On 27 November 1895, Alfred Nobel signed his last will and testament, giving the largest share of his fortune to a series of prizes, the Nobel Prizes, awarded for outstanding contributions in chemistry, physics, literature, peace, physiology and medicine. As described in Nobel's will one part was dedicated to «the person who shall have made the most important chemical discovery or improvement». The most common research field for Nobel Laureates in Chemistry is biochemistry.

The Nobel Prize in Chemistry 2014
was awarded jointly to **Eric Betzig, Stefan W. Hell and William E. Moerner**
«for the development of super-resolved fluorescence microscopy».

***Eric Betzig, Stefan W. Hell** and **William E. Moerner** are awarded the Nobel Prize in Chemistry 2014 for having bypassed a presumed scientific limitation stipulating that an optical microscope can never yield a resolution better than 0.2 micrometres. Using the fluorescence of molecules, scientists can now monitor the interplay between individual molecules inside cells; they can observe disease-related proteins aggregate and they can track cell division at the nanolevel.*

In what has become known as nanoscopy, scientists visualize the pathways of individual molecules inside living cells. They can see how molecules create synapses between nerve cells in the brain; they can track proteins involved in Parkinson's, Alzheimer's and Huntington's diseases as they aggregate; they follow individual proteins in fertilized eggs as these divide into embryos.

When scientists in the 17th century for the first time studied living organisms under an optical microscope, a new world opened up before their eyes. This was the birth of microbiology, and ever since, the optical microscope has been one of the most important tools in the life-sciences toolbox. Other microscopy methods, such as electron microscopy, require preparatory measures that eventually kill the cell.

For a long time, however, optical microscopy was held back by a physical restriction as to what size of structures are possible to resolve. In 1873, the microscopist Ernst Abbe published an equation demonstrating how microscope resolution is limited by, among other things, the wavelength of the light. For the greater part of the 20th century this led scientists to believe that, in optical microscopes, they would never be able to observe things smaller than roughly half the wavelength of light, i.e., 0.2 micrometres. The contours of some of the cells' organelles, such as the powerhouse mitochondria, were visible. But it was impossible to discern smaller objects and, for instance, to follow the interaction between individual protein molecules in the cell. In order to fully understand how a cell functions, you need to be able to track the work of individual molecules.

Eric Betzig, Stefan W. Hell and William E. Moerner are awarded the Nobel Prize in Chemistry 2014 for having taken optical microscopy into a new dimension using fluorescent molecules. Theoretically there is no longer any structure too small to be studied. As a result, microscopy has become nanoscopy. The story of how Abbe's diffraction limit was circumvented runs in parallel tracks; two different principles are rewarded, which have been developed independently of each other.

In Turku Stefan Hell worked on so-called fluorescence microscopy, a technique where scientists use fluorescent molecules to image parts of the cell. For instance, they can use fluorescent antibodies that couple specifically to cellular DNA. Scientists excite the antibodies with a brief light pulse, making them glow for a short while. If the antibodies couple to DNA they will radiate from the centre of the cell, where DNA is packed inside the cell nucleus. In this manner, scientists can see where a certain molecule is located. But they had only been able to locate clusters of molecules, such as entangled strands of DNA. The resolution was too low to discern individual DNA strings.

When Stefan Hell read about stimulated emission, he realized that it should be possible to devise a kind of nano-flashlight that could sweep along the sample, a nanometre at a time. By using stimulated emission scientists can quench fluorescent molecules. They direct a laser beam at the molecules that immediately lose their energy and become dark. In 1994, Stefan Hell published an article outlining his ideas. In

the proposed method, so-called stimulated emission depletion (STED), a light pulse excites all the fluorescent molecules, while another light pulse quenches fluorescence from all molecules except those in a nanometre-sized volume in the middle. Only this volume is then registered. By sweeping along the sample and continuously measuring light levels, it is possible to get a comprehensive image. The smaller the volume allowed fluorescing at a single moment, the higher the resolution of the final image. Hence, there is, in principle, no longer any limit to the resolution of optical microscopes.

Stefan Hell's theoretical article did not create any immediate commotion, but was interesting enough for Stefan Hell to be offered a position at the Max Planck Institute for Biophysical Chemistry in Göttingen. In the following years he brought his ideas to fruition; he developed a STED microscope. Two laser beams are utilized; one stimulates fluorescent molecules to glow, another cancels out all fluorescence except for that in a nanometre-sized volume. Scanning over the sample, nanometre for nanometre, yields an image with a resolution better than Abbe's stipulated limit. In 2000 he was able to demonstrate that his ideas actually work in practice, by, among other things, imaging an E. *coli* bacterium at a resolution never before achieved in an optical microscope.

The STED microscope collects light from a multitude of small volumes to create a large whole. In contrast, the second principle rewarded, single-molecule microscopy, entails the superposition of several images. Eric Betzig and W. E. Moerner (who always has been called by his initials, W. E.) working separately have independently of each other contributed different fundamental insights in its development. They laid the foundation for the second method, *single-molecule microscopy*. The method relies upon the possibility to turn the fluorescence of individual molecules on and off. Scientists image the same area multiple times, letting just a few interspersed molecules glow each time. Superimposing these images yields a dense super-image resolved at the nanolevel. In 2006 Eric Betzig utilized this method for the first time. The foundation was laid when W. E. Moerner succeeded in detecting a single small fluorescent molecule.

In most chemical methods, for instance measuring absorption and fluorescence, scientists study millions of molecules simultaneously. The results of such experiments represent a kind of typical, average molecule. Scientists have had to accept this since nothing else has been possible, but for a long time they dreamt of measuring single molecules, because the richer and more detailed the knowledge, the greater the possibility to understand, for instance, how diseases develop.

Therefore, in 1989, when W. E. Moerner as the first scientist in the world was able to measure the light absorption of a single molecule, it was a pivotal achievement. At the time he was working at the IBM research centre in San Jose, California. The experiment opened the door to a new future and inspired many chemists to turn their attention to single molecules. One of them was Eric Betzig, whose achievements will be covered below. Eight years later Moerner took the next step towards single-molecule microscopy, building on the previously Nobel Prize-awarded discovery of the green fluorescent protein (GFP).

In 1997 W. E. Moerner had joined the University of California in San Diego, where Roger Tsien, Nobel Prize Laureate to be, was trying to get GFP to fluoresce in all the colours of the rainbow. The green protein was isolated from a fluorescent jelly-fish and its strength lies in its ability to make other proteins inside living cells visible. Using gene technology scientists couple the green fluorescent protein to other proteins. The green light subsequently reveals exactly where in the cell the marked protein is positioned.

W. E. Moerner discovered that the fluorescence of one variant of GFP could be turned on and off at will. When he excited the protein with light of wavelength 488 nanometres the protein began to fluoresce, but after a while it faded. Regardless of the amount of light he then directed at the protein, the fluorescence was dead. It turned out, however, that light of wavelength 405 nanometres could bring the protein back to life again. When the protein was reactivated, it once again fluoresced at 488 nanometres.

Moerner dispersed these excitable proteins in a gel, so that the distance between each individual protein was greater than Abbe's diffraction limit of 0.2 micrometres. Since they were sparsely scattered, a regular optical microscope could discern the glow from individual molecules — they were like tiny lamps with switches. The results were published in the scientific journal Nature in 1997. By this discovery Moerner demonstrated that it is possible to optically control fluorescence of single molecules. This solved a problem that Eric Betzig had formulated two years earlier.

Just like Stefan Hell, Eric Betzig was obsessed by the idea of bypassing Abbe's diffraction limit. In the beginning of the 1990s he was working on a new kind of optical microscopy called near-field microscopy at the Bell Laborato-

ries in New Jersey. In near-field microscopy the light ray is emitted from an extremely thin tip placed only a few nanometres from the sample. This kind of microscopy can also circumvent Abbe's diffraction limit, although the method has major weaknesses. For instance, the light emitted has such a short range that it is difficult to visualize structures below the cell surface.

In 1995 Eric Betzig concluded that near-field microscopy could not be improved much further. In addition, he did not feel at home in academia and decided to end his research career; without knowing where to go next, he quit Bell Labs. But Abbe's diffraction limit remained in his mind. During a walk a cold winter day a new idea came to him; might it be possible to circumvent the diffraction limit by using molecules with different properties, molecules that fluoresced with different colours?

Inspired by W. E. Moerner, among others, Eric Betzig had already detected fluorescence in single molecules using near-field microscopy. He began to ponder whether a regular microscope could yield the same high resolution if different molecules glowed with different colours, such as red, yellow and green. The idea was to have the microscope register one image per colour. If all molecules of one colour were dispersed and never closer to each other than the 0.2 micrometres stipulated by Abbe's diffraction limit, their position could be determined very precisely. Next, when these images were superimposed, the complete image would get a resolution far better than Abbe's diffraction limit, and red, yellow and green molecules would be distinguishable even if their distance was just a few nanometres. In this manner Abbe's diffraction limit could be circumvented. However, there were some practical problems, for instance a lack of molecules with a sufficient amount of distinguishable optical properties.

In 1995 Eric Betzig published his theoretical ideas in the journal Optics Letters, and subsequently left academia and joined his father's company.

For many years Eric Betzig was entirely disconnected from the research community. But one day a longing for science sprang to life again, and returning to the scientific literature he came across the green fluorescent protein for the first time. Realizing there was a protein that could make other proteins visible inside cells revived Betzig's thoughts of how to circumvent Abbe's diffraction limit. The real breakthrough came in 2005, when he stumbled across fluorescent proteins that could be activated at will, similar to those that W. E. Moerner had detected in 1997 at the level of a single molecule. Betzig realized that such a protein was the tool required to implement the idea that had come to him ten years earlier. The fluorescent molecules did not have to be of different colours, they could just as well fluoresce at different times.

Just one year later, Eric Betzig demonstrated, in collaboration with scientists working on excitable fluorescent proteins, that his idea held up in practice. Among other things, the scientists coupled the glowing protein to the membrane enveloping the lysosome, the cell's recycling station. Using a light pulse the proteins were activated for fluorescence, but since the pulse was so weak only a fraction of them started to glow. Due to their small number, almost all of them were positioned at a distance from each other greater than Abbe's diffraction limit of 0.2 micrometres. Hence the position of each glowing protein could be registered very precisely in the microscope. After a while, when their fluorescence died out, the scientists activated a new subgroup of proteins. Again, the pulse was so weak that only a fraction of the proteins began to glow, whereupon another image was registered. This procedure was then repeated over and over again.

When Betzig superimposed the images he ended up with a super-resolution image of the lysosome membrane. Its resolution was far better than Abbe's diffraction limit. An article published in Science in 2006 subsequently presented the ground-breaking work.

The methods developed by Eric Betzig, Stefan Hell and W. E. Moerner have led to several nanoscopy techniques and are currently used all over the world. The three Laureates are still active researchers in the large and growing community of scientists spearheading innovation in the field of nanoscopy. When they direct their powerful nanoscopes toward the tiniest components of life they also produce cutting-edge knowledge. Stefan Hell has peered inside living nerve cells in order to better understand brain synapses. W. E. Moerner has studied proteins in relation to Huntington's disease. Eric Betzig has tracked cell division inside embryos. These are just a few of many examples. One thing is certain, the Nobel Laureates in Chemistry 2014 have laid the foundation for the development of knowledge of the greatest importance to mankind.

***Sources*:**

http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/

http://www.nobelprize.org/nobel_prizes/facts/chemistry/index.html

http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/popular-chemistryprize2014.pdf

Alfred Nobel had an active interest in medical research. Through Karolinska Institutet, he came into contact with Swedish physiologist Jöns Johansson around 1890. Johansson worked in Nobel's laboratory in Sevran, France during a brief period the same year. Physiology or medicine was the third prize area Nobel mentioned in his will.

The Nobel Prize in Physiology or Medicine 2014
was awarded with one half to **John O'Keefe**
and the other half jointly to
May-Britt Moser and **Edvard I. Moser**
«for their discoveries of cells that constitute a positioning system in the brain».

Photos: D. Bishop, UCL and Geir Mogen/NTNU

Three foreign-born scientists who have all worked in the UK have jointly won this year's Nobel Prize in Physiology or Medicine for their pioneering research into how an inner "GPS" of the brain creates internal maps of the real world and makes it possible to orient ourselves in space, demonstrating a cellular basis for higher cognitive function.

The three researchers share this year's prize "for their discoveries of cells that constitute a positioning system in the brain" according to the citation from the Nobel Assembly at the Karolinska Institute in Stockholm, which pointed out that philosophers have argued for centuries about how we know where we are. How can we find the way from one place to another? And how can we store this information in such a way that we can immediately find the way the next time we trace the same path?

An attack on the brain's positioning system occurs in some neurological illnesses, such as Alzheimer's disease where the progressive degeneration of the neurons of the brain results in patients becoming disoriented after losing their memory of spatial locations.

The discoveries of John O'Keefe, May-Britt Moser and Edvard Moser have solved a problem that has occupied philosophers and scientists for centuries — how does the brain create a map of the space surrounding us and how can we navigate our way through a complex environment?

The sense of place and the ability to navigate are fundamental to our existence. The sense of place gives a perception of position in the environment. During navigation, it is interlinked with a sense of distance that is based on motion and knowledge of previous positions.

Questions about place and navigation have engaged philosophers and scientists for a long time. More than 200 years ago, the German philosopher Immanuel Kant argued that some mental abilities exist as a priori knowledge, independent of experience. He considered the concept of space as an inbuilt principle of the mind, one through which the world is and must be perceived. With the advent of behavioural psychology in the mid-20th century, these questions could be addressed experimentally. When Edward Tolman examined rats moving through labyrinths, he found that they could learn how to navigate, and proposed that a "cognitive map" formed in the brain allowed them to find their way. But questions still lingered — how would such a map be represented in the brain?

John O'Keefe was fascinated by the problem of how the brain controls behaviour and decided, in the late 1960s, to attack this question with neurophysiological methods. When recording signals from individual nerve cells in a part of the brain called the hippocampus, in rats moving freely in a room, O'Keefe discovered that certain nerve cells were activated when the animal assumed a particular place in the environment. He could demonstrate that these "place cells" were not merely registering visual input, but were building up an inner map of the environment. O'Keefe concluded that the hippocampus generates numerous maps, represented by the collective activity of place cells that are activated in different environments. Therefore, the memory of an environment can be stored as a specific combination of place cell activities in the hippocampus.

In 1971, John O'Keefe discovered the first component of this positioning system. He found that a type of nerve cell in an area of the brain called the hippocampus that was always activated when a rat was at a certain place in a room. Other nerve cells were activated when the rat was at other places. O'Keefe concluded that these "place cells" formed a map of the room.

More than three decades later, in 2005, May-Britt and Edvard Moser discovered another key component of the brain's positioning system. They identified another type of nerve cell, which they called "grid cells", which

generate a coordinate system and allow for precise positioning and pathfinding. Their subsequent research showed how place and grid cells make it possible to determine position and to navigate.

The two researchers, working at the Norwegian University of Science and Technology in Trondheim, found that these locations formed a hexagonal grid, with each "grid cell" within the entorhinal cortex reacting in a unique spatial pattern — collectively the grid cells form a coordinated system that allowed spatial navigation through a complex maze.

The two findings, together with other discoveries, allowed the scientists to discover how mammals, including humans, were able to build up an internal map of the environment to locate their position and work out how to get from one place to another.

May-Britt and Edvard Moser were mapping the connections to the hippocampus in rats moving in a room when they discovered an astonishing pattern of activity in a nearby part of the brain called the entorhinal cortex. Here, certain cells were activated when the rat passed multiple locations arranged in a hexagonal grid. Each of these cells was activated in a unique spatial pattern and collectively these "grid cells" constitute a coordinate system that allows for spatial navigation. Together with other cells of the entorhinal cortex that recognize the direction of the head and the border of the room, they form circuits with the place cells in the hippocampus. This circuitry constitutes a comprehensive positioning system, an inner GPS, in the brain.

Recent investigations with brain imaging techniques, as well as studies of patients undergoing neurosurgery, have provided evidence that place and grid cells exist also in humans. In patients with Alzheimer's disease, the hippocampus and entorhinal cortex are frequently affected at an early stage, and these individuals often lose their way and cannot recognize the environment. Knowledge about the brain's positioning system may, therefore, help us understand the mechanism underpinning the devastating spatial memory loss that affects people with this disease.

The discovery of the brain's positioning system represents a paradigm shift in our understanding of how ensembles of specialized cells work together to execute higher cognitive functions. It has opened new avenues for understanding other cognitive processes, such as memory, thinking and planning.

John O'Keefe was born in 1939 in New York City, USA, and holds both American and British citizenships. He received his doctoral degree in physiological psychology from McGill University, Canada in 1967. After that, he moved to England for postdoctoral training at University College London. He has remained at University College and was appointed Professor of Cognitive Neuroscience in 1987. John O'Keefe is currently Director of the Sainsbury Wellcome Centre in Neural Circuits and Behaviour at University College London.

May-Britt Moser was born in Fosnavåg, Norway in 1963 and is a Norwegian citizen. She studied psychology at the University of Oslo together with her future husband and co-Laureate Edvard Moser. She received her Ph.D. in neurophysiology in 1995. She was a postdoctoral fellow at the University of Edinburgh and subsequently a visiting scientist at University College London before moving to the Norwegian University of Science and Technology in Trondheim in 1996. May-Britt Moser was appointed Professor of Neuroscience in 2000 and is currently Director of the Centre for Neural Computation in Trondheim.

Edvard I. Moser was born in born 1962 in Ålesund, Norway and has Norwegian citizenship. He obtained his Ph.D. in neurophysiology from the University of Oslo in 1995. He was a postdoctoral fellow together with his wife and co Laureate May-Britt Moser, first at the University of Edinburgh and later a visiting scientist in John O'Keefe's laboratory in London. In 1996 they moved to the Norwegian University of Science and Technology in Trondheim, where Edvard Moser became Professor in 1998. He is currently Director of the Kavli Institute for Systems Neuroscience in Trondheim.

Sources:

http://www.nobelprize.org/nobel_prizes/medicine/laureates/2014/advanced-medicine-prize2014.pdf

http://www.nobelprize.org/nobel_prizes/medicine/laureates/2014/press.html

http://www.independent.co.uk/news/uk/home-news/nobel-prize-2014-british-scientist-john-okeefe-among-winners-of-prestigious-award-for-medicine-after-discovery-of-brains-internal-gps-9777000.html

Prepared by
A. Vinogradova