Histology & Embryology Periodical

Department of Histology and Embryology
Third Faculty of Medicine, Charles University in Prague

November
2014
Volume 2, Issue 2

The Internal Newsletter, 3rd Faculty of Medicine, CU

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What's up

In the month of November we begin the second course of the module Cellular Basis of Medicine, called **Energy for cells.**

However several practicals related to the first course remain to be completed such as on exocrine and endocrine glands, epithelial tissue as barriers and a revision practical. Although the practicals are officially listed as the course two, their topic will be focusing primarily on the cell structures.

MUDr. Maňáková will present a **lecture on mitochondria** in week seven, about their structure and origin. She and the mitochondria look forward to you and your curious questions!

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Reminders

One reminder from the Secretariat, a thought inspired by Johann Wolfgang von Goethe:

"One ought every day at least to hear a little song, read a good poem, see a fine picture, and, if it were possible, to speak a few reasonable words in order that worldly care may not obliterate the sense of the beautiful which God has implanted in the human soul."

Wednesday, December 10th 2014

The Teratology Information Center at the Third Medical Faculty in Prague will present a lecture entitled:

"Trends in Developmental Toxicology and Teratology"

The lecture will be held in Czech however handouts in English will be available.

Developmental toxicology is a "hot field" these days. The U.S., for example, has currently more than 85,000 chemicals in commerce. The California Department of Toxic Substance Control states that there are approximately 2,500 "high production volume" (HPV) chemicals, which are manufactured at a rate of more than one million pounds annually, with "nearly 45 percent of these HPV chemicals lacking adequate toxicological studies conducted to evaluate their health effects on humans and wildlife"[1]. Further, from the same governmental source, about 2,000 new chemicals are introduced into commerce annually in the U.S., at a rate of seven new chemicals a day. If you choose developmental toxicology as a career, you will always find topic for your research! See you at the lecture!

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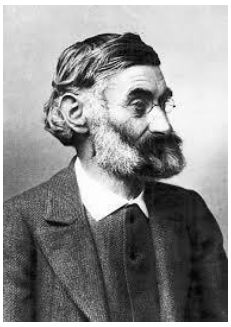
The Nobel Prize in Chemistry 2014

"for the development of super-resolved fluorescent microscopy"[2]

The majority what we know in biology comes from microscopy. In the late 1600 and early 170 0**Robert Hooke** with his microscopic observations and **Antonie Van Leeuwenhock** using his handcrafted microscopes observing and describing the single-cell organisms, were the pioneers of microscopy and biology.



Microscope technology and their resolution has improved greatly over the following centuries until it reached the impasse. The problem in optical microscopy is that the images are made by light therefore the resolution – in other words, the information gained from an image - cannot be greater than the wavelength of light. This fact was first concluded by **Ernst Abbe**, the German physicist and optical scientist, in 1873, who described the so called **diffraction limit** of the optical microscopy in the Abbe´s formula[a]. The resolution of light microscopy (200nm) is a fantastic tool because it allows to see images about 100 times smaller than is size of a cell size (10 to 100μm). On the other hand, viruses, most intracellular structures and molecules are much below the resolution limit!

In the 1920´ the physicists came in help with shorter wave length and images in the nanometer level in **electron microscopy**. However, optical microscopy has never really lost its´ practical impact. First and foremost, optical microscopes are simple to use and care for bur most importantly, there are two main advantages: a/ optical microscopy allows for **non-invasive imagining** of dynamics in the living system (vs. for the electron microscopy water in the sample has to be replaced with resin or the sample must be deep frozen), b/ optical microscopy allows imaging of specific proteins and sub-cellular structures and functions, in other words specific and highly sensitive **detection of cellular constituents through fluorescent tagging** is possible[3]. These advantages has always been the major drawback for electron and scanning probe microscopy - although providing nanoscale resolution down to the atomic level they cannot overcome their inherent incompatibility with living matter.

So now what next, what technology would combine the advantages of optical microscopy and still achieve the resolution of the electron microscopy?

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The Nobel Prize in Chemistry 2014 is equally shared by three outstanding man in the field of physical chemistry for „the development of super-resolved fluorescence microscopy".

Stefan W. Hell

Honorary professor of experimental physics, University of Göttingen, Germany
Adjunct professor of physics, University of Heidelberg, Germany

Stefan W. Hell is a physicist and a scientific member of the Max Planck Society and a director at the **Max Planck Institute for Biophysical Chemistry** in Göttingen, where he currently leads the Department of NanoBiophotonics.

Stefan W. Hell is credited with having **conceived, validated and applied the first viable concept for breaking Abbe's diffraction-limited resolution barrier in a light-focusing microscope**.

However, it was not an easy path for a young physicist with a fresh diploma from Heildelberg and such a revolutionary idea. It took many years and many knocks on the doors to find the listening ears at last. In spite of serious offers from London, Harvard and many other prestigious scientific destinations, Prof. Hell

[a] $d = \lambda / 2NA$, here d in the resolution, λ – wavelength and NA stands for numerical aperture

remains working at the Max Planck Institute that was the first to trust Hell´s revolutionary idea, and where he, in his own words, gets the most freedom and opportunities for his further research. [4]

Research of Prof. Hell's team

The **Department of NanoBiophotonics** under leadership of prof. Hell has been on the forefront of the developments of methods to surpass the diffraction barrier for several years. A strong background in optics helps the team to develop new microscopes with enhanced resolution, faster image acquisition, higher sensitivity and greater flexibility. With the chemical tools available in the department, the team of Prof. Hell strives to understand the switching mechanisms of **fluorophores** and develop tailor-made organic dyes. Genetics is used to create new and improved fluorescent proteins. Finally, in their laboratories, the team combines all these efforts in high-end imaging applications, e.g. for biological research. [5]

Nanoscopy: the Concept

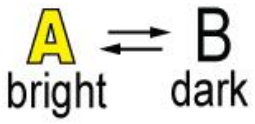

Figure: Basic principle of nanoscopy

In short, **all realized nanoscopy concepts utilize a transition between two distinguishable states of the fluorophore** which is operated as a fluorescence switch. In one state the molecule is fluorescent in the other state the molecule is non-fluorescent; between both states can be switched via light of a characteristic wavelength. The key idea is that fluorescent objects which are very close together can still be separated if they can be registered one after another. This can be achieved by switching the ability to fluoresce on adjacent objects which is realized either in a stochastical or in a determined mode. The figure above illustrates the different targeting and read-out modes.

Implementation of these concepts are Stimulated emission depletion (**STED**) microscopy, ground state depletion (**GSD**) microscopy and Single Molecule Switching (**SMS**) microscopy.

A detailed introduction into fluorescence nanoscopy can also be found in the article on **far-field optical nanoscopy** by the Nobel laureate Prof. Stefan W. Hell[6].

William E. Moerner

Harry S. Mosher Professor
in Chemistry
and
Professor, by courtesy,
of Applied Physics
at Stanford, CA USA

Prof. Moerner is credited for **inventing a single-molecule spectroscopy and imaging, a new field of science.**

His field of interests is astonishingly wide; he majored in electrical engineering, physic and mathematics, and his current research area covers physical chemistry, chemical physics, single-molecule biophysics, super-resolution imaging and nanoparticle trapping.

*

Prof. Moerner explains why he is interested in single-molecule optical nanoscience? [7]

"Complex systems - including molecules - can contain hidden heterogeneity produced by different local environments, different conformational states, or even different protein folds. **Single-molecule studies** allow us to explore hidden heterogeneity because we measure the distribution of behavior by recording the properties of each member of the ensemble, one by one. There are several specific ways single-molecule measurements can provide new information."

The macromolecule Prof. Moerner has focused on most of his professional life is the **green fluorescent protein (GFP)**. GFP serves in biological research as a universal "tagging" protein. Via knowledge where a certain protein or a molecule is located in a certain time allows the biologists to find out what is going on? In 1997 Prof. Moerner discovered a specific type of GFP that can be switched on and off using light of specific wavelength - a **photoswitchable single-molecule fluorophore.**

"Many functional nanomachines present within cells operate one by one, thus the ability to observe single copies provides a new way to try to understand how the system works. In collaboration with the molecular biology and biochemistry communities, we work to

discover how much can be learned with such single-molecule biophysical measurements. Our studies have explored various genetically encoded fluorescent proteins like **GFP, kinesin molecular motors, Ca++ ion concentration sensors, chaperonins assisting protein folding, transmembrane proteins of the immune system in and out of living cells, and genetic regulatory proteins in bacteria**. To enable further single-molecule imaging in cells, we are actively involved in the development of new **fluorophores**."

Prof. Moerner says about his future research: "Single molecules also provide a window into a growing new field, **nanophotonics**. …. On a deeper level, a single molecule can be viewed as a probe of its immediate local nanoenvironment on the scale on the order of the molecular size (~1 nm). Because single molecules are nanoscale emitters, when active control is used to turn molecules on an off, it is possible to build up a super-resolution image of the object under study, far beyond the optical diffraction limit. Several advanced techniques for obtaining three-dimensional information from single-molecule photoswitching are under development in the Moerner lab."[7]

*

Eric Betzig, PhD

Eric Betzig is a physicist, inventor and engineer from Michigan, USA, currently affiliated with the Howard Hughes Medical Institute (**HHMI**). He is a group leader of the **Janelia Farm Research Campus** in Ashburn, Virginia.

Before he moved to the Janelia Research Campus, Eric Betzig didn't have a lab. As he says in his Biography[8], the tools of his trade amounted to "a laptop and a couple of really good ideas".

His first affiliation in the early 90' however, was with the Bell Laboratories where he did have a lab and there he began to pursue work regarding **near-field fluorescent microscopy**. He was able to produce his first super-resolution images using a newly developed probe that point by point illuminated the object of interest.

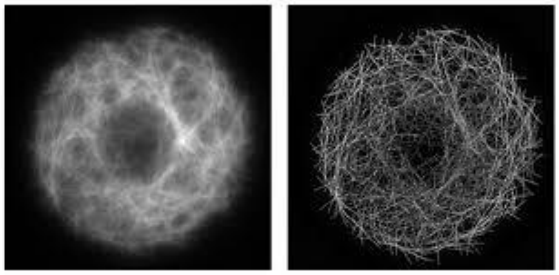

Figure: *Comparison of super-resolution and conventional images of microtubules in a Drosophila S2 cell*[9]

At about the same time Dr. Moerner invented the single-molecule imaging. Eric Betzig was brought independently in the same field however, the time "was not ripe" for Betzig and his further scientific achievements in the field of microscopy. As soon as the near-field fluorescent microscopy became a "hot field" in the mid 1990', Betzig turned his back to academic science, and returned to his hometown, Ann Arbor in Michigan. He began to develop a machine tool based on "flexible adaptive servohydraulic technology" in his dad's company. The technology was not commercially successful and Betzig felt he is ready for a come-back. He settled himself in a cottage on the Lake Michigan, and with his "laptop and a couple of really good ideas" came up with a design for a microscope using multiple beams to multifocal microscope. Along with his colleague Harald Hess, they combined the microscope with the technique of photoswitchable fluorescent proteins and produced the first **PALM** (photo-activated localization microscopy) images.

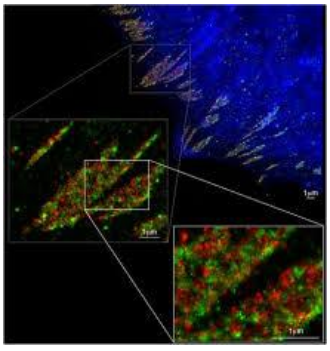

Figure:
Zoomed-in view of proteins within the human skin cells using PALM

H.Shroff et al. Proceedings of the National Academy of Sciences 104. 20308 (2007)

At Janelia Research Campus, Eric Betzig worked first with the PALM technology, later he and his team developed a digital scanned laser **light sheet microscopy** (DSLM).

However, Betzig's dream ever since his graduation had been to see and make an imagining possible of what's really going on in cells, not just on their surface.
One day, he heard Scott E. Frazer, Ph.D. saying that "biology is about trying to understand the rules of the game" and just like in football you need to watch game in action to be able to understand the rules, still photographs are not sufficient. "You need movies to truly appreciate what's going on", Betzig says in one of his presentations[3].

And that made him to switch the field and made him to ask "is there a way how we can build a microscope that improves 3D live cell imaging in some metric as much as PALM improves special resolution?" To make a long story short, in 2011 the team under the leadership of Eric Betzig designed and constructed the **Bessel Beam illumination microscope** – a high-speed, high-resolution, 3D technology that gives extraordinarily detailed views of cellular processes in action[10]. And just recently, they improved the technology by dividing the Bessel beam creating the **lattice light sheet microscope**. Betzig's team freely shares its designs, providing detailed instructions to scientists with the expertise to build their own version of the instrument. **Zeiss** (a company founded by Carl Zeiss and co-owned by Ernst Abbe, to round up our story) has licensed the Bessel beam and lattice light sheet microscopy. "It takes a huge amount of effort to move from a successful high-tech prototype to broader adoption of an imaging technology", Betzig says. "Ultimately, commercialization is the crucial last step to ensuring that these technologies can have broad impact in the research community."[11]

*

The amazing short movies produced by Betzig microscopes, are available e.g. at:
http://vimeo.com/109405410
http://vimeo.com/109406489
http://vimeo.com/109403880 etc.

*

One other article about the new microscopic technique from the lab of Eric Betzig containing several images and short movies, e.g. a fascinating 3D video on cell division, was published in **Scientific American** in May 2013 and is available at:
http://www.scientificamerican.com/article/no-kill-high-resolution-3d-movies-cells-now-possible/

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In his lesson in front of an audience of young scientists at MF Symposium, Singapore Eric Betzig delivers a personal message on success:

- Do what you love to do
- Have a good reason for doing it
- Work VERY hard
- Don't let others dilute your convictions
- Be your own toughest critic
- Stay just a bit scared
- Failure is the best teacher
- Success comes in many forms[3].

***

Repetitio, mater studiorum....

APOPTOSIS

The term "apoptosis" was coined in:

a) the ancient Greece
b) Aberdeen
c) The Royal Mint

In their article in the **British Journal of Cancer in 1972**[12], Kerr, Wyllie and professor Currie suggested the adoption of the term "apoptosis" that was proposed by Professor James Cormack of the Department of Greek, University of Aberdeen. The word "apoptosis" is used in Greek to describe the "dropping off" or "falling off" of petals from flowers or leaves from trees.

What, at that time, in 1972, the authors could have known about "controlled cell deletion", or apoptosis as they started calling it?

- that apoptosis is an **active**, inherently programmed phenomenon
- that apoptosis can be **initiated** or **inhibited** e.g. estrogens inhibiting the expected Műllerian ducts regression in genetic males, and androgenic steroids promoting regression in genetic females
- the authors described **two discrete stages** of apoptosis: first, the condensation in both nucleus and cytoplasm and breaking up of the cell into **apoptotic bodies**, and in the second, the apoptotic bodies being shed from surfaces or phagocytized
- that focal appearance of apoptosis at specific times happen during normal **ontogenesis** – either in development e.g. lumina of tubular structures or the fashioning of limbs (the formation of interdigital clefts) and the involution of phylogenetic vestiges
- they stated that controlled cell death plays an opposite but **complementary role to mitosis**. In fact, in the adult life cell death exactly balances cell division[13]

What can you tell about **apoptotic bodies?**

a) Apoptotic bodies maintain their cell membrane to prevent a local inflammatory reaction (vs. necrosis)
b) Apoptotic bodies can be found in histological sections of many healthy tissues
c) A few of apoptotic bodies is taken up by **histocytes** but the majority are rapidly phagocytized by intact parenchymal cells (e.g. in the neoplastic cells) [5]
d) all of the above is correct

D is correct!

The Nobel Prize regarding apoptosis occurred in

a) 2002
b) 1996
c) yet has not happened

The Nobel Prize in Medicine or Physiology in 2002 was given to **Sydney Brenner**, **H. Robert Horowitz** and sir **John. E Sulston** "for their discoveries concerning genetic regulation of organ development and programmed cell death". During their collaborative effort in the MRC Laboratory of Molecular Biology they explained the precise order in which cells in roundworm *Caenorhabditis elegans* divide and succeeded in tracing the nematode's entire embryonic cell lineage.

*

Proving Eric Betzig´s words about importance of movie sequences in biology research and understanding processes in organisms, watch an exciting short video posted by **„The Cell: an image library"** on apoptosis. A time-lapse series using digital holographic microscopy shows living human prostate cancer cells induced to undergo of apoptosis following treatment with etoposide (a cytotoxic drug):
http://www.cellimagelibrary.org/images/43705

*

Make sure you remember that apoptosis is one of the **four basic morphogenetic processes** along with...

- Proliferation
- Migration
- Association

"MAPA" is an acronym, and a Czech word for a map, a map throughout your classes on embryology.

*

And now it´s time to open your textbooks and learn about mediators of apoptosis, initiation via either extracellular or intracellular signals, Bcl2 and IAP, the huge chapter on caspases, Fas and TNF pathways, mitochondrial regulation and many other aspects of apoptosis. As for maintaining your sanity, it might help to keep reminding yourself that it is the **dynamic balance** between anti-apoptotic and pro-apoptotic factors that determine whether a cell lives or dies.

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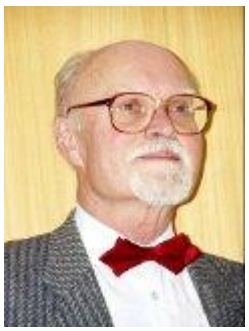
Prof. MUDr. Richard Jelínek, DrSc.
(October 8th 1934 – October 27th 2008)



Prof. Richard Jelínek, founder of Czech experimental teratology, a histologist, embryologist and anatomist, the head of Department of Histology and Embryology of the Third Medical Faculty, Charles University in Prague for fifteen years, **died on October 27th 2008**.

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Professor Richard Jelínek possessed many talents. When he had to decide what to study his major interests where mathematics, medicine and music. He chose medicine and math. After two semesters of both his interest shifted to medicine exclusively. But he did not forget his old love and studied math later in life in a course "Applied Logic" at the Philosophical Faculty, Charles University. And he didn´t betray music either; played piano, recorder and guitar. He enjoyed singing and sang well.

As a medical student of the General Medicine Faculty, Charles University in Prague he became an assistant at the Anatomic Department. Prof. Borovanský influenced his professional interest in brain structure and embryology. During his eight semester of med school, he published his first piece of science. And then 300 more. He put his last publication efforts into the Czech translations of a histology textbook by L. C. Junqueira et al.: Basic Histology in 1993 and embryology textbook by K. L. Moore, T. V. N. Persaud: The Developing Human in 2000. Those textbook translations were meant as an intrinsic debt repayment to his students for the twenty years he was forbidden to teach. All that as a consequence of his statement, proclaimed during a student happening on August 21, 1968, that the 200 000 Warsaw Pact troops and 2000 tanks invading Czechoslovakia was, in fact, an „act of aggression".

His enormous academic achievements were not without the downside, such as too little time for his family. His daughters would enjoy daddy only once they were capable of prolonged bike trips, Spartan camping and long hikes on the most challenging mountain trails. Biking, Nordic skiing, swimming, basketball, fencing in the early years, and canoeing with the dachshund in the lap of his wife were his regular, lifelong recreational activities. Many students were puzzled when they caught sight of their respected professor wearing an admiral's cap.

Prof. Jelínek was a man of great character, hardworking and demanding hard work, with a good sense of humor and an ability to see a situation or a problem in context and from different perspectives. He was charming and graceful.

The Czech poet Karel Šiktanc in his book "Horoskopy" wrote a characteristic of people born in the sign of zodiac of Libra: *"I am made of wings. Of the spread out feathers"*.

He was, indeed.

Ivana Jelínková
In Prague, October 14th 2014

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