The 2014 Nobel Prize in Chemistry

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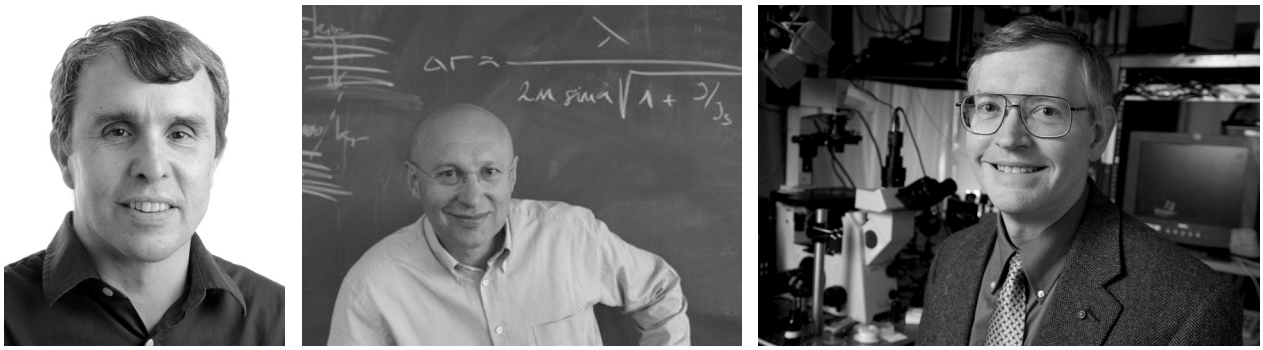
The Royal Swedish Academy of Sciences awarded the 2014 Nobel Prize in Chemistry to ***Eric Betzig***, ***Stefan W. Hell***, and ***William E. Moerner*** of the Janelia Farm Research Campus at the Howard Hughes Medical Institute in Ashburn, Virginia, the Max Planck Institute for Biophysical Chemistry in Göttingen and the German Cancer Research Center in Heidelberg, and the Chemistry Department at Stanford University in California, respectively, *for the development of super-resolved fluorescence microscopy*. The Nobel Prize-winning microscopy techniques have allowed scientists to visualize precise molecular mechanisms inside living cells, opening new windows to how life can be studied.

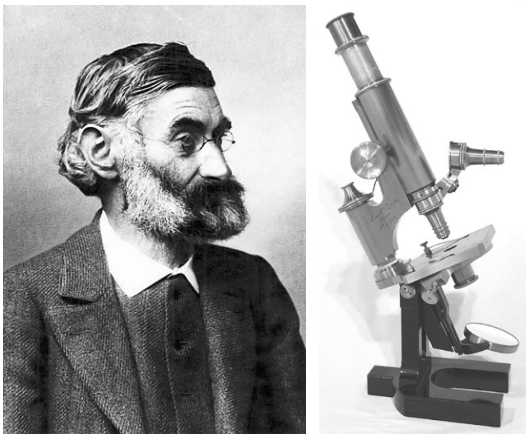


Left: Eric Betzig (courtesy Dr. Betzig); centre: Stefan W. Hell. © Max Planck Institute for Biophysical Chemistry, with permission; right: William E. Moerner (courtesy Stanford University)

Until recently, optical microscopy has been limited because it was presumed that it could give a resolution no better than to half the wavelength of light. The 2014 Nobel Laureates have ingeniously circumvented this limitation using fluorescent molecules in a new technique called super-resolved fluorescence microscopy and brought optical microscopy into the nano dimension by combining physics and molecular biology. Examples of the methods include photo-activated localisation microscopy (PALM) developed in 2006 by Eric Betzig and Harald Hess at Janelia and by Samuel Hess at the University of Maine; stochastic optical reconstruction microscopy (STORM) developed at the Howard Hughes Medical Institute; stimulated emission depletion (STED) microscopy by Stefan Hell at the Max Planck Institute in Göttingen; and saturated structured illumination microscopy (SSIM) at Janelia and the University of California, San Francisco.[1]

The work that has gained the 2014 Nobel recognition comes from a two-pronged ground-breaking approach that has taken optical microscopy into the nano dimension.[2] It marks the fifth time that the Nobel Prize has been awarded for an advance in microscopy and, in the view of this author, it could have gained either the chemistry or physics awards. Termed *nanoscopy*, this technique now allows scientists to visualise the pathways of individual molecules inside living cells by using fluorescent proteins. One can now see how molecules create synapses between nerve cells in the brain, track proteins involved in Parkinson's, Alzheimer's and Huntington's diseases as they aggregate, and follow individual proteins in fertilised eggs as these divide into embryos.

In 1873, Ernst Abbe defined the physical limit for the maximum resolution of traditional optical microscopy no better than around 0.2 µm for visible light. The ability to resolve objects with optical microscopy had been limited by the wavelength of light – anything smaller than about 0.5 µm appeared somewhat blurry because of diffraction effects. Despite electron microscopy and X-ray technologies going beyond this level of detail, those techniques kill any living cell in making the

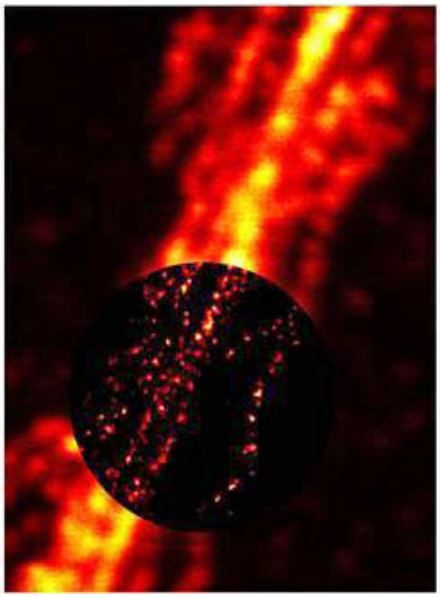


Left: Ernst Abbe; Right: 1879 Zeiss Microscope with optics by Ernst Abbe

observation and this same limit also applied to the diffraction fluorescence microscopy frequently used in biology and medicine. Betzig, Hell and Moerner were awarded their prize for having surpassed this limit and taken optical microscopy into the nano world to enable examination of living cells.

The developments are based on two separate principles. The first was developed by Stefan Hell, a 51-year-old physicist at the Max Planck Institute for Biophysical Chemistry. It radically overcame the resolution limit of

light microscopes with an entirely new concept. It was whilst at the University of Turku in Finland that Hell developed the principle for a new form of microscopy. Then, after transferring to the Max Planck Institute in 1997 to study sub-diffraction-resolution microscopy, he and his co-workers published the 1999 results obtained from his new microscope. These showed that he had translated his idea into an instrument which produced an image of a common bacterium with a resolution higher than the Abbe limit.[3] This 2000 paper named the technique STED microscopy. Hell's apparatus employs one laser beam to the fluorescent molecules in, for example, a cell nucleus, and another to blank out all the fluorescence except that occurring in a defined nano-size volume. Moving over the nucleus nanometer by nanometer, taking a snap at each stop, the microscope then produces an image of the nuclear cell molecules. The technique was not simply invented by Hell but also developed by him to application readiness. It was the first focused light microscopy method no longer limited by diffraction and allows up to ten times greater detailed observation in living cells than previously. It made structures visible that are much smaller than 200 nm.



STED microscopy (circular inset image) provides approximately ten times sharper details of filament structures within a nerve cell compared to a conventional light microscope (outer image); © G. Donnert, S. W. Hell, Max Planck Institute for Biophysical Chemistry, with permission.

In order to overcome the phenomenon of light diffraction, Hell and his team applied a donut-shaped STED beam to the focal spot of the fluorescence excitation beam. It switches off fluorophores at the spot periphery effectively confining them to the non-fluorescing ground state. In contrast, molecules at the donut centre in the fluorescence beam fluoresce freely. The resolution is typically improved by up to ten times compared with conventional microscopes. This means that labelled protein complexes with separation of only 20-50 nm can be discerned. As the brightness of the STED beam is increased, the spot in which molecules can fluoresce is further reduced in size and, in principle, the resolution of the system can be increased to molecular dimensions.

By developing special fast recording techniques for STED microscopy, Hell and the team further succeeded in recording fast movements within living cells. The exposure time for single images was reduced in such a dramatic way that they can film movements within living nerve cells in real-time and with a resolution of 65 to 70 nm - some three-to-four times better than conventional light microscopes.[3]

The second principle was established independently by Eric Betzig and William Moerner working separately. They arrived by a far more circuitous route at the other prize-winning technology now known as *single-molecule microscopy*. This method relies, in part, on the ability to turn the fluorescence of individual molecules on and off, a trick that Moerner had accomplished with green-fluorescing proteins in 1997 when at the University of California in San Diego. Betzig learned that other fluorescing proteins could be switched on and off at will. With that information, he realized that the Abbe limit could be exceeded by taking multiple images of the same area, allowing just a few interspersed molecules to fluoresce each time. By superimposing the images on each other a denser super-image is obtained, resolved at the nano level. This method was used for the first time by Eric Betzig in 2006. Today, nanoscopy is used worldwide and new knowledge of great benefit to mankind is produced daily.

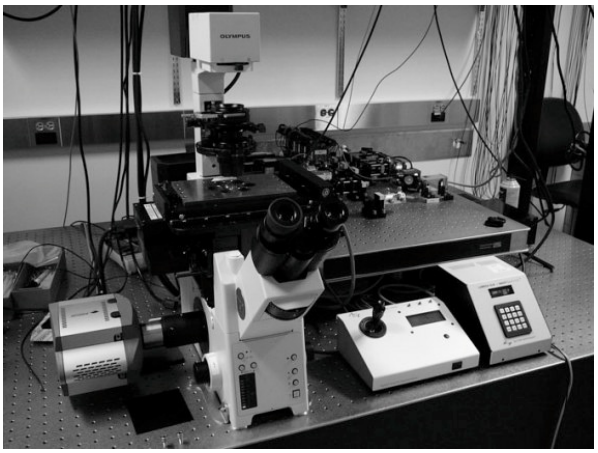
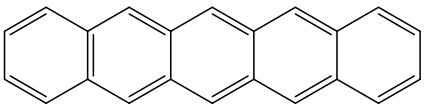


Photo-activated localisation microscope (PALM) developed by Eric Betzig and Harald Hess in 2006 (courtesy of Dr Betzig)

It is obvious that scientists need to study living cells in the smallest molecular detail and have the ability to dive deeper and deeper into human biology visualising the inner workings of cells at a molecular level. W.E. Moerner, the Harry S. Mosher Professor of Chemistry at Stanford, provided the ground-breaking work to observe molecules at the smallest scales, opening up new possibilities for discovery in areas ranging from disease management to drug development. From 1981 to 1995, he was a research staff member at IBM, and it was there that he made the first of two major discoveries key to his role in the Nobel-winning work. In 1989, he used laser-based techniques to allow the first visualisation of a single molecule, namely pentacene.



pentacene

"Prior to W.E.'s work, we all believed in molecules, but no one had ever seen one," said long-time colleague in the Stanford Chemistry Department, Richard Zare. "It opened up all sorts of new experiments in which you can see how cells divide, how the ribosomes can make proteins, and how the cells work" he said.[4] Moerner joined the chemistry faculty at the University of California-San Diego in 1995, and it was there that he made the second major discovery, albeit somewhat by accident. With colleague Robert Dickson he found that when looking at cells tagged with a green fluorescent protein, instead of staying brightly lit, the tags turned the fluorescence on and off (blink) at different wavelengths "like mini beacons, or flashlights, telling us where the structure is and in precise detail going far beyond the optical limit of diffraction" Moerner said.[4] This led to an entirely new way of looking at living cells as one can now go to factors of ten or more below the former level using fluorescence.

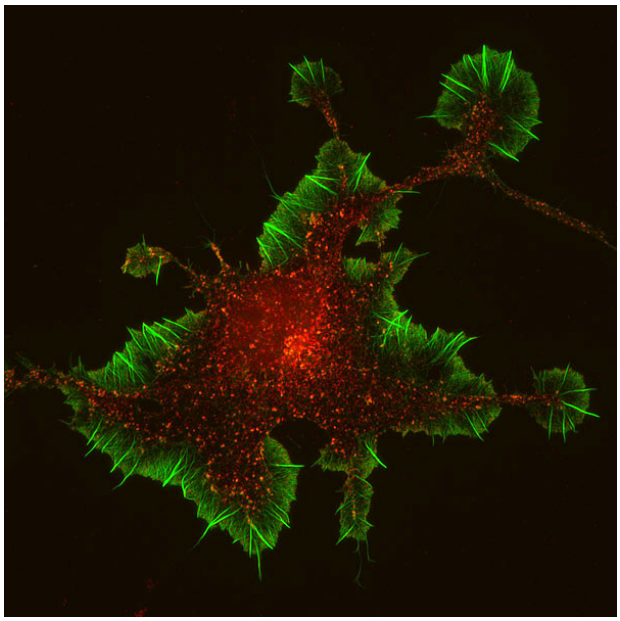


Image of a specialised brain cell captured using a structured illumination super-resolution microscope (Photo by Brad Zuchero and Andrew Olson, Stanford University School of Medicine; with permission)

In contrast to William Moerner, Eric Betzig is a physicist, inventor, and engineer. After two and a half years of theoretical research, he took his theories into the laboratory, where he applied them to the challenges of biological research aimed at developing a microscope that allows biologists to peer inside living cells with unprecedented resolution.[4] Trained as an experimental physicist, Betzig made waves in his field early on by helping to develop the technique now known as near-field microscopy that allows for the identification of nano-sized features with chemical contrast. It brought into focus structures that scientists had long considered too small to see with a light microscope. He made it more practical for biologists by allowing powerful imaging of dead cells.

The technique developed by Betzig and Moerner involves illuminating the sample with a weak laser pulse to ensure that only a tiny fraction of the fluorescent molecules will blink at a given time. Because of this it is extremely unlikely that any of these blinking molecules are separated by distances less than the diffraction limit. As each molecule emits a number of photons during a blink, they are detected as an intensity peak that has a normal distribution and a width that is limited by the diffraction limit. However, since the light comes from a single molecule, its location can be put at the centre of the normal distribution with high probability. Since the uncertainty in the location of the molecule falls as the inverse of the square root of the number of photons detected, an individual image can show only the locations of a few molecules. However, by repeating the process many times, a composite image of all the molecules can be created.[5]

Both prize-winning technologies are now used daily in biology and medicine worldwide for myriad purposes as noted at the outset. With the two technologies having given rise to nanoscopy, and in accord with earlier major innovations in scientific instruments, the field has blossomed. It now encompass other branches of research, generated improvements and variations, and stimulated a budding industry. All of this because Hell, Betzig, and Moerner circumvented the Abbe limit – in the accolade of the Nobel announcement: *ingeniously*.

References

1. See: http://www.hhmi.org/news/eric-betzig-wins-2014-nobel-prize-chemistry (accessed 21 October 2014).
2. See: http://www.nobelprize.org/nobel_prizes/chemistry (accessed 10 October 2014).
3. See: http://news.stanford.edu/news/2014/october/moerner-nobel-prize-100814.html (accessed 17 October 2014).
4. See: http://www.mpibpc.mpg.de/14734047/pr_1429 (accessed 21 October 2014).
5. Johnston, H. *Chemistry Nobel awarded for super-resolution microscopy*, Physics World, Institute of Physics, 8 October 2014; see: physicsworld.com (accessed 21 October 2014)