conformism pressure is less but it is certainly present. In my opinion for an university allowing diversity in characters with a random crack pot in between is better for attracting and retaining the best people. And it is a lot more fun. To cite from the inaugural speech of Marileen Dogterom from 2002 “*But don’t you think that all the conferences, committees and networking is all that exciting for me. The larger the numbers and the larger the homogeneity in these groups the larger the urge to run away screaming.”*  Apparently times are a changing, as she is now department head of Bionanosciences here in Delft – therefore I plan to reflect on my urges in 10 years from now.

 Once you see that all these scientists have diversity in character you realize that they therefore must be people. And once you see that you need to invest in people skills, because you deal with people where you came to understand physics. It turned out that TU Delft is very well aware of the fact that most scientists here are not people persons to begin with. So the university organizes courses on different aspects of coaching and leadership, and that really helped in my experience – which also surprised me to me honest. I want to encourage the university to keep this costly assistance, as it is essential to the oil in the machinery of the organization of the university.

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[S32] When I read a call from minister Bussemakers for 100 extra female full professors this year, I suspected that she meant to promote persons who perform task typically associated with women. Getting children, caring for them, working part time, cooking and doing the laundry to name just a few conceptual biases. Then I thought I fit all of them expect the first one and should be eligible and the rector should put me on the list. On second thought, I could not remember anytime I was asked on how I combine the full child care at home and only working 4 days a week with my potential promotion. Maybe just nobody suspected it? Maybe it was because my appointment was not via an open vacancy? I do not know.

 Then I had a look at the percentages of female professor at Dutch universities over the last years. And if you take an average of 15 years from assistant professor to full professor you see that gap is closing, but there is still some loss in the promotion of women.

After reading up on the literature on possible reasons for this, I read about selection bias of male hiring committees and many more. The potential career breaker that I did not read about but witnessed quite often [S33] is the kitchen table and the happiness of the mean. At the kitchen table career paths are discussed within a partnership and the spoken and unspoken decision making could be as following: First, most women like to marry smart (men), the husband then has at least an equally good education, but more likely a higher income than a starting academic (if he is not one himself).

Second, the happiness of the mean as I witness it, comprises for the men to have employment for 5 days a week and 3 days for women once children are in the picture. Only after the youngest child enters school that shifts to 4 days a week. And a volia the career of the husband has gained priority in a crucial period for promotion.

[S34] Here you see all female faculty at Applied Sciences, where the line indicates the separation between the Dutch women and the foreigners.

[S35] Drawing the same separation for the male professors shows a more than inverted ratio of Dutch and foreigners, giving some support for my hypotheses.

Why am I telling you this? We as academic community can do little about the happiness of the mean, but we want to have the best people, not wasting any potential talent. So what can we do?

[S36] The starting salary we pay for beginning assistant professors is just too low. In red you see the range of starting salaries from people I asked, where the salary just scales with age.

We ask a lot from them. More than is required from a senior scientist in a R&D department, that is in the hours they have to put in but also the responsibilities. Therefore I propose to shift the starting scale from 11 to 12 – not because academics are in for the money, but to have a better bargaining position at the kitchen table. To pay for this investment I suggest removing the last 3 steps in the full professor scale. [I am happy to take feedback on this idea after the talk especially from the people who salary I just cut].

 In addition, I suggest to the dean and rector installing a cross university mentorship. That is having an experienced female scientist from e.g. Leiden from the same discipline mentor a starting female in Delft. The cross university is important as it allows people from the same discipline to discuss careers without being direct colleagues or competitors.

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[S37] Room at the bottom. Just before I tell you about that, I want to ask the questions: Why is my speech here not as good as the speech of Feynman. Maybe a bit broader when was the last time you heard one as good from any academic? Not only showing a vision, but also putting numbers to it, thinking it through to a reasonable extend. My suspicion is that nobody has time to spend on such a talk, but also no time to think deeply on something else than what is directly at hand. Running from one meeting to another is a killer for creativity and doing something outside of your current mental box. I would argue that for scientists to have good ideas an empty agenda is better than for most other people – and taking really long showers for that matter. At universities the former is getting harder and harder. That might discourage the best young people more than anything else to join us, but instead seek inspiration elsewhere.

Now finally let’s have a look in the *Room at the bottom*

To really see biological processes, I mean really see, we would want to build microscopes and develop methods to make things visible at a spatial resolution of about one nanometer, with a time resolution of a millisecond in all 3 dimensions. Not only in a single cell but in cell culture or better a tissue. Putting numbers to that:

Imaging a cube of 100mu side length @ 1 nm resolution @ 1000fps gives 10^15 pixels per volume (more than a million giga bytes) and 10^18 pixels data per second (more than a billion giga bytes).

That seems a very ambitious amount of data, but much more problematic is to get some useful information on all these pixels even if we could acquire it.

Because to maintain a constant SNR per pixel, the total light collected from the sample has to increase by the same amount as the number of pixels.

Apparently there is a tradeoff triangle between the spatial resolution, temporal resolution and the SNR. You can only exchange one for the other, but never have the best of everything. Even now people like Harlad Hess at Janilea Farm try to image one entire brain of a fly with nanometer resolution with an electron microscope, but they also estimate that it takes 8 years to do so. And after 8 years they have *one* brain! So what could be the developments in the next years to cut some corners in this triangle?

We must do more with less. With that I mean we must be able to obtain the same or more information density from images with a lot less light. In fact get information from images that look like noise. Why is that important?

[S38] First of all now we shine the equivalent of about 1000 suns on a bright day on our cells for localization microscopy. You can imagine that this is not good at all and hampers imaging of living cells.

Also we often overexpress proteins in cells to have sufficient labeling and light, going to natural expression levels will again decrease the information density. That leaves us with even more noise and less signal.

From the labeling side I expect to see that DNA PAINT labeling approaches as we used for the TUD logo will be possible inside living cells, potentially by the use of unnatural amino acids, which would allow specific labeling with small bright dyes at normal expression levels.

Further inclusion of prior knowledge into localization microscopy has seen a conceptual step forward by Stefan Hell and his group last year with the MINFLUX paper in Science. However, be prepared if you read it, it is the worst paper ever written by him, I think it is hard to follow as the idea does not fit in the on-off game he likes to play, but it is just a matter of extra prior knowledge they managed to get in. When further developed this idea will give the same localization precision but with ~10x less light as we use today. Potentially it could be as fast as imaging today, which would truly cut a corner.

The combination of information I have shown you for the TU logo is just the beginning of the idea to reveal structure by combining many images to reconstruct one of very high quality. This technique will become as important as it is electron microscopy today. The use of electron microscopy and light microcopy in a combined fashion will also be everywhere in a few years from now once we have overcome the countless technical problems of really making it happen.

Let met finish with a wild speculation that could really cut a corner of the triangle. Currently our signal to noise ratio in imaging scales as square root of the detected number of photons *N*. If we now could use not *N* independent photons but *N* entangled photons than the SNR would scale as *N*! That would be a tremendous gain. Even if we would only use pairs of entangled photons that can be easily made today, we would gain a factor of sqrt(2) better SNR for free. Now the problem is that fluorescence is not a coherent process and up to now I could not figure out how to combine it with this idea although it seems not physically impossible.

My take on the future of microscopy is that we should not be afraid to acquire a lot worse images to begin with but in the future the immense computation power will allow us to see much more in the noise than we see today.

I want to conclude by thanking people who have been important.

[S39] First of all Lucas van Vliet who has been my PhD advisor and gave my then but also later all the freedom and stimulation to enjoy doing science. Then Tom Jovin, my postdoc advisor in Göttingen, who still breathes science with every step and lives it by the example. And to the right Sjoerd Stallinga my roommate since a few years, we are actually developing the science on localization microscopy together, which is a lot of fun – with and without the use of people skills.

[S40] A couple of people have been influential in different way. Ted by living that education matters, Piet for his endless curiosity, Mike for his friendship, Frans and Cees for being an inspiration.

[S41] A thanks not to a person but the social security system in Germany that allowed me to have a proper university education and only having a small loan afterwards.

[S42] Thanks for the fun. Here just showing a few people that made it worth the while over the last years.

[S43] Thanks to the different people never making it on to a publication, but who are essential for the scientists to be doing their job properly. And thanks for the patience they have with us, for being not people persons most of the times.

[S44] Manon vandag vooral bedankt voor je steun voor mijn werk en dat we de agenda’s uitgelijnt krijgen ondanks twee drukke banen, het vele reisen en de extra zorg voor Moritz. Maar vooral dat we het ondertussen nog steeds erg gezellig met elkaar hebben.

Otto, schoen dass du heute auch dabei bist. Es freut mich sehr dass du meine Begeisterung um Sachen rauszufinden teilst. Da werden wir noch viel Spass dran haben.

Ik heb gezegd.

Optional part:

This story exemplifies a real problem still present in modern science. Conformation bias – you see what you expect to see. And if you see something new or different you, either discard it yourself or the reviewers will do it for you. For something new you have to work twice as hard to prove to others that you are right and to show that something established is wrong 10x as hard. This attitude is very good to not get fooled by vague new claims, but very bad for fixing an established error in the community.

Another option would have been Hugo de Groot who was born in Delft and is well known as founding father for international law. He even has a statue on the market square in Delft, where Antoni van Leeuwenhoek has a relative small plate on this house.

SKIP?[S19] What we can do now is image the Nuclear Pore Complex in the light microscope. That is the gateway between the cell nucleus and the rest of the cell. Here you see the regular light microscopy image in the top left corner and the super-resolved image down here. We could reconstruct one such complex and overlay it onto an electron microscopy image here in gray. You can clearly see the 8-fold symmetry of the complex and the resolution far below the wavelength of light of about 600 nm.

Another thing we cannot do but Feynman thought was easy is real 3D imaging. Our imaging volumes look typically like pan-cakes not like cubes. That also has to do with the inability to collect information time efficiently and that the quality of imaging deteriorates by the sample itself. Here I see adaptive optics approaches from astronomy making a large contribution in the next years.

[So on what are we spending our time at university apart from our main tasks of education, research and valorization? Meetings? We spend too much time on (unsuccessfully) competing from research funding. That is not that our research is bad, but that there are too many good researchers in the Netherlands competing for too little money. That results in a prior success rates of 10-20% where probably 2/3 of all submitted projects are fundable. At least that has been my experience when reviewing grant proposals. How often do you encounter something that is really not good enough? These low rates are a consequence of not increasing research funds from the government while all universities hire more and more scientific staff members in a rat race for research funding and to enable teaching for more and more students.

 Studying at university level has been transformed from an elite to a common practice increasing the number of students … At the same time

* committee meetings, stw etc.
* grant writing, 10%, 20% is considered good, explain NOW sheet, not 80% bad science
* administrative rules (controlling like in grammar school and health care, DE school e.g.)
* educative more students. That would not cost per se a lot of more time if only classical lecture and more students would not mean more requests for exceptions and emails from students. But group work etc.]

Today manipulation of single atoms can be done as Feynman predicted, here by IBM and tiny machines can be engineered from only a few atoms as has been done by Ben Feringa from Groningen who made a car like molecule that was drive by electric pulses.