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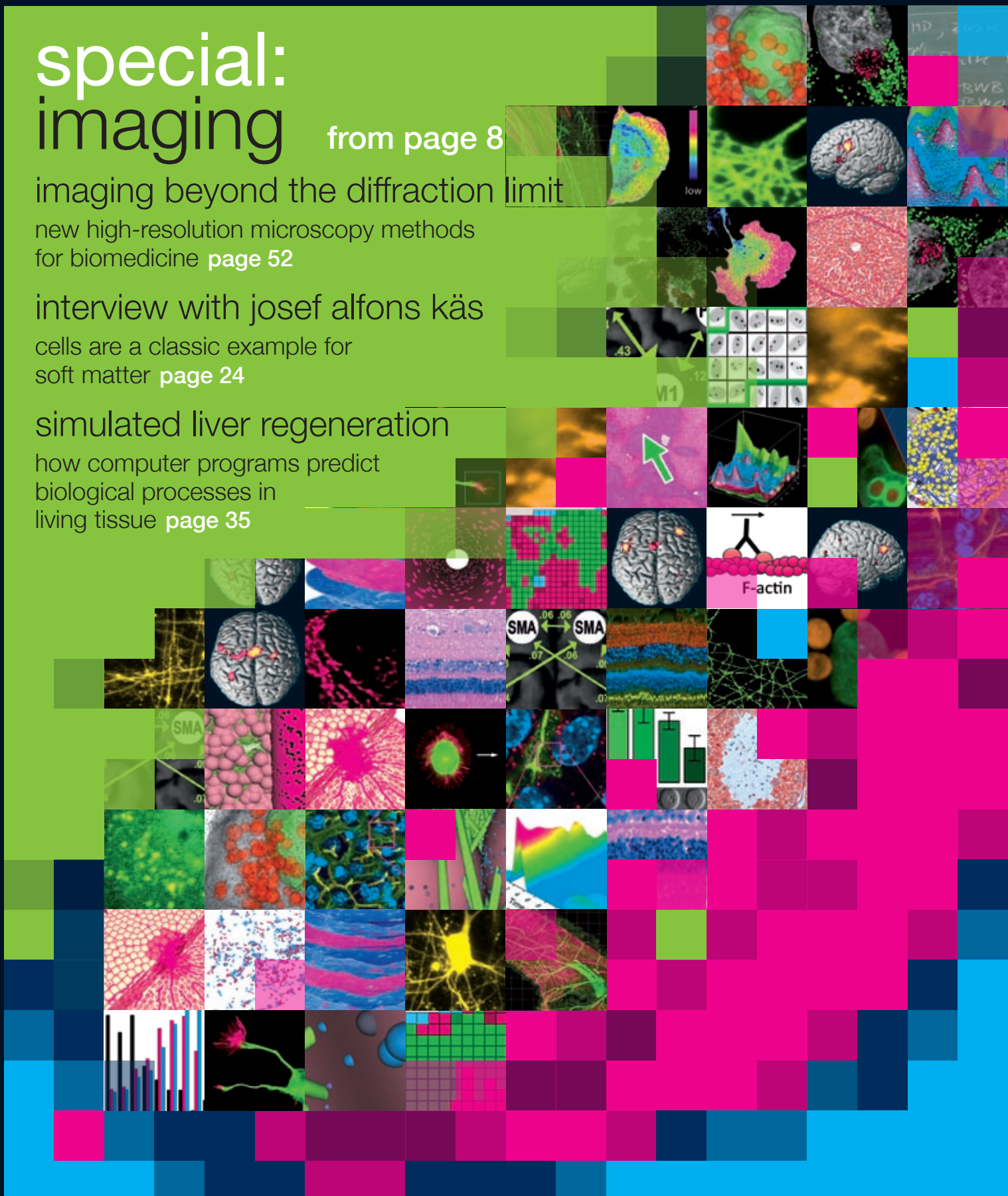

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Systems biology is a young and dynamic discipline that sees the whole picture. As part of the life sciences it builds a bridge between sophisticated laboratory experiments and mathematical modelling, between high-tech data measurements and computer-aided data evaluation. Its research subjects are the network-like entangled activities of signal transduction and metabolism in cells, tissues, organs and organisms. Systems biology research deals with this complexity by organising itself into interdisciplinary networks. Experience this fascinating, upcoming branch of science and what answers it provides to previously unresolved questions about human life in this international 3rd edition of systembiologie.de.

Cover photo: Collage of images from the third issue of systembiologie.de
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welcome note

Dear Reader,

The task of systems biology is to understand biological processes as a whole. The numerous possibilities for applying this unique research approach make it a key field of the life sciences. Systems biology combines the strengths of biotechnology and information technology, generates interdisciplinary synergies, and creates sustainable solutions to global challenges.

The German Federal Ministry of Education and Research (BMBF) has given intensive support to this branch of research over the past six years, and Germany is now one of the world's leading centres of systems biology research. An understanding of life processes and the development of predictive mathematical models stimulate ideas and innovations in health research and bioeconomics. In future we expect systems biology to contribute especially toward the development of new drugs, new pharmacologically and diagnostically relevant biomarkers, and individual therapeutic approaches. Along with medical findings, a further main emphasis is on solving pressing questions of energy supply using renewable resources.

At the same time, the collection of experiment data will be accelerated considerably by developing and optimising new methods and technologies. For example, in order to facilitate the quantitative analysis of complete tissue sections, we must achieve the transition from the semi-quantitative microscopic methods customarily in use at present to new, image-based methods. Special attention is therefore being given to imaging methods that enable molecules to be depicted in their natural surroundings and within intact tissue.

This third issue of *systembiologie.de* magazine gives insight into the development of imaging methods and their applications, and features current contributions being made by German scientists to this field of research. I hope readers will make many interesting discoveries and gain important insights into the future of systems biology.

I hope you will find it stimulating reading!

Prof. Dr. Annette Schavan, MdB
Federal Minister of Education and Research

welcome note

Dear Reader,

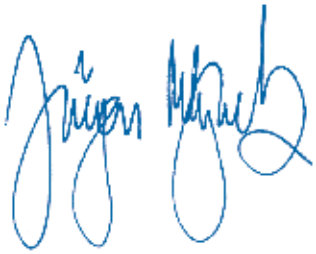
Following its mission, the Helmholtz Association contributes to solving grand challenges which face society, science and industry. This includes regularly asking ourselves whether we are doing the right things and whether we are approaching them in the right way. Within the Association, this process is supported among other things by the Helmholtz Think Tank, of which some of our best scientists are members. Their task is to identify key topics for the future in the areas of strategy and science, to subject them to critical discussion, and to scrutinize them with a view to a future role for the Association.

One of the first topics chosen by the Think Tank for intensive discussion is synthetic biology, a branch of systems biology, which it has identified as a potential interdisciplinary research topic. Interdisciplinary collaboration between biologists, chemists and engineers in this young discipline could make it possible to selectively design molecules and cells in future, thereby opening up completely new possibilities for application and use. In order to facilitate a wide discussion of opportunities and risks, the Think Tank initiated a workshop in January 2011. Along with scientists from the Helmholtz research fields Health, Earth and Environment, Key Technologies, and Energy, the participants also included international specialists in this new field.

A detailed analysis of the potential for synthetic biology in the individual research fields, and the Helmholtz Association's research portfolio in general, enabled the workshop to draw up initial recommendations for topics and for suitable means of integrating this new field of research.

One of the most important outcomes of the workshop is the recognition that synthetic biology can provide crucial contributions and stimuli in all the above mentioned research fields and would therefore complement the Helmholtz Association's portfolio as an initiative across research fields extremely well. For example, one fascinating possibility for this future technology is efficient production of ethanol with the help of specially developed microorganisms. This would save valuable cropland currently required to generate biofuels. Another application gains attention in medicine, in the field of optogenetics, where light is used to activate genetic processes. This opens up completely new possibilities for the targeted manipulation of molecular processes within individual cells.

I hope you enjoy reading this issue of systembiologie.de!



Yours, Jürgen Mlynek

President of the Helmholtz Association

foreword

Between the lines,...

Dear readers, is not where you will have to read in this issue of *systembiologie.de* in order to grasp the significance of imaging in systems biology. Although you may not find any new model of systems biology in this issue, it will provide diverse insights into the fascinating world of imaging methods. During the past two decades, these methods have taken quantitative biology by storm. In the long term they will have a greater impact on life sciences than the currently much-lauded high-throughput molecular technologies. These "silver bullets" of molecular biology generate a flood of data, but it is data that defies intuitive interpretation. Imaging methods may not deliver findings *per se*, but they do generate images that lend themselves much better to intuitive interpretation than do endless chains of letters or numbers.

Gottfried Böhm, professor of contemporary art history at the University of Basel and publisher of leading works on image theory, refers to a deep-seated human need for visual images. He says that the question "What is an image?" and questions about the significance of visual images are as old as our cultural history itself. *Homo pictor*, the cave painter, predates the *zoon logon echon*, the being that thinks in concepts and is endowed with reason. The image, of which even Plato was generally suspicious, has long become an important tool of modern natural and life sciences and plays an increasingly definitive role in our daily and scientific lives.

So it comes as no surprise that systems biology, a discipline that deals by definition with complex concepts and correlations, is making more and more use of imaging methods. In the spirit of his *Sophist* dialogue, in which Plato describes making things visible as "taking something to its being", systems biology uses images to make biological forms visible, indeed to endow them with life and being.

On venturing into the microcosm of cells and the nanoworld of molecules, pioneers of imaging methods find themselves confronted with major challenges. A high degree of tenacity, inventiveness and engineering skill has revolutionised high-resolution light microscopy. For nearly a century and a half, it was considered impossible to move beyond the central optical theorem concerning the spatial limit to resolution. However, smart ideas coupled with theoretical deliberations led to the overcoming of limits to resolution in light microscopy that were previously believed to be set in stone (see Stefan Hell's article, page 52). We can be justifiably proud that these developments were driven forward by researchers in Germany, the country of poets and philosophers, but also of inventors and innovators. The playful spirit that is otherwise attributed mainly to children is abundant in the field of systems biology. This spirit of invention is opening up fascinating new fields of application for systems biology, from stem cell research to the biomechanics of tumour cells.

As regards the anthropological question "What is an image?", Böhm says that every image draws its determining power from its liaison with the indeterminate. The indeterminate and the potential is the supporting base for the visual image, he says. Thus, in systems biology images derive their fascination from the promise to render visible that is invisible and to make the incomprehensible comprehensible.

Welcome to the brave new world of systems biology images!

Yours, Roland Eils

Editor in Chief

index

the power of image analysis for systems biology

by Marino Zerial and Yannis Kalaidzidis

Systems biology aims at the understanding of how many different molecules work together to build cells, organs and, ultimately, an entire organism. The potential for scientific discoveries as well as for economics is enormous because the understanding of a biological system in terms of its engineering properties and functional circuitries allows predicting how a given perturbation, such as a disease or drug, can impact on the physiology of an organism. Recognizing this enormous potential, the BMBF has invested heavily in systems biology focusing on a multi-scale model of the liver (Virtual Liver Network, VLN). In the VLN, one of the key approaches to collect data for systems biology is imaging. Recent developments in light microscopy and image analysis have been revolutionizing the way scientists can interrogate biological systems and unravel their underlying design principles.

The importance of who, when and where

In the past two decades, the sequencing of various genomes in combination with a number of analytical technologies (*omics*) has enabled the systematic analysis of cellular components. Functional genomics techniques (e.g. RNA interference, RNAi) applied at large scale have addressed the function of individual genes with respect to a particular cellular process. Cataloguing the individual molecules expressed in a cell is, however, not sufficient to reconstruct the mechanisms whereby individual molecules interact in functional networks or assemble into organelles to form cells and tissues. The "synthetic biology" approaches can complement such studies with the biochemical reconstitution of molecular constituents in minimal systems capable of recapitulating a given biological process (Ohya *et al.*, 2009).

The present modelling approaches largely focusing on signal transduction and metabolic pathways can capture the complexity of cell and tissue organization only to a very limited degree. One of the reasons why cell and tissue morphogenesis remains an unsolved problem is that we lack detailed and accurate information on the spatio-temporal patterns of molecules (the *where* and *when*) in the context of their function in cells and tissues. Imagine if we could observe in space and time every single component of a cell or tissue: This would improve our understanding of biological mechanisms and allow more accurate predictions of the systems function. It would shed light into how interactions between molecules are orchestrated to generate complex structures (e.g. organelles) and how cells collectively form tissues, organs and organisms. Therefore, the spatio-temporal analysis of cellular components needs to be incorporated in systems biology.

Light microscopy is a major source of detailed information on spatio-temporal organization of biological systems, ranging from single molecule to sub-cellular structures up to whole organ level. In recent years, light microscopy techniques have been developed to such an extent that they can now provide an unprecedented level of resolution, dynamic range and throughput for the imaging and analysis of biological samples. For example, various microscope techniques (STED, PALM-STORM) have been developed, which can break the diffraction resolution limit, something that was thought to be impossible to achieve for more than 100 years. These techniques allow the visualization of cellular structures down to 20-30 nm. The development of SPIM (selective plane illumination microscopy) allows the 3D visualization of entire organs and organisms. Novel light detectors and illuminators, new generations of Charged Coupled Devices (CCD) and photomultipliers have increased the sensitivity and dynamic range of detection of (sub) cellular structures. This is very important for live cell imaging to avoid toxic light doses. Computerized microscopes are available that automatically acquire hundreds of thousands of high-resolution, multi-color images per day. These examples provide an idea of the ongoing revolution in light microscopy promising unprecedented data quantity and quality in systems biology.

However, if imaging cells and tissues is a necessary step to explore biological processes, mechanistic information needs to be extracted from such images in an unbiased and accurate

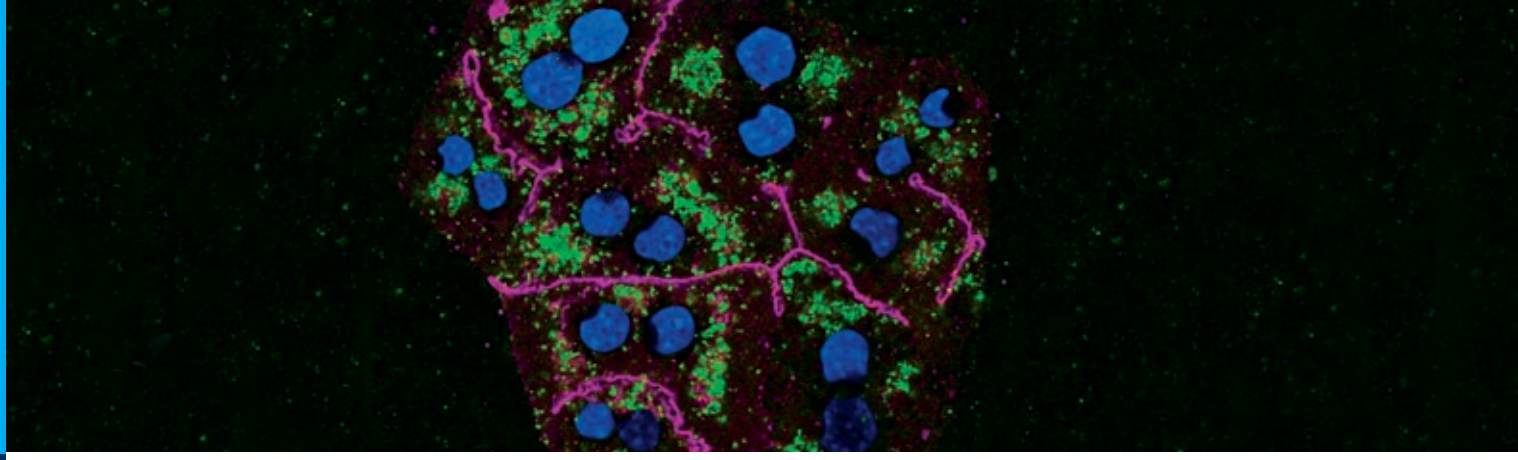
Prof. Marino Zerial and Dr. Yannis Kalaidzidis combine *in vitro* and *in vivo* experimental approaches at different scales with state-of-the-art image analysis and mathematical modeling in a multidisciplinary approach to advance our understanding of key cellular processes at the system level.

manner. An example of detailed images of primary liver cells (hepatocytes) grown *in vitro* or under native conditions in the organ (in a tissue section) is shown in Fig.1. The image illustrates sub-cellular organelles called endosomes (they appear as dot-like structures) which concentrate and distribute nutrients and signalling molecules selectively imported into the cell from the environment. This uptake process or *endocytosis* is absolutely essential for our cells (Schenk *et al.*, 2008) and it is easy to comprehend why it must be mechanistically elucidated to develop a systems understanding of cell and tissue organization and whole organ function. Yet, the field of signal transduction has traditionally ignored the spatio-temporal distribution of signalling molecules, hence the necessity of the VLN to integrate the process of *endocytosis* with metabolic and signalling mechanisms, cell polarity and tissue formation.

State-of-the-art image analysis of endosomes in liver cells

Imaging and quantifying cellular organelles such as endosomes is a major challenge. Endosomes range in size between 100nm and 1-2µm (Fig.1) and the amount of specific molecular components can vary more then 2-3 orders of magnitude. The human eye can appreciate major changes but cannot provide an unbiased, accurate and quantitative estimate of the multitude of structures imaged and their molecular content. For this, a computer-assisted approach is necessary. Although image processing is being done in biology, most of the techniques used are still inadequate to extract quantitative parameters from image data. Pixel-based algorithms, which are widely used both in commercial and academic software, are either prone to miss faint structures or merge bright ones in clumps. At the same time, these algorithms are inaccurate to estimate quantitative parameters of individual structures, when their size is close to the diffraction limit of conventional microscopes.

To solve this problem, we have applied a segmentation approach, traditionally used for imaging single molecules, which consists of fitting fluorescence intensity by single base function, i.e. point spread function of the microscope. We expanded the fitting approach by using a sum of base functions to overcome the limitation of "sub-diffraction" size and provide accurate description of "free-shape" objects. Such an intensity fit approach has been applied to multiple (hundreds-to-thousands) objects in the Motion-Tracking/Kalaimoscope software. In essence, for each cell represented in an image the software can provide accurate statistics of the number of structures, size, amount of molecular markers and intracellular distribution in relation to cell and tissue organization. The software has been further expanded to estimate with high precision cell perimeter, cell shape and cell-cell contacts. These features are extremely important for the 3D representation of liver tissue organization. This image analysis platform combined with mathematical modelling allowed the identification of specific design principles of endocytosis (del Conte-Zerial *et al.*, 2008) with implications for a broad range of biological processes in various cellular systems. Applied to a genome wide RNAi screen, it has provided proof-of-principle for the analysis of endosomal transport (Collinet *et al.*, 2010) and cell shape (Marsico, Collinet, Kalaidzidis and Zerial, in preparation). We deduced design principles of the endocytic system that allow us to predict the function of several new genes. This systems survey revealed for the first time that cells control very accurately the distribution of signalling cargo within endosomes. This is in line with the idea that transmission of signals from the surface to the nucleus is regulated by the endosomal system. This example illustrates the importance of performing a spatio-temporal analysis of cellular components in order to aim at a systems understanding of cell organization and tissue formation.



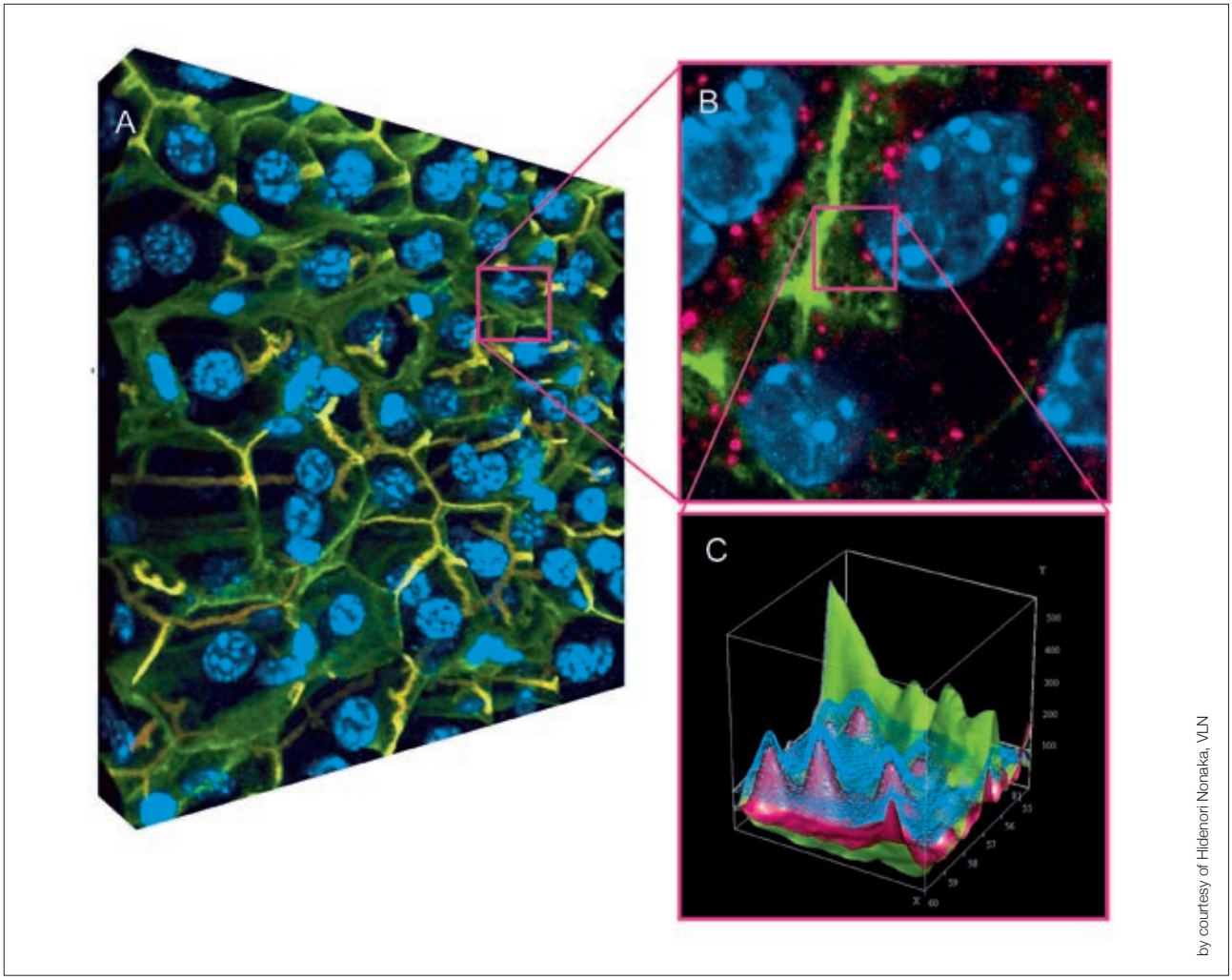
Primary hepatocytes after six days in a 3D Collagen Sandwich Culture System, labelled by the lysosomal marker Lamp 1 (green), Tight Junction Marker ZO1 (shows bile capillaries in red), cell nuclei are labelled in blu.

Image analysis and the development of new therapeutic approaches

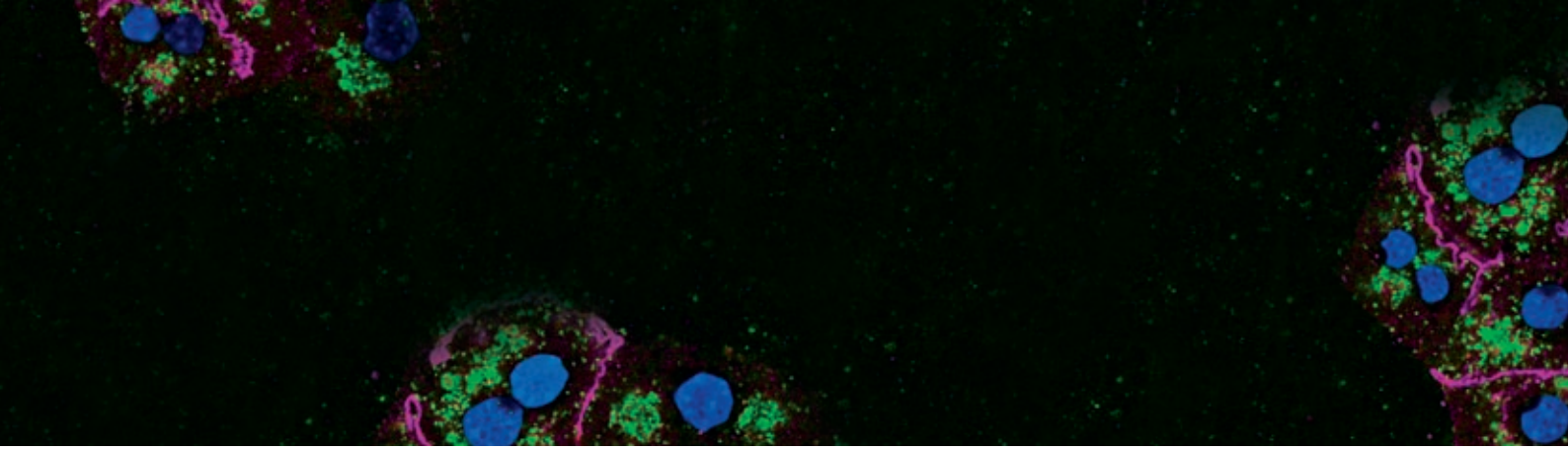
Modern microscopy does not just provide a descriptive representation of biological samples but offers a new way of extracting functional information from images. Perturbations such as genetic mutations, effects of chemicals and pathogens (viruses, bacteria) can be analyzed taking advantage of the multi-dimensionality of data. The quantitative data can therefore be used to generate mathematical models that describe the process observed. When applied to high-throughput studies, this technology has the potential to explore the complexity of cellular circuits and discriminate between

Fig. 1: 3D reconstruction of a liver section

Cells are labelled by 4-colour markers: Cell nuclei, blue (DAPI); actin filaments, green (phalloidin), apical membranes (bile canaliculi), yellow (CD3); endosomes, red (EEA1). The insets show 1) a higher magnification of cells and their organelles and 2) the image analysis of the fluorescence signals depicting the original intensity of actin filaments (green) and the endosomal marker (EEA1, red). The blue mesh indicates the fit of the marker distribution on the endosomes by a set of base functions.



by courtesy of Hidenori Nonaka, VLN



possible mathematical models by testing their predictions (Collinet *et al.*, 2010). We anticipate that this approach will reveal a new generation of biomarkers for diagnostic purposes. Especially considering an important organ like the liver, quantitative image analysis applied to this organ has the potential to innovate drug discovery, by providing a more predictive mean of assessing the properties of novel bioactive molecules but also their potential side effects on cellular and organ toxicity. This is of high importance in the context of liver detoxification and, thus, a deliverable of the VLN of high relevance for the pharmaceutical industry.

The research project in brief:

The German Virtual Liver Network is a national initiative funded by the Federal Ministry of Education and Research (BMBF). The network is made up of seventy research groups distributed across Germany, and is establishing links with research groups and international initiatives to supplement its work.

The Virtual Liver will be a dynamic model that represents, rather than fully replicates, human liver physiology, morphology and function, integrating quantitive data from all levels of organization. Adriano Henney is the Program Director of the network.
www.virtual-liver.de

www.mpi-cbg.de/research/research-groups/marino-zerial.html

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drawing a map of life and death

Quantitative approaches to study the routes of cell death
The Research Group "Translational Systems Biology" in Heidelberg

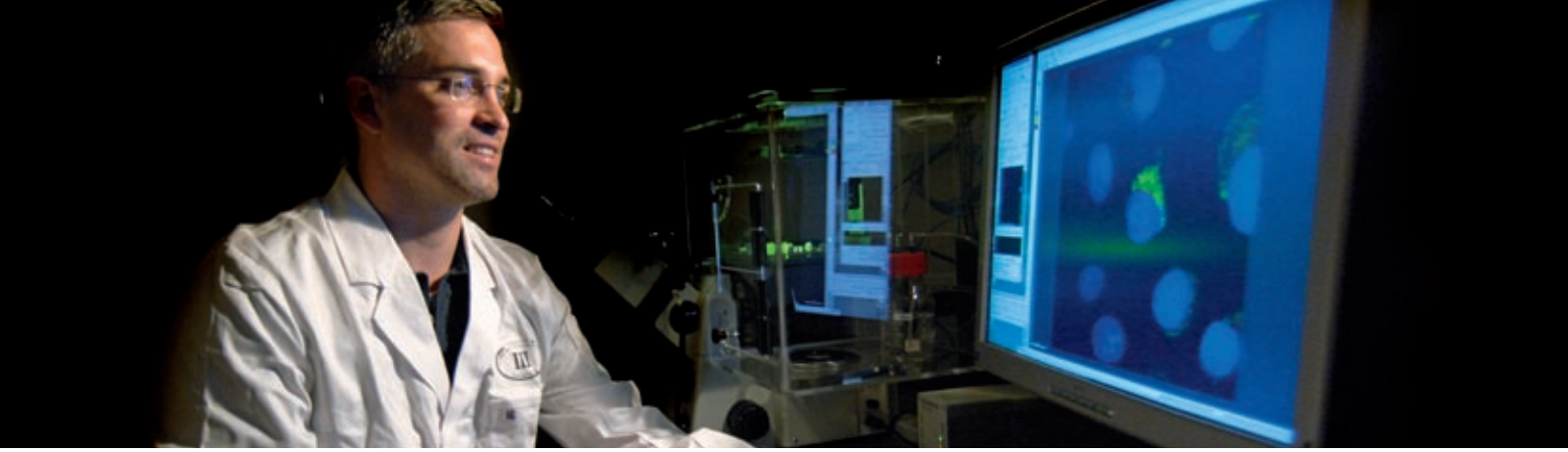
by Nathan Brady

Multicellular organisms, from simple slime mold to man, require that in order to live, certain cells in the body must die. This mechanism is known as programmed cell death (aka PCD) and is crucial for countless processes, such as development of organs and extremities. Faults within the decision processes leading to death or survival of cells are at the root of many diseases. In cancer cells, imbalances in death and survival signals are a major cause of tumor development and the lack of effective therapies. In order to improve therapies, we must better understand the nature of these faults, and how PCD pathways can be therapeutically targeted. A main challenge to understanding this most fundamental of biological processes is in our ability to observe and accurately describe highly complex events occurring not only within single cells, but also within cell populations and whole organs. High-resolution microscopy gives us the unique ability to monitor the machinery controlling the different ways a cell decides to die. Mathematical modeling helps to generate new insights into the processes. With these methods we aim at finding optimal treatment strategies to make cells sensitive towards death signals again, or to directly induce cell death in aberrant cells.

All cells within our bodies contain varied and potent mechanisms to enact different programmed forms of cell death. Loss of regulation of *programmed cell death* (PCD) underlies many diseases. In chronic and acute disease such as heart failure and neurodegeneration, undesirable PCD results in loss of irreplaceable cells. Alternatively in cancer, uncontrolled proliferation is in part due to failed engagement of cell death mechanisms. As such PCD regulation represents a fundamental focus in biology. Our new research group entitled "Translational Systems Biology" at the German Cancer Research Center (DKFZ) and the Heidelberg University Medical Faculty focuses on the systemic elucidation of cell death mechanisms in pancreatic, breast and neuronal cancer cells. Through a fusion of quantitative experimental and theoretical approaches, our lab aims to gain a more sophisticated understanding of fundamental regulatory mechanisms of PCD, and how dysfunction within survival and death pathways contributes to cancer. In doing so, we hope to elucidate new therapeutic strategies to optimize cellular PCD decision processes.

Mitochondria can be converted into inducers of cell death

Apoptosis, the most studied mode of PCD, is activated via two routes. The extrinsic pathway is triggered through extracellular signaling molecules, which activate so called death receptors on the cell membrane. The intrinsic (mitochondrial) pathway is an internal mechanism driving cells with severe aberrations into suicide, thereby ensuring that they cannot propagate (Fig. 1). Both routes are acting via caspases, a family of cysteine proteases, which disassemble and kill the cell. Mitochondria, which under normal conditions produce the majority of ATP, the cell's energy currency, play a pivotal role in this process. Their role in PCD is our main research focus. Under stress conditions they undergo *mitochondrial outer membrane permeabilization* (MOMP) leading to the leakage of proteins from the mitochondria into the rest of the cell. The MOMP converts mitochondria into executers of PCD, and is governed by the Bcl2-protein family which contains both, pro-apoptotic and pro-survival proteins.



Nathan Brady uses high-resolution microscopy to elucidate mechanisms of cell death (Image: DKFZ).

When the cell decides to eat itself

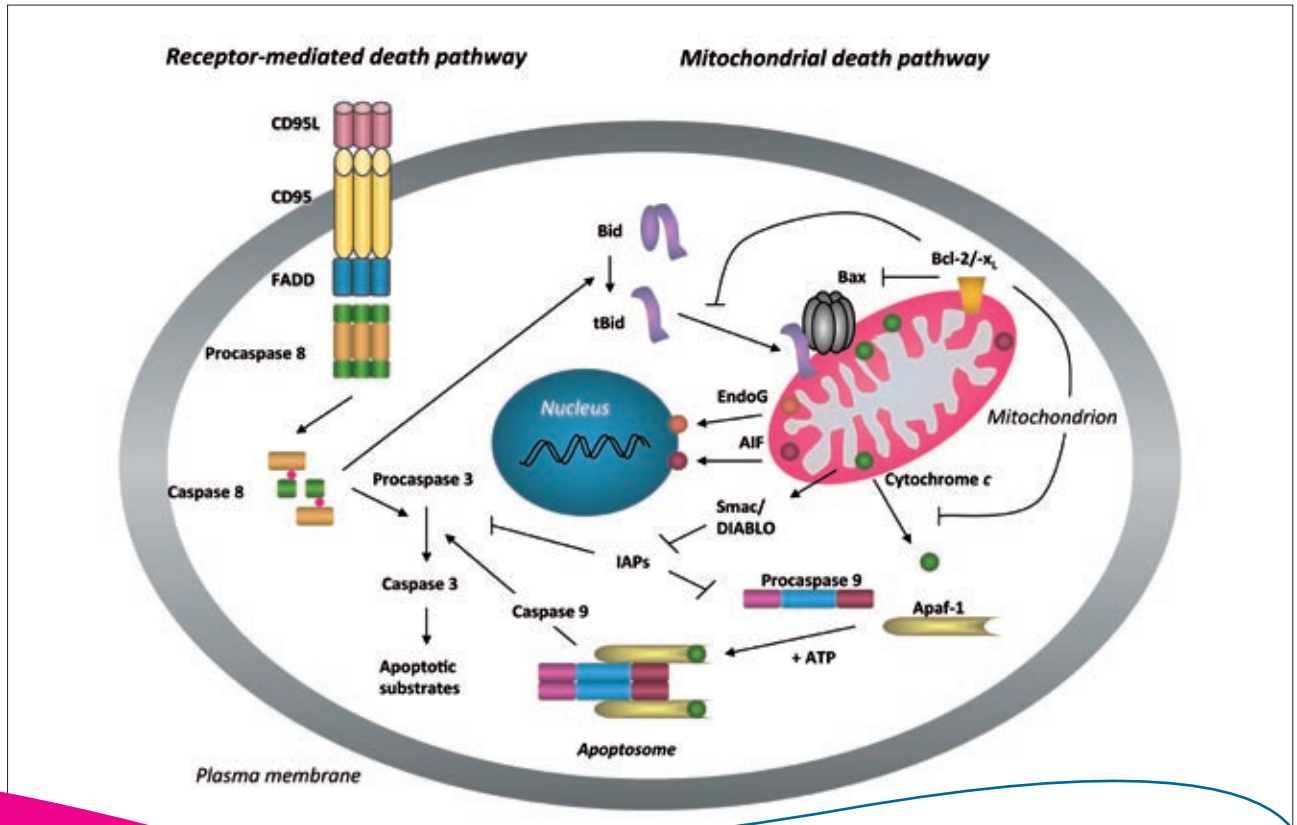
Importantly, apoptosis is only a part of a larger picture in PCD. Macroautophagy, aka autophagy, ("self-eating") has emerged as an important counterpart to apoptosis in last few years. Autophagy is a process by which intracellular components are sequestered by autophagosomes, which then fuse with and are degraded by lysosomes. The autophagy degradative pathway serves alongside of the proteasome as a critical modulator of protein homeostasis, but also of lipids and organelles. Autophagy was once referred to as a common and alternative form of cell death, but is now understood to be a precise and potent signaling survival mechanism (Fig. 3).

Cancer cells don't like their eating to be disrupted

PCD sub-networks do not function in isolation; they rather exist within highly interconnected networks (Fig. 1&3). It is our working hypothesis that crosstalk, at the level of lipids, ions, proteins and organelles, and between classically-defined pathways of survival and death, represents the most attractive target for inducing cell death. As autophagy is a potent survival mechanism for cells, we are actively screening compounds for their impact on different aspects of the autophagy pathway.

Fig. 1: Schematic of death receptor and mitochondrial apoptosis pathways

The Bcl-2 family members can be divided into three sub-families: the anti-apoptotic/pro-survival proteins (e.g. Bcl-2 and Bcl-xL), the multidomain proapoptotic proteins (e.g. Bax and Bak), and BH3-only proteins, which serve as upstream sensors of cellular stresses. BH3-only proteins inhibit the anti-apoptotic Bcl-2 family members, which normally function to heterodimerize with Bax/Bak to suppress their pro-apoptotic action. Upon activation, Bax/Bak initiate MOMP upon which pro-apoptotic cytochrome c is released to the cytosol to trigger caspase-mediated cell death. Taken from Cardiovasc Drugs Ther. Hamacher-Brady, 2006; 17149555.



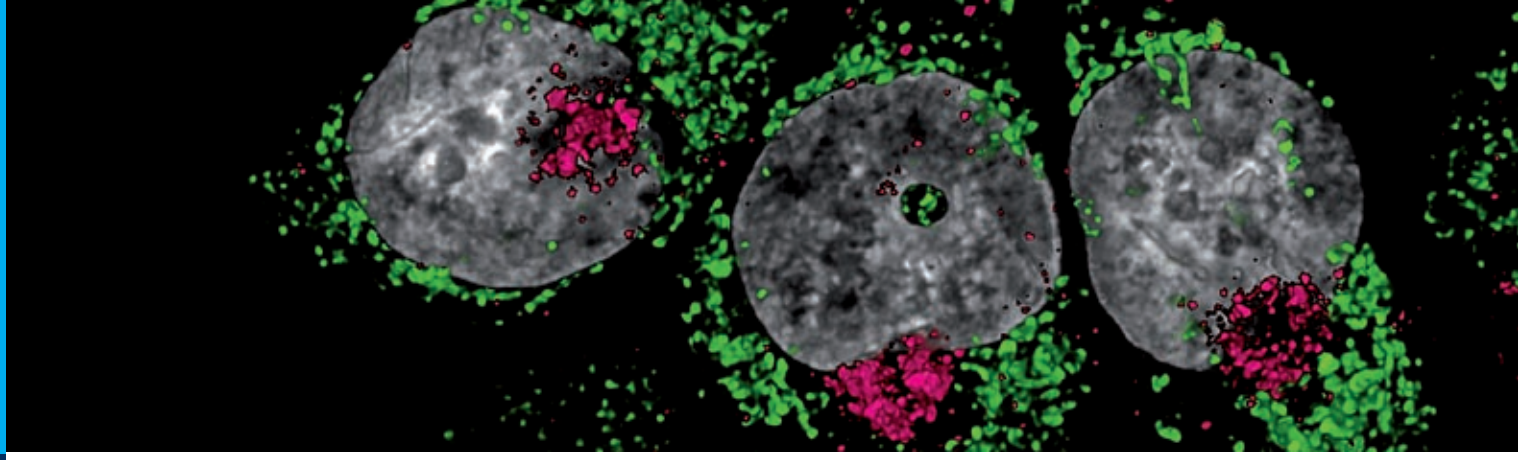


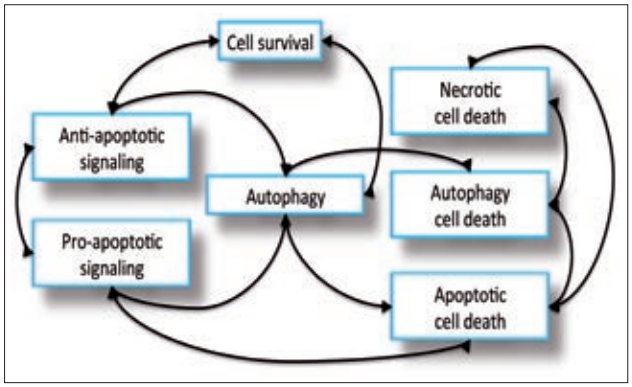
Fig. 2: 3D rendering of mitochondria (green), lysosomes (red) and nuclei (grey). In response to artesunate, lysosomes cluster asymmetrically at the nucleus. Pro-death signaling from lysosomes induces mitochondrial fragmentation and convert mitochondria into inducers of cell death via cytochrome c release (Image: Nathan Brady).

Attacking cancer cells with a drug against malaria shows a crucial role for iron

In a joint project with the group of Roland Eils, we focused on artesunate, a compound derived from artemisinin, which is extracted from the plant *Artemisia annua* and is commonly used to treat malaria. Recently it was also described to have potent cancer killing capabilities. Intriguingly, artesunate targets the food vacuole inside the unicellular malaria parasite, which is analogous to the lysosome in mammalian cells. Lysosomes are small vesicles, full of highly-active enzymes degrading whatever is inside them - be it proteins, lipids or other compounds.

This inspired us to determine whether the effect of artesunate on cancer cells involves lysosomes. To that end, we treated a panel of breast cancer and non-cancer cells with the drug and studied the effect on different organelles, including endosomes, lysosomes and mitochondria. Strikingly, we found that artesunate treatment resulted not only in profound changes to the morphologies and activities of lysosomes, but also on mitochondria and cell nuclei (Fig. 2). After treatment with artesunate, lysosomes became generators of reactive oxygen species, which are highly reactive and toxic molecules.

Fig. 3: Crosstalk during cell fate decisions

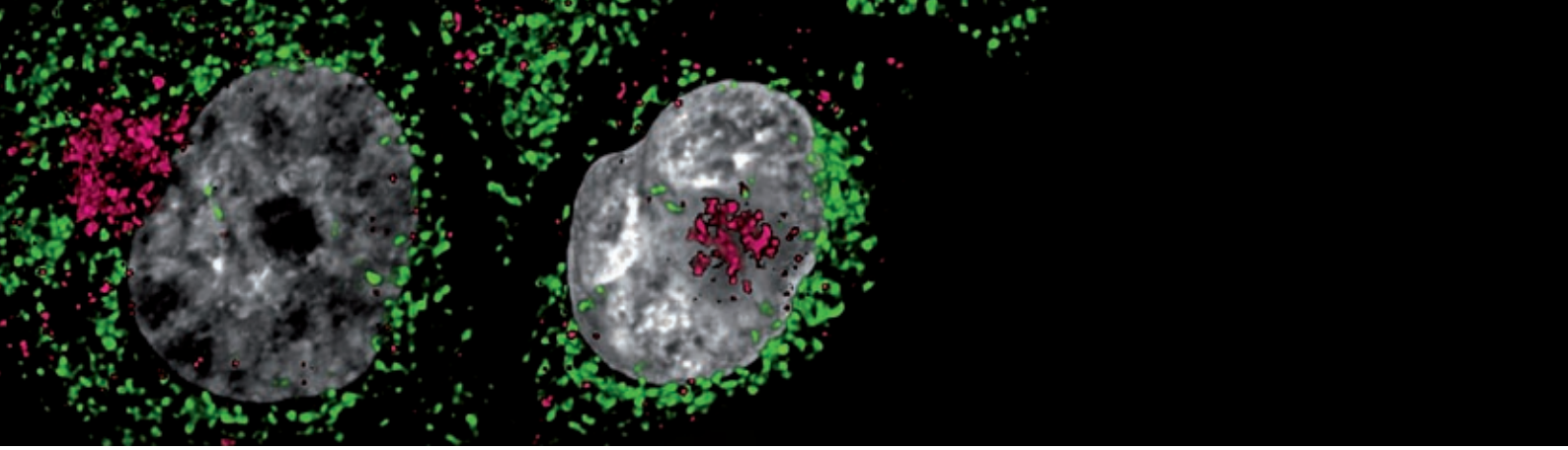


Cell fate decisions involve extensive and multi-directional crosstalk between pro-apoptosis, anti-apoptosis and autophagy pathways (Chart: Nathan Brady).

Through an as-yet-unidentified mechanism, these reactive molecules signaled mitochondria to release cytochrome *c*, i.e. activation of mitochondrial apoptosis. By systematically comparing different cell lines, we found that several breast cancer cell lines were more sensitive to artesunate treatment than non-cancerous breast epithelial cells. Intriguingly, we also found that the cancer cells could be rendered more sensitive by feeding them iron destined for their lysosomes. By reducing this iron content via so-called chelating agents, these cells became resistant to the treatment, showing that the lysosomal iron content renders the cells sensitive to artesunate. This finding has important implications for further clinical use: cancer cells are generally metabolically more active than normal body cells and therefore require more cellular iron, and apparently resulting in higher iron concentrations in lysosomes. Therefore, targeting lysosomes in an iron-dependent manner, may be a fantastic opportunity to battle cancers cells (Hamacher-Brady *et al.*, 2011). Current work in our lab has shown that this pathway is also present in pancreatic cancer cells and we are now focusing on the identity of the mechanism which transfers death signal from the lysosome to mitochondria.

Producing the image of a dying cell

Research approaches as the one outlined above produce insight that hopefully in the near future can be translated into clinical use. However, truly optimized killing of cancer cells will require integrating many types of quantitative and qualitative data. Here, mathematical modeling helps to get further understanding and to uncover previously concealed interrelations. Indeed, the past decades of PCD research have shown that the contributing processes are exceedingly complex and nearly impossible to summarize – over 200,000 reports have been published concerning apoptosis, yet much of the basic knowledge is still lacking! For example, it is assumed that autophagy is protective for cells. However, earlier we could demonstrate that Bcl-2, a protein promoting survival, suppresses autophagy (Brady *et al.*, 2007). To make matters even



more complex, the pro-apoptotic BH3-only proteins target mitochondria for degradation via autophagy, as a protective response to cell death signaling (Hamacher-Brady *et al.*, 2007).

The most important component of our approach is the use of high resolution fluorescence imaging. By attaching fluorescent proteins like the Green Fluorescent Protein (GFP) to key players, sub-cellular events, including protein-protein interactions, degradation, translocations, and organelle movements can not only be observed, but more importantly be quantified. This is an important consideration, as many imaging-based studies of molecules and pathways involved in PCD are based on a low number of snap-shot images. Often, these snap-shots do not give a true representation of such highly complex processes – a good analogy would be trying to predict the ending of the recent movie "Inception" from a few darkened pictures taken with a bad camera phone...

While we probably will never entirely overcome our lack of knowledge concerning basic mechanisms, we do seek to gain deeper insights into the inner workings of the cell by heavily relying on accurate, time-resolved imaging. By scrutinizing subcellular events in cells undergoing specific (and not so specific) perturbations we thereby illuminate the rules of PCD. Surprisingly, one constant result of our research is that the responses observed are never as simple, as in ON versus OFF. Very likely, if one looks close enough, binary responses do not exist in the mammalian cell. From a population of cells, to a population of organelles or proteins, a cellular response is always defined by heterogeneity. Another important revelation for us is that with quantitative imaging approaches, we rapidly become saturated with information, and require assistance in the form of mathematical modeling. Experimentally we are able to reveal individual rules, but mathematical modeling provides the means to write and understand the rulebook for PCD. It is this combination of experiment and theory that we hope will allow us to push towards a more rational design of therapeutic strategies to optimize cellular PCD decision processes.

The research project in brief:

The Junior Research Group "Translational Systems Biology" led by Dr. Nathan R. Brady is part of the Helmholtz Alliance on Systems Biology. The group is part of the German Cancer Research Center (DKFZ) and the Heidelberg University Medical Faculty (Department of Prof. Büchler) and is located in BioQuant, Heidelberg University's center for systems biology.

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systems biology goes multicellular

Caenorhabditis elegans is one of the most elegant model organisms

by Marlon Stoeckius

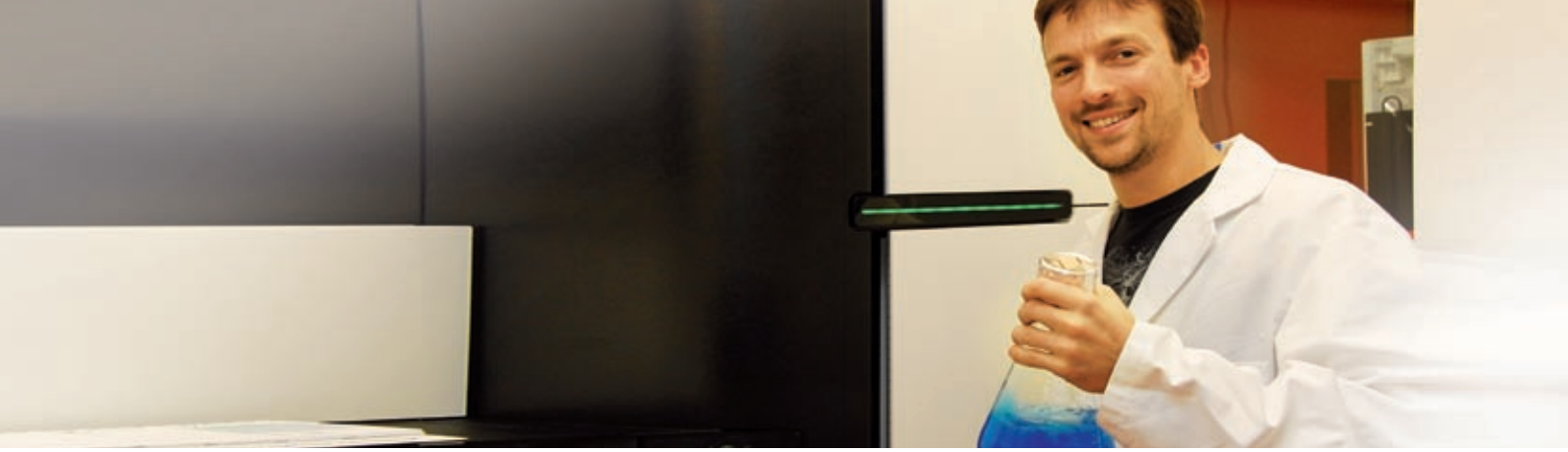
Caenorhabditis elegans is a transparent nematode about 1 millimetre long that lives in the soil (Fig. 1). In the 1960s, biologist Sydney Brenner first used it as a model for researching nerve development. In the years that followed, *C. elegans* became established as a model system for cell division and embryonic development. Many fundamental mechanisms in this tiny worm function in a way that is similar to the way they function in humans. As a result, it is now one of the most popular model organisms for developmental biologists and is an equally widely used model system for researching the molecular bases of many diseases, including cancer, diabetes, Parkinson's and Alzheimer's. Another, fairly recent field of research is research into the function of small RNAs, such as microRNAs, which were first discovered in *C. elegans*. Molecular biological methods of examining the nematode are very well established and offer many approaches for analysing fundamental phenomena and mechanisms. This has already resulted in the awarding of three Nobel prizes for discoveries in *C. elegans.*

A toolbox filled to the brim for systems biologists

In recent times, *C. elegans* has also become a modelorganism of systems biology – mainly because its molecular biological and biochemical toolbox is so lavishly equipped. A consortium of scientists laid the foundations in 1998 when they fully decoded the worm's genome. *C. elegans* was the first multicellular organism to have its genome sequenced (Sequencing Consortium, 1998). Even now, ten years after the human genome was sequenced, the nematode's comparatively small genome (roughly 100 mega base pairs) is still the best-mapped genome of a multicellular organism. Using modern methods, the worm's genome can be manipulated relatively easily. This means that foreign or modified genes can be inserted accurately into the genome (MosSCI; Frøkjær-Jensen *et al.*, 2008). To modify genes, researchers using the worm can make use of "gene banks" where almost all genes (ORFeome; Lamesch *et al.*, 2004) and regulators (Promoterome; Dupuy *et al.*, 2004. 3'UTRome; F. Piano laboratory) from the nematode's genome have been isolated. They can then be freely combined in the manner of a construction kit. Genes in *C. elegans* can thus be comparatively easily disabled by feeding the worm special bacteria (RNAi feeding; Boutros and Ahringer, 2008). Likewise, specific sections can be deleted from the genome (MosDEL;

Fig. 1: *Caenorhabditis elegans*

The one millimetre long nematode *Caenorhabditis elegans* is a popular model organism (Image: Marlon Stoeckius).



Marlon Stoeckius is using the nematode for research into the molecular bases of early embryonic development (Photo: Alexander Baltz @ BIMSB).

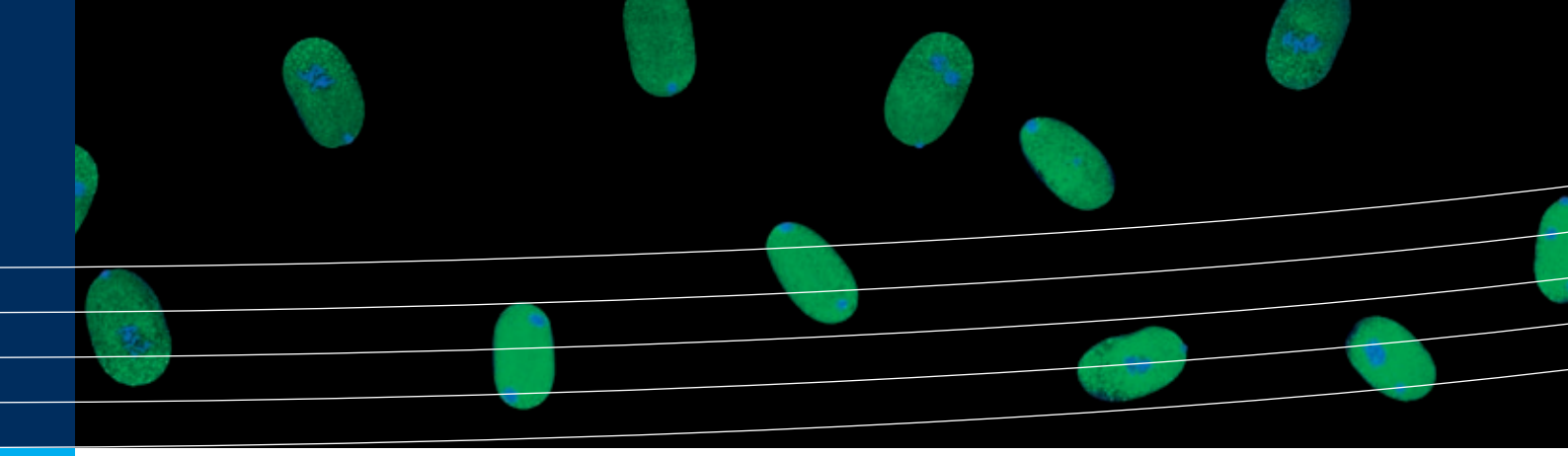
Frøkjær-Jensen *et al.*, 2010). The worm can be cultivated on agar plates with up to 384 chambers, as if in separate habitats. This makes it possible to carry out parallel, automated, high-throughput screening whereby approximately every gene of the nematode is disabled individually in just one week. The *C. elegans* embryo and its fully-grown worm are both transparent, and it always develops stereotypically. That makes it possible to observe individual cells in the living embryo and nematode under a microscope. In this way, the origin and the fate of every cell has been described, from the single-cell stage via the hatching larva with 558 cells to the adult worm with 959 somatic cells (Sulston *et al.*, 1983). Nowadays, embryonic development can be monitored automatically with the help of imaging methods. When specific genes in the embryo are deactivated, changes in the characteristic pattern of cell division can thus be recognised automatically. This makes it possible to construct hypotheses about the gene's function. Scientists have generated more than 13,000 genetically manipulated nematodes (CGC, March 2011) in recent decades, with individual genes deleted, mutated or marked with a fluorescent marker. This has produced a huge resource that is shared among scientists in line with the principle of open source access. Unlike many other model organisms, these worms can simply be stored in a freezer until further use. In addition to laboratory method resources, hundreds of datasets from high-throughput experiments are available in databases. A recently completed project for the systematic mapping of regulators in the genome, in addition to establishing new methods, produced several terabytes of data that is now freely available (ModENCODE; Gerstein *et al.*, 2010). Altogether, these resources make *C. elegans* an ideal multicellular model for systems biology.

What is (was) lacking

Modern DNA sequencing technologies permit quantitative analysis of all activated genes (RNA expression) in all organisms. So far, in most model organisms it has not been possible to quantitatively measure proteins as has been done for several years in cell culture (SILAC; Ong *et al.*, 2002). Until now, therefore, hypotheses about the function of certain genes could only be constructed on the basis of microscopic changes in the worm, of changes in RNA expression, and by measuring a small number of selected proteins.

Ultimately, in order to understand biological processes, the quantity, identity and dynamics of the metabolic products in an organism must be determined quantitatively. Metabolism both in single-celled and in multicellular model systems is an insufficiently developed area of research (Metabolomics; Oliver, 2006). High-throughput research projects involving *C. elegans* require both the optimisation of methods and an innovative approach to data analysis. After the worm has hatched, it can be cultivated in great numbers synchronously throughout its life cycle. As a result, sufficient experimental data (e.g. RNA and proteins) is available for analyses of worms of the same age. Nonetheless, previous quantitative analyses have been based mainly on measurements of the whole organism, which has led to mixing of the different tissues. This makes it difficult, for example, to distinguish which genes are specifically activated in muscles or in nerves. In future, methods must be established that permit selective high-throughput measurements in individual tissues and cells.

Similar hurdles must be overcome for research into embryonic development. The adult nematode carries embryos of different ages inside its body, from the single-celled zygote to the hatching larva with 558 cells. More material-intensive measurements have always been based on heterogeneous populations of embryos at different cell-development stages. Until now, in order to examine the sequence of chronological events during embryonic development, embryos had to be sorted by age under a microscope. When sorting manually in this time-consuming manner it is impossible to isolate the quantities of embryos required for analysing RNA expression or protein expression. With a steady hand, it is possible to collect around 500 embryos a day. However, modern high-throughput analyses require more than 100,000 embryos.



Single-celled *C. elegans* embryos (Image: M. Stoeckius).

Automatic sorting of embryos of the same age

We have developed a method of isolating tens of thousands of embryos at the same stage of development within a short time with the help of a conventional cell sorter (FACS) (Fig. 2). The fundamental prerequisite for this method, which we christened eFACS, is a strain of worm that specifically produces a fluorescent protein only at one unique cell stage. The fluorescence serves as a marker for the desired stage and is specifically recognised by the cell sorter. We use this method to enable us to sort large quantities of single-celled and two-celled embryos. In principle, however, it can be used to isolate embryos at any stage of development. eFACS thus opens the door to systematic research on embryonic development in *C. elegans* using modern high-throughput methods (Stoeckius *et al.*, 2009).

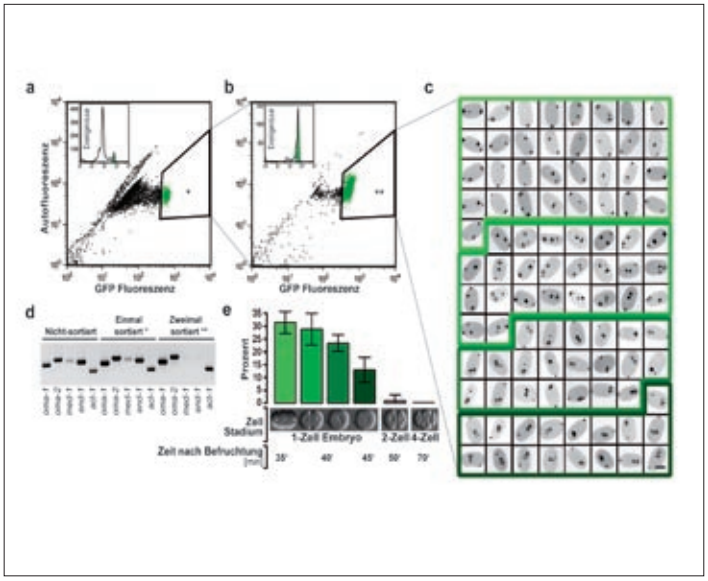
Quantifying thousands of proteins

In collaboration with the laboratory of Matthias Selbach (MDC Berlin) we have developed in the laboratory of Nikolaus Rajewsky (BIMSB@MDC) a new method that enables us to quantify the activity and quantity of thousands of proteins by comparing two samples of *C. elegans* (Thierfelder *et al.*, in preparation). For the first time, this provides insights into changes in the quantities of thousands of proteins, thereby permitting one to draw conclusions about their function in the living organism. It is now possible to examine how protein quantities change throughout the worm's development and how certain proteins behave when a specific gene is deactivated. This method also enables the quantitative measurement of proteins activated by chains of signals (phosphoproteome) and makes it possible to improve the sensitivity of other methods used with the nematode (e.g. Co-IP).

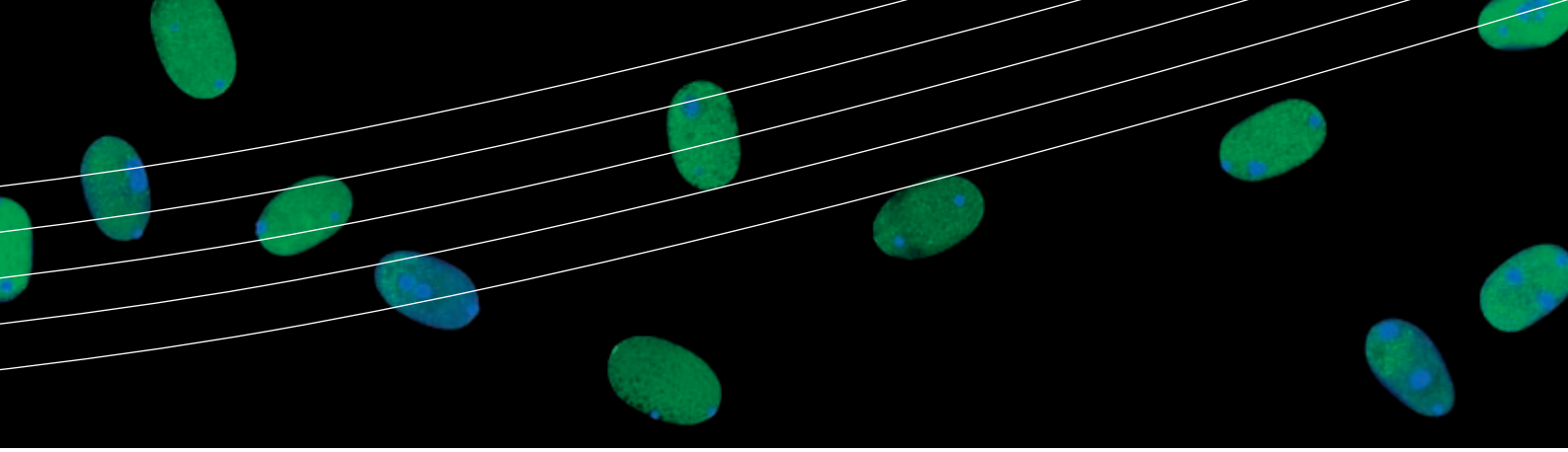
How do a sperm and an egg become an embryo?

The fusion of two highly specialised cells, a spermatozoon and an oocyte, leads within a short time to the emergence of a self-dividing cell, the zygote, which then develops into a complete organism. How this fundamental process in the development of every living creature functions is to a large extent unknown. True, many fundamental factors are known. For example, around 600 genes are essential for the embryo of *C. elegans* to develop (Sönnichsen *et al.*, 2005). However, the global processes are yet to be illuminated. Interestingly, in all creatures stud-

Fig. 2: eFACS



We use eFACS to sort large quantities of single-celled and two-celled embryos. For this we use a strain of nematode that expresses a gene that is specific to the single-celled embryo and has been linked to a fluorescent marker (OMA-1-GFP). This method routinely results in a recovery rate of several tens of thousands of embryos with purity in excess of 98% (a). The scatter plot shows the first stage of sorting of single-celled OMA-1-GFP embryos. The GFP-positive population (~3–5% single-celled embryos) is selected and sorted (b). After the first sorting, the GFP-positive population has a purity of roughly 70% and must be sorted again. The purity of the GFP-positive population that has been sorted twice is then checked by examination under a microscope (c and e) and by RT-PCRs (d). These confirm a purity of more than 98% of single-celled embryos. (Chart from Stoeckius *et al.*, 2009. Copyright Nature Methods).



ied, these "early" processes are controlled by factors that are present in oocyte and spermatzoon prior to fertilisation. That leads one to ask how big and how essential is the part played by gene products of oocyte and sperm.

In the laboratory of Nikolaus Rajewsky (BIMSB@MDC) we use *C. elegans* and the methods established here to learn about these fundamental reorganisation and reprogramming processes, taking a genome-wide approach. For this project, we measure both coding and non-coding RNAs and proteins in oocytes, spermatozoa, single-celled and two-celled embryos. This data provides us with the first-ever glimpse into a time frame covering the first minutes in early embryonic development. We are observing dynamic changes in the quantities of RNAs and nearly 5,000 proteins. RNAs and proteins behave very differently, which leads one to conclude that there is a high degree of post-transcriptional gene regulation. We are also observing dynamic changes within and between all known short, non-coding classes of RNA (miRNAs, 21U-RNAs, 22G-RNAs, 26G-RNAs, and other endo-siRNAs). The data permits the hypothesis that a large number of RNAs, mRNAs and proteins in the embryo likely originate in the sperm. This points to an unexplored paternal contribution to early embryonic development. The data also suggests that the single-celled *C. elegans* embryo may already be transcriptionally active. These enormous datasets are currently being analysed and validated in independent tests.

The research project in brief:

"High-throughput research into the proteome and transcriptome during the reprogramming of an oocyte into a zygote and the early embryonic development of *C. elegans*" in the group of Nikolaus Rajewsky, BIMSB Berlin. Funded since 2009 by the BIMSB/NYU PhD exchange programme (www.mdc-berlin.de/en/bimsb/index.html). This work is part of a longstanding collaboration with Fabio Piano (NYU, USA).

Thanks:
This project is part of my doctoral research in the laboratory of Nikolaus Rajewsky. I would like to thank my colleagues Jonas Maaskola (co-lead author of the Nature Methods publication) and Dominic Grün (co-lead author of the publication in preparation) for all their bioinformatics and statistical analyses.

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fluorescence nanoscopy with photoswitchable fluorophores

Super-resolution fluorescence microscopy and single-molecule detection offer new insights into cellular processes

by Sebastian Malkusch, Ulrike Endesfelder, Meike Heidbreder and Mike Heilemann

Imaging biological samples is crucial for the understanding of biological function. Within the toolbox of techniques available, fluorescence microscopy is outstanding because of a number of reasons: availability of strategies for specific labeling of target structures and a large selection of fluorophores, compatibility with experiments in live cells, as well as high sensitivity which makes even the observation of single molecules possible. The newest developments in single-molecule fluorescence microscopy apply photoswitchable fluorescent probes and single-molecule localization with high precision to generate images of cellular structures with subdiffraction and near-molecular resolution and to derive quantitative data. In addition, this approach allows following dynamics of biomolecules in live cells over a long observation time, which for example allows studying the influence of an external stimulus.

Fluorescence microscopy is realized by selectively labeling a target structure with fluorescent probes and allows for the noninvasive observation of biological samples. As any light microscopy technique, the spatial resolution of fluorescence microscopy is limited to about 200 nm in the imaging plane, and to >500 nm along the optical axis. A comparison of these numbers with the biomolecular length scale illustrates that this limit in resolution hampers to resolve small (sub)cellular structures, the composition of molecular machines or viruses, or to map the precise spatial arrangement of biomolecules. This fact has been a motivation for many research groups which focus on microscopic techniques to develop new concepts that can bypass the resolution limit in light microscopy.

Among the different concepts for super-resolution fluorescence microscopy, single-molecule localization-based techniques which employ stochastically switchable fluorescent probes are of particular interest: the experimental realization is rather simple, a spatial resolution in the range of 20 nm

Fig. 1: Single-molecule localization microscopy

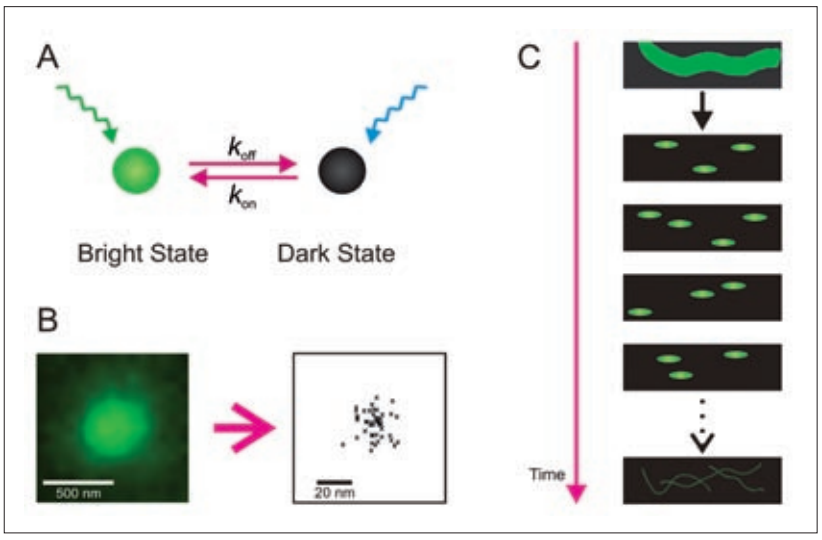

Chart: Prof. Mike Heilemann, Julius-Maximilians-University Würzburg

Photoswitchable fluorescent probes (A) can be switched between a fluorescent and a non-fluorescent state. Combining nanometer-precise localization of single molecules (B) and a temporal separation of the overall fluorescence signal (C) provides a large number of single-molecule coordinates which can be used to reconstruct a super-resolution image.

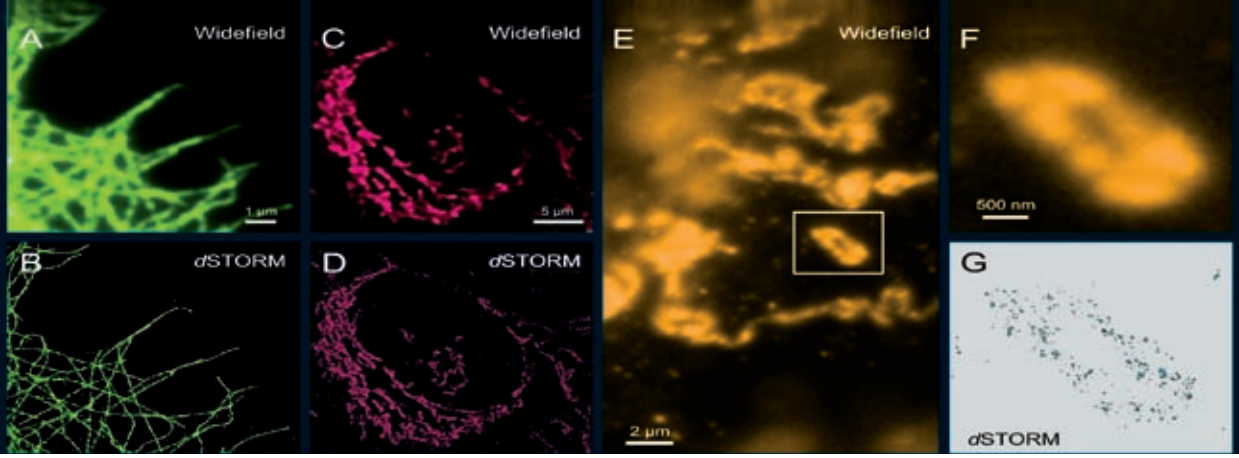

Fig. 2: ***direct*** **Stochastic Optical Reconstruction Microscopy (dSTORM) of cellular structures.**
Cellular structures (microtubulin (A, B), endoplasmatic reticulum (C, D)) as well as protein distributions (in mitochondria (E-G)) can be imaged with near-molecular resolution using the *d*STORM approach. Labeling of biological samples is performed via immunofluorescence using conventional fluorophore-labeled antibodies (Chart: Ulrike Endesfelder, Julius-Maximilians-University Würzburg).

can be achieved, and a large selection of suitable fluorescent probes and labeling techniques are readily available. As single-molecule techniques, static and dynamic heterogeneities can be resolved, and a high quality of quantitative information is provided. In analogy to classic microscopy techniques, single-molecule localization microscopy can be performed in multicolor mode and in living cells. In addition, single-molecule localization microscopy provides accurate information on both the spatial localization and time for each single molecule, which is useful beyond the generation of high-resolution images, for example to map spatial distributions of biomolecules ("biomolecular mapping", topology), in cluster algorithms or to generate of topological networks.

Super-resolution fluorescence microscopy with photoswitchable fluorescent probes

Single-molecule localization microscopy combines three main tasks:

- **1.** the application of fluorescent probes that can be photoactivated or photoswitched,
- **2.** the localization of single fluorophores with a precision of a few nanometers, and
- **3.** the temporal separation of the overall fluorescence signal of a sample (Figure 1).

The actual experiment starts with a "dark" sample (i.e. with all probes in their non-fluorescent state or "switched off"). In a first step, only a few fluorescent probes are activated ("switched on") and their fluorescence signal read out, hereby making sure that the density of fluorophores per image is low enough that they are detected as single emitters. In a second step, the position of each single fluorophore is determined by approximation with a Gaussian function. The precision of localization increases with the number of photons detected, reaching <10 nm for 1000 photons. These two steps are now repeated until a sufficient number of single-molecule coordinates have been collected from stochastically activated fluorophores, and can now be used to generate a reconstructed image (a "pointillistic" image) which exhibits subdiffraction spatial resolution (e.g. about 4,000 images à 20 ms for a densely labeled two-dimensional structure, or 100 images for one-dimensional filaments).

Single-molecule localization microscopy requires suitable fluorescent probes that can be switched between a fluorescent ("bright") and a non-fluorescent ("dark") state, e.g. by irradiation with light, through intrinsic photophysical processes or photochemical reactions. Examples for such probes are photoactivatable or photoconvertible fluorescent proteins that become fluorescent after irradiation with UV light (e.g. photoactivatable GFP, paGFP). Organic fluorophores are a second group of well-suited fluorescent probes, as they can be cycled between a "bright" and a "dark" state through photophysical or photochemical processes. One approach to photoswitch a large spectrum of organic fluorophores has been introduced with "*direct* Stochastic Optical Reconstruction Microscopy" (*d*STORM), which paved the way to use conventional fluorophores that are used for a long time in cell biology e.g. in combination with immunofluorescence. *d*STORM allows imaging of cellular structures in fixed cells with subdiffraction spatial resolution (Figure 2A-D), mapping of the spatial distribution of proteins (Figure 2E-G), following dynamics of single filamentous structures with a temporal resolution of 1 Hz and even super-resolution imaging inside a living cell.

Next to suitable fluorescent probes, single-molecule localization microscopy requires specific labeling strategies. While fluorescent proteins can be genetically co-expressed and integrated, labeling a biological sample with organic fluorophores can be realized with immunofluorescence or with small molecules that specifically bind to a target (e.g. phalloidin to label actin filaments). In addition, an increasing number of specific

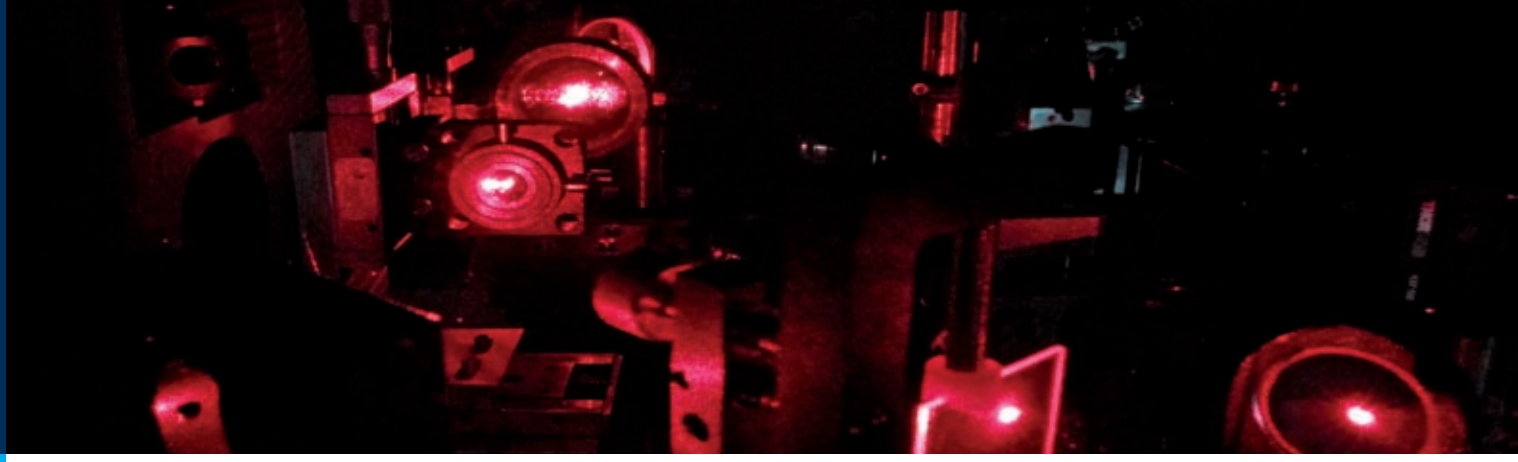

Image: Heilemann group, Würzburg

tag proteins is becoming available, which allow specific and stoichiometric labeling of biomolecules with organic fluorophores.

Localization of single molecules: more than images

Among the different super-resolution fluorescence microscopy methods available today, single-molecule localization is of additional interest because of the nature of the data: for each single molecule detected, nanometer-precise spatial coordinates as well as a temporal coordinate are determined. This primary data can be used straight-forward to reconstruct super-resolution images (Figure 2). Beyond that, single-molecule coordinates can further be processed by various algorithms, and information on clustering of biomolecules or trajectories in living cells can be obtained (Figure 3).

Photoswitchable fluorescent probes and single-particle tracking

Studies of the dynamics and the mobility of biomolecules as well as of intermolecular interactions can provide useful experimental data to establish new or to refine existing models in systems biology. An interesting and novel approach is the combination of single-particle tracking and photoswitchable fluorescent probes: different to "classical" single-particle tracking, a large pool of non-activated ("dark") probes is available from which a small subset is activated at a given time and followed. This approach allows tracking the mobility of a fluorophore-labeled biomolecule over a long time (many minutes up to one hour), with a temporal resolution of tens of milliseconds and a spatial resolution of a few tens of nanometers. For example, the mobility of a membrane receptor before and after activation by a signal initiating molecule can be de-

Fig. 3: Single-molecule localization microscopy primary data

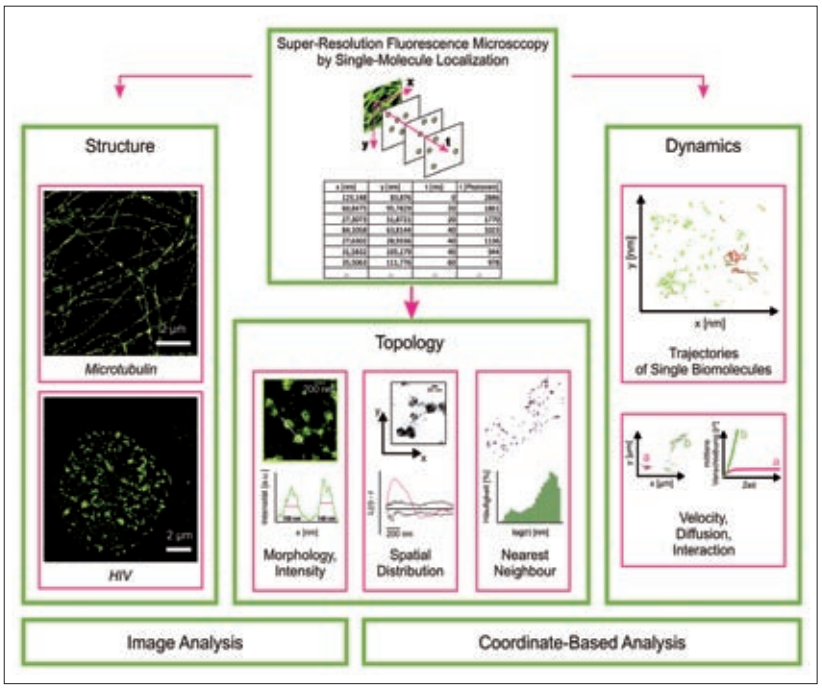

Chart: Sebastian Malkusch, Julius-Maximilians-University Würzburg

The primary data that is produced in single-molecule localization microscopy can be used to generate reconstructed images with subdiffraction resolution (left). In addition, localization data can further be processed with topological or morphological algorithms (middle) and provide quantitative information on biomolecular assemblies and clustering. On top, dynamic processes in live cells can be observed and single-molecule trajectories can be generated (right).

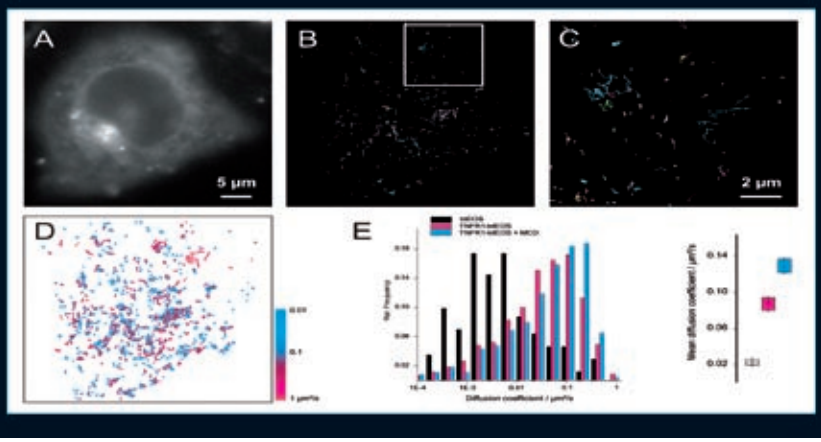

Fig. 4: Photoswitchable fluorescent probes in combination with single-particle tracking. Biomolecules that are labeled with a photoactivatable fluorescent probe ((A), TNF receptor 1 labeled with the fluorescent protein tdEOS) can selectively be activated, their movement be followed in a live cell and single-molecule trajectories calculated (B, C). Further dynamic parameters such as diffusion coefficients (D, E) can be calculated from single-molecule trajectories (MCD = methylcyclodextrin). (Chart: Meike Heidbreder, Julius-Maximilians-University Würzburg).

termined and information on aggregation, internalization and protein-protein interaction can be extracted. In addition, the mobility of membrane molecules with respect to membrane heterogeneity can be studied, and a map of diffusion coefficients can be generated (Figure 4).

Outlook: towards molecular resolution

The development of novel microscopic techniques has paved the way for imaging cellular structures with near-molecular resolution. The combination of super-resolution localization microscopy with single-molecule detection is a particular useful one, as it can provide a new quality of quantitative data and provide new insights into cellular structures and dynamics, biomolecular interactions, heterogeneities and subpopulations.

The research project in brief:

Project name: FORSYS-PARTNER Young Investigator „Fluorescence Techniques for Quantitative Studies of Virus-Cell Interactions“

Partner:

ViroQuant, Heidelberg University

Cooperations:

Prof. Christian Kaltschmidt, Prof. Barbara Kaltschmidt,
Dr. Darius Widera, Bielefeld University;
Dr. Jean-Baptiste Sibarita, Dr. Deepak Nair, Prof. Daniel Choquet,
University of Bordeaux;
Prof. Hans-Georg Kräusslich, PD Dr. Walter Muranyi,
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cells are a classic example for soft matter

Portrait Josef Alfons Käs

Josef Alfons Käs first and foremost sees one thing in biological cells: soft matter. On the other hand, they also are fascinating systems and a genuine challenge for the physicist who does research at the University of Leipzig (Germany). He traces the cell's biomechanical properties with experimental techniques derived from physics to learn what distinguishes the various cell types. Why this is useful, and what cell biomechanics can teach us about cancer, he explains in this interview with systembiologie.de. And he tells us why for him research is a fantastic playground for letting off steam.

Mr. Käs, what made you as physicist examine cells, i.e. biological systems?

I traditionally come from a field called soft matter physics. This has to do a lot with polymer and liquid crystal physics. And biological cells are a classic example for soft matter. Initially, I had begun dealing with lipid membranes and vesicles but I realized very quickly that a cell is clearly more than its membrane. Below it you find the cytoskeleton. This was very interesting to me and, to begin with, I examined the polymeric properties of individual actin filaments. My mentor, Nobel Prize winner for physics Pierre-Gilles de Gennes, said if I really wanted to stay with biophysics I should take up a medical aspect. I took this very seriously and turned to tumour biology.

How did you approach molecular biology?

I studied physics in quite a traditional way. Later I worked at Harvard Medical School in a mostly medical environment. In addition I studied molecular biology there. Then I went to Austin, University of Texas, where I held two chairs, one in physics and one in molecular biology. And yet I do not see myself as molecular biologist. There are excellent scientists in this field. I want to understand biology but I also want to make a contribution from a physical perspective.

What is your contribution as a physicist?

The molecular biological knowledge of the processes occurring within the cell is getting better and better. But I feel such a complex biological network, interconnecting more than 140 molecules, is extremely confusing. Taken to its extreme: If I only have to associate two individual components, with regard to a car I would have to conclude that the door handle is the most essential part of the motor, since when I cannot open the car I normally cannot start the engine. Regarding their functions, however, they have nothing to do with each other. So, in systems biology we define functional units and analyse these modules. This adds structure to the object. The module of our interest is the biomechanics of a cell and its material properties – How rigid the cell is and how such property influences division and motion.

Why do you need a biomechanical approach in the first place?

Biomechanics sounds sexy such as "nano" and "laser manipulation" but that's not the point. The crucial point is: Minor changes in the cytoskeleton have major impact on the cell because they potentiate non-linearly. So, on the protein level, we can monitor effects that otherwise can be difficult to detect or cannot be investigated at all.

How do you measure this effect?

We have developed an optical cell stretcher, a really great toy for physicists. We point two laser beams on a cell which causes a stretching. This way we determine its flexibility. And on top, the whole process is self-aligned because cells are dielectric. They like electrical fields and they very quickly align in the electrical field of laser light. So we have a relatively high flow-rate of at least 30 cells per minute and – with a little patience – we can monitor enough cells to obtain significant data.

What is revealed by cell elasticity?

Different cells feature different elasticities. Stem cells, for example, are undifferentiated and have almost no cytoskeleton, meaning they are particularly soft. One of the projects here at

Josef Alfons Käs (Photo: Stefanie Reinberger).

the University of Leipzig is to grow scaffold material in a bioreactor. So cell elasticity helps to determine the level of cell differentiation and to choose adequate cells for further steps.

However, we mainly focus on cancer cells. We want to know how their biomechanics change when a tumour is developing and progressing. Though many different types of tumours exist, the principle of disease is always similar: It begins with uncontrolled proliferation. Then the tumour cells have to become invasive against the surrounding tissue. And finally the cells have to be capable of leaving the tumour to form metastases. And here the softness or rigidity of a cell is essential. We have found typical biomechanical features needed by every solid tumour to meet these three requirements.

And these are?
Vigorously proliferating cell populations are soft and can be deformed easily by light compression because their actin cortex dissolves when dividing. But if you apply greater counter pressure the tumour cells stiffen. They stiffen particularly well and displace the softer normal tissue. And finally, we find cells in far progressed tumours that cannot be stretched with the optical cell stretcher. Instead, they contract and are very mechanosensitive. And so they downsize their surface and become less adhesive. We think these are the cells, which leave the tumour and form metastases.

How will cancer research profit from these findings?
We do have good diagnosing methods and it would be ridiculous if I as a physicist came along and said we now needed a laser for this purpose. But we can make a contribution to a more differentiated diagnosis. At present two clinical studies are under way for breast cancer and cervical carcinoma. Prospects for being able to identify metastasising cells are looking good – without having to resect lymph nodes. And then, biomechanics can be used for early detection of tumours in the mouth and pharyngeal zone. This is of great interest for China and India where these cancers are increasing considerably with growing prosperity.

How do biomechanics and molecular biology team up?
Together with Roland Eils from the German Cancer Research Center in Heidelberg we have started a wide screening approach searching for genes and key positions, which are crucial for mutations of biomechanical properties. I will be able to say more in a couple of years. At the moment it simply means a lot of work.

What does interdisciplinary work mean for you?
It means that in our department physicists, physicians, veterinarians and, of course, engineers all work together, but, to my regret, no biologists at the moment. We have molecular biology labs and can do our own basics. But if we really have to go to the core, like in our big screening, we need specialists who also have the appropriate equipment for something like this, i. e. robotic lines etc. The great thing about such collaborations is the fact that you don't need to be a specialist in every field. It is alright to ask stupid questions and perhaps point out new aspects this way. Biology is very open for impulses from other disciplines.

What else do you need to be creative in your work?
Freedom to live out my drive to play. On principle I'm still a little boy who just loves to puzzle. For instance, I never read instructions for use of a new technical device; I always try to find out how it works by myself. And this is how I see my work, too. I want to know how things function and experiment until I find out. And then I need people who contradict, who have other conceptions and ideas. This is the only way to a fruitful interchange.

Interviewed by Stefanie Reinberger.

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interactions between areas of the brain determine what we do

Modelling contributes toward understanding of brain function

by Simon B. Eickhoff and Karl Zilles

How can we understand the organisation and networked systems of such a complex organ as the brain? Finding an answer to this question is essential both for basic research and for diagnosing and treating neurological and psychiatric illnesses. One prerequisite for gaining a better understanding of the causes and peculiarities of disease-related changes is to see the organisation of the healthy brain as a system, not just as an assembly of isolated structures and mechanisms. To find out more about how the brain functions, the Institute of Neuroscience and Medicine (INM) at the Forschungszentrum Jülich (Jülich Research Centre), in collaboration with partners in the Human Brain Model network of the Helmholtz Alliance on Systems Biology and in the Jülich-Aachen Research Alliance JARA Brain, combines non-invasive methods of measuring human brain activity with systems biology methods of modelling brain function.

Concepts of the organisation of the human brain – How do areas of the brain interact?

There have long been two fundamental concepts of how the brain is organised: *segregation* and *integration*. *Segregation* means specialisation of the brain, particularly the cerebral cortex, into individual modules or areas that differ in structure and function, while *integration* denotes interaction between different areas, reciprocal influence and interplay between components. Research conducted in recent decades has shown that motor and cognitive functions are not localised in a single region, cell or molecule. Rather, they originate in a com-

Fig. 1: Overview of dynamic causal modelling (DCM)

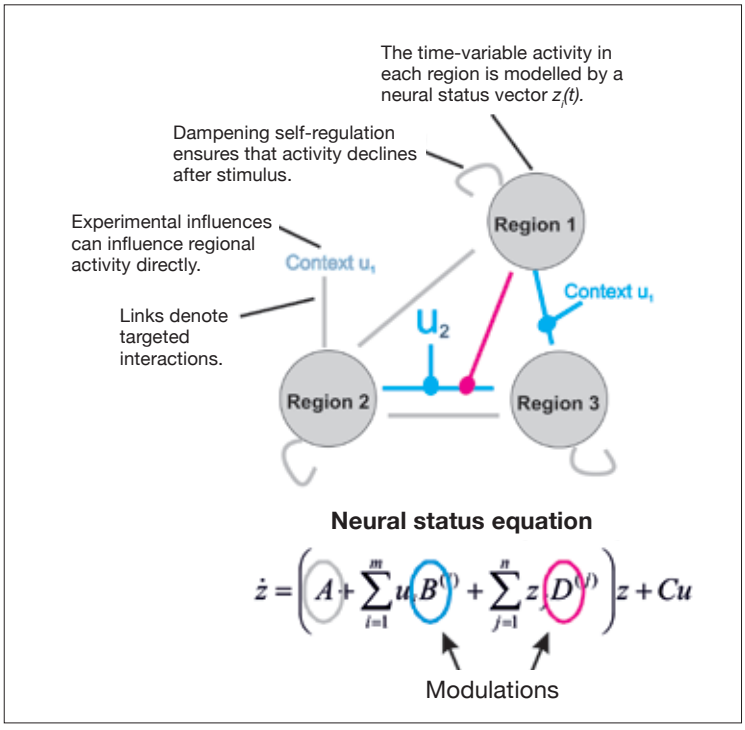


Chart: Simon B. Eickhoff

Dynamic causal modelling is based on a non-linear, deterministic system model of interaction between neural areas, whereby the dynamics of the system are a function of the current state (represented by the neural status vector *z*), external influences *u* and the (neural) system parameter (intrinsic or endogenous connectivity: *A*; context-dependent modulation: *B*; direct effects of experimental influences: *C*; non-linear interaction effects: *D*).

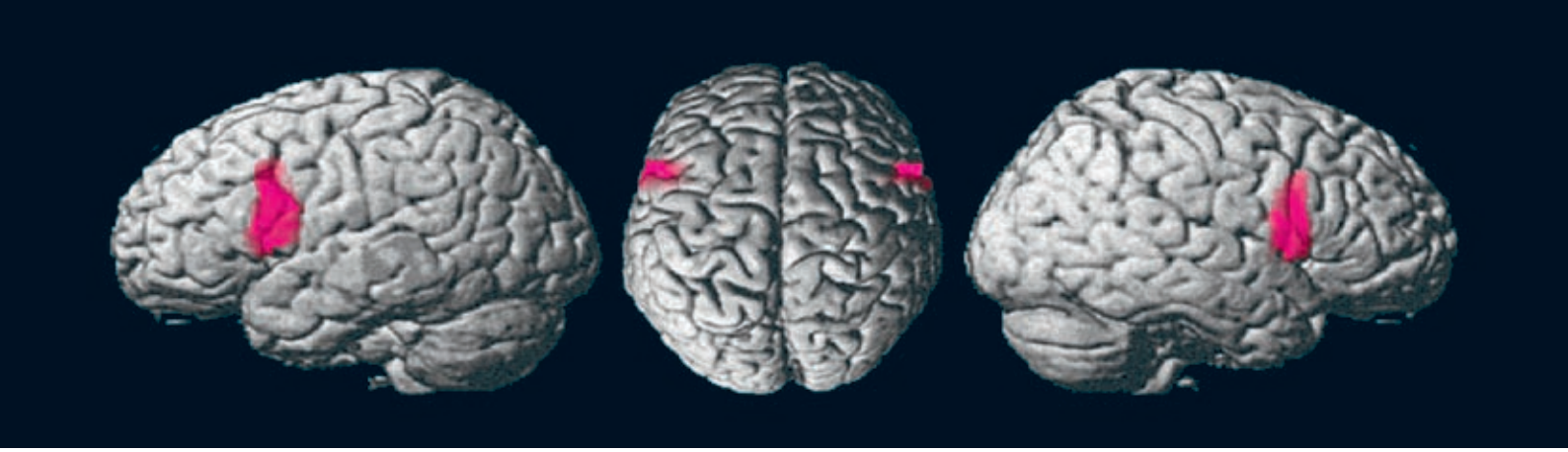


Fig. 2a: The microscopically defined Broca area (Brodmann Area BA44)
One area, many functions? The Brodmann Area (BA) 44, the anatomical position of which is shown in red in the above illustration, is often equated with a part of Broca's speech area. Examined via functional imaging, it shows activity during many other functions in addition to speech. Thus Broca's speech centre contains areas of the cortex that preclude simplistic equation of this region's function with speech motor control. This suggests a need for a systems approach and to explain cognitive processes and behaviour as interactions between different regions (Image: Simon B. Eickhoff).

plex network and on very different scales – from molecular transmitters and neurons to entire areas of the brain – via a dynamic exchange of information. The concept of integration also presupposes a regional segregation of brain structures and mechanisms, since it requires the interaction of specialised sub-functions. It is therefore impossible to arrive at an understanding of the complex structure and function of the brain without a systems biology analysis.

Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) can be used to localise the neural activities underlying cognitive processes in the human brain non-invasively. This has resulted in the localisation of increasingly specialised neuropsychological processes becoming the dominant paradigm. Moreover, new approaches to mapping the microstructures of the human cerebral cortex (Zilles and Amunts, 2010) make it possible to explore questions about the relation between anatomy and function in the brain. Yet when findings from functional imaging are compared with population-based probability maps, e.g. on the position and extent of microstructurally (cytoarchitectonically) defined areas in the cortex, two apparently contradictory phenomena are observed. On the one hand, there is evidence for a good correspondence between areas and functions, making it possible to allocate different functions to adjacent areas. On the other, it is clear that an area can be activated in different contexts. For example, a number of different linguistic functions are attributed to Broca's speech area, a specialised region of the cerebral cortex, and can be mapped in system models. Interestingly, however, activity is observed in the same area when actions are observed and controlled, and in working memory and visual search functions (Fig. 2). Thus, in addition to the main function ascribed to it, language production, Broca's area is also involved in numerous other functions (Caspers *et al.*, 2010).

This apparent contradiction between segregation and integration highlights the need for a systematic study of the brain whereby cognitive processes and behaviour are explained as structural and functional interactions between individual regions and interpreted with the aid of computer models. This method represents a clear move away from the prevalent approach in experimental psychology and medicine, which is to divide a problem into tiny units and mechanisms and to examine these in isolation. Explaining a mode of functioning on the basis of its dynamic network structure is only possible using a systems biology approach. As in so many cases, the whole is more than the sum of its parts. Systems biology modelling thus also plays a crucial role, as with today's methods it is often impossible to measure brain activity and interactions between different areas directly. Rather, functional MRI (fMRI) measurements merely reflect changes in local blood flow that are induced by neural activity. A system model of the measured data opens up the possibility of characterising neural activities that cannot be measured directly and the causal interactions between the regions involved.

Dynamic causal modelling: a system model of the brain

The most important approach to describing functional networks mathematically is dynamic causal modelling (DCM), which describes the brain as a dynamic input-output system (Friston *et al.*, 2003). The system elements are (i) individual regions of the brain, (ii) inputs – experimental manipulations such as proffering a stimulus or requesting that the test subject move a hand – and finally (iii) outputs – the (indirectly) measured brain activity. The model contains

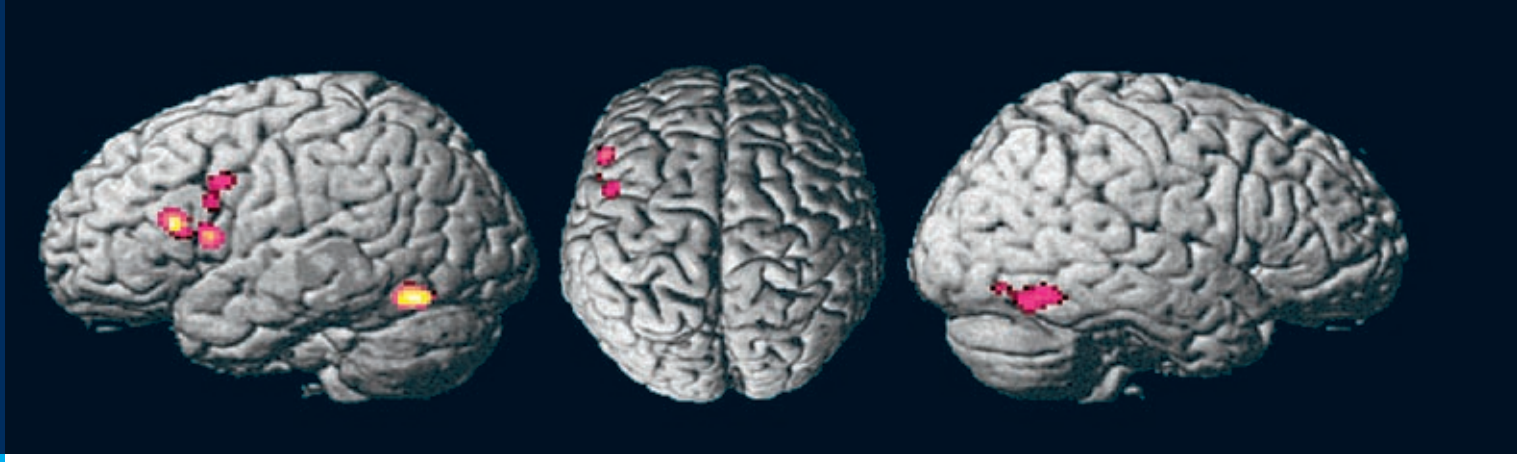
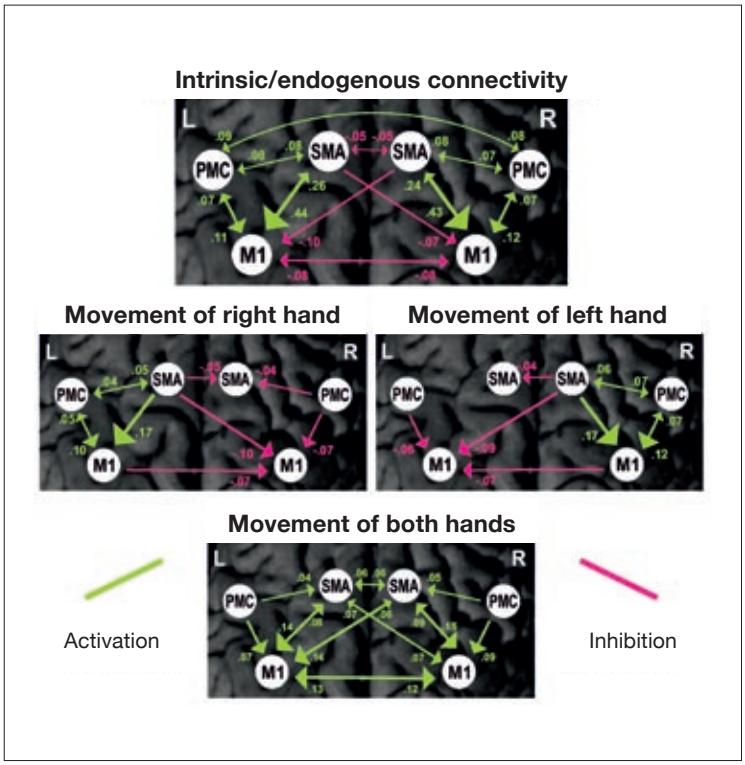


Fig. 2b: Functional activation on visualising actions.

a neural and a haemodynamic (bloodflow-related) aspect. Crucial to the understanding of functional integration is the neural level, in which the activity of every region is represented by means of a status variable $z_i(t)$. This does not represent a direct physiological state of the brain, but serves as an overall measure of the activity of the area represented. The network dynamics are determined by the interactions between regions and the experimental influences. The latter can influence the system by directly inducing activity in an area, as well as modulating interactions between regions. In the context of DCM, the dynamics of the system are mapped as a function of the neural states (that is, of the current status vector z), the modelled influences u and the interactions between the regions (Fig. 1).

The neural model is then linked to the haemodynamic forward model, which describes the connection between neural activity and measured values. Naturally, the validity of a system model such as DCM depends on the assumptions it makes, though in practice it is difficult to assume, or rule out, definitively a link between two regions or their modulation. As a rule, several neurobiologically plausible models exist, representing alternative hypotheses about the structure and dynamics of the examined network. However, the approach taken in DCM makes it possible to estimate not only parameters of a model, but also its inherent *a posteriori* probability. This enables one to identify the model that constitutes the best compromise between accuracy and complexity and thus to decide between alternative hypotheses.

Fig. 3: Systems model of hand movement



DCM illustrates the great significance of hemispheric specialisation and of the interaction between the two hemispheres. Intrinsic connectivity is organised symmetrically, with bidirectional positive intra-hemispherical links (green arrows) and predominantly inhibiting interhemispheric links (red arrows). This dynamic balance is modulated noticeably when one hand alone is moved. When it is moved, there is an increase in connectivity between all areas of the contralateral hemisphere, while all connections to the primary motor cortex controlling the hand that is not moved are modulated negatively. The same pattern is produced for the other hand, but inverted. In contrast, however, when both hands are moved simultaneously, is a positive connection, that is an interaction between the hemispheres.

Image: Simon B. Eickhoff

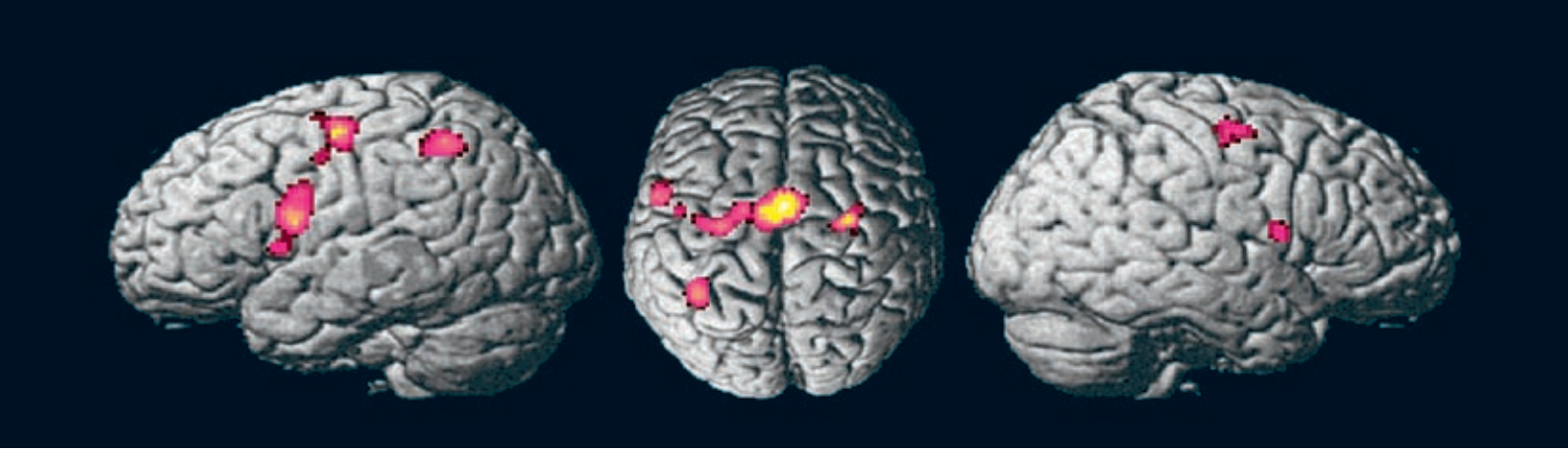


Fig. 2c: Functional activation on naming images.

Dynamic interactions control hand movements

The benefit of modelling neural systems in order to understand effective connectivity can be demonstrated very clearly using the example of the motor system. For example, when one hand is moved, the primary motor cortex (M1) on the opposite side of the brain is activated and sends orders to contract muscles to the appropriate motor neurons in the spinal cord. Functional imaging studies have made it possible to show that additional regions in the motor system control the execution of movements. Thus, the premotor cortex (PMC) and the supplementary motor area (SMA) also play a part in steering or controlling movements on the opposite side. Very little is known about the interactions between these regions and what role interhemispheric links play. Yet precisely this interaction is adjudged to play a crucial role in reorganisation processes after a stroke or a peripheral injury to the locomotor system. Systems biology investigations using DCM can make a crucial contribution toward a deeper understanding of these interactions. To this end we conducted tests on healthy subjects and subsequently on different groups of patients (Grefkes *et al.*, 2008; Eickhoff *et al.*, 2008). The test subjects lay in the fMRT scanner and were instructed via a monitor to move only their left hand, only their right hand, or both hands simultaneously. The brain activity induced was recorded continuously during the experiment. A significant increase in activity was found on active movement, in each case in the opposite cerebral hemisphere to the hand that was moved.

When DCM is used to model interactions in the motor system, the most probable model shows that hemispheric specialisation and interhemispheric interaction are highly significant (Fig. 3). Intrinsic or endogenous connectivity shows highly symmetrical positive links between areas in one hemisphere (M1, PMC, SMA) and inhibiting interhemispheric connections (e.g. M1-M1). Thus, an increase in activity in one hemisphere effects a reduction in the level of activity in the other hemisphere. If either the right or the left hand is moved, there is a significant increase in connectivity between all areas of the opposite hemisphere. In contrast, all connections in the direction of the primary motor cortex that controls the hand that is not being moved are modulated negatively: in a manner of speaking, the brain concentrates its attention on the unilateral movement. A completely different interaction results when both hands are moved simultaneously. The interhemispheric links are reversed, and there is now a positive interaction between the hemispheres. In other words, while the two sides of the brain are normally in a mutually inhibitory balance, one that is further strengthened when there is a need for unilateral movement, there is closer cooperation between the hemispheres when both hands are moved in coordination (Grefkes *et al.*, 2008).

These dynamic processes in the brain must naturally be based on structural organisations in the form of information tracts, of which more or less use is made depending on need. For a long time, few details were known about these fibre tracts in the human brain. New imaging methods such as MRT-based diffusion tensor imaging or high-resolution imaging of the fibre tracts by means of polarisation microscopy are now delivering increasingly detailed information about the basis of these neural interactions.

Together, these structural and functional data and models will open up new prospects for understanding the organisation of the healthy and the sick brain and enable knowledge-based diagnosis and treatment of neurological and psychiatric illnesses. Research is currently concentrated on two crucial issues: how to integrate different aspects of connectivity in the brain into common models, and how to distinguish pathological processes from normal variance or changes during healthy ageing.

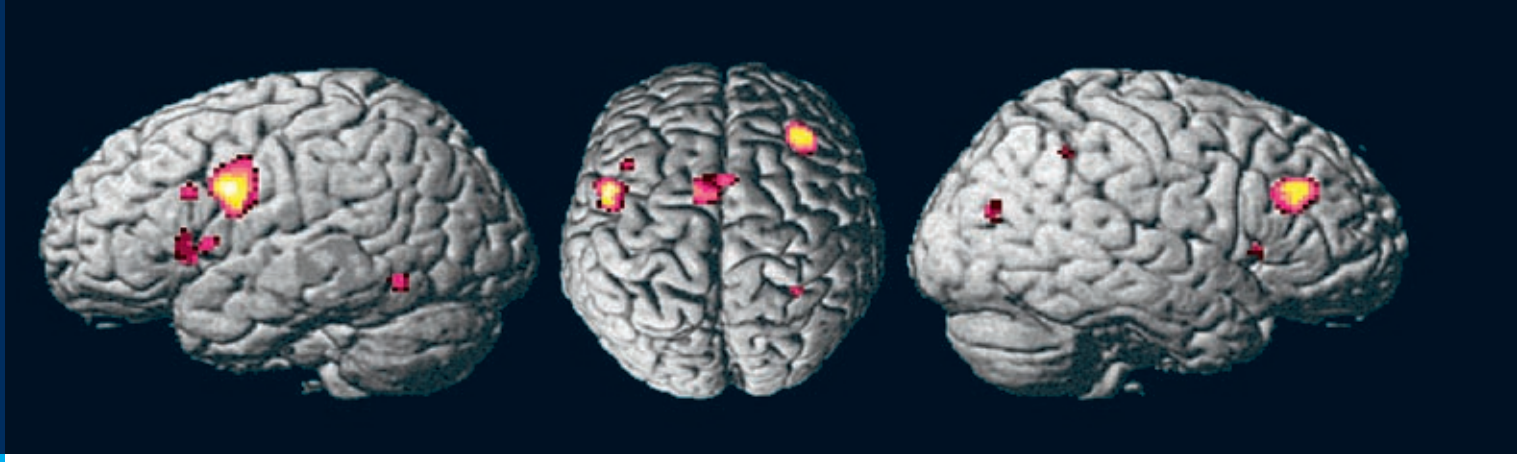


Fig. 2d: Functional activation during working memory tasks.

The research project in brief:

As part of the JARA-BRAIN collaboration, scientists and physicians at the Forschungszentrum Jülich and at Aachen University Hospital are working on new strategies for the prevention, diagnosis and treatment of psychological and neurological brain disorders. In this project, basic research, clinical research and technical-methodological skills are closely interlinked.

The Human Brain Model network of the Helmholtz Alliance on Systems Biology is investigating at the Forschungszentrum Jülich the relationships between structure and function in the human brain and the integration between brain functions. The aim of its interdisciplinary approach is to understand the organisation of this complex system and to devise models to describe it.

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how the retina functions

Systems biology leads to new findings

by Stephan Meding and Axel Walch

Despite decades of research on the retina, the part of the eye responsible for vision, we know little about its function. Hence only few theoretical models for its mode of operation exist, and they are also not based on experiments that examine the intact tissue. The aim of the network project "IMAGING – Multimodal Proteome Imaging: an entry to biomedical tissue systems biology" is to describe the retina as a model system based on tissue examined *in vitro* and *in vivo*. For this it employs the layered structure of the retina to develop and integrate novel imaging methods for a systems biological description of complex tissue states. These innovative methods are complemented by automated image analysis.

Over the last century or more, the retina has been described in detail – its structure, its cellular components, the functions of individual cell types and their interaction. Yet such seemingly simple, everyday processes as adaptation to light intensity, or adaptation to light and dark, cannot be described at the tissue level. Though the individual biochemical processes have been explored, the overall context is not yet understood.

The adaptation of the retina – an ideal model for systems biology

The retina is responsible for converting light into nerve impulses and for transmitting them correctly to the brain. The clear, layered structure of the retina makes it an ideal research object and model system for developing novel tissue-based approaches in systems biology. Light is converted into nerve impulses in the cone and rod layer. Above this layer there are several other ones that are responsible for connecting the signal and transmitting it to the brain (Fig. 1). The adaptation takes place mainly in the cone and rod layer.

Until now, many limitations have made it difficult to understand adaptation to light and dark at the tissue level. First and foremost, cell culture systems with limited suitability for exploring this question, sub-optimal measuring methods and a lack of modelling approaches have impeded understanding of the

Fig. 1: Layered structure of the retina

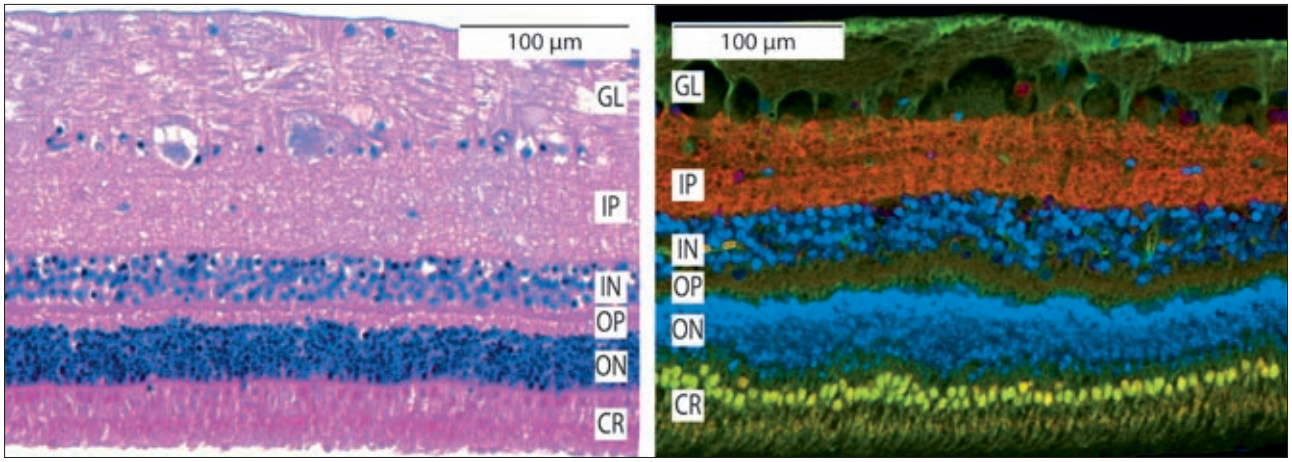


Image: Institute of Pathology, Helmholtz Zentrum München

An optical microscope (left) and a fluorescence microscope (right) image of the retina: the individual layers of the retina are clearly recognisable. The distinctly layered structure makes the retina an ideal model system for systems biological tissue analyses. (GL: ganglion cell layer, IP: inner plexiform layer, IN: inner nuclear layer, OP: outer plexiform layer, ON: outer nuclear layer, CR: cone and rod layer – main site of adaptation).

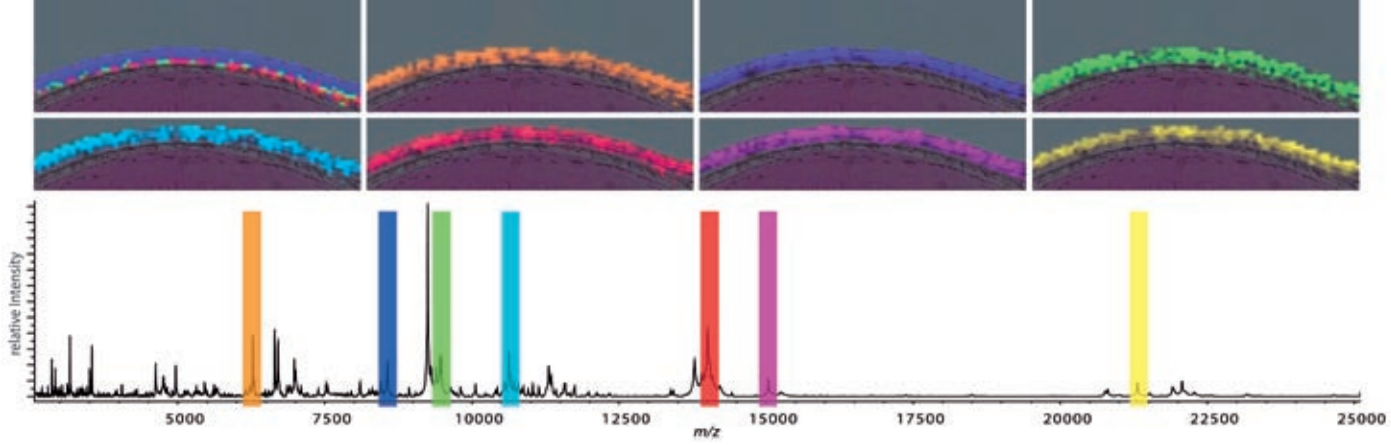
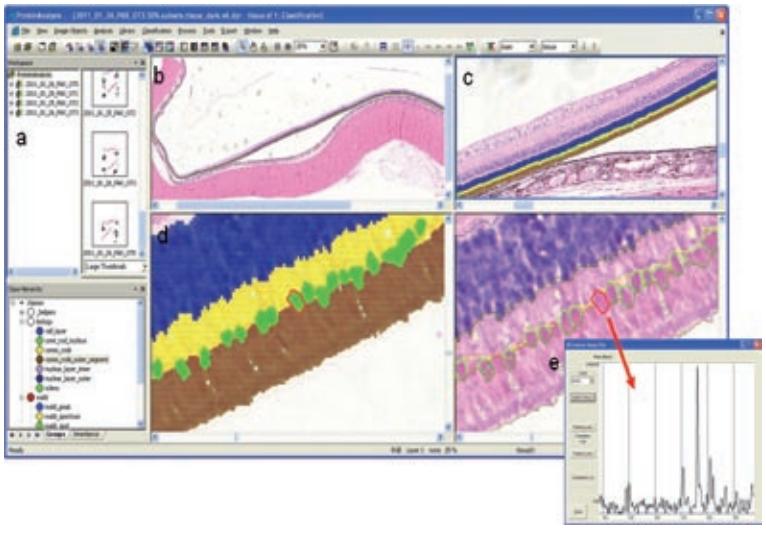


Fig. 2: MALDI Imaging of the retina
MALDI Imaging makes it possible to observe complex protein patterns in the retina and therefore to assign proteins to individual layers of the retina. An example of this is the classification of the spectra (top left) and the distribution of seven proteins in the retina, along with the corresponding mass spectrum (below).
(Image: Institute of Pathology, Helmholtz Zentrum München)

general context. Great advances have been made in methodology in recent years, but no comprehensive theoretical model of adaptation has resulted. The main reason for this was that researchers tried to draw conclusions from a given molecular detail about the next higher level. This made it impossible to gain an overall view of tissues, like that of the retina. Systems biology takes a different approach. It tackles the problem holistically, in this case starting from the level of the tissue. Only this approach enables a systems biological understanding of adaptation, with imaging methods playing a central role. Understanding the retina as a tissue and analysing it as a whole is necessary to gain a general understanding of adaptation. Until now, this approach has not been possible due to a lack of methodology. Now, by combining molecular and imaging methods, the retina can be examined at the level of the individual cell. By this, it can be examined as a whole and analysed by systems biological methods for the first time.

Along with a systems biological, integrative approach that combines a variety of methodologically complementary, molecular and imaging methods, the project requires close cooperation with modelling specialists both in planning experiments and during subsequent mathematical modelling. These prerequisites are fulfilled for the first time by the network project "IMAGING – Multimodal Proteome Imaging: an entry to biomedical tissue systems biology", part of the SysTec Initiative of the German Federal Ministry of Education and Research (BMBF). This network project unites four research institutes of the Helmholtz Zentrum München (the Research Unit Protein Science, the Institute of Pathology, the Institute of Biological and Medical Imaging, and the Institute of Biomathematics and Biometry) and two industry partners (Definiens AG, Munich, and Bruker Daltonik GmbH, Bremen). Together, we can now study the adaptation of the retina by various methods *in vitro* and *in vivo* and use the experimental findings to generate and validate a theoretical model.

Fig. 3: The Definiens software platform for management and analysis of image data



The chart shows a) project management for the individual slides of the retina; b) a view of a histological section of the retina; c) automatically identified layers of the retina (blue, yellow, brown); d) a detailed view of automatically identified structures (green) in the cone and rod layer; e) a diagram of an assigned spectrum of a mass-spectrometry imaging measurement.

Image: Definiens AG, Munich

MALDI Imaging – a key technology

MALDI (matrix assisted laser desorption/ionisation) Imaging (mass spectrometry) combines microscopy and mass spectrometry and is a key technology in this network project. It adds a complex, molecular dimension to histology, the microscopic examination of tissue. It renders the distribution of proteins in tissues visible at a cellular level, thereby enabling detailed, exact tissue analysis (Walch *et al.*, 2008). Preparation for MALDI Imaging involves several simple steps. First, tissue sections are treated with a matrix, and the proteins present in the tissue are transferred to the matrix in a spatially resolved manner. A laser is then used to desorb the matrix-protein mixture spotwise and by mass spectrometry the protein composition at each individual measuring point is analysed. After measuring, the matrix is removed and the tissue, which is not damaged by measurement, is histologically stained. The final stage is to superimpose a high-resolution microscope image of the histological section onto the spatially resolved protein pattern on a computer so that both the morphology and distribution of individual proteins in the tissue can be observed simultaneously (Fig. 2). The resolution of commercially available mass spectrometers was previously limited to 100–200 µm. However, further developments in methodology and technology (in which Bruker Daltonik GmbH played a leading role) have increased the spatial resolution to a cellular level (20 µm) (Lagarrigue *et al.*, 2010). Now, MALDI Imaging makes it possible to overcome this crucial limitation to the analysis of proteins in tissue. For the first time, this method can be used to assign individual proteins and protein patterns to certain cell types which is of utmost important in the case of the retina. Without single-cell resolution it is impossible to assign processes that take place during adaptation, a majority of them in the cone and rod layer, to individual layers.

Synergism of imaging and proteomic methods

MALDI Imaging is complemented by other imaging methods and image-based analysis methods as well as by comprehensive proteomic analysis of the retina after adaptation to light and dark. Modern, proteomic methods are used for this purpose. All proteins in the retina and their respective modifications are identified after adaptation and quantified comparatively (Hauck *et al.*, 2010). With respect to systems biological analysis, these proteomic signatures can now be combined for the first time with MALDI Imaging so as to map the adaptation in a molecular, spatially resolved form. Selective immunohistochemical staining of tissues (*in situ*) completes these analyses at the molecular level and can be quantified by comparative image analyses. In order to examine the dynamics of adaptation, the *in vitro* findings are complemented with *in vivo* experiments. This facilitates better understanding of the retina by contributing dynamic, location-specific parameters. In this project, ophthalmological examination techniques are used and linked to molecular imaging via multispectral methods, which make it possible to measure several fluorescent markers simultaneously (Themelis *et al.*, 2008). The aim of doing so is to track the proteins marked with fluorescent molecules over extended time periods in order to describe their spatial and temporal dynamics. To improve the quality of measurement, microscope images are analysed by automatic image analysis. Computerised, automated identification of the individual layers of the retina enables layer-specific protein patterns to be defined with a high degree of accuracy for further modelling. A software platform from Definiens AG (Fig. 3) is used to analyse and combine all imaging and mass-spectrometry data obtained during the course of the project. In doing so, the aim is to establish interfaces between the different test methods and use them to construct a systems biological model of the retina that is as reliable and comprehensive as possible.

Medical benefit and clinical relevance of molecular imaging

The prerequisite for understanding diseases of the retina is to understand the retina itself in its normal, healthy state.

The participating partners
Ralf Schönmeyer, Axel Walch, Alice Ly, Stephan Meding, Karin Radrich, Marius Ueffing, Günter Schmidt, Detlev Suckau, Sandra Rauser, Stefanie Hauck, Laurent Demaret, Vasilis Ntziachristos, Martin Storath (from left to right). (Photo: Partners of the IMAGING alliance)

After investigating the adaptation, the IMAGING alliance will therefore start with doing research on diseases of the retina. A better understanding of pathological changes in the retina can open up new methods of treatment and enable effective prevention.

Because it integrates a variety of tissue imaging methods, systems biology is not restricted to examination of the retina. The developed models and scientific approaches are transferable to other tissues, such as tumour tissue, and inflammatory reactions. This makes it possible to bridge the gap between molecular research and clinical application, especially in regard to human medicine.

The research project in brief:

Project name:
"IMAGING – Multimodal Proteome Imaging: an entry to biomedical tissue systems biology", as part of the SysTec Initiative
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Project partners:
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(5) Definiens AG, Munich: Dr. Günter Schmidt, Dr. Ralf Schönmeyer
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simulated liver regeneration

How computer programs predict biological processes in living tissue

by Dirk Drasdo, Stefan Hoehme and Jan G. Hengstler

Models identify a so far unknown order mechanism

What processes take place in living tissue? How do individual cells in a tissue manage to work together to form functional units? Biologists have been pursuing this question for more than a century. Knowledge about complex tissue architecture and interaction between groups of cells is important for understanding multi-cellular diseases. We recently developed a technique that can be used to record the architecture of a tissue structure quantitatively and to simulate developments. Taking the example of liver regeneration, we were able to show how this technique can improve understanding of the complex mechanisms of interaction between hepatocytes, the main cell type found in the liver, and endothelial cells, the cells of small blood vessels in the liver. In ongoing dialogue between experimenters and modellers we discovered a previously unknown order mechanism that helps the liver to regenerate after intoxication (Hoehme *et al.*, 2010).

The liver

In Greek mythology, Zeus punished Prometheus by sending an eagle to peck out part of his liver every day. The liver regenerated, only to be eaten anew the following day. In fact, the liver does have a remarkable capacity to regenerate. The human body can rebuild more than half of its liver mass after damage.

As the largest human internal organ, the liver plays an important role in metabolism throughout the body. It is responsible not only for absorbing nutrients, but also for detoxifying the blood.

The human liver is made up of two main lobes that are each assembled into basic functional units comprising approximately 500,000 lobules. Altogether, we have around a million liver lobules, through which blood rich in oxygen and nutrients flows. The special architecture of a liver lobule ensures an optimal exchange of materials between the blood and the hepatocytes, the liver's "workhorses".

Medical relevance

The regenerating liver is called on to restore a highly complex tissue architecture. Liver damage results from viral infections, alcohol, or certain medicines. One of the most common causes of acute liver failure is an overdose of paracetamol (also known as acetaminophen). This drug causes a characteristic pattern of damage, mainly affecting the centre of the liver lobules.

We used carbon tetrachloride as the compound for our experiments on mice, as it creates a pattern of damage very similar to that caused by paracetamol. After only one week, the laboratory animals had completely regenerated their liver mass. Liver function was also quickly restored. We were interested to know how the liver managed to regenerate in this way. So we started by developing a process chain of experiments, image analyses and computer simulations that enabled us to analyse the regeneration process quantitatively.

From experiment to model

As a starting point we needed a reference object showing the situation before damage to the liver occurred. We therefore quantitatively characterized the microarchitecture of the undamaged mouse liver with the help of optical section im-

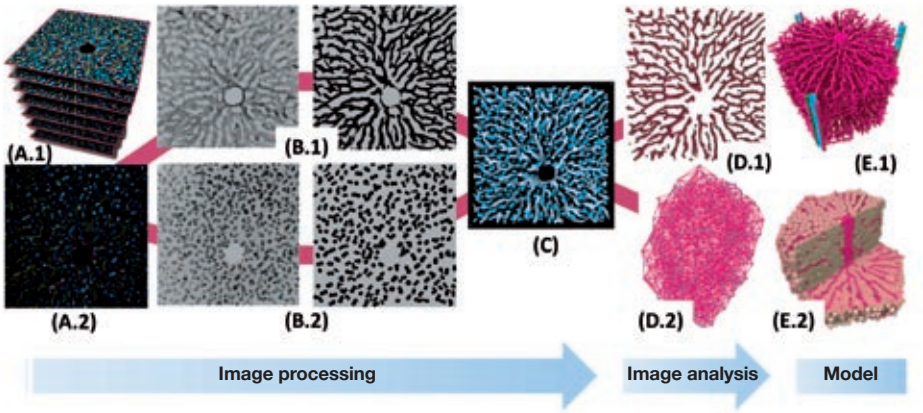
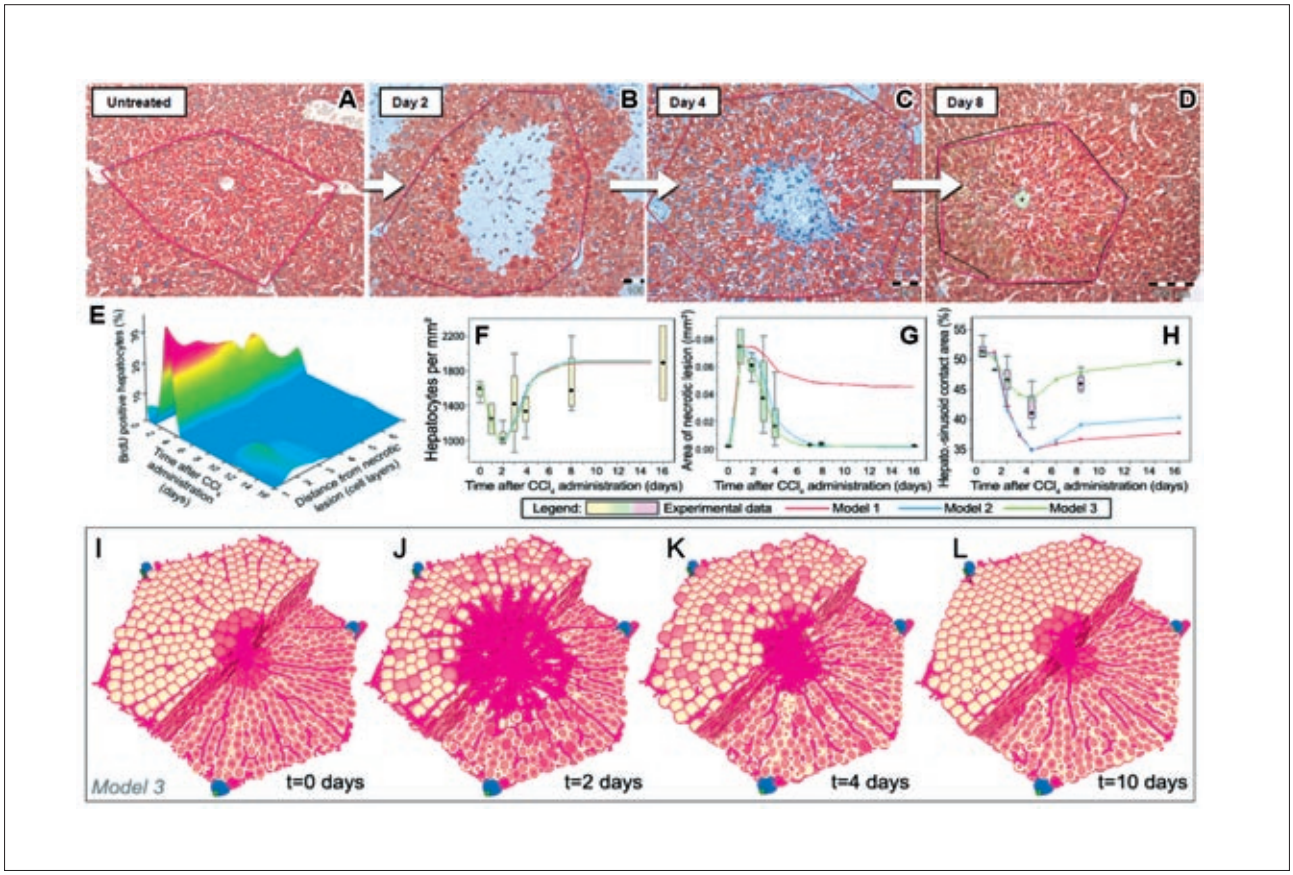


Fig. 1: From original image to mathematical model
(A.1) shows a stack of confocal images. The colour blue marks the cell nuclei, green the sinusoids (blood vessels in the liver lobules) and bile ducts, and red the sinusoids and endothelial cells. The superposition of the red and green channel (yellow) marks the sinusoids alone. (A.2) is a typical original section image prior to image processing. The B images show the separation of yellow and blue-stained image elements (B.1: sinusoids, B.2: cell nuclei) that were processed in a number of image processing steps and assembled by computer into (C) a three-dimensional volume data set of the liver lobule, which was then further processed for the purpose of quantifying the image information during image analysis (D) so as to construct a representative lobule for the mathematical model (E).
(modified from: Hoehme *et al.*, 2010)

ages using a confocal microscope (Fig. 1 A). In order to make individual structures visible, we used special fluorescent dyes to stain selected areas (Fig. 1 A.1). These were then assembled into a three-dimensional computer image by means of a set of image processing and analysis steps (Fig. 1 C). We quantified the information in the image by further processing the location, position, density and shape of the cells and the blood vessel's architecture numerically (Fig. 1 D). The end product of the image processing was a representative lobule that served as a starting configuration for the subsequent computer simulation (Fig. 1 E).

Fig. 2: Section of a liver lobule



Section of a liver lobule before administering carbon tetrachloride (A), and two, four and eight days after administration (B–D). (E–H) Quantitative characterisation of the regeneration process via process parameters (see article for details). The symbols denote the results of experiments after image processing; the curves the results of simulation using different computer models (see key). (I–L) Regeneration process in the computer simulation for the model denoted by the green curve in F–H. (modified from: Hoehme *et al.*, 2010)

Dirk Drasdo

The regeneration process in figures

Around a week after intoxication the lobule mass had been fully restored. Figure 2 shows a direct comparison between the regeneration process in the experiment (top row) and the computer simulation (bottom row). In order to establish comparability between biological reality and the model we identified four representative parameters – the pattern of cell division in the lobule, the number of cells per section, the amount of dead tissue, and the area of contact between hepatocytes and blood vessels. The figures show clearly that regeneration of the mass precedes regeneration of the architecture, which takes two weeks to complete.

The regeneration process in the computer model

The computer model comprises both every individual hepatocyte and the blood vessels (sinusoids) as a network of tensile, tube-shaped objects. Every hepatocyte is described by means of an equation of motion that is used to calculate its exact position at any time. The equation includes the hepatocyte's active movement along with all the forces exerted on it – the forces from other hepatocytes and from sinusoids, and that exerted on the hepatocyte by the extracellular matrix in the small empty spaces between cells and sinusoids. In principle, all cell parameters in the model are measurable. An equation of motion equivalent to that for the cells was also constructed for the sinusoids.

In order to ensure that the model reflects reality, we compared the computer data repeatedly with the biologists' measurements. The model was further developed in three stages, which evidences the importance of feedback from the laboratory experiments. In the first version of the model, cells divided in random directions and cell migration was also undirected. The model lacked explanatory power, since only the number of regenerated liver cells was described correctly (red curve in Fig. 2 D). Closer study of the microscopy sections showed that hepatocytes on the periphery of the dead regions were putting out extensions, like little feet into the dead tissue. Could the cells perhaps migrate in a directed manner collectively, even though no evidence of isolated hepatocytes was found in the dead parts of the liver? The second version of the model took account of directed cell movements, and this resulted in a considerable improvement in the computer prediction. Model 2 made it possible to also describe the closure of the dead zone correctly (blue curve in Fig. 2 G). Nonetheless, this model still failed when it came to the architecture (Fig. 2 H), because this requires a high degree of structure. The hepatocytes have to be organized along the sinusoids like beads on a string in order to maximise their contact area. In fact, the breakthrough came with Model 3, in which we assumed that when hepatocytes divide they align themselves along sinusoids. This mechanism was able to explain all process parameters correctly (green in Fig. 2 E–H). But did the explanation provided by the model correspond to the reality of living tissue?

Our prediction is confirmed: HSA is a order mechanism

In a final experiment we determined in three dimensions the angle between the two daughter cells after division and the closest sinusoid. The result agreed with the values predicted theoretically by Model 3. This confirmed that hepatocytes align themselves to sinusoids. Without this structuring mechanism, which we called HSA (hepatocyte-sinusoid alignment), there can be no proper regeneration of the liver's lobule architecture.

This work enabled us to show by example how imaging information from histological slides can be used to build models for predicting spatial/temporal organisational processes in tissue. The principle of our method is easily transferable and is highly

versatile. For example, it is currently being used to understand cancer and other diseases of the liver and lung. This work also aids in understanding liver regeneration after partial removal by surgery, which is a frequent therapy in liver cancer.

Since the model represents each individual cell, intra-cellular molecular regulation mechanisms can easily be included (Ramis-Conde *et al.*, 2008). When different biological levels, in this case the behaviour of the individual cell and its internal molecular processes, are linked mathematically, the model is said to be a multiscale model. Multiscale models of the liver are currently being developed within the *Virtual Liver* network project. Our models form the interface between molecular and whole-tissue models. In the long term, it will be possible to predict changes in cell mass and tissue architecture when molecular changes take place.

The research project in brief:

The project grew out of collaborations within the German HepatoSys network and the EU-funded initiatives PASSPORT and CancerSys.

Further information:

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www.msysbio.com
www.ifado.de/forschung_praxis/projektgruppen/susceptibility/index.php

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Participating institutes:

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how many cells make up a liver?

New image analysis methods enable the quantitative analysis of complete histological tissue sections

by André Homeyer, Andrea Schenk, Uta Dahmen, Michael Schwier, Tobias Preusser and Olaf Dirsch

Your child feels ill and you put your hand on its forehead. Fever? To make sure your partner feels again. The child seems to be healthy! But only a thermometer can provide certainty.

Important parameters, crucial for diagnosis, often cannot be determined accurately without technical devices. This is particularly true for the quantification of tissue properties in pathology. Hence, Fraunhofer MEVIS develops innovative methods for analysing entire tissue sections in close collaboration with physicians from Jena University Hospital. The resulting technology has the potential to answer open scientific questions and improve clinical diagnosis significantly.

Histology, i. e. the microscopic study of tissue, provides a wealth of spatially resolved information on tissue structures and their underlying cellular processes like no other imaging method. Pathologists use this information together with clinical details for their diagnosis.

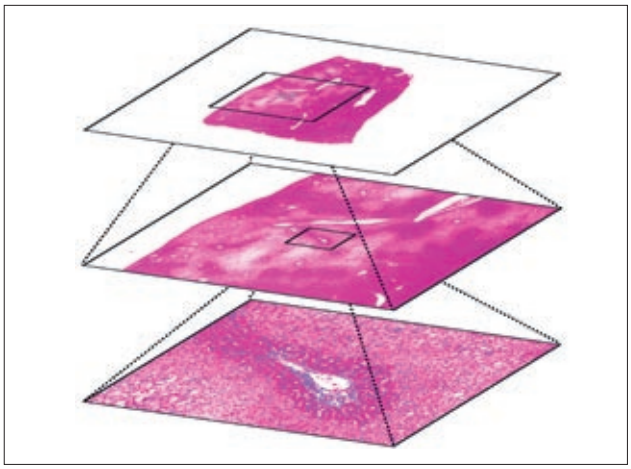
In contrast to other fields in medical imaging, like radiology, histology is still practised mainly without computer support. Fuelled by the development of novel digital slide scanners, however, histological diagnosis will experience a surge of innovation that will effectively improve the efficiency and quality of this diagnostic method. In this context, automatic image analysis will contribute significantly to the efficient, accurate and reproducible measurement of tissue properties.

From local evaluation to global analysis

Up to now, the analysis of tissue properties was confined to a few small tissue areas whose results were extrapolated to the whole organ. Considering that many tissue properties are distributed unevenly, this simplification can lead to severe errors. Through the development of novel slide scanners which digitise whole slides at high magnification it is now, for the first time, possible to apply automatic image analysis methods to entire tissue sections.

This technology, however, imposes new challenges for automatic image analysis. Since many relevant details are only visible when highly magnified the complete slide has to be digitised at this magnification level. Furthermore, pathological examinations depend on a quick change between magnifications. Therefore, virtual slides have to be stored at multiple resolutions (Fig.1). Thus, one single virtual slide comprises

Fig. 1: Virtual slides



Virtual slides are digitised at highest magnification and stored in various resolutions. This results in gigabyte-sized image files that are particularly challenging to process (Source: Fraunhofer MEVIS / U. Dahmen & O. Dirsch, Exp TxChir Jena).

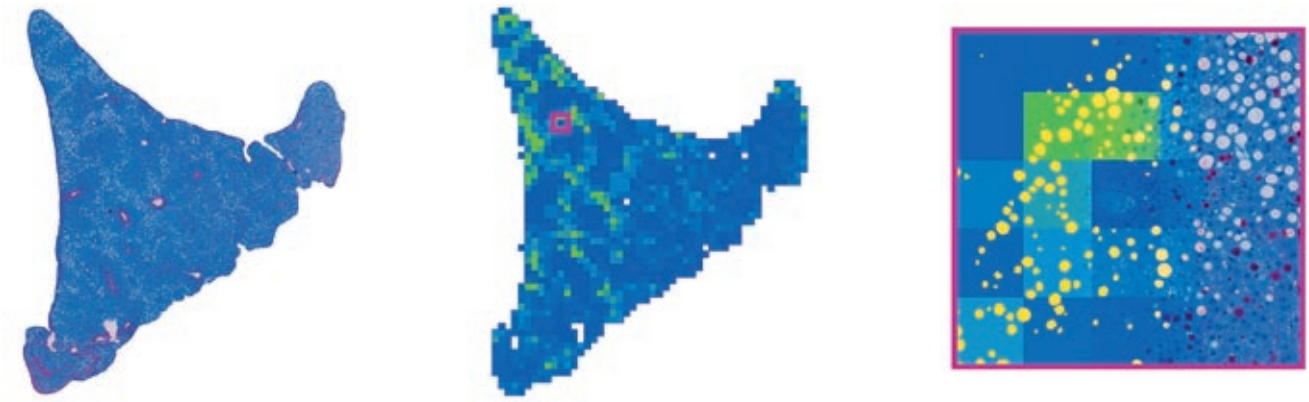


Fig. 2: Image analysis of complete tissue sections
Fraunhofer MEVIS develops automated image analysis methods for determining and spatially depicting microscopic tissue properties, like the fatty degeneration of individual cells, across entire tissue sections (Source: Fraunhofer MEVIS / U. Dahmen & O. Dirsch, Exp TxChir Jena).

up to 25 gigapixels, equalling the quantity of 2,500 pictures of a modern digital camera. A whole set of slides quickly fills a terabyte hard disk.

With conventional methods, the analysis of virtual slides can take several days. So most of the available image analysis methods are still limited to evaluating just a few small tissue areas and ignore the potential of the new technology. Fraunhofer MEVIS, however, has specialised on the analysis of complete virtual slides right from the beginning (Fig. 2). While alternative approaches try to cope with the huge data volumes through increased processing power, Fraunhofer MEVIS develops intelligent strategies to quickly process these "gigantic pictures" on standard computers.

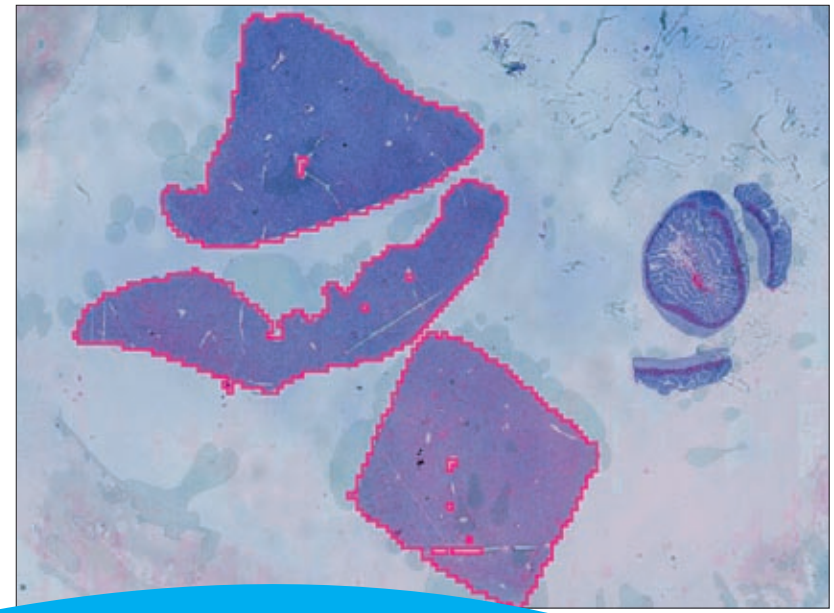
The idea: Just like a pathologist, the computer first searches for relevant image areas at low magnification and only changes to higher magnifications if more detailed information on these image areas is required. In comparison to a complete analysis at the highest resolution such multi-scale evaluations require considerably less computing time.

Digital histology in medical research

First practical applications of this novel image analysis technology enable the automatic discrimination between vital and dead tissue in liver sections. With further applications, different cell types and cell structures can be counted to determine hepatic regeneration activities or the level of fatty degeneration. Each of these parameters is computed automatically per tissue section – even if multiple sections of different tissue types are placed on one slide or if the slide is contaminated with dirt (Fig. 3). These methods are continually upgraded and improved within the *Virtual Liver* project.

The distinctive feature of the newly developed image analysis algorithms is that they do not only evaluate small tissue areas but entire tissue sections. The considerably increased number of samples guarantees a higher statistical validity of the resulting parameters. Furthermore, the parameters can not only be output in numerical form but also be visualised as coloured overlays on top of the original image. In this manner, structural patterns are revealed, which remain hidden in purely quantitative analyses. Initial validations have

Fig. 3: Automated detection of tissue sections



The developed image analysis methods work even if the slide contains multiple sections of different tissue types or contaminations with dirt. This figure shows the automated recognition of rat liver sections on a slide that additionally contains intestinal sections and contaminations (Source: Fraunhofer MEVIS / U. Dahmen & O. Dirsch, Exp TxChir Jena).

"Experimental Transplantation Surgery Group"
at the Department of General, Visceral and Vascular Surgery, University Hospital Jena (f.l.t.r.: Anding Liu, Olaf Dirsch, Uta Dahmen, Haoshu Fang, Hao Jin, Jian Sun, Wei Dong).

confirmed that the examined tissue properties are not evenly distributed across the sections but, for instance, aligned along the course of vascular structures.

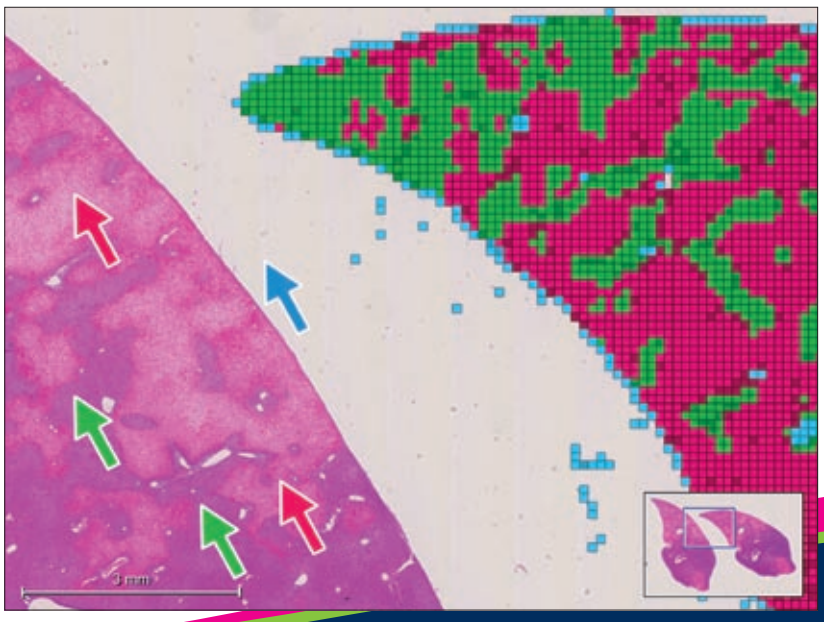
The wide variability of the captured images is a major challenge in histological image analysis. Even slight differences in the preparation of the slides can alter the digital image so severely that static image processing methods fail. For this reason, Fraunhofer MEVIS has made the computer capable to learn. All the user has to do in order to adapt the algorithm to new staining characteristics is to point out examples of the different tissue types (Fig. 4). The computer then adjusts automatically and classifies additional slides with similar characteristics without further interaction.

3D histology

Up to now, histological analyses were limited to two spatial dimensions. For many new issues, however, like the modelling of liver regeneration or blood flow regulation within the research network *Virtual Liver*, tissue has to be examined in its actual three-dimensional context. Hence, Fraunhofer MEVIS carries out research into innovative image processing methods that combine the powers of computer tomography and histology, in an interdisciplinary discourse with experimental surgeons, pathologists, and radiologists.

In an initial application, a comprehensive three-dimensional model of a remnant liver after surgical resection is created. For this purpose, the vessels of a mouse liver are filled with contrast medium and subjected to a CT scan in order to produce a 3D image of the organ structure and the vessel tree. Afterwards, histological serial sections of the complete organ are produced in order to determine different tissue properties and the expression of various molecular markers. In this context, Fraunhofer MEVIS develops image analysis algorithms that integrate the radiological and histological information into a three-dimensional organ model (Fig. 5). Within the *Virtual Liver Network* this novel method of 3D histology will contribute greatly to the holistic understanding of liver morphology and function.

Fig. 4: Simple and intuitive user interface



The developed analysis methods can be quickly adapted to varying image characteristics via an easy user interface. The user only has to choose examples of the relevant tissue types (arrows) (Source: Fraunhofer MEVIS / U. Dahmen & O. Dirsch, Exp TxChir Jena).

"Virtual Liver Image Processing Group"
at Fraunhofer MEVIS (f.l.t.r.: Michael Schwier, Tobias Preusser, André Homeyer, Andrea Schenk).

The research project in brief:

The Virtual Liver project, funded by the BMBF (Federal Ministry of Education and Research) is a network comprising 70 research groups. The network's goal is to create a dynamic multi-scale model of the human liver. In cooperation with the "Experimental Transplantation Surgery" group and the liver and vascular pathologist from Jena University Hospital, Fraunhofer MEVIS develops macroscopic models of liver morphology and methods for the automated determination of tissue properties in experimental image data. Here, Fraunhofer MEVIS benefits from more than 15 years of experience in medical image processing and modelling.

Fig. 5: 3D histology

The three-dimensional spatial correlation of various tissue properties is one of the current research topics. For this purpose, histological serial sections are alternatingly stained with different methods, digitised and returned to their original spatial structure with novel image processing methods (Source: Fraunhofer MEVIS / U. Dahmen & O. Dirsch, Exp TxChir Jena).

Additional reading:

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News from the BMBF

Science year 2011 – research for our health

Knowledge is the best medicine: that was the motto used in Berlin in January at the launch of Science Year 2011 – Research for Our Health. Numerous exhibitions, competitions and discussion events provide insights into current challenges and research findings. At the same time, the Science Year serves as a forum for dialogue between the world of science and the general public. Along with rare diseases and individualized medicine, the Science Year's focus is on widespread diseases and on prevention by means of nutrition and physical activity. "Our aim is to ensure that new results and findings are incorporated into medical practice as quickly as possible," German Federal Research Minister Schavan explained at the opening ceremony.

New media such as YouTube, Twitter or Facebook are being used, mainly in order to reach younger people. As part of the Research Exchange (www.forschungsboerse.de), school classes can invite scientists to their classroom or visit them at their places of work.

Further information at:
www.forschungsboerse.de

Health research framework programme

New or improved diagnostic processes and therapies are being developed in healthcare research in order to help sick people more effectively. The search is on for new approaches and methods of prevention to help ensure that diseases do not occur in the first place. The aim of this health research, funded by the German Federal Government, is to enable everyone to benefit from current research results. To this end, the Cabinet approved the Federal Government's new Health Research framework programme. It defines the strategic course of medical research for the years to come and is the basis of funding for medical research at universities, university hospitals, non-university research facilities and in the commercial sector.

In addition to setting up German Health Research Centres to deal with widespread diseases, the research programme focuses on another five fields of action: (i) research into individualized medicine, (ii) prevention and nutrition research, (iii) health services research, (iv) research into optimization of the healthcare sector and (v) global cooperation in healthcare research.

The German Federal Ministry of Education and Research (BMBF) is investing a record sum of more than €1 billion in healthcare research in 2011.

Further information at:
www.bmbf.de/de/gesundheitsforschung.php
www.bmbf.de/press/3014.php

Federal budget 2011

Germany's future lies in first-rate education and training of its citizens on the widest possible basis. "The quality of Germany's research and innovation system will have a decisive influence on our ability to compete internationally," says German Federal Research Minister Annette Schavan. Only with excellent initial and continuing

Aiming to take the bio-economy forward: (from the left) Reinhard Hüttl, Thomas Rachel, Robert Kloos, Holger Zinke and Helmut Born (Source: BIOCOM)

vocational training will Germany be able to maintain and extend its leading position in the world. These statements are reflected in the BMBF's 2011 budget, which is about €782 million up on the previous year to a total of €11.646 billion. "Germany is in a unique position today by having set a clear priority on investing in the future," Federal Minister Schavan comments, "both throughout the course of its history and by international comparison."

Further information at:
www.bmbf.de/press/3001.php

BioEconomy 2030 – strengthening bio-based business

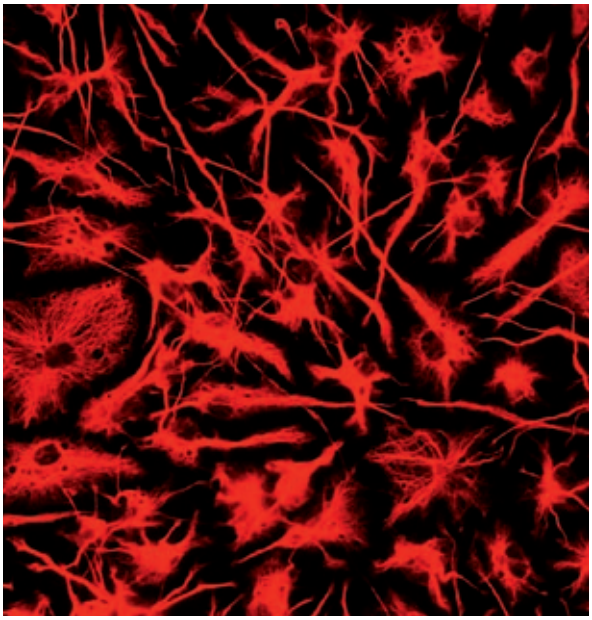
BioEconomy is defined as sustainable commercial use of biological resources such as plants, animals and microorganisms. Very wide-ranging, it includes a large number of industries in the commodities and food sector, mechanical engineering and plant construction, the automobile industry, environmental technology, construction and numerous service industries. In the National Research Strategy BioEconomy 2030, the German Federal Government has laid the groundwork for the vision of a sustainable, wide-ranging, bio-based economy by 2030 that provides the world with healthy food in sufficient quantities and high-quality products from renewable raw materials.

Funding will total more than €2 billion over the next six years. One of the first measures will be an innovation initiative on white biotechnology. The BMBF is making up to €100 million in funds available over five to ten years to promote business and science networks.

Further information at:
www.bmbf.de/de/1024.php
www.bmbf.de/press/2991.php

BMBF funds German-American stem cell research

Regenerative medicine is developing into an area on which great hopes are pinned for neurodegenerative diseases and others, such as diabetes or cancer. Scientists are conducting research into special properties of stem cells, which can be used to activate the body's self-healing power and to grow tissue outside of the human body.



Astrocytes from embryo stem cells
(Source: German Federal Institute for Risk Assessment, ZEBET)

"Only by means of cooperation between the best researchers can stem cell therapies become a reality," says German Federal Research Minister Annette Schavan. In keeping with this maxim, the BMBF is investing up to €12 million in cooperation between German and US researchers. Starting this year, this has enabled German scientists to take part in calls for proposals by the California Institute for Regenerative Medicine (CIRM), which specializes in stem cell research.

Three teams with German participants were chosen in the current process. The team led by Prof. Oliver Brüstle, University of Bonn, is developing a treatment for patients with the neurodegenerative condition Canavan disease. Dr. José Tomás Egana, Technische Universität München, whose research aims to accelerate frequently problematic wound healing in diabetics, is a participant in the second project. In the third project, scientists led by Prof. Andreas Hochhaus, Jena University Hospital, are working on cancer stem cells.

Further information at:
www.bmbf.de/press/2995.php

Developing sustainable land use

For humankind, the Earth's surface is our primary living space, which we have influenced for centuries and shaped as the most important basis of our life. Global change and the growing world population are increasing competition for land use all over the world. "We must find solutions that continue to ensure supplies of food and energy while maintaining both an urban habitat and an intact environment," explains Dr. Georg Schütte, State Secretary at the German Federal Ministry of Education and Research.

As part of the BMBF framework programme Research for Sustainable Development, the Sustainable Land Management funding measure addresses aspects of optimal land management. Projects focus on model regions because the repercussions of global change on living conditions are especially apparent at the regional level. The Ministry is providing €100 million in funding between now and 2015. Worldwide projects combine the expertise of engineers, social and natural scientists as well as representatives of authorities and regional and local governments.

Further information at::
www.bmbf.de/press/2992.php

The European Spallation Source ESS – a super-microscope for unique insights into matter

The unique European Spallation Source (ESS) will enable research scientists to gain insights down to the smallest levels. Bombarding materials with minute, uncharged neutrons enables the internal structure of materials or the movement of particles to be investigated. In future, scientists will be able to see the movement of proteins or other processes in cells, for example.

With 16 European countries taking part, construction of the new neutron source is scheduled to begin in Lund, Sweden, in 2013. After completion in 2018 the first neutrons will probably start

GeoEn pools the outstanding competences of these three major research facilities in the geosciences and energy research
(Source: Laurence Gough - Fotolia)

to flow at the ESS in 2025. Scientists from the Forschungszentrum Jülich (Jülich Research Centre) are coordinating the project, in which the Deutsches Electronen-Synchrotron (DESY), the Helmholtz Centre Dresden-Rossendorf, the Helmholtz Centre Berlin for Materials and Energy, the Helmholtz Centre Geesthacht, the Karlsruhe Institute of Technology (KIT) and other Helmholtz Centres (HZIs) are taking part. The Technische Universität München is also a partner in the project.

Further information at:
www.bmbf.de/press/3000.php
www.weltderphysik.de/ess
www.youtube.com/watch?v=5hi7zXj3xug
http://ec.europa.eu/research/esfri

GeoEn – climate-friendly energy supply research in Potsdam and Cottbus

One of the global challenges of our time is to ensure universal availability of energy supplies. Geo-resources in Germany have an important part to play in the quest for energy generation concepts that conserve the environment and the climate. In the GeoEn project a research network from Brandenburg is dealing with important aspects of geothermal energy, developing unconventional natural gas deposits and the capture, transportation and storage (CCS technology) of carbon dioxide from fossil-fuel power stations. Recipients of the €5.77 million in funding are the German Research Centre for Geosciences Potsdam, the Brandenburg University of Technology Cottbus and the University of Potsdam. “GeoEn pools the outstanding competences of these three major research facilities in the geosciences and in energy research. With its second grant approval letter, the BMBF is ensuring the further development of GeoEn into a powerful regional research network,” commented Dr. Helge Braun, Parliamentary State Secretary to the Federal Minister of Education and Research, in Cottbus.

Further information at:
www.bmbf.de/press/3024.php

Contact

For information about this and other interesting aspects of the High-Tech Strategy for Germany, please visit:
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THE CHANGES CHEMICALS TRIGGER IN OUR CELLS –

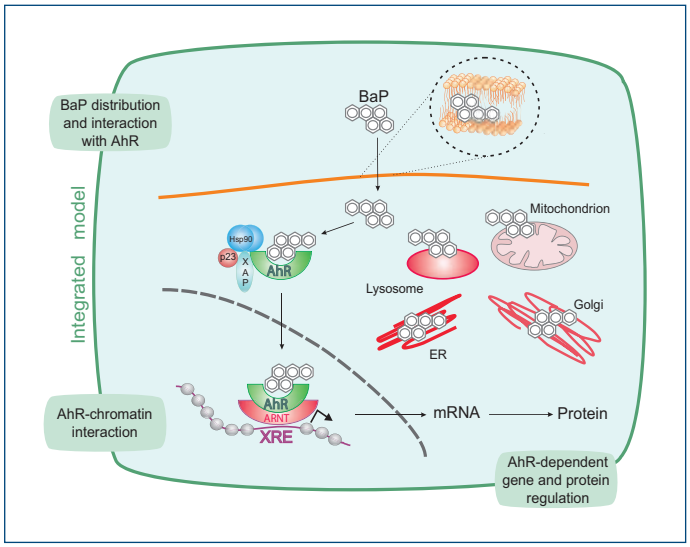
Network of the Helmholtz Centre for Environmental Research (UFZ)

In our everyday life we come into contact with a wide variety of chemicals, and their impact on our organism is not yet fully understood. If a chemical penetrates a cell, it triggers a complex cascade of processes. One key element in this process is the aryl-hydrocarbon (Ah) receptor, which interacts with a large number of environmental chemicals. Once activated by binding of a chemical, the cytoplasmic receptor protein acts as a transcription factor regulating a large number of genes. This regulation perturbs important cellular functions and may even lead to the death of the cell. It is not yet possible to predict whether every chemical that binds to the Ah receptor has the same effects, or which concentration leads to which cellular phenotype.

The network "From contaminant molecules to cellular response: system quantification and predictive model development" is part of the Helmholtz Alliance for Systems Biology, coordinated by the Helmholtz Centre for Environmental Research (UFZ). This network is developing models designed to facilitate a better understanding of cellular processes in response to chemical exposure with the final aim to predict the resulting cellular fate. In the long term, such predictive models may not only be able to limit the number of experimental tests that are required to assess the risks of chemicals, but may also reduce the number of animal experiments needed. In addition, our project aims to deduce possibilities for averting negative side effects from the mathematical models developed.

We started with the assumption that the impact of AhR ligands on gene regulation, and consequently cellular damage, varies with the number of receptor-ligand complexes reaching the nucleus and subsequently binding to the DNA. Essential for a model describing such processes are the cellular distribution of the chemical (ligand) and its interaction with the Ah receptor. To take into account such parameters in the final predictive models (Fig. 1) and link them to the regulation of gene and protein expression, the relevant parameters for the distribution of chemical and receptor in the cell, and their interaction have to be determined. Fluorescence microscopy data provide the basis for the deduction of these parameters.

Fig. 1: An integrated model of cellular response to exposure to a toxin



The aim is to explain and render predictable the cellular response to exposure to a chemical via the use of an integrated model. To do so, the interaction and distribution of both, the chemical (BaP) and the aryl-hydrocarbon receptor (AhR) as well as their effects at the gene and protein level are investigated. Image: UFZ

How molecules are distributed within a cell

Modern microscopy methods allow the observation of processes at the cellular level. By using confocal fluorescence microscopy in our model system we are able to both examine the Ah receptor and its activating chemical (in our case benzo[a]pyrene, BaP) spatially and temporally resolved in the living cell. The polycyclic aromatic hydrocarbon BaP, which can be found, among other things, in automobile or industrial exhaust, serves as a model chemical for an entire class of AhR binding chemicals.

Using a so called FRAP (fluorescence recovery after photobleaching) experiment (Fig. 2) we derive data that can be analyzed by mathematical models, to deduce the relevant parameters for the movement, distribution and the interaction of the chemical and the receptor within a cell (Mai *et al.*, 2011). Fluorescence microscopy also enables us to observe cellular structures, from which true-to-life three-dimensional cell geometries can be reconstructed. By integrating the mathematical reconstructions of real cells with the parameters of the BaP/AhR interaction, the dynamics of that interaction can be followed or predicted in simulations (Fig. 3).

What are suitable parameters for prediction?

A chemical can trigger a complex cellular response, comprised of different aspects. The resulting change in the phenotype of a cell is not due to the perturbation of an individual signalling pathway,

Fig. 2: Laser microscopy for measuring particle movements in the cell

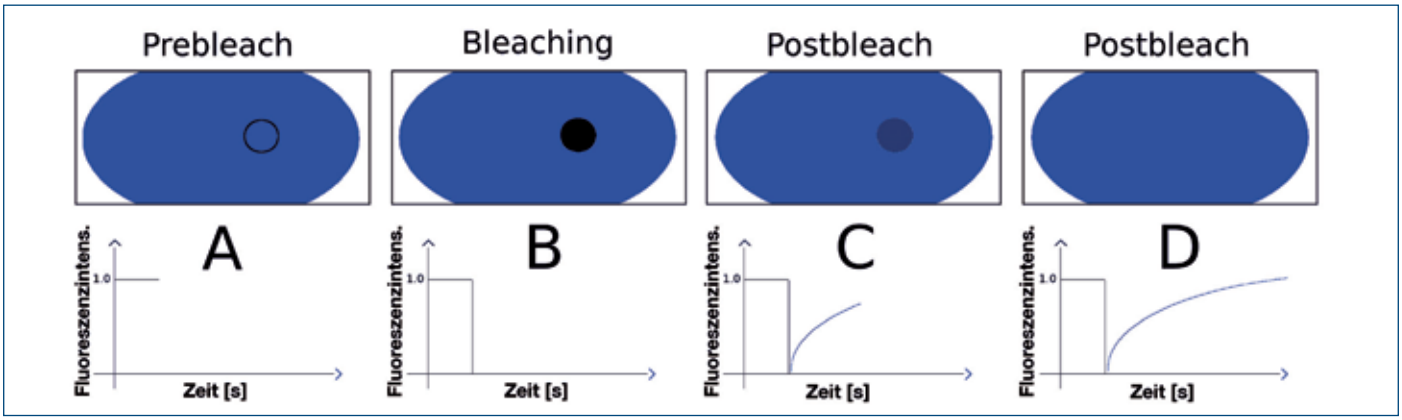


Diagram of a fluorescence recovery after photobleaching (FRAP) experiment. At the start of the experiment, a high-intensity laser beam is used to destroy irreversibly the fluorescence of the particles in a localised area. As time passes, the increasing fluorescence in that area provides information about the movement and reaction characteristics of the fluorescent particles being observed

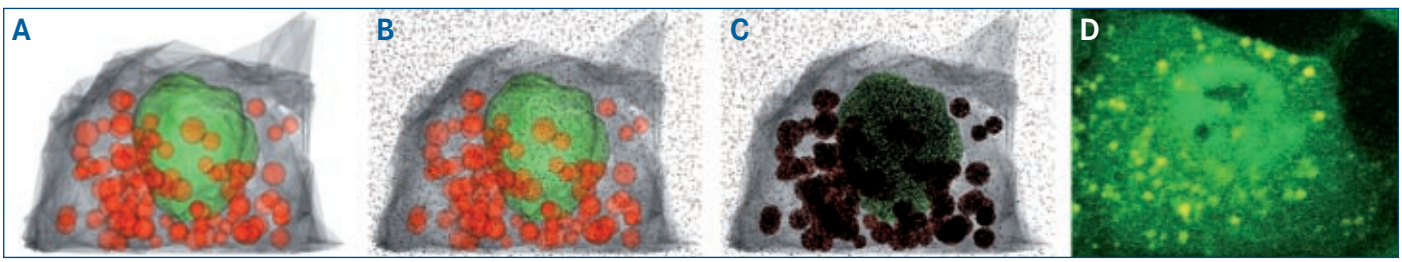
Image: UFZ

but is always the result of multiple disturbed signalling pathways. How does the binding of the receptor-ligand complex to the DNA affect gene and protein regulation? Which signalling pathways are influenced? What are the consequences on the cell's phenotype? First, molecular biology methods are used to detect the sites in which the receptor binds the DNA and alters its structure. Combined with information about the differentially regulated genes and proteins, as well as a particular phenotype (e.g. loss of adhesion or cell death), this provides the basis for extracting the essential components describing and predicting the cellular response. But how are these individual components linked? A newly developed method for modelling gene and protein networks, based on Zhegalkin polynomials, can be used to address this question (Faisal *et al.*, 2010). This special type of polynomial makes it possible to apply continuous optimisation methods even in the case of discrete data, such as gene and protein expression kinetics.

Model integration makes it possible to predict effects

After obtaining information on the dynamic interaction of the chemical and the receptor, as well as identifying the essential components for cell fate decision at the gene and protein level, it is possible to construct an integrated model that predicts concentration- and time-dependent effects on the cellular level. Not only environmental chemicals mediate their effects via the Ah receptor, but also drugs and endogenous metabolites, which play a significant role in the regulation of immune responses associated with sepsis,

Fig. 3: Model and observed results in harmony



Simulations in true-to-life 3-dimensional cell geometries illustrate the absorption and distribution of the chemical (benzo[a]pyrene, BaP) and its interaction with the aryl-hydrocarbon receptor (A–C) as can be observed in living cells (D). Reconstructed cell with cell nucleus and chemical-absorbing intracellular structures (A), simulation immediately after adding BaP (B), after 1 hour of exposure to BaP (C), matching observation in a real cell with the aryl-hydrocarbon receptor (green) after 1 hour of exposure to BaP (yellow) (D).

Image: UFZ

asthma and tumorigenic processes, can bind and activate the Ah receptor. As such, our models are not only significant for predicting toxic effects of chemicals. Prediction of the required therapeutic dosage for drugs modulating immune responses via the Ah receptor is only one of the many other possible applications.

Supporting the next generation of researchers

The HIGRADE graduate school at the Helmholtz Centre for Environmental Research (UFZ) runs an introductory course in systems biology. Students are introduced to both experimental and theoretical principles, and the course attracts keen interest from other Helmholtz centres.

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On the basis of a resolution adopted by its Assembly of Members in 2004, the Helmholtz Association promotes open access to research results and encourages its scientists to publish their findings in accordance with open access principles.

For text publications there are two options. Using the so-called “gold road” to open access, research results are published in primary open access journals, which in systems biology include Molecular Systems Biology[1] and BMC Systems Biology[2]. After a quality check by peer review, submitted manuscripts are published electronically with free access, usually in return for payment of a publication fee. Many publishers also permit secondary publication of articles from subscription-based journals[3]. Using this “green road” to open access, authors can deposit a manuscript of their publication in an online repository, thereby also making it freely accessible. Overall, open access increases the visibility of research and facilitates a swift discussion of current findings.

The Helmholtz Open Access Project[4] is supporting the implementation of open access with information and advice. Helmholtz Association scientists are involved as authors or editors of open access journals. A majority of the 17 Helmholtz Centres operates institutional repositories for secondary publication. Another focal point of the project is open access to research data, which especially in systems biology is of particular importance for the integration of many records and different types of data.

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imaging beyond the diffraction limit

New high-resolution microscopy methods for biomedicine

by Stefan Hell

Imaging processes are among the most important sources of data contributing to a deeper understanding of life processes. However, complex biological systems can only be described quantitatively if the dynamic aspects of those systems within a cell are also taken into account. This requires high-resolution microscopy methods which make it possible to pinpoint and track cellular processes accurately. Only the accurate spatial and temporal description of cellular processes can enable realistic, mathematical modelling of those processes. For precisely this reason, the light microscope has long been a key instrument of life science research, even though until recently its diffraction did not permit precise observations to be made at the nanometre level. However, latest developments are revolutionising the performance of this key instrument. The research group led by Prof. Dr. Stefan W. Hell is investigating at the Max Planck Institute for Biophysical Chemistry in Göttingen and at the German Cancer Research Center & BioQuant in Heidelberg new methods of optical far-field microscopy with diffraction unlimited resolution that make it possible to examine cellular structures and processes on a nanometre scale.

Diffraction limits the visibility of cellular structures

There is no question that light-wave diffraction is a fundamental physical phenomenon. That is why for more than a century the dogma prevailed that diffraction fundamentally limits light-microscopy resolution. Ernst Abbe systematically uncovered the princi-

Fig. 1: The STED principle

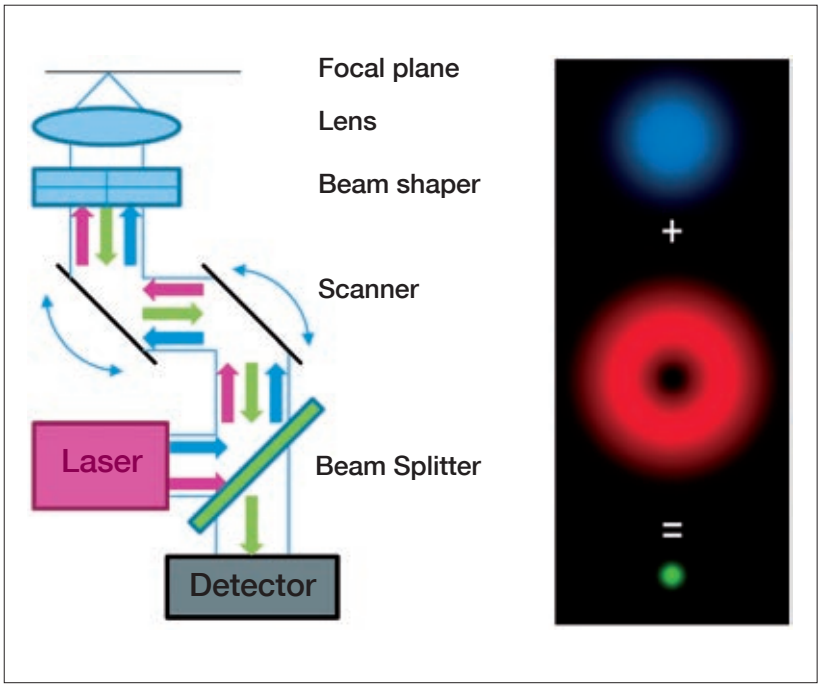


Image: Dr. J. Engelhardt, DKFZ

A laser system delivers an excitation beam (blue) to excite fluorescence and a depletion beam (red) to quench fluorescence. The beams are conducted via a colour beam splitter and a scanner through a colour-dependent beam shaper and focussed by the lens on the object in the focal plane. The beam shaper selectively changes only the red depletion light so that at the focal plane it is distributed in a ring around the blue focal spot. This ring-shaped depletion light switches off the fluorescence at the edge of the diffraction-limited excitation spot so that only molecules in a much smaller central area fluoresce (green area). The fluorescent light (green) is collected by the lens, passes through the colour beam splitter and is observed by the detector. The scanner scans the beams over the object, thereby generating a high-resolution image of the object.

ples of optical resolution in a microscope around 150 years ago and went on to construct the formula for diffraction-limited resolution that appears in physics and biology textbooks everywhere:

$$d \approx \frac{\lambda}{2n \sin(\alpha)}$$

In this formula, d stands for the minimum distance required between two similar details of an object in order for them to be seen as distinct under a microscope, n is the refractive index of the medium and a the angle of aperture of the lens.

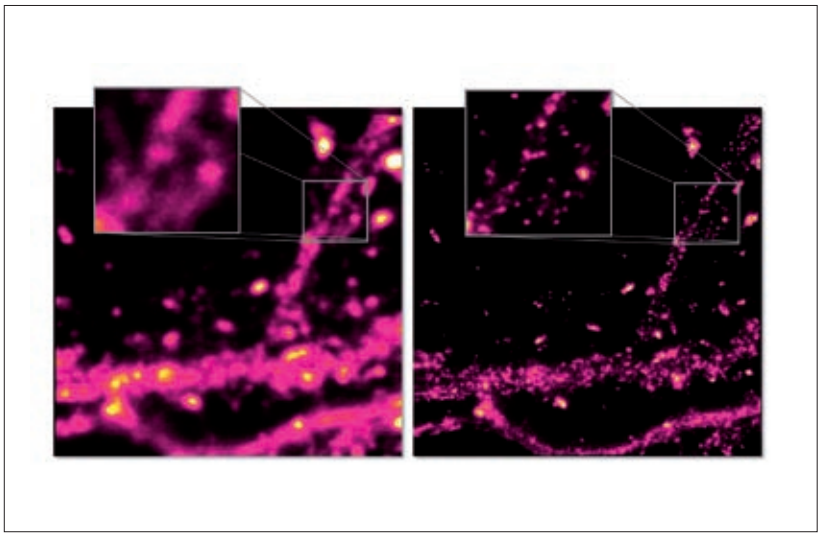
The problem…

As the formula reflects that a ray of light cannot be focused on a spot smaller than approximately 200 nm. Consequently, all fluorescence molecules on that spot fluoresce simultaneously. Moreover, no matter how small an object may be, due to the limits of diffraction, it will appear as a large spot of light about 200 nm in size. So how is it possible, despite this omnipresent phenomenon of diffraction, to observe, that is to resolve separately, similar details of an object that are close together?

…and its solution

The answer seems simple. Some kind of mechanism is needed to ensure that individual details of an object within a diffraction-limited spot no longer light up simultaneously, but rather sequentially, so that they can be detected separately. Though this approach sounds straightforward, it was not realised until 1994, in the STED microscopy method developed by Stefan Hell. STED, or stimulated emission depletion (Hell, 2007 and 2008), means disallowing the excited state of a fluorescent molecule. In order to do this, the molecule is irradiated with light so that after excitation it reverts immediately to its ground state.

Fig. 2: Confocal image from the hippocampus of a mouse brain



The left-hand picture is a confocal image (10x10 µm) of a sample (T. Dresbach, University of Heidelberg) from the hippocampus of a mouse brain. The presynaptic protein bassoon was stained with ATTO565 dye. In the right-hand image the STED beam was switched on. The inset in particular shows clearly that the STED method makes it possible to resolve much finer structures and cellular details of the synapses.

Image: Dr. M. Reuss, DKFZ

A molecule excited to fluorescence is actively forced back to its ground state, and thus depleted, when irradiated with a sufficiently intense light pulse at a wavelength within the emission spectrum of the dye. The surplus energy is picked up as an additional light particle in the beam, but that need not concern us here. The crucial factor is that the fluorescence molecule can no longer fluoresce, even if hit by an excitation beam. In other words, a depletion beam can be used for selective quenching of the dye molecule's ability to fluoresce. In order to increase the resolution of the fluorescence microscope, the depletion beam is positioned in a ring shape around the diffraction-limited spot. This depletion ring quenches the fluorescent molecules on the periphery of the diffraction-limited spot so that only molecules right in the centre can glow. The excitation and depletion beam do thus only need to be scanned across the object in order to pick up the fluorescent molecules successively. That automatically produces a higher-resolution image of the specimen (Fig. 1). In order to describe the new resolution quantitatively, a root term must be added to Abbe's formula to include the intensity of the quenching light I and the saturation intensity I_s at which the depletion beam quenches half of the fluorescent molecules. The new formula

$$d \approx \frac{\lambda}{2n \sin(\alpha)\sqrt{1+\frac{I}{I_S}}}$$

shows that the STED microscope resolution can be determined via I and is no longer limited.

Application

Researchers currently using STED are already achieving a resolution on biological samples that is ten times better than that achieved with a conventional light microscope. The process is often used to show structures of cell nuclei, microtubules and vesicles, but also of viruses and receptors. STED helps in itemising protein distribution for the purpose of clarifying neurological questions, viral infection pathways and numerous other questions where conventional fluorescence microscopy fails due to lack of resolution. Figure 2 shows an example of the in-

Fig. 3: Molecular orientation made visible

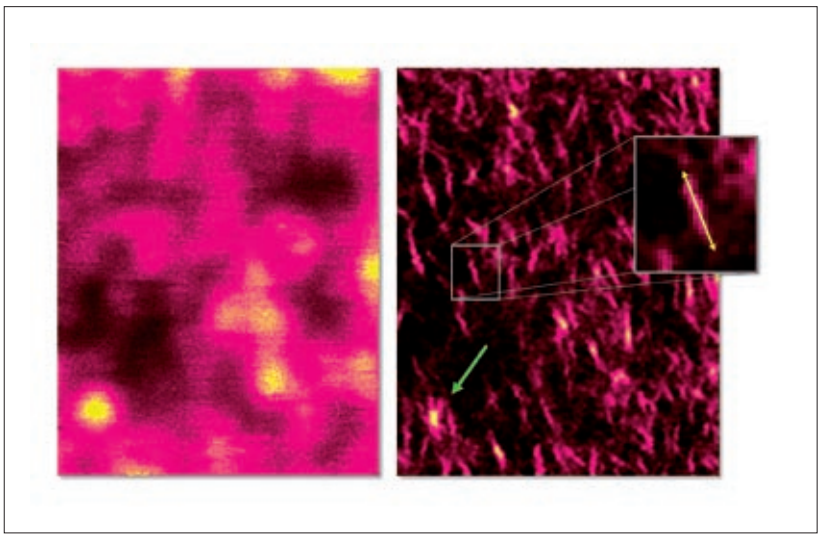


Image: Dr. M. Reuss, DKFZ

MOM (molecular orientation microscopy) STED (right) is used to map the dipoles of individual molecules as fine lines, thereby indicating the molecules' orientation (yellow arrow). If several molecules are close together (green arrow), they appear star-shaped due to their differing orientations. In contrast, in the confocal image (left) the individual molecules can neither be resolved, nor is it possible to determine their orientation individually.

crease in resolution. The higher definition makes it possible to show smaller objects separately and to quantify them. In multicolour applications, co-localisation of substances can now be directly detected or reliably ruled out down to 20 nm. Certain questions can thus even be clarified more quickly and reliably via direct observation of the molecular participants rather than by drawing indirect biochemical conclusions.

But STED can do even more. For example, other newly developed beam-shaping methods developed at the BioQuant Center (Fig. 3) can be used to indicate the orientation of dye molecules directly (Reuss *et al.*, 2010).

The future

The latest developments by the Hell research group at the BioQuant Center show that the systems for STED, which were originally still complex, can be constructed much more simply and virtually without any loss in performance. That is a decisive step toward making this new process fit for standard use, and a prerequisite for disseminating it worldwide and making it available to large numbers of scientists for the purpose of clarifying pressing questions in biomedicine.

The research project in brief:

Prof. Dr. Stefan W. Hell is director of the Max Planck Institute for Biophysical Chemistry in Göttingen, where he heads the NanoBiophotonics department. Since 2003 he has also headed the Optical Nanoscopy unit of cooperation between the Max Planck Society and the German Cancer Research Center in Heidelberg. Stefan Hell is the inventor of the STED method. The optical processes developed by him and his colleagues for high-resolution imaging of cellular and sub-cellular structures delivered the first-ever far field light microscopy images with resolutions down to the 20 nm regime.

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what makes cells move?

Activity sensors provide insight into the regulation of cellular dynamics

by Perihan Nalbant

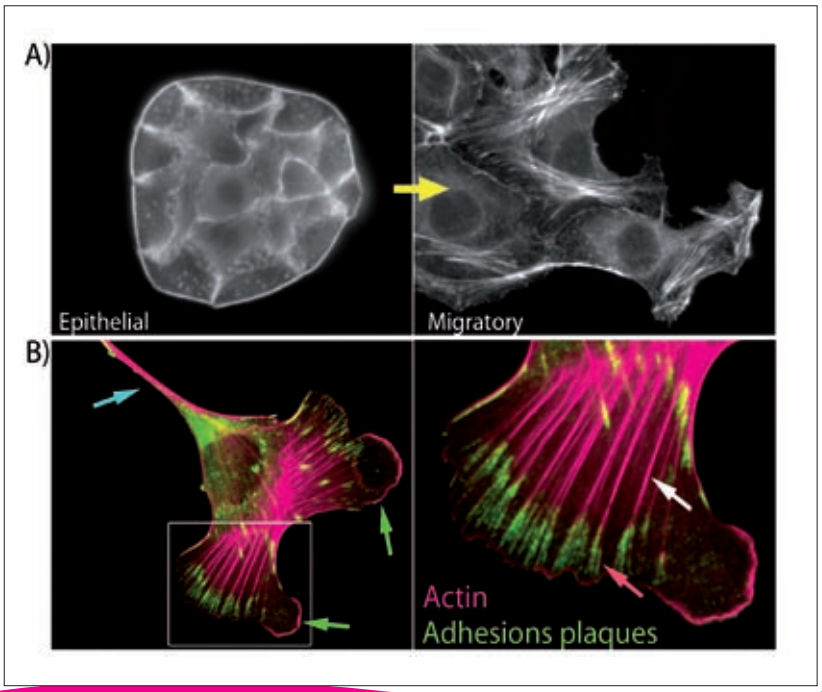
Many physiological processes, such as the development of organs in an embryo or wound healing, require the reorganisation and migration of cell populations. The dynamic movements of the individual cells involved in these processes must be precise and efficient in order to ensure proper organisation of the overall migratory programme. Aberrant regulation of the temporal and spatial coordination of this dynamic system can lead to uncontrolled pathological migration, such as during tumour metastasis (Yilmaz and Christofori, 2009).

During the transition from a quasi-motionless (epithelial) state to a migratory behaviour, cells undergo dramatic changes in shape (Fig. 1A). The actin cytoskeleton, a polymer system that continuously undergoes dynamic changes and can form a variety of higher-order structures, plays a pivotal role in this morphological transition. Particularly, the leading edge of migrating cells develops membrane protrusions to actively enlarge the cell surface, while contractile actin filaments in the rear area pull the cell body in the direction of migration. At the same time, the cell is anchored to the 3-dimensional environment via special points of adhesion (Fig. 1B). Controlled assembly and disassembly of these focal adhesions enables the cell to maintain its polarised shape during the migratory process and to simultaneously regulate the direction of migration (Yilmaz and Christofori, 2009).

Rho GTPases: master regulators of cell migration

Our work focuses on understanding how dynamic cell structures are coordinated during cell migration by intra-cellular signalling cascades. It focuses specifically on the Rac1, RhoA and Cdc42 proteins of the Rho GTPase family as they are the master regulators influencing the overall behaviour of actin-based cell structures (Yilmaz and Christofori, 2009). Specifi-

Fig. 1: The organisation of the cytoskeleton in migrating cells



A) Epithelial cells embedded in clusters of cells can be stimulated to migrate by external signals, such as growth factors. The actin cytoskeleton has been stained.
B) (Left) Migrating cells form a polarised shape with dynamic protrusions at the leading edge (green arrow) and a contractile rear area (turquoise arrow). (Right) Traction forces are generated by long actin filaments (white arrow) and transferred to the extracellular matrix via specialised focal adhesions (orange arrow).

Image: Perihan Nalbant

AG Nalbant. From left to right: Diana Moser, Nina Schulze, Olga Müller, Perihan Nalbant, Vera Schultz, Melanie Gräßl, Johannes Koch, Bettina Wagner (Photo: Perihan Nalbant).

cally, Rac1 regulates cell protrusions, while RhoA is essential for the formation and organisation of contractile actin filaments, which provide traction forces. Finally, Cdc42 is associated with the generation of antenna-like dynamic projections known as filopodia and is essential for the orientation of the cell during the migration process. The Rho GTPases are not permanently active throughout the cell. Rather, they act like molecular switches that can be switched on or off. Spatio-temporal activation of these switch proteins is determined by a complex interplay between upstream regulatory proteins and among the three Rho GTPases themselves. However, the underlying principles that coordinate such reciprocal interactions and the overall dynamics of cell migration are still unknown.

Measuring and manipulating the activity of Rho GTPases

In order to better understand this molecular network, we and our collaborators have developed fluorescence-based biosensors that make it possible to visualise the cellular distribution of activated Rho GTPases in individual living cells (Kraynov *et al.*, 2000; Nalbant *et al.*, 2004; Pertz *et al.*, 2006). Essentially, these biosensors report cellular activity by the local change in their fluorescence signal depending on the quantity of active protein (Fig. 2). Precise measurability of such local activities within a cell makes it possible to correlate the corresponding protein functionalities temporally and spatially with the dynamic assembly and disassembly of cellular structures involved in the migration process (Machacek *et al.*, 2009; Nalbant *et al.*, 2009).

Fig. 2: Two different biosensor strategies for visualising active Rho GTPases in living cells

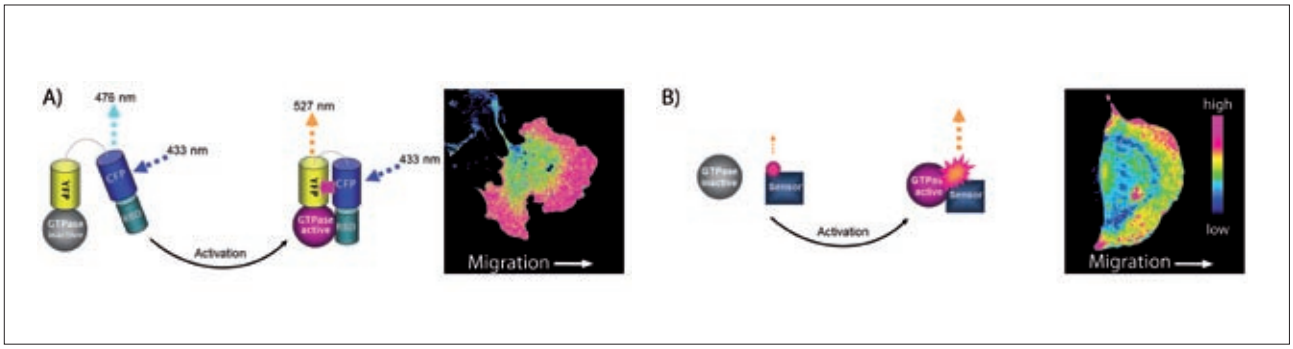


Image: Perihan Nalbant

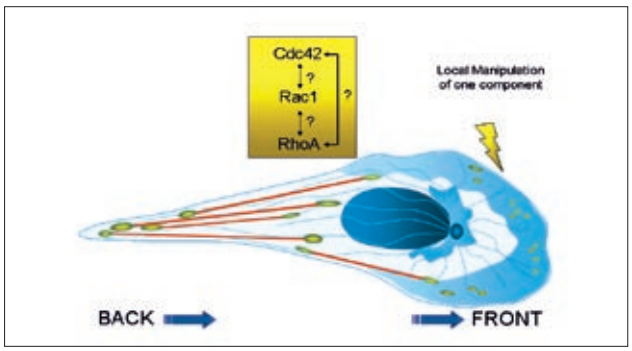
A) The RhoA-biosensor is based on the photophysical process of fluorescence resonance energy transfer (FRET). For detection, the specimen is irradiated with blue light (458 nm) under the microscope. The energy of this light is absorbed by a part of the molecule (CFP). When the Rho GTPase is in its inactive form, the energy absorbed during irradiation is released again, mainly in the form of turquoise-coloured light (476 nm). When the Rho protein is in its active form, the overall structure of the sensor changes, bringing the two fluorophores (CFP and YFP) in the sensor closer to each other. Depending on the distance between them, energy can be transferred between the fluorophores. Due to this process, light at a wavelength of 527 nm is generated (yellow light). Microscopic imaging of the cell and measurement of light at this wavelength enables us to measure the spatio-temporal distribution of the active Rho protein in the cell (Pertz *et al.*, 2006). The activity is shown in false colours, with warmer colours representing increased activity.

B) The biosensor for measuring Cdc42 activity consists of a protein fragment that can only bind to the active GTPase. The fragment is coupled to a fluorophore that, upon interaction with the active GTPase, reacts to the change in its environment with a significant change in its fluorescence intensity. Measurement of the amount of fluorescence in different areas of the cell using the microscope thus enables visualisation of the corresponding quantity and distribution of active Cdc42 protein (Nalbant *et al.*, 2004).

Measurement of such correlations can provide important evidence for the role of Rho GTPases in the coordination of the cell shape. Very recently, photoactivatable and photodeactivatable versions of individual Rho proteins have been developed to gain more direct insight into the complex interactions of Rho protein signal networks. These engineered versions of Rho proteins can be selectively switched on and off by light, thereby allowing direct control of the spatio-temporal distribution of the activated form (Wu *et al.*, 2009). In combination with computational modelling, such a direct experimental strategy makes it possible to decipher signal networks that determine the spatio-temporal regulation of Rho GTPases and the dynamic behaviour of migrating cells (outlined in Fig. 3).

Finally, a deeper understanding of the underlying signalling networks involved in cell migration can provide a foundation for therapeutic approaches in medicine, for instance in the treatment of cancers.

Fig. 3: Diagram of a migrating cell



In order to form and maintain the polarised cell shape and a directional forward movement, several cellular reactions must be coordinated. Reciprocal control by the Rho GTPases enables spatio-temporal control of dynamic structures during this migratory movement. Local manipulation of selected GTPases makes it possible to examine the logic of complex, spatio-temporally organised Rho GTPase networks (Image: Perihan Nalbant).

The research project in brief:

Perihan Nalbant is a junior professor at the Centre for Medical Biotechnology (ZMB) at the University of Duisburg-Essen. Her research group is investigating the molecular mechanisms that regulate cell migration.

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the dynamics of the cellular skeleton

How molecular "speckles" can reveal cellular building blocks

by Leif Dehmelt

The development of multicellular organisms, including humans, is characterised by numerous changes in the shape of individual cells. These changes are driven by cellular structures, collectively known as the cytoskeleton. Unlike the rigid macroscopic skeleton of animals, the cytoskeleton is very flexible and dynamic. It consists of a multitude of different filamentous structures that are assembled and disassembled inside the cell via polymerisation and depolymerisation of individual soluble components (Fig. 1, top section).

Actin filaments and microtubules, which are made of the protein building blocks G-actin and tubulin, play an especially important role in this process. These types of filaments have an asymmetric structure along which molecular motor proteins drive directional transport. On the one hand, such transport processes are fundamental for the spatial organisation of the cell interior. In addition, the movement of these motor proteins also generates forces that push the cytoskeleton against other cell components, thereby effecting changes in cell shape. In recent decades, many details of the mechanisms by which individual components of the cytoskeleton are regulated were uncovered. However, higher-level mechanisms, that direct how different components of the cytoskeleton affect each other and how these mechanisms cause the filamentous structures in the cell to organise and influence the cell shape, are still largely unknown. Nerve cells (Fig. 1, below) are especially interesting in this regard, due to the dramatic changes in cell shape that occur during their development.

Measuring the dynamics of the cytoskeleton

In our research group, we are pursuing a modern, systems biology approach that combines quantitative experimental studies with computer modelling in order to gain a deeper understanding of cellular processes. The quality of such models depends upon the quality of measurable experimental data, which typically represent cellular chemical reactions or interactions between individual proteins. Studying changes in cell shape also requires precise measurements of the mechanical processes driven by the dynamic cytoskeletal rearrangements. A special technique known as speckle microscopy is often used in experiments investigating these dynamic properties of the cytoskel-

Fig. 1: Spatial organisation of the nerve cell cytoskeleton in the early stages of development

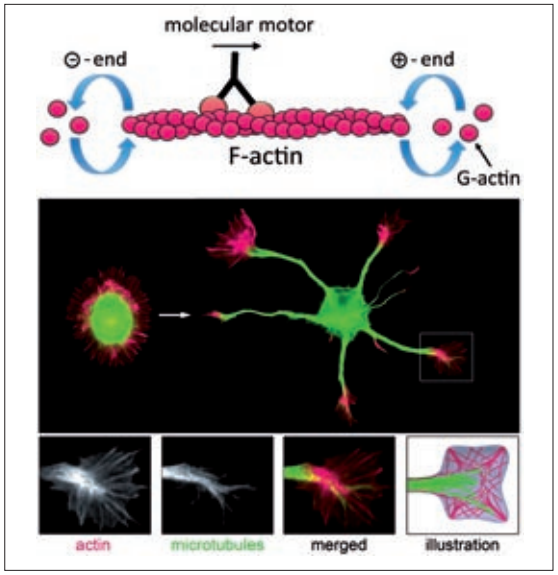


Image: Leif Dehmelt

Above: Diagram of an asymmetric actin filament (F-actin) formed by polymerisation of individual, soluble G-actin building blocks. The asymmetric structure of the filament facilitates directional transport by means of motor proteins.
Below: Two kinds of cytoskeletal filaments are stained in a developing nerve cell – actin (red) and microtubules (green). A structure in the peripheral area of the cell, known as a growth cone, has been magnified (reproduced from Dehmelt *et al.*, 2003 with the permission of The Society for Neuroscience).

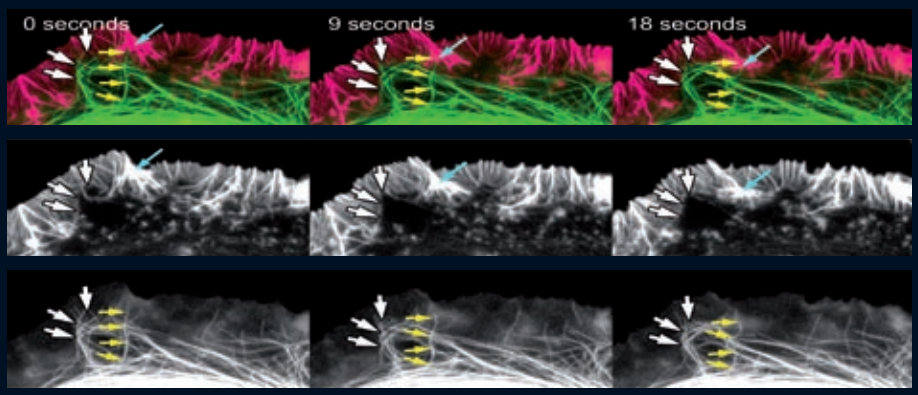


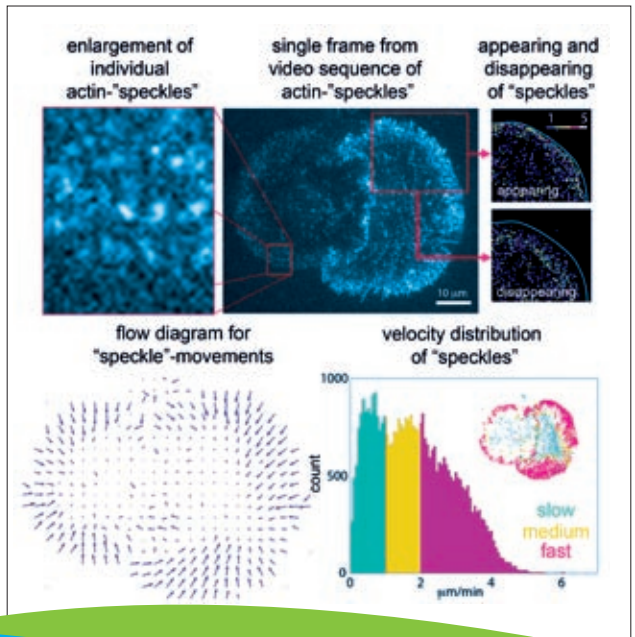
Fig. 3: Interactions between cytoskeletal components in cells
Actin filaments (red) are subject to constant flow (turquoise arrows), which also shifts microtubule filaments (green) towards the cell centre. Under the influence of this force, the microtubules are often swept back towards the cell center by the actin cytoskeleton (yellow arrows). However, a large group of microtubule filaments (white arrows) is able to counteract this force, which thereby influences the spatial arrangement of the actin cytoskeleton and indirectly alters cell shape (Dehmelt *et al.*, 2003). The entire film sequence can be viewed via the internet link www.mpi-dortmund.mpg.de/forschungProjekte/AGs/Dehmelt/forschung/index.html (Image: Leif Dehmelt).

eton (Danuser and Waterman-Storer, 2006). With this method, individual filament components are marked with a dye, such as the fluorescent green protein from the jellyfish *Aequorea victoria*, and a very small quantity is introduced into cells. These individually marked components can be detected as tiny speckles inside cells. As long as the components are present in a soluble form, they move very fast with typical thermal motion (molecular diffusion). As a result, in speckle microscopy they are not confined to a small region of the cell and thus not detectable as individual molecules. However, if these individual components are incorporated into dynamically reorganising cytoskeletal filaments, their mobility is dramatically reduced because of the larger size of these cytoskeletal structures, enabling more precise location in cells. Careful microscopic analysis can even track individual marked filament components in cytoskeletal filaments in living cells (Watanabe and Mitchison, 2002). The sudden appearance of one of these speckles corresponds to the incorporation (polymerisation), while the disappearance of a speckle corresponds to the loss (depolymerisation) of a filament segment. Lateral shifting of individual speckles are a result of movements within the cytoskeletal structure, which are often not precisely measurable with conventional techniques. Figure 2 shows how this technique can be used to measure the continuous flow of the actin cytoskeleton in living cells.

Organisational principles of the cytoskeleton: models for the integration of experimental data

To improve our understanding of the spatiotemporal organisation of the cytoskeleton, it is not sufficient to measure only the dynamic characteristics of an individual cytoskeletal component, such as actin filaments, since these components communicate with a multitude of other components via complex mechanical and chemical mechanisms (Dehmelt and Bastiaens, 2010). Figure 3 illustrates how the interaction between microtubule- and actin-mediated forces impact cytoskeletal structure and thereby influence the cell shape (Dehmelt *et al.*, 2003; Dehmelt *et al.*, 2006). Unfortunately, the number of components that can be tracked simultaneously in a single cell is limited by the number of available distinguishable

Fig. 2: Measuring filament dynamics via speckle microscopy



The actin cytoskeleton was marked with individual molecular speckles in neural precursor cells. Computer-aided analysis of the dynamic characteristics of these speckles makes it possible to track and quantify the spatial distribution of polymerisation and depolymerisation as well as lateral movements of actin filaments. (Image: Leif Dehmelt, Tomáš Mazel).

Dehmelt research group: (left to right and top to bottom) Leif Dehmelt, Nicolas Brauckhoff, Julia Arens, Melanie Grässl, Olga Müller, Tomáš Mazel, Johannes Koch, Anja Biesemann, Silke Gandor, Pia Jeggle, Magda Krejczy, Verena Hannak.

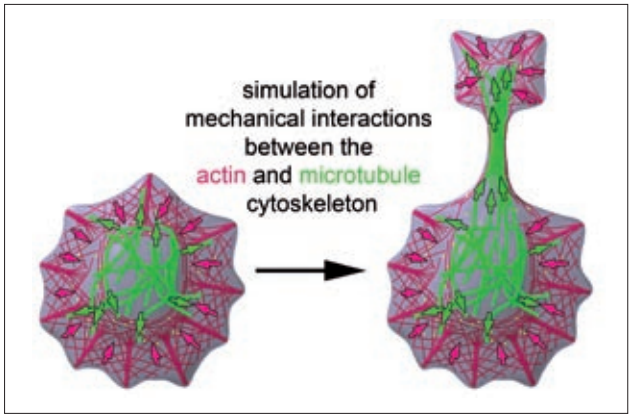
The Max Planck Institute for Molecular Physiology in Dortmund.

dyes. Consequently, not all possible interactions can be measured simultaneously. In order to overcome this limitation, we integrate observations from various experiments into dynamic, mathematical models of the main cytoskeletal components (outlined in Fig. 4). Predictions about cell dynamics derived from such models are checked by means of selective experimental manipulations. Through close coordination between theory and experiment we can thus decipher the fundamental principles that determine how the cytoskeletal structure is organised by means of mechanical and chemical interactions with other cell components, and how these processes lead to changes in cell shape. Direct observation of molecular speckles helps us in this process to gain insight into such complex biological processes in cells.

The research project in brief:

This work is funded by the FORSYS Partner Initiative of the German Federal Ministry of Education and Research under the heading "Interactions of cellular systems in the development of nerve cells" and carried out in the Department of Systemic Cell Biology at the Max Planck Institute for Molecular Physiology and at the TU Dortmund.

Fig. 4: Diagram of a simple model for simulating changes in cell shape



Three components – actin (red), microtubules (green) and the plasma membrane (grey) influence each other via mechanical forces and spontaneously form a cellular outgrowth. For simplicity, numerous other components that produce mechanical forces and regulate the chemical and mechanical characteristics of the cytoskeletal filaments are not depicted (Image: Leif Dehmelt).

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the man who brings light into the black box

Portrait Timm Schroeder

Timm Schroeder has seen a lot – and not only on our globe. In the manifold fields of methods essential for his work, he also moves like a globetrotter who feels at home in many places. Born in South Africa, he mainly grew up in Erlangen and Munich, Germany, where he also went to university. His researcher's career later took him to Harvard Medical School in Boston, USA, and again and again to Japan. As a PhD student and young postdoc he was in Kyoto several times and later spent two years in Kobe.

He seems to have taken a particular liking for the land of the rising sun, even though he often had to hurry through the lab on tiptoes because the mandatory safety shoes were too small and bigger ones could not be organized on-site. Perhaps it was the charm of the foreign and the unknown that fascinated the scientist. "In Japan, even getting to work is an adventure in itself; you cannot read a thing and you have to rely on the help of others and hope that somehow everything will turn out well", Schroeder recalls. Anyway, he has picked up enough of the Japanese language to make himself understood in the most important areas of life.

His enthusiasm for Nippon is also reflected in the calligraphies and photos that are waiting to be hung on the walls of his new office at Helmholz Zentrum in Munich. Here, the 40-year-old leads an independent research group at the Institute for Stem Cell Research since 2004. His objective: understanding haematopoiesis, i. e. the development of our diverse blood progenitor cells. However, classical molecular and cell biology will not do for him. For him it must be computer technology and sophisticated modern imaging procedures.

The young scientist dealt with this particular subject as early as when writing his PhD thesis. His focus then was on the so-called notch signalling pathway which is crucial for various developmental processes. "Initially, we knew notch from the fruit fly *Drosophila*, where the protein blocks the differentiation", explains Schroeder. "But then we found that this factor can effectuate just the opposite in the development of blood progenitor cells."

A finding that amounted to a change in paradigm at that time, it has been broadly accepted long since and also holds true for skin and nerve cells. But Schroeder learnt quite a different lesson from this fact: "Actually, I always found just the opposite from what everybody expected", he says. Then he realized that quite often his recorded data amazingly resembled those of his colleagues. "This owes to the fact that in the traditional biological approach often a starting point is described and then again an endpoint." All steps in between virtually vanished in a black box leaving a lot of room for interpretations.

Schroeder gives an example: "If we have one solitary cell at the starting point and a few days later, at the next reading point we have four cells, what do you think has happened?" In all probability two steps of division have occurred. But no one can really be sure. "There also could have been three or four divisions and, on top, a few cells have died. Or the initial cell itself has not divided at all but other cells have wandered into the area of investigation." The same is true for molecular processes. Instead of evaluating starting and endpoints he wants to observe these processes continuously on the single-cell level. This way the black box can be filled with knowledge and high-quality data can be obtained in the end, which can be utilised sensibly for systems biology models.

"In bone marrow you find just one haematopoietic progenitor cell within a total of 50,000, so if you average the whole population you learn nothing about stem cells", says Schroeder. Via microscope he focuses on individual cells over a longer time span instead, and follows how they change, when, for instance, they develop special characteristics or which role different molecules are playing in this process. This observation demands high-quality image processing – sometimes for as long as two weeks.

Very soon, however, Schroeder had to learn that standard computing tools were not able to master this task. So, the biologist

Timm Schroeder (Photo: Helmholtz Center München).

started writing his own software. He had already acquired the necessary know-how during his academic studies – driven by the charm of the new and the desire to learn. The result of these efforts was Timm's Tracking Tool. This name, putting a smile on the blond scientist's face, arose from the necessity to find a three-letter file extension for labelling the data: .ttt. "Initially, this was just for internal use, but then the name stuck." By now Schroeder makes his software available to other research teams via his group's homepage.

In some issues of course, Schroeder collaborates with specialists from other fields, despite all his interdisciplinary know-how. "I am able programme but if we want to generate mathematical models based on our data, we certainly need mathematicians and bioinformaticians", he explains. Within the Helmholtz Alliance on Systems Biology he works together with Fabian Theis. The mathematician and physicist heads a group specialised in computational modelling at the Institute for Bioinformatics and Systems Biology at Helmholtz Zentrum in Munich and holds a chair for mathematics of systems biology at the Technische Universität München (TUM). They virtually do research door by door. "This proximity is an incredible advantage, since our personnel are in close communication this way – not only via telephone and e-mail but also in joint meetings and by lunching together." He feels this is very important, because finding a common language is not the only stumbling block in doing interdisciplinary research. Different cultures of publishing have to be considered, too. "Here it is of advantage if such issues can be discussed from person to person."

Schroeder's wish to become a scientist became apparent early in his life. His mother once handed him one of his old drawings from art class. The task then was as follows: Draw a picture of what you want to become when you grow up. "On the picture I'm sitting in a forest exploring something unidentified", he remembers with a smile. When starting his university studies, the direction he would take would long be settled, and Schroeder already passed his civilian service in the laboratory. He learnt a lot of practical things there. As a student he mainly focused on understanding the facts and their interrelations.

The worst experience during his university studies: "I had the impression to be thwarted every time I wanted to do more than was required – for instance, I was often rejected when I inquired on student internships because nobody had any use for a student from the lower semesters." That is why Schroeder always keeps his door wide open to young academics. An intern is just as welcome as a computer science student looking for a job as student assistant. "Every motivated person can contribute to the issue with his individual capabilities" is the scientist's credo.

Schroeder' s career was focused on molecular cell biology and modern technologies right from the beginning. And yet he allowed himself an excursion into classical biology during his basic studies: "I once spent a semester break in a whale research station in Canada. This was an incredible experience and yet, I did not see my future in this field."

But his love for nature persisted. He acts out this love in his holidays together with his wife in far away countries. And then he also devotes himself to his hobby of nature photography – with the same demand for high-quality pictures as in the lab.

The interviewer was Stefanie Reinberger.

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BioQuant

Interdisciplinary Research Center for Systems Biology at Heidelberg University

by Angela Oberthür and Roland Eils

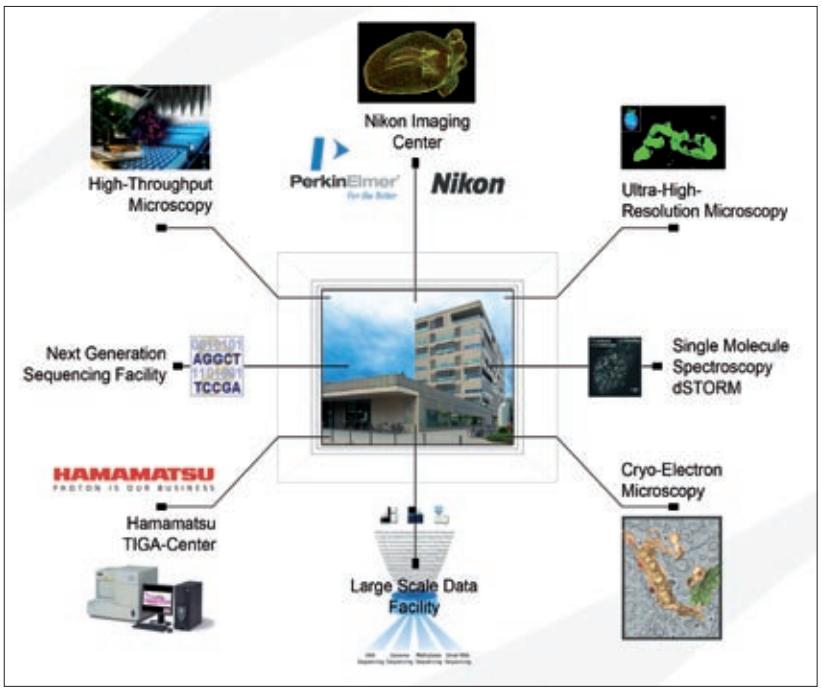
300 staff members, 30 research groups, more than 10 independent junior groups

There is no other place in Germany where so many internationally renowned scientists from a wide variety of disciplines exclusively deal with questions of systems biology. BioQuant integrates these successful systems biology research activities in Heidelberg in the setting of a university-based research center, crossing both faculties and research institutions borders. Established in 2007 as the first center for systems biology in Germany, BioQuant belongs to the leading systems biology centers in Europe.

The core area of scientific work of BioQuant lies in the quantitative and multiple-scale description of cellular processes with particular emphasis on medically relevant topics.

With the implementation of the international Curriculum Systems Biology at Heidelberg University and numerous independent junior scientist groups, BioQuant has set standards for systems biology education & training in Germany.

Fig. 1: BioQuant technology platforms



The technology platforms of BioQuant mainly focus on quantitative live-cell imaging methods

Image: BioQuant

Research focus: Cellular systems

Since the BioQuant Center's inauguration in 2007, a leading research community in the field of systems biology has been established on the Heidelberg campus, primarily dealing with medically relevant cellular processes, such as virus-host cell interaction[1], signal transduction[2], apoptosis[3], gene regulation[4] & epigenetics[5], and cell architecture[6].

So, the FORSYS-ViroQuant center (co-ordination: Prof. Roland Eils/Prof. Jürgen Wolfrum), sponsored by the Federal Ministry of Education and Research, has been an integral part of BioQuant since 2007. The interdisciplinary research programme addresses SYSTEMS BIOLOGY OF VIRUS-HOST CELL INTERACTIONS and pools the expertise of acknowledged virologists, molecular biologists, mathematicians, physicists, computer scientists and engineers at the BioQuant center. FORSYS-ViroQuant focuses on the causative agents (pathogens) of AIDS (HIV), and hepatitis C (HCV).

This effective, interdisciplinary collaboration within ViroQuant was the starting point for the international collaborative project PathoSys (co-ordination: Prof. Roland Eils/Dr. Lars Kaderali), sponsored by the European Commission since 2010. Within PathoSys, international academic and industrial partners develop new mathematical methods for the understanding of virus and host-cell interactions, based on the hepatitis C virus. BioQuant co-ordinates the EU network PathoSys.

Other core research activities of BioQuant are the numerous scientific projects dealing with SYSTEMS BIOLOGY OF CANCER, part of the Helmholtz Alliance on Systems Biology, initiated by the Helmholtz Association (network "Systems Biology of Cancer", co-ordination: Prof. Eils/Prof. Klingmüller).

THE CENTER FOR MODELLING AND SIMULATION IN THE BIOSCIENCES (BIOMS, co-ordination: Prof. Ursula Kummer/Prof. Willi Jäger) is a theoretically oriented research programme, established as early as 2004. Mainly supported by the State of Baden-Württemberg and the Klaus-Tschira-Foundation as well as the participating research institutions (University of Heidelberg, European Molecular Biology Laboratory and Max-Planck-Institute for Medical Research), a research programme was launched, at the time unique in Germany, supporting the development of new theoretical approaches and methods for systems biology in Heidelberg. This local programme specifically concentrates on the support of junior scientists in systems biology. Major parts of the CELLNETWORKS EXCELLENCE CLUSTER (Coordination: Prof. Hans-Georg Kräusslich), forwarded under the excellence initiative, are based at BioQuant, also.

In addition to these current research programmes, numerous new projects have formed at BioQuant, supported by the FORSYS partner programme, SysTec, MedSys, GerontoSys, CancerSys, Virtual Liver, SysMo and also by the ERANet"EraSysBio".

Focus on technologies: *in vivo* imaging & data management

Extracting quantitative and spatio-temporally detailed data is essential for a realistic, mathematical description of biological systems. For this purpose high-performance IT infrastructures and a comprehensive technology platform were established at BioQuant, at present mainly focussing on imaging methods. The repertoire offered ranges from high-content to high-resolution live-cell microscopy and high throughput approaches up to cryo electron microscopy. The "RNAi Screening Facility" and the recently established "Deep Sequencing Facility" complete this sector. The Nikon Imaging Center (see page 68 in this issue) and the Hamamatsu Tissue Imaging and Analysis Center-TIGA (see contribution in systembiologie.de, issue 2) are both integral parts of BioQuant's technology platform. To cope with the large quantity of data that is generated by these sophisticated technologies, a Large Scale Data Facility (LSDF) has been established at BioQuant with financial support from both the Federal government and

BioQuant with its award-winning architecture offers ideal conditions for top-level interdisciplinary research.

the State of Baden-Württemberg. In 2012, the LSDF will have a data storage capacity of approximately 6 petabyte. It will be one of Europe's largest data storage units exclusively available for life sciences research.

Focus on the Promotion of junior scientists

To meet the great demand for interdisciplinarily trained young scientists in systems biology, BioQuant has established its own interdisciplinary education programme shortly after its foundation. The interdisciplinary Major Curriculum of Systems Biology (co-ordination: Prof. Ursula Kummer/ Prof. Ursula Klingmüller) has been an integral part of the international Master Programme "Molecular Biosciences" at Heidelberg University since the winter semester 2008/2009. The faculty includes highly renowned scientists from both theoretical and experimental disciplines.

More than one third of the research groups at BioQuant are independent junior groups, supported by distinguished extramural funding programmes. For example, two junior groups (Dr. Anne Marciniak and Dr. Ilka Bischofs-Pfeifer), supported by the European Research Council with *ERC Starting Grants*, find ideal conditions at BioQuant for realising their research projects in systems biology. Most recently, the first BioQuant junior group leaders have been offered full professorship positions after working at the center for just a few years.

Contemporary architecture supports interdisciplinary research

The basis for the Center's active systems biology research community was laid in Heidelberg as early as 2001. Then, a network uniting various disciplines and research institutions with the topic "Quantitative Analysis of Cellular and Molecu-

Fig. 2: BioQuant research programmes

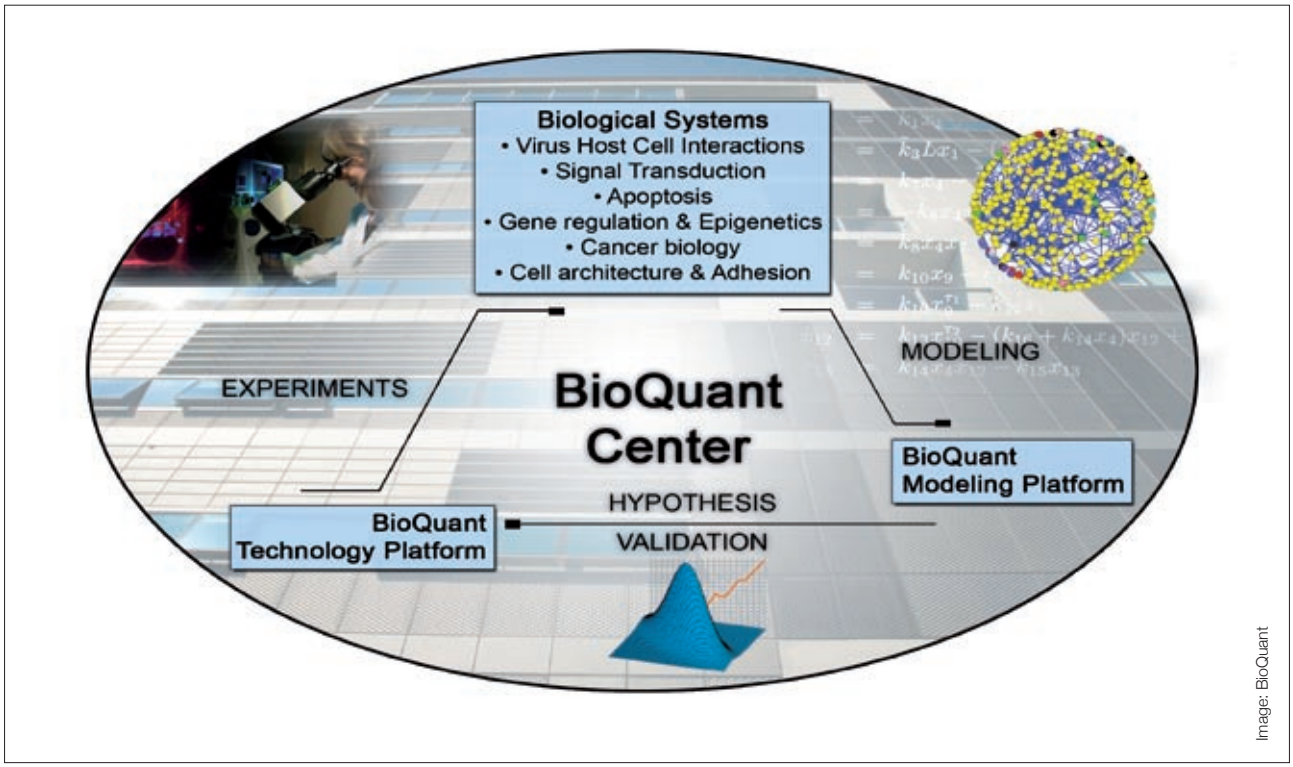


Image: BioQuant

lar Bio-systems" evolved within a competition launched by the Baden-Württemberg government to establish interdisciplinary life sciences centers. The University of Heidelberg successfully bid for the formation of a central structure for this interdisciplinary network, merging the internationally renowned core research fields of the Heidelberg campus, molecular biological and biomedical research, and the expertise in scientific computing. Since the opening ceremony in spring 2007, approximately 5,000 square metres solely dedicated to research and education in systems biology are at disposal at BioQuant.

This ample space is situated in a building of architectural excellence. Only recently has Bioquant been awarded the Hugo-Härling Prize for exemplary buildings in the state of Baden-Württemberg by the Association of German Architects (BDA) This architecture encourages scientific exchange between the various disciplines with its central communication area and offers ideal conditions for the center's experimental and theoretical research groups.

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BioQuant
MODEL base of LIFE

the nikon imaging center at heidelberg university

A facility for advanced light microscopy as a collaboration between Academia and Industry

by Peter Bankhead and Ulrike Engel

Light microscopy has undergone a breathtaking development during the last quarter of a century. Advanced light microscopes for biomedical research share little resemblance with the microscopes that everybody encounters during biology courses in school. For example, confocal laser scanning microscopes, which employ a focused laser beam to scan through living cells and obtain a full 3D-view from this information within seconds, are among the microscopes most widely used by biologists today. These instruments and their siblings, the spinning disk confocal microscopes, are completely controlled by software to synchronize optics, lasers, shutters and detectors (Fig. 1). At most, the researcher usually needs only to take a glimpse through the eye pieces at the beginning of an experiment, before turning around to look at the computer monitor. The Nikon Imaging Center at Heidelberg University was founded five years ago to make techniques such as these widely accessible to researchers on campus.

Confocal laser microscopy needs fluorescent tags

All the microscopes in the facility are based on detecting fluorescence. The components of animal cells, however, are not naturally fluorescent – or are only slightly so. As a result, researchers have to stain what they want to see with fluorescent tags. For fluorescence imaging in live cells, researchers largely depend on fluorescent tags that are made by the cell itself. Although most animal cells do not provide us with fluorescence, there are a few shining exceptions – like the luminescent jelly fish *Aequoria victoria*. We use a protein from this jellyfish called Green Fluorescent Protein (GFP) as a fluorescent label. The first biological experiment published with GFP involved its introduction in neurons of the worm *Caenorhabditis elegans*, and was published by Chalfie's lab in 1998 in Science (Duggan *et al.*, 1998). Thanks to a subsequent innovation, yellowish, orange, red and far-red fluorescent proteins are now available to researchers (Shaner *et al.*, 2007). The Nobel Prize in Chemistry 2008 was awarded jointly to Osamu Shimomura, Martin Chalfie and Roger Y. Tsien (who will be one of the Honorary Speakers at ICSB 2011, see also page 98) for the identification of these fluorescent proteins and their adaptation for use in biological research. Our ability to perform live imaging owes as much to this discovery as to any other technical development. By introducing the genetic coding information (DNA) into cells, researchers can use this color palette to make cellular components visible in the microscope. The fluorescent proteins are like glowing tags that highlight only one thing in the household of the cell, if engineered to do so by the biologist. To image their fluorescence we use higher energy light to excite the fluorescence: the green fluorescence is triggered with blue light, the red fluorescence with green light.

These tags then allow us to record multiple images focused at different depths and at different times, and thereby produce multicolour, 3-dimensional movies that show how dynamic processes evolve over time. In Figure 2 we see a cell that is expressing a green fluorescent protein as it engulfs a smaller cell, which is a yeast that has been tagged with a red dye. This was imaged by laser confocal microscopy in different focus planes over time. Without cutting physically, the instrument has optically sliced the cell in many optical sections, after which a computer program allows us to reassemble vertical sections (as seen in Fig. 2B) or any other arbitrary section (Fig. 2C). This is important to clearly determine whether the yeast cell has really been internalized into the other cell, or if it might just be hiding behind the cell a few micrometers away.

Challenges in light microscopy: detecting details in live cells

Animal cells are tiny: with typical diameters around 0.02 mm, they are too small to be seen by the naked eye. Nevertheless, viewed up close such cells resemble vast spaces, divided into

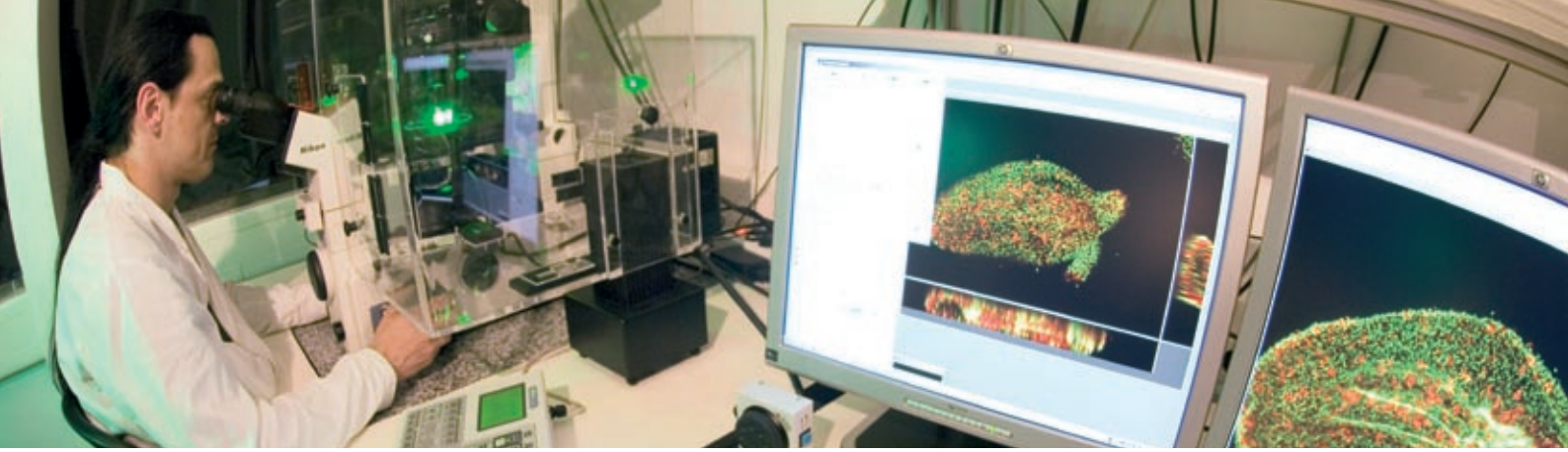


Fig. 1: Laser scanning confocal microscope at the NIC@Uni-HD (Photo: Markus Winter).

many sub-compartments like the rooms in a house. Each of these sub-compartments then contains further, much smaller objects and machinery, which in turn have specific functions that we would like to understand better as a means to understanding the whole. So the question is how much of this detail we can visualize by microscopy.

Fortunately, unlike the walls of a house, the membrane of the cell provides only a limited hindrance to seeing the activity inside. For the fluorescent light produced by the fluorophores, the cell membrane and adjacent cells are more or less transparent. However, when relying upon light to try to see smaller and smaller things one soon encounters a fundamental problem. This is that anything smaller than a few hundred nanometers - which is around the order of the wavelength of visible light - ends up looking like a small, diffuse blob, regardless of the object's true shape. Increasing the magnification further does not help: we have reached the so-called 'diffraction limit'. This prevents us seeing all the detail that we would like, and inevitably causes the images of very small structures to look blurry.

Other microscopy techniques are far superior in resolving structures. For example, electron microscopy uses beams of electrons instead of light to image much smaller structures close to molecular resolution (fractions of a nanometer). However this comes at a cost of requiring that the sample is 'fixed' (and therefore dead) and very thinly sliced before imaging, so cannot be used to view living cells. Yet while achieving a high resolution is often very desirable, it is not always necessary or the main requirement for understanding. As an analogy: if we had never seen a pair of scissors before, from their shape alone we may or may not correctly guess what they are used for - but if we could acquire a blurry view of the scissors actively cutting up paper then we would immediately know the function, even if the shape might remain unclear. And so one of the major benefits of light microscopy is its ability to record live cells moving and acting, but this then brings us to the challenge of speed, and being able to see really fast processes. While you are seated at a table and not moving at all, the cells inside your body are bustling with activity, moving and dividing. Inside the cells themselves there is even more activity. In Figure 3 we show a cell that has ingested

Fig. 2: Time lapse sequence of a cell (*Dictyostelium*) expressing green fluorescent protein engulfing a red fluorescent yeast

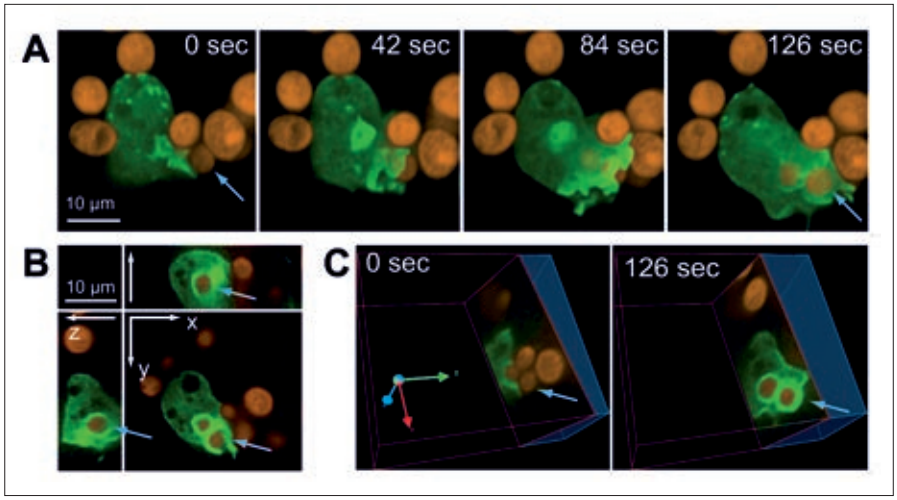
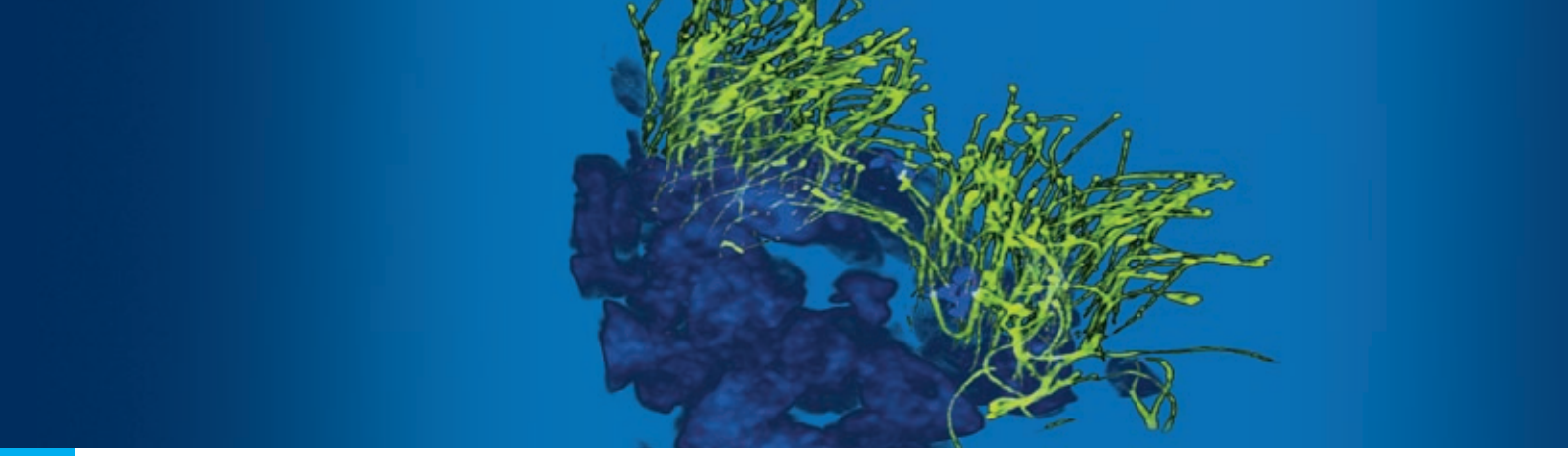


Image: Ulrike Engel

For each time point 26 optical slices were acquired with a confocal laser microscope. (A) Within 2 minutes the cell has internalized the yeast (arrow). (B) Vertical and horizontal slices of the last time point. (C) Arbitrary sections showing that the yeast (arrow) is first outside, and than inside the green cell.



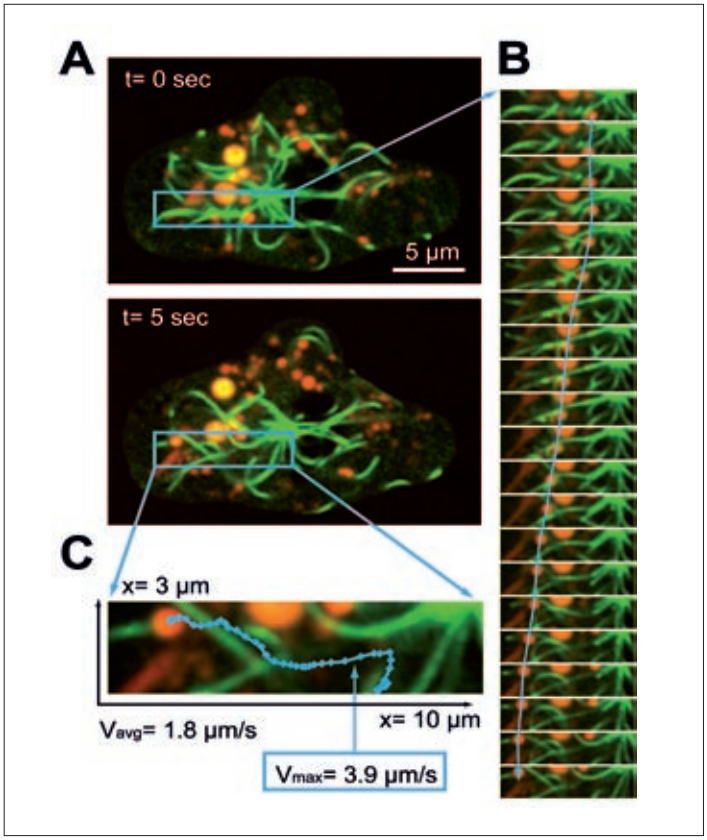
Bundle of motile cilia in the epithelium of a frog embryo. Microtubuli staining in yellow, nuclear staining in blue (Image: Ulrike Engel).

material and is now moving this back and forth. These little food packages, called vesicles, are transported at up to 3 micrometers per second. This is three million times slower than a cyclist traveling at 30 km/h, but because it happens at such a small scale that it is fast enough that we need 10 frames per second in image acquisition to see what is going on. Taking such time lapse movies, we can follow the transport along the cellular transport rails, called microtubules. The vesicles can be automatically traced by specialized computer programs, and their paths analyzed to extract their direction and velocity (Fig. 3C).

Where do we go: Light microscopy goes super resolution

The aim in light microscopy is to achieve higher resolutions and fast live imaging together. The last 5 years have brought tremendous advances, where several sophisticated techniques have allowed us to 'see past' the diffraction limit, and view smaller details in living cells (reviewed in Chi *et al.* 2009). All major microscopy companies are now trying to make these instruments available to biologists. Nikon has launched two new kinds of super-resolution microscopes: STORM and SIM, which use very different principles. While STORM relies on single molecule imaging approach to assemble super resolution from 10,000–100,000 images, SIM uses a striped illumination pattern to transport high frequency information into the realm visible to light microscopy. For the Nikon SIM, the Nikon Imaging Center in Heidelberg was chosen as the first installation site outside the factory worldwide. While a lot of optimization still needs to be done, we are now producing images of cells that have less blur and more structural detail that had been possible before (Fig. 4A).

Fig. 3:



A cell where microtubules are highlighted by GFP (green) has taken up food and packaged it into vesicles (red). (A) Both cellular components have moved within 5 seconds of observation. (B) In a time lapse captured with a confocal laser microscope vesicles can be followed. (C) Automated tracking reveals the path of the vesicle and its velocity.

Image: Ulrike Engel

About the NIC at Heidelberg University

The instrumentation for advanced light microscopy is both elaborate and expensive, and often too expensive to be afforded by a single lab. The applications of the technology, however, cover research on isolated cells, developmental biology and areas of medicine. Providing access to these advanced microscopes and their use is the mission of the Nikon Imaging Center at the University of Heidelberg (NIC@Uni-HD).

The NIC@Uni-HD was inaugurated in 2005 as a central core facility of the University, representing a collaboration between Industry and Academia. This new way of providing researchers with cutting-edge technology was initiated by Prof. Thomas Holstein at the Zoology department (now Center of Organismal Studies) and the general manager of Nikon Messtechnik Germany, Jörg Kukulies, based on an existing NIC at Harvard Medical School in Boston. In this joint effort, Nikon Instruments provides cutting-edge microscopes, while the University provides staff and rooms. Other companies contribute to setups with cameras, filters, and even complementary confocal technology. The NIC@Uni-HD was the first imaging facility on the Heidelberg Campus where researchers could obtain training on microscopes before then using them by themselves. Starting with 5 microscopes, the number of setups at the centre has quickly doubled, and most of these serve different purposes: there are two laser scanning confocal microscopes, two spinning disk confocal microscopes, two upright fluorescence microscopes, a microscope for total internal reflection fluorescence (TIRF), two inverted fluorescence microscopes dedicated to live imaging and multipoint acquisition, a 2-photon microscope for imaging deep into living tissue, and the N-SIM mentioned above for super resolution microscopy.

In the last 5 years, the NIC staff, now a group of 4 people, has trained 600 researchers on their systems, resulting in more than 100 publications so far. The projects include the analysis of human heritable mental retardation, the study of how neurons migrate in brain formation, how cells polarize and divide, and the role of calcium waves in signal transduction. Even the interaction of two proteins can be monitored by

Fig. 4:

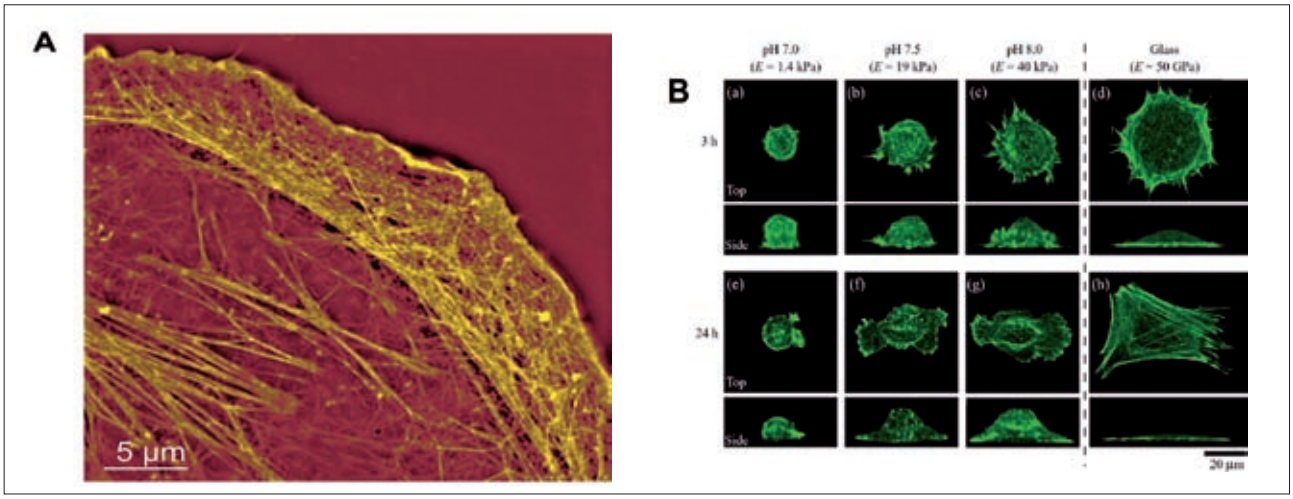
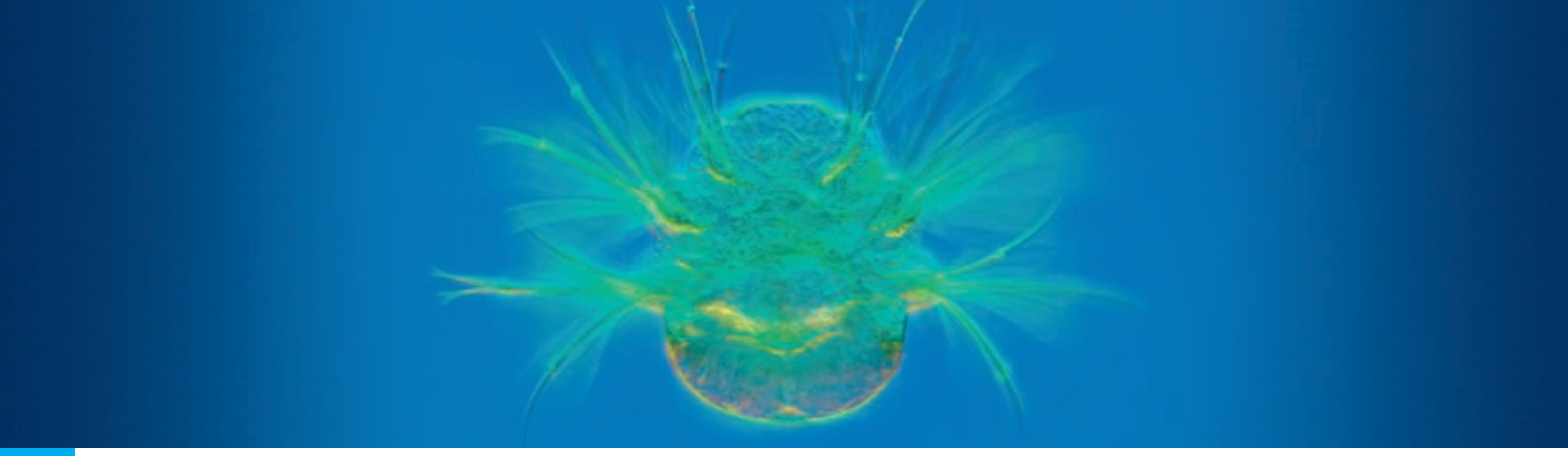


Image: A Ulrike Engel, B Copyright 2011 ACS

(A) Super resolution image by structural illumination microscopy of an epithelial cell. The staining for actin shows the dense meshwork of actin at the periphery of the cell and prominent stress fibers in the interior. (B) Adhesion of epithelial cells to substrates of variable stiffness after 3 hours (a-d) and 24 hours (c-h). On glass which has the highest stiffness, cell spreading is most pronounced (Yoshikawa *et al.*, 2011). Reproduction of this figure with kind permission of the Journal of the American Chemical Society.



Juvenile stage of the marine worm *Platynereis* in in differential interference contrast (Image: Ulrike Engel).

fluorescence microscopy. Microscopy is never the *only* tool that leads to the discovery and understanding of a mechanism, but together with molecular biology, biophysics and biochemistry, clever assays can be developed to prove that a protein acts as a pair of scissors in a cell, or that a component is responsible for dragging the spindle into a daughter cell. Figure 4B shows how a combination of chemical engineering and microscopy can help us to understand how cells attach to a substrate. On a soft substrate the cell cannot spread. However, as the substrate was designed to change its stiffness by modification of the pH in the surrounding medium, it can be shown, that at a higher stiffness the same cell spreads on the substrate and attaches well (Yoshikawa *et al.*, 2011).

In summary, the Nikon Imaging Center allows research groups whose main expertise is not imaging to make use of advanced microscopy to solve complex questions. The cutting-edge instrumentation is one necessary ingredient, while the expertise offered by the facility on how to operate the systems and plan experiments is the other.

Acknowledgments:
We would like to express our gratitude to Thomas Holstein (Center of Organismal Studies), who initiated the NIC, and to thank Günther Gerisch, MPI Munich, for providing cells and handling for the Dictyostelium experiments, and also Nikon and the DFG for ongoing support.

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microscopy at the highest level

The Life Imaging Center (LIC) at the University of Freiburg's Center for Biological Systems Analysis (ZBSA)

by Roland Nitschke

Over the last 25 years, due to the use of new illumination and detection methods and the resulting possibilities for carrying out experiments, light microscopy has become one of the most important methods used in life sciences. Today's modern optical technologies permit unimagined insights into biological details and mechanisms, often by the use of fluorescent protein markers. High-resolution spatiotemporal imaging has enabled to reveal the sequence of many biological processes for the first time, and in some cases to be mathematically modelled as well through subsequent analysis. This has significantly improved perception and understanding of even complex signal transduction pathways.

The problems associated with the use of new optical techniques are the technical expenditure required, the complexity of applying them, and the analysis of the images. Only properly trained and supervised scientists can make full use of the capabilities these techniques offer, which is why they are sometimes either under- or overestimated, or not even taken into consideration because of their complexity. It is not always possible to predict which technique will lead to the optimal result. The purchase of modern, specialised microscopes for a single laboratory is only conceivable, and only makes sense, if their capacity will be utilised in the long term. For this reason it is advisable to centralise microscopy techniques in cross-disciplinary, cross-faculty core facilities.

The Life Imaging Center (LIC) in Freiburg was established by Roland Nitschke and Wolfgang Driever in 2001 as a facility of the Collaborative Research Centre 592 (SFB 592). In 2008, the LIC, along with other central facilities for genomics, proteomics and metabolomics, moved into the Center for Biological Systems Analysis (ZBSA), which is overseen jointly by the rector's office, the life sciences faculties and the faculty of engineering.

State-of-the-art equipment

In an area of 400 m^2, the LIC has nine confocal microscopes, also for specialised methods (two-photon, high-speed, photomanipulation and photoactivation, spinning disk, laser ablation, multi-position) and seven wide-field microscopes for ratio imaging, FRET, TIRF and time-lapse images. A computer lab with twelve workstations, image analysis and visualisation software, and a further eight workplaces enables analysis up to publication quality and hosting of internal training courses with partners from industry. At present, in addition to the head of the centre, the LIC has five employees (computer scientists and technical assistants), who fill the equivalent of 3.5 full-time positions.

Usage, areas of expertise and training

The LIC has 295 users from all natural science faculties. It is also open to users from other universities as long as they are working in joint projects with research groups from Freiburg. Equipment capacity utilisation at the facility is on average more than 80%. Many LIC users work with living organisms or cell culture systems that are used in developmental biology, cell signaling research and systems biology. Some examples are model organisms such as the *Danio rerio, Arabidopsis, Drosophila, Xenopus, C. elegans* or mice, various parts of plants (root, leaf, bud), and cells from primary and long-term cultures and cell clusters (cysts, biopsies, brain sections). Most instruments at the LIC are specialised in long-term observation of up to ten days (including cell cultures under flow conditions) (Fig. 1; Boehlke *et al.*, 2010), in large-format, high-resolution images (Fig. 2; Tay *et al.*, 2011; Emmenlauer *et al.*, 2009), in object identification and tracking (Lienkamp *et al.*, 2010), and in the automation required for these purposes. Via the BIOSS toolbox, the resource and information centre of the BIOSS Excellence Cluster (EXC 294), more than 150 fluorescent proteins are available to users for their experiments. Since 2002, over 285 users have taken a one-week course in "Advanced Imaging Techniques in Microscopy" to introduce them to the theory and practical use of high-end microscopy.

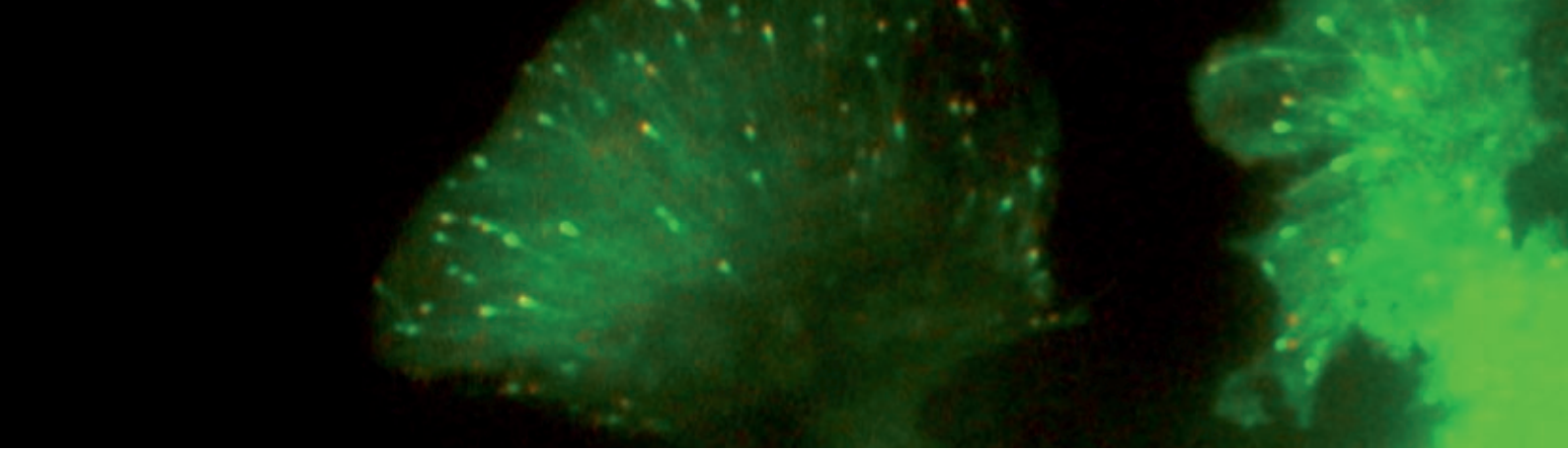


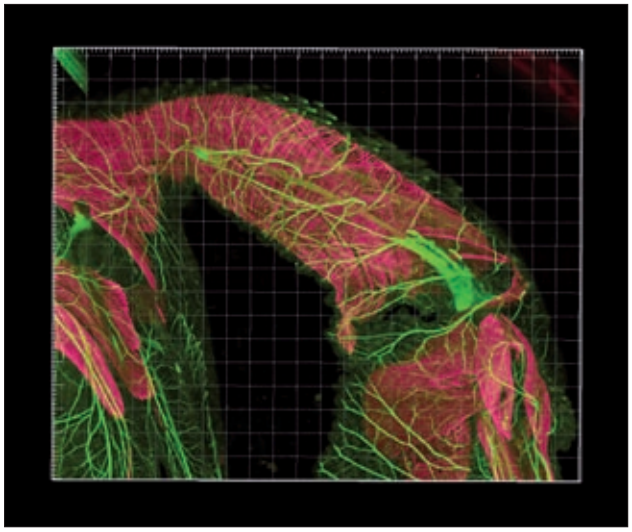
Fig. 1: Visualisation of the growth of microtubules by means of TIRF
Time-lapse image from an image series of stable transfected EB-1 YFP MDCK cells. The increased distance has been dyed red (Image: C. Böhlke, Med. Clinic IV, University of Freiburg).

The LIC is financed by the University, the SFB 592 and the BIOSS Cluster of Excellence. New equipment is acquired on the basis of funding applications by groups or individual researchers, who then transfer the equipment to the LIC.

Success through networking

In the BIOSS Cluster of Excellence, the LIC is working with the 11th Faculty of Engineering on new, intelligent microscope systems (4D analysers) that integrate microfluidics, automation and image processing. Its successful collaboration with the faculty of biology and the faculty of medicine is reflected in numerous high-quality joint publications (references 1–5). The LIC has collaborations with the Imaging Facility at the Max Planck Institute in Freiburg and at the Friedrich Miescher Institute in Basel.
The LIC is a member of the European Light Microscopy Initiative (ELMI) and an associate partner in Euro-BioImaging. Roland Nitschke, together with Elisa May, is German coordinator for EuroBioImaging and the German BioImaging network established in 2010 (**http://bioimagingde.org**). The LIC has longstanding partnerships with companies such as Carl Zeiss MicroImaging, Jena and Göttingen, and ibidi GmbH, Munich. Together with the German Federal Institute for Materials Research, Sigma-Aldrich GmbH and Schott AG, it has developed and patented tools for calibrating fluorescence microscopes (Resch *et al.*, 2008).

Fig. 2: Confocal 3D stack of a 7-day-old chicken embryo



Immuno-fluorescent dyes (green: nerve cells; red: muscle cells)
Size of dataset of 32 GB with 100 sub-images, each with 160 z-levels (Image: R. Nitschke, LIC, and S. Theiss, UCL London).

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tracking plant growth

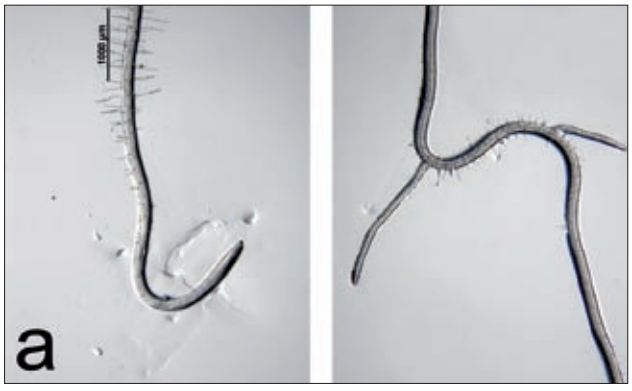
New microscopy technique renders root formation processes visible

by Klaus Palme, Bingshan Wang, Franck Ditengou, Roland Nitschke, Alexander Dovzhenko, Rainer Uhl and Olaf Ronneberger

Genetic techniques are indispensable for uncovering the details of complex biological signalling processes. However, the mapping of precise reaction mechanisms in a three-dimensional environment is best achieved by light microscopy. The 4D Analyzer platform delivers accurate quantitative new insights at high-throughput into the three-dimensional molecular networks of cells. For our model system, we used roots of the plant *Arabidopsis thaliana* to explore the interaction between environmental signals and endogenous (i.e. internally produced) development programmes in the *de novo* ('from scratch') formation of plant organs.

Unlike mammals, whose organs develop within a very short time frame during embryonic development, plants develop new organs throughout their life cycle. This enables them to adapt to continuously changing environmental conditions, to make optimal use of sunlight, and to use their roots to look for nutrients and water in the soil. But how do environmental signals induce the *de novo* formation of lateral roots, and how is this growth regulated? We have developed an approach combining genetics, biochemistry, imaging techniques, information technology and systems biology modelling to help us understand the molecular mechanisms involved.

Fig. 1: Mechanical stimulation of the *Arabidopsis* root tip by flexure with forceps induces lateral root formation



It has long been known that mechanical forces provided by wind and other environmental cues play a pivotal role in the development of plant organs. Growing roots are efficient at exploring the soil volume and growing around stones and into cracks. During this process, mechanical stimuli determine growth and permit the development of new roots. Remarkably detailed descriptions of this process have existed for more than a century (e.g. Darwin, 1880; Noll, 1900). However, only recently have we been able to map the molecular mechanisms behind these processes (for overview see Teale *et al.*, 2006; Santos *et al.*, 2010). The development of the 4D Analyzer platform and computerised methods of four-dimensional cell analysis play a crucial role in this achievement. Until now there has been no convincing molecular proof that mechanical forces reprogram plant development. The first indications came from locally inducible expression of the protein expansin, which normally only controls cell elongation. Surprisingly, local production of expansin led not to the expected cell elongation, but to the formation of a new leaf (Pien *et al.*, 2001). This indicated that changes in the biomechanics of the cell division zone at the tips of shoots could lead to the development of a new organ. Now, using molecular, genetic and optical methods, we have demonstrated that the creation of lateral roots is induced by mechanical flexure (i.e. bending) of root tips (Fig. 1).

Insights into the architecture of the gene networks involved

The development of lateral roots is determined by the coordinated expression of multiple genes. Transcriptom analyses of the temporal course of gene expression in lateral roots reveal

Klaus Palme tending thale cress, *Arabidopsis thaliana* (Photo: Klaus Palme).

that many of the genes involved play a role in the biosynthesis, transport, action and dismantling of the plant hormone auxin (Ditengou *et al.*, 2008; Paponov *et al.*, 2008). These different activities are summarised in a gene network model in Figure 2. We were able to prove the central role of auxin and its polar transport in controlling the growth of lateral roots (Paponov *et al.*, 2008). All gene expression data indicate that diversion of the flow of auxin (caused by uneven cell division) leads to the formation of a new root primordium, where new lateral roots then develop. In this process, the repressor protein IAA14 controls the auxin-specific transcription factors ARF7 and ARF19. If the proteins ARF7 and ARF19 are absent, no lateral roots can develop. As these two proteins block lateral root development at a later stage, we examined the reactions that are activated after mechanical stimulus.

Chronology of reactions in root tissue

In order to track at the cellular level the signalling processes that induce the formation of roots from tissue capable of division (pericycle cells), reporter genes for root growth processes were introduced into the plants. This made it possible to obtain information about the temporal and spatial sequence of processes that take place in a single cell after mechanical flexure of part of a root. Initially the reporters used were the proteins PIN1 and PIN2, which are polarly localised in cells and are thought to be responsible for directing the cellular outflow of auxin. Subsequently we used a promoter (DR5) that can be activated by auxin to find out which cells respond to the auxin signal and how strong this activation is. As shown in Figure 3, PIN1 (green) and PIN2 (red) are localised in cells of differing polarity. After mechanical bending, PIN2 does not alter its position, whereas PIN1 changes its position about three hours later, when it can be found on the opposite side of developing vascular cells. As a result of this reorganisation, auxin transport in these cells is blocked. With the help of the DR5 reporter, we were able to detect a local auxin blockage microscopically two hours after PIN1 changed its position. The auxin transport protein AUX1 then pumps auxin into the neighbouring pericycle cells, where it induces cell division and the creation of a new lateral organ.

The 4D Analyzer – a microscopy platform for live cell imaging

Biological signalling systems are tremendously complex: they involve highly mobile chemical signals and a flexible localisation of proteins, not to mention reactions composed of many

Fig. 2: Gene network model of the *Arabidopsis* root

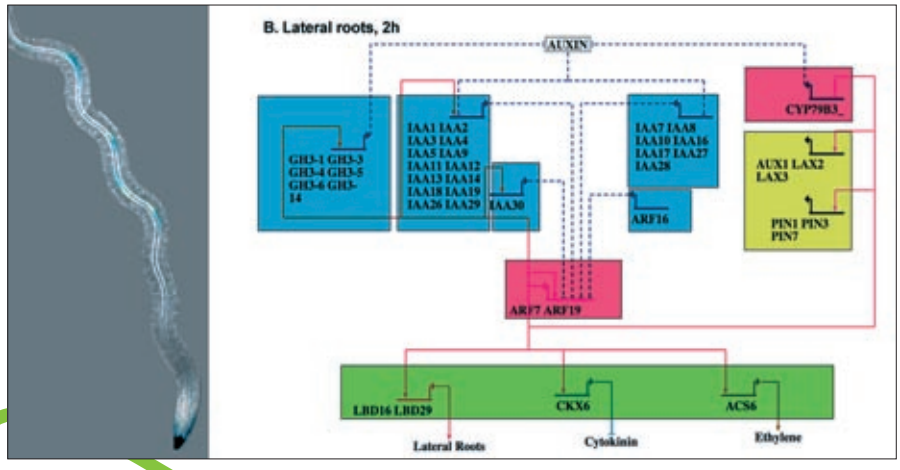


Chart: Klaus Palme

Left: Periodic oscillation of the auxin reporter DR5 in flexure zones.

Right: Gene network model of the regulation of genes that play an important role in auxin response.

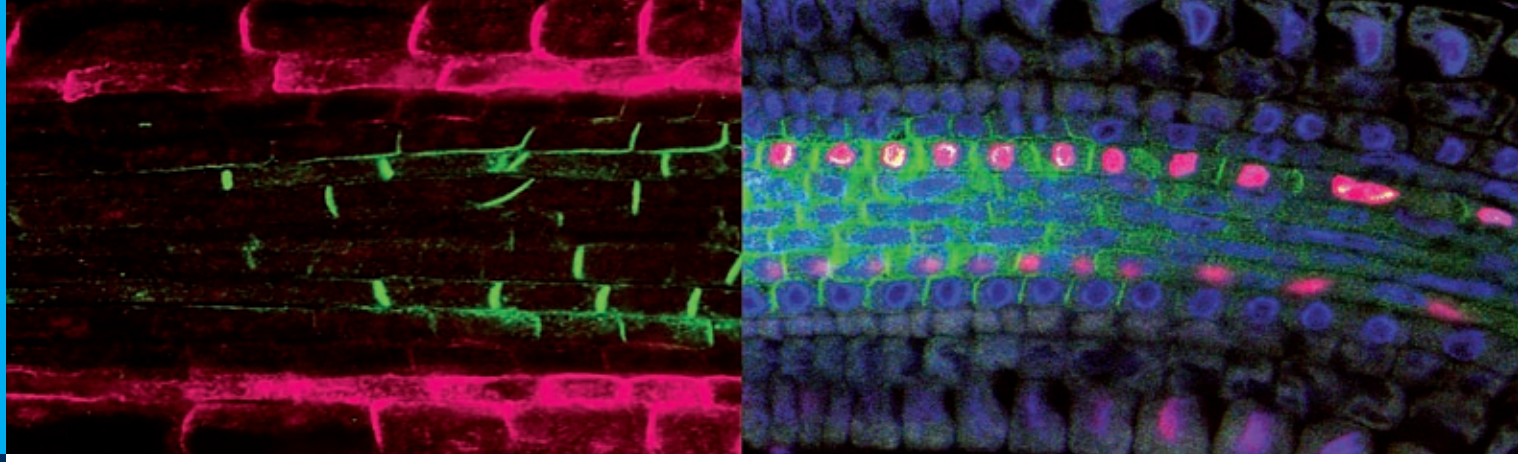
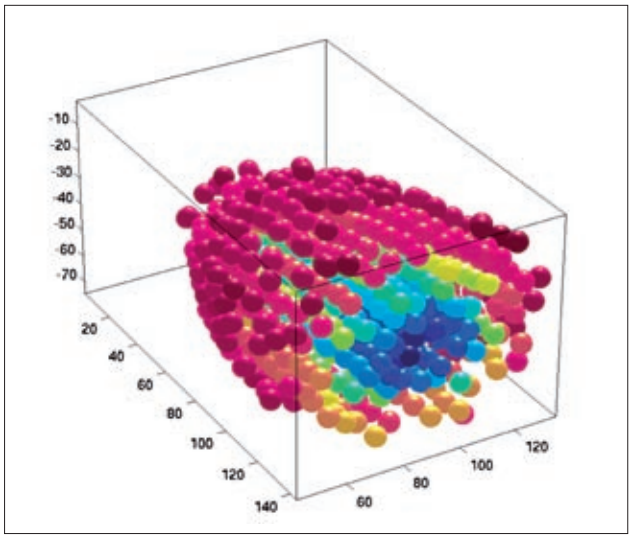


Fig. 3: Localisation of the polar-localised proteins PIN1 (green) and PIN2 (red) in mechanically flexed *Arabidopsis* roots (A). The rearrangement of the PIN proteins leads to a microscopically detectable blockage of the hormone auxin, which can be proven with the help of a pink-dyed auxin reporter in transgenic lines that contain the construct PIN1::PIN1- GFP, pDR5rev::3XVenus-N7 (Copyright: PNAS).

variable stages that can affect a large number of cells, usually with rapid information exchange. Though snapshots showing the reaction to mechanical stimuli, as described above, provide an initial insight into complex reaction events, they cannot map entire signalling processes. Only dynamic imaging techniques can deliver almost unimpaired observation and analysis of life processes in individual cells. Innovative optical techniques are therefore needed to understand complex living processes and reaction cascades. The close linking of molecular biology with optical technology has enabled us to develop new techniques and to integrate them into a new kind of light microscopy tool.

The 4D Analyzer is an automated microscope platform that combines spatially (3D) and temporally (+1D) resolved imaging with automated, intelligent image analysis. Its key function is to conduct fully automated experiments with living cells in real time, at maximum imaging speed, with minimal damage to samples. The integrated design makes it possible to switch between various applications such as FRET, FRAP, structured illumination, TIRF and wide-field fluorescence – all within milliseconds. It combines these applications with online analyses and interfaces for post-processing images producing visualisations of multidimensional datasets. This unprecedented efficiency makes it possible to record and visualise cellular structures and functions in real time and enables spatial representation, in 3D, of molecules in their signalling cascades. One can work through a complex set of measurements at many different sites in a specimen, then return to any site and retain a high level of reproducibility, making it ideal for conducting long-term observations in parallel. The resulting datasets can also be subjected to quantitative analysis via three- and four-dimensional pattern recognition algorithms, combined with self-learning strategies for intelligent segmentation (Schulz *et al.*, 2006). Using these techniques, we have developed an intrinsic system of coordinates for the *Arabidopsis* root and are able to make direct, quantitative comparison of the cells of different roots (Schmidt, Pasternak *et al.*, 2011; in preparation – Fig. 4).

Fig. 4: Visualisation of the cell nuclei at the tip of the *Arabidopsis* root



In designing the 4D Analyzer platform, one of our main concerns was to minimise the exposure of specimens to the device's observation light at each phase of measurement. Only then could we ensure that no artefacts would intrude, and that specimens would remain in as natural a state as possible, even during long-term study. One must, however, also take into account a kind of "biological uncertainty principle": every observation of a biological system interferes with that system, and that interference grows larger as precision of measurement and duration of exposure increase. For example, confocal measurement (i.e. illumination of a small area of a specimen by a scanning light source) delivers better 3D resolution than wide-field measurement, which involves illuminating the entire assay area. But the more one tries to accelerate confocal measurement, the more the specimen is

exposed to light. A key future priority, therefore, is to refine this technology in order to combine the resolution of a confocal microscope with the speed of wide-field measurements.

The 4D Analyzer microscope platform is a groundbreaking new tool, and not only for plants. We expect this method to have applications as a diagnostic instrument in many fields where quantitative visualisation of molecular processes in a cell opens up a new plane of investigation into biological systems.

The research project in brief:

Project name: The research project "4D cell analysis – quantitative 3D and 4D cell analysis in living organisms – novel instrumentation, computational tools, proof of concept applications" is a project coordinated by Klaus Palme, carried out within the framework of the German Federal Ministry of Education and Research (BMBF) funding programme "New Methods in Systems Biology". It forms part of the general programme "Biotechnology – Using and Creating Opportunities",
AUTOSCREEN for cell-based high-throughput and high-content gene function analysis and drug discovery screens. Sixth EU framework programme, thematic priority LifeSciHealth,
www.autoscreen.eu.
BIOSS – Centre for Biological Signalling Studies (research centre supported by the excellence initiative of the German Research Foundation DFG [EXEC 297])
www.bioss.uni-freiburg.de.
FRISYS – Freiburg Initiative for Systems Biology,
www.frisys.biologie.uni-freiburg.de.
FORSYS – Systems biology research units supported by the German Federal Ministry of Education and Research, www.forsys.net.
Albert-Ludwigs-Universität Freiburg – Center for Biological Systems Analysis (ZBSA), www.zbsa.uni-freiburg.de.

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distribution of therapeutic antibodies in tumour tissue

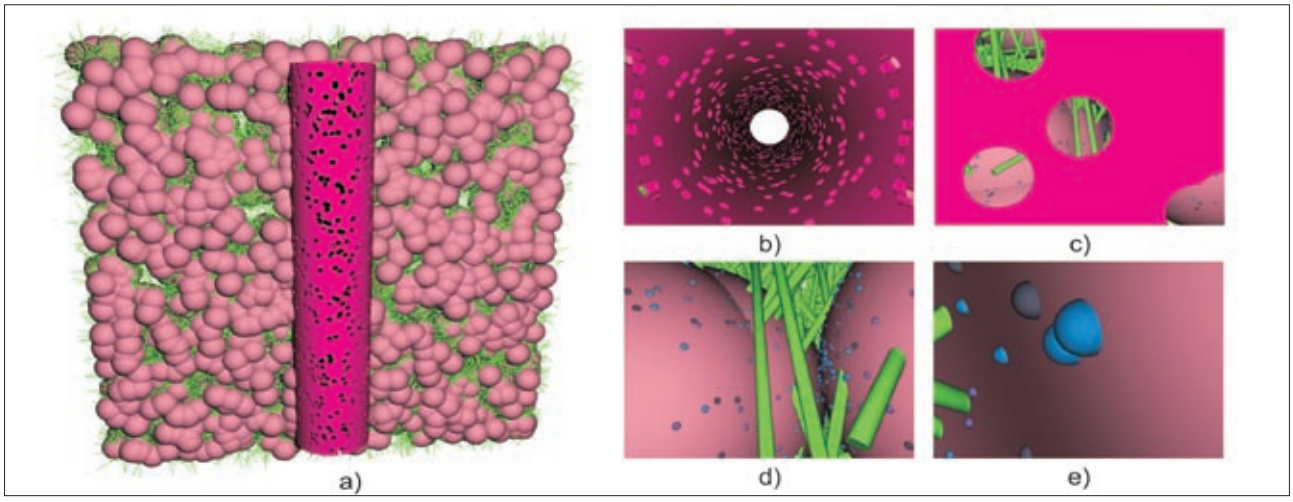
Spatio-temporal multi-scale methods for modelling and simulation of tumour therapies

by Holger Perfahl and Matthias Reuss

At the start of therapy most tumours already are of greater diameter and possess a well-developed vascular system. Treatment is aimed at destroying as many tumour cells as possible and preserving the healthy cells in the process. An interdisciplinary research team at the CSB (Center Systems Biology) of Stuttgart University works on mathematical modelling and simulation of tumour growth and drug delivery in tissue. Their research is focused on a multi-scale description of the interaction between three-dimensional tumour structures and the efficiency of various therapies.

Avascular tumours are tumours that do not contain any blood vessels and they constitute the start of solid tumour growth. They usually stop growing when they measure just a few millimetres in diameter. At this point, the same number of cells die within the tumour, as new cells develop by proliferation. This seemingly stable condition ends when the malnourished cells inside the tumour secrete growth factors that stimulate the adjacent vascular system to develop new blood vessels, which then grow into the hitherto avascular tumour. After successful angiogenesis the tumour can continue growing. Apart from supplying the tumour with nutrients, these blood vessels serve as "highways" enabling the transport of drugs into the tumour. Hence, this vascular system has crucial bearing on the efficiency of tumour therapy.

Fig. 1: Distribution of drugs in tumours



A small tissue section is used to investigate drug transport and diffusion. On the left you see the complete tissue (a). Spheres constitute coarse structures such as cells. One sphere does not necessarily represent one cell; several balls can illustrate one cell also. This reconstruction generates a virtual tissue section, which has the same specific features as real tissue. The green structures represent small-scale tissue details, for instance extra-cellular matrix. The four illustrations on the right show the course of a drug particle through the vascular system (b) and its extravasation through the vessel fenestration into the interstitial space (c). Fig. d shows the motion of drug particles through the interstitial space, fig. e their reaction with the receptors on the cell surface (Chart: Holger Perfahl).

Holger Perfahl (Photo: CSB / sven cichowicz photography).

How do drugs get into the tumour and how do they diffuse?

The drug particles have a long way to go after venous injection. First, they distribute in the major vessels until they pass into the capillaries and then enter the interstitial space. They gradually accumulate in various organs and are, for instance, metabolised in the liver or excreted through the kidneys. Only a minor part of the total drug volume arrives at the tumour. In interstitial space, the molecules diffuse through the tissue; they are, however, slowed down in their free diffusion by tissue structure. The extracellular matrix, the cells and the tumour stroma all prevent the drugs from rapid and uniform distribution in the tumour. Hence, it is crucial to incorporate detailed information on tumour structure into the simulation of drug delivery. Holger Perfahl's and Matthias Reuss' team works on replicating various tissue segments, as detailed as possible, to examine drug diffusion through simulation (Fig. 1). The crucial question: To what extent do shape and size of drug molecules influence drug motility and consequently their efficiency. Another question: At which size do drug particles loose their capability to leave the vascular system, or when do molecules get stuck in dense tissue sections and thus cannot reach the receptors on the surface of the tumour cells.

Multi-scale modelling: Which scales are involved?

Regarding biological processes in the human body, length scales range from nanometres to metres. Time scales range from around one hundredths of a second to decades. Thus, rapid processes in the body (e.g. opening and closing of ion channels) as well as extremely slow ones (ageing process) can be mapped. Tumour growth comprises processes that range from the level of the intracellular scale to the individual tumour cell, to the tumour itself, to the afflicted organ up to the complete organism. Interactions of the individual scales do not only run in one direction from cell to organism, but multi-fold interactions between the various scales occur. One goal of current research is to determine the relevant effects on the various scales and to couple them with other scales to eventually develop a comprehensive model of the growth and structural changes of tumours under the effects of various therapies.

Modelling and simulation of tumour growth

Investigating simulation of vascular tumour growth, Holger Perfahl's and Matthias Reuss' team intensely cooperates with the universities of Nottingham and Oxford* to delineate three-dimensional vascular tumour growth (Fig. 2). The employed multi-scale model (Perfahl *et al.*, 2010) interconnects intracellular processes with cell movement and angiogenesis. Diffusing substances like oxygen or drugs extravasate through the vascular system and are absorbed by the cells. Hypoxic cells secrete VEGF (vascular endothelial growth factor), stimulating the surrounding vascular system to grow new vessels. In turn the oxygen concentration field influences the duration of cell cycles and cell division rates. To achieve a closer correlation between experiments and the vascular networks obtained through simulation, we chose a hybrid approach. This approach combines imaging data and simulations. Experimentally obtained vascular systems are the basis of hybrid simulation. With these simulations we attempt to predict the further growth of the tumour. For validation Holger Perfahl's and Matthias Reuss' research group and their partners in Nottingham and Oxford cooperate with scientists from the Moffit-Cancer-Center** in the U. S., who provide experimental data.

Drug flow in tumours

Once the structure of the vascular system is at hand, more detailed information on the molecules' motility in the tissue

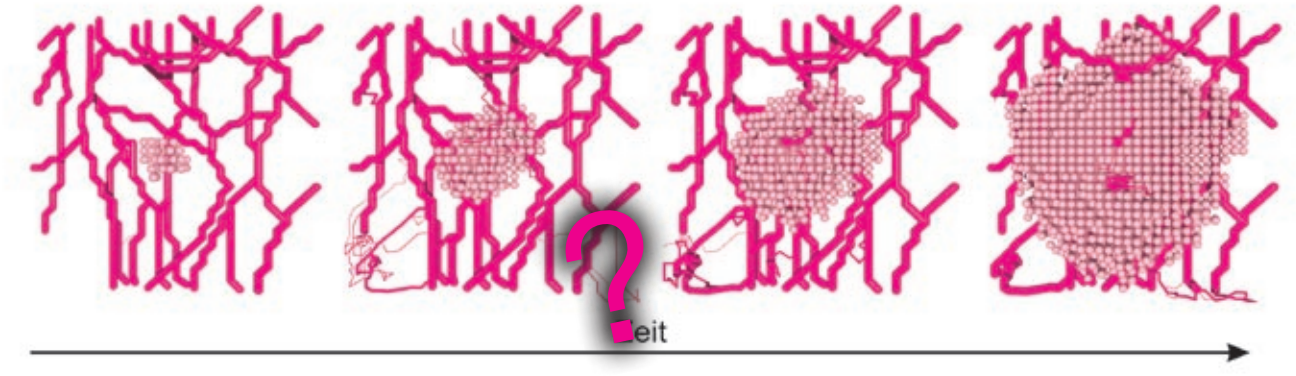


Fig. 2: Vascular tumour growth
The vascular structure of the simulation model is based on *in vivo* imaging data** from a mouse model. After implantation of several tumour cells into the virtual tissue, the further development of the complete system is simulated. For reasons of clarity the healthy cells surrounding the tumour are not shown. The simulation demonstrates the growth of the tumour cells and its impact on the vascular system. With this simulation a hybrid modelling approach (combination of actual and virtual structures) could be realised (Chart: Holger Perfahl).

is required for predicting the effectiveness of a therapy. An agent-based approach is used to follow the movement of every individual molecule in the tissue (Fig. 1). Reconstructions of various tumour tissues, as detailed as possible, are the basis for simulations. Segments of the vascular system are modelled as conjoined tubular systems for the blood flow; openings, randomly arranged in the vascular wall, specify its permeability. The cells and the extra-cellular matrix are arranged to exhibit the same characteristics as actual tumour tissue (volume fraction, cell-to-cell spacing).

Fig. 3: Interlinking of scales

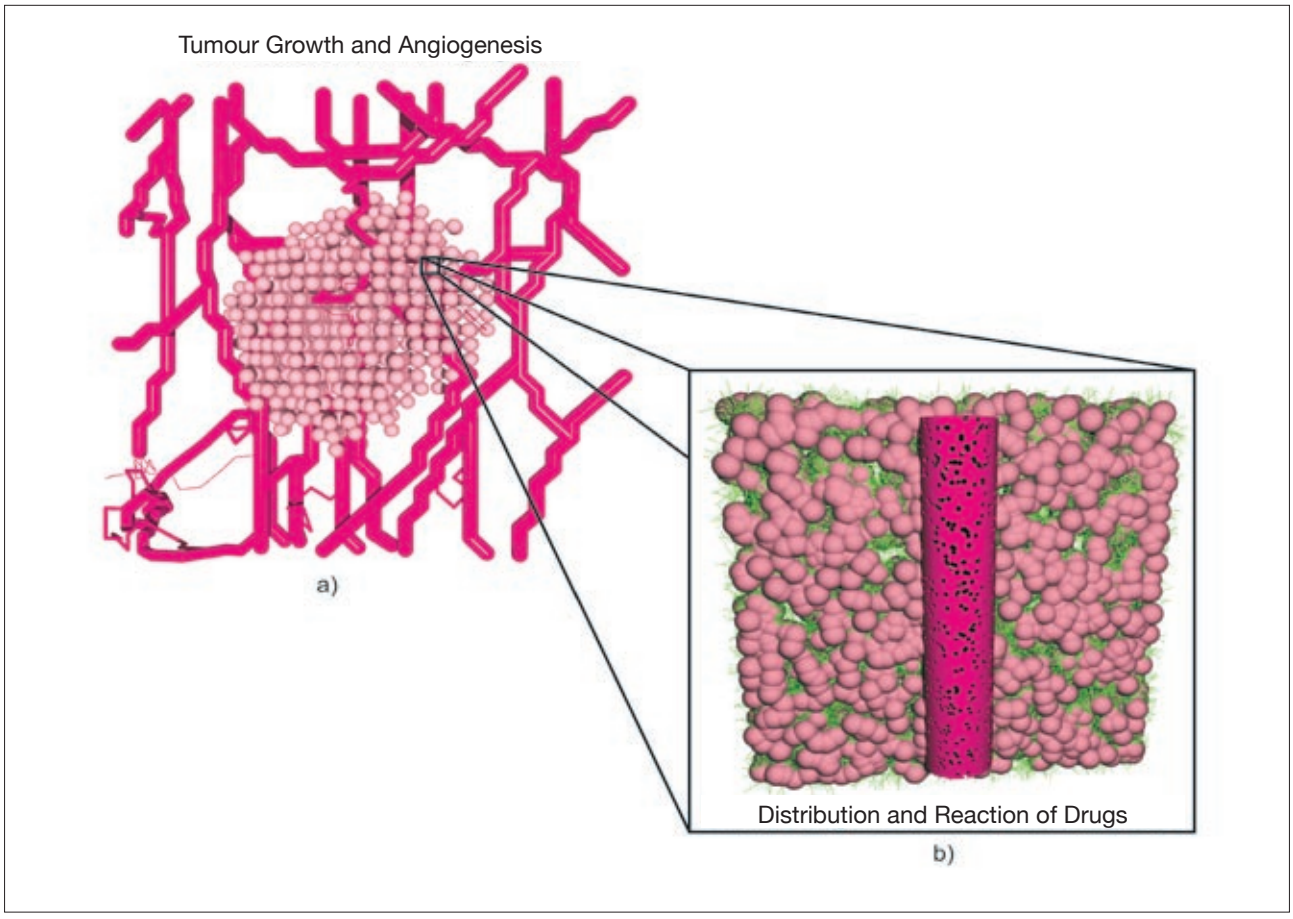


Fig. 3 demonstrates the interaction of the tumour growth model with the random walk model and provides the opportunity to investigate the effect of various therapies on the tissue. Detailed information on tissue structure on the smaller length scale (b) allows following the circulation of individual drug molecules through the tissue and to simultaneously observe the effect of tissue structure in the process. Rates of extravasation, diffusion and reaction, measured on the smaller length scale, are the basis for assessing the drug diffusion on the greater length scale (a) (Chart: Holger Perfahl).

Matthias Reuss (Photo: CSB / sven cichowicz photography).

From model to individual cancer therapy

Present research aims at reconstructing realistic tumour structures from histological slices and their application as basis for evaluation. With these simulations we can investigate the influence of tissue structure on the drugs' motility. These findings can then be integrated in larger-scale models (Fig. 3). Multi-scale techniques help describing and investigating interactions and effects of various scales on one another. Linking imaging data and simulations is a first step towards "personalised medicine" – a modern medicine that takes into account the interindividual variability of patients resulting in an optimised therapeutic approach for particular tumour types and their specific geometry, aided, for instance, by imaging examinations.

The research project in brief:

Forsys Partner Project: „A Systems Biological Approach to Predictive Cancer Therapy". University of Stuttgart, Center Systems Biology (CSB); Institute of Cell Biology and Immunology: Klaus Pfizenmaier (coordinator), Roland Kontermann, Peter Scheurich; Institute for Systems Theory and Automatic Control, Frank Allgöwer; Chair for Hydromechanics and Modelling of Hydro Systems: Rainer Helmig; Center Systems Biology: Matthias Reuss; University of Tübingen, Laboratory for Preclinical Imaging and Imaging Technology: Bernd Pichler; Dr. Margarete Fischer-Bosch Institute for Clinical Pharmacology: Matthias Schwab; Institute for Automatic Control and Systems Theory, University of Magdeburg: Rolf Findeisen; Celonic GmbH: Andreas Herrmann; Bayer Technology Services: Jörg Lippert.

Participating partners:

Holger Perfahl, Matthias Reuss, Alexei Lapin, Bernd-Simon Dengel, Center Systems Biology, University of Stuttgart

*) Helen M. Byrne, Markus R. Owen, Universität Nottingham, UK; Philip K. Maini, Universität Oxford, UK; Tomás Alarcón, Centre for Research on Mathematics, Spain.

**) Mark C. Lloyd, Veronica Estrella, Tingan Chen, Robert A. Gatenby, Robert J. Gillies, H. Lee Moffitt Cancer Center and Research Institute, Tampa, USA.

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innovative techniques in light microscopy

Company profile of TILL Photonics GmbH

by Frank Lison

TILL Photonics GmbH develops, manufactures and sells innovative modular components and complete solutions for the study of living cells using fluorescence microscopy. The company was founded in 1993 by Dr. Rainer Uhl, now Head of the BioImaging Centre (BIZ) at the Ludwig-Maximilians-Universität (LMU) in Munich. It grew rapidly into an internationally recognised enterprise, identified both with German quality and innovation. After a short period as a subsidiary of the US group Agilent Technologies (2008–2009), TILL Photonics was subsequently taken over by a consortium led by laser manufacturer TOPTICA Photonics AG.

The first TILL Photonics products were fast-switching light sources for fluorescence microscopy. In the years after the company was founded, its portfolio was gradually expanded. It focused mainly on complete imaging systems comprising all components that turn a microscope stand and a camera into a complete real-time microscope system. In the field of living cell microscopy, during that period the name TILL Photonics became synonymous with equipment for the study of rapid processes in a manner which causes little damage to the cells.

Customers and products

TILL Photonics' customer base today consists of scientists in the field of neurobiology and cell biology as well as OEM partners and systems integrators who use TILL Photonics components as an opto-mechanical basis for their own product configurations. The company's products are mainly used in laboratories where basic biological and pharmaceutical research is conducted, but increasingly also in fields of applied research and in the search for active ingredients as well.

TILL Photonics products are protected by several families of patents, which are continuously developed further in collaboration with the BioImaging Centre (BIZ) at the Ludwig-Maximilians-Universität in Munich and TILL I.D. GmbH (Martinsried, Munich).

In a third, still ongoing phase, TILL Photonics began expanding its product portfolio to include its own microscope stands. It launched its first proprietary microscope, the octagonal iMIC, in the market in 2003. The opto-mechanical concept of the iMIC platform, which has now been installed more than 200 times, represents a paradigm shift. What this means specifically is a systematic implementation of the principle of a central optical axis in constructing the microscope, so as to realise short distances to the optical axis. This concept, which has won several awards, is modular and scalable and can be optimally supplemented with now indispensible components such as cameras, lasers, scanners, etc. The iMIC platform (Fig. 1) by TILL is a versatile tool that can be used to combine and fully automate a multiplicity of highly demanding microscopy techniques such as EPI, TIRF, FRAP, FRET, structured illumination, confocal spinning disk, or even FCS in a single, compact and highly integrated instrument. Yet experienced users can still adapt the configuration to their own requirements, or add additional methods.

The *more* platform – high-end microscopy with minimal space requirements

TILL Photonics' second microscope platform is designed to address an entirely new market segment. It is no longer intended only for scientists for whom dealing with a highly complex instrument is paramount to their work, but also for biologists or medical professionals who wish to concentrate entirely on their biological research without despairing at the complexity of their equipment. The ***more*** platform combines the functionality of various greatly specialised high-end microscopes in a single instrument, while delivering a performance in no way inferior to that of specialised instruments. Indeed, thanks to a high degree of integration, it out performs them in many respects. Its combination of speed and precision and the fact that all functions of the microscope can be automated are unique in the field of high-end microscopy.

Fig. 1: The iMIC platform in three different configurations, for screening, with optical illumination, and with a small stage, the most common microscopy platform.

During the development phase, numerous prototypes and pilot instruments were built in a wide variety of configurations and tested extensively in house, at the BioImaging Centre, and at the premises of selected customers. Along with routine applications in cell biology, this made it possible to gain experience in the observation of individual rapid processes in physiology as well as mass studies using *high-content screening*.

The core of the ***more*** platform is formed by a mineral cast body that functions as a three-dimensional optical table where all opto-mechanical components can be interrelated. The mineral cast material ensures high intrinsic stability and, due to its outstanding absorption properties, very good short and long-term stability compared with standard materials such as aluminium.

The ***more*** platform enables the integration of functionalities otherwise found only in considerably larger and more cost-intensive apparatus within the smallest of space constraints. It is also scalable, but - unlike with the iMIC - the production process must take into account which of the numerous features are ultimately to be integrated.

The current product range of TILL Photonics thus includes two microscope platforms, iMIC and ***more***, as well as intelligent, fast-switching and tunable light sources, microscopy accessories (e.g. TIRF extensions) and photometry systems. All these systems feature constantly available real-time control, enabling the synchronisation of fluorescence activation and imaging on microsecond timescales. Photo damage caused to the living cells being examined is thus minimised.

TILL Photonics GmbH in brief:

Forty people are currently employed at the company headquarters in Gräfelfing near Munich, one third each in Research & Development, in Sales, Marketing and Product Management, and in Production. The core production team consists of engineers from universities of applied science, skilled workers and specially trained production workers. The supply chain is located almost entirely in Germany, mainly in the south of the country. The development unit is staffed by highly qualified academics from the natural sciences, electrical engineering and mechanical engineering.

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For further information visit:
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where high-end light microscopes are both designed and used

The BioImaging Zentrum (BIZ) of the Ludwig-Maximilians-University in Munich

von Rainer Uhl

At the BIZ the light microscope occupies centre stage. This centuries-old instrument - not long ago considered obsolete and relevant only for routine tasks or for teaching - has experienced an unanticipated renaissance in recent decades. No other instrument allows seeing into living cells and tracking inter- and intra-cellular processes on a sub-micrometre scale, in three dimensions and in real time. Moreover, overcoming the limits to diffraction - a very recent accomplishment - has advanced the realm of the instrument into molecular dimensions, a domain previously reserved for highly invasive methods such as electron microscopy.

Most major advances that have brought light microscopy to its current levels of performance have originated in an academic environment. The dialectics between biological problem and methodological solution, i.e. the interplay between "wanting" and "being able to", has functioned especially well in the case of light microscopy, repeatedly giving a competitive edge to biologists who didn't have to rely on available instrumentation but were part of or close to developments pushing the envelope of existing technology. In keeping with this tradition, researchers at the BIZ take up their own biological questions or abstract from the problems of partner groups and serve as a starting point for methodological development, driven forward in close collaboration with partners in industry.

The five subject areas at the BIZ

Man machine interface:

The task here is to design a novel user interface for controlling automated *high-end* microscopes, to implement it with partners in industry and to validate it in a variety of applications. Goal is to maintain the ease of use, robustness and speed of simple automated microscopes while controlling sophisticated high-tech microscopes. This will enable a wide circle of users to execute complex imaging tasks and protocols for the study of living cells. Until now, these methods have been reserved for methodologically oriented specialists.

Intravital microscopy:

In this field we collaborate closely with Professor Jochen Herms' research group at the Centre for Neuropathology and Prion Research (ZNP) of the Ludwig-Maximilians-Universität in Munich (LMU), which is investigating the growth and destruction of nerve cells deep in the brains of "Alzheimer's mice" that have been under observation for months (Fig. 1), and with Professor Giovanni Galizia at the University of Konstanz, who "watches live flies and bees smelling." The BIZ has developed new concepts that have considerably enhanced the photon collecting efficiency of a multi-photon intravital microscope, and is currently working on new digital drive concepts to enable a scanning femtosecond laser beam to be moved across a slide in three dimensions at unprecedented speed. Open-source software developed at the BIZ for controlling a multi-photon microscope is already being used successfully in a number of partner laboratories.

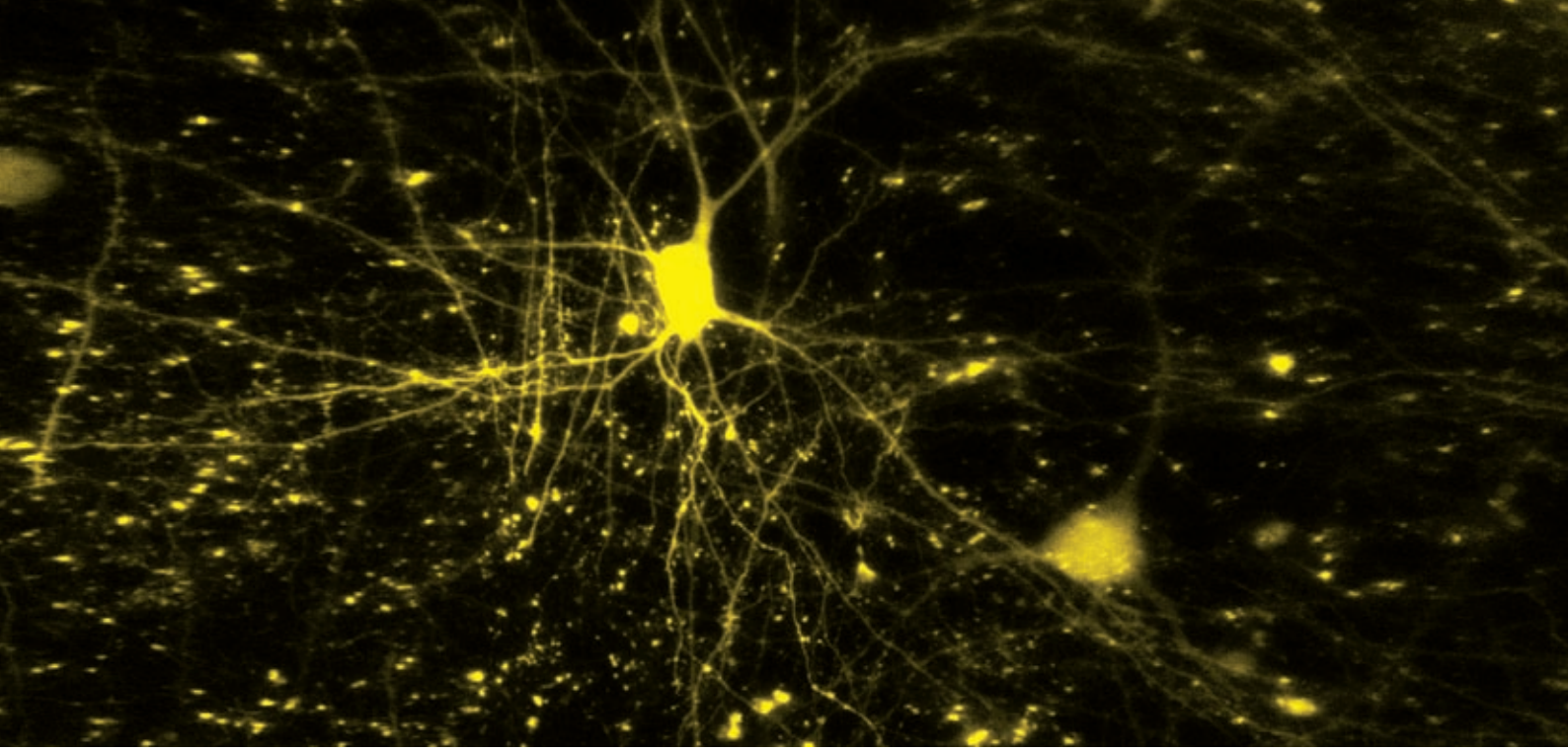

Fig. 1: Projection of an image stack of a YFP-dyed neuron taken in the brain of a live mouse (Image: Sabine Scheibe).

Structured illumination:

This technique delivers 3D tomography without the use of laser scanning techniques, as well as permitting increased resolution down to approximately 100 nm. Researchers at BIZ are currently working on simple alternatives to the extremely expensive instruments available on the market.

Spinning disc microscopy:

The spinning disc method is currently the sole option for carrying out confocal measurements in real time and keeping a live specimen alive even during longer-term observation. New methodological approaches to this are also being developed at the BIZ and are currently in the testing phase.

Super-resolution microscopy:

On the basis of single-molecule localisation, the resolution of light microscopy can be increased to the 10-nanometre scale. Researchers at the BIZ are currently devising new techniques for a three-dimensional multicoloured version and testing them in collaboration with partners in Hamburg and Nijmegen.

In addition to the development projects mentioned, the BIZ has further cutting-edge instrumentation in the fields of FRET (fluorescence resonance energy transfer), TIRF (total internal reflection fluorescence), FRAP (fluorescence recovery after photobleaching), FLIM (fluorescence life-time imaging) and FCS (fluorescence correlation spectroscopy). All are based on the microscope platform developed in close cooperation with TILL Photonics.

The research project in brief:

The **BioImaging Zentrum (BIZ)** at the LMU in Munich was established on the initiative of the Faculty of Biology and has set itself the task of encouraging interdisciplinary research and teaching in all areas concerned with the application of microscopic imaging techniques for biological subjects. Sixteen scientists currently work at the BIZ, including biologists, chemists, physicists and several engineers. The BIZ is part of the Munich Cluster of Excellence in Medicine. In 2004, along with TILL Photonics GmbH, it won a Bavarian Innovation Prize for its contribution to the development of the iMIC, the "microscope of the future". A year later, this microscope concept also won the respected Prism Award presented by *Photonics Spectra* magazine.

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genome-based systems biology

University of Bielefeld introduces Germany's first Master's programme in systems biology

by Frank-Jörg Vorhölter, Alf Pühler and Karsten Niehaus

In October 2005 when the first students arrived to take the Mathematical Methods in Systems Biology module at the University of Bielefeld, it marked the start of Germany's first Master's degree programme in systems biology. The departing point for establishing the programme was the demand from suitable graduates of Bachelor's programmes in the life sciences. Systems biology is an interdisciplinary science and requires contributions from different areas of specialty. The Bielefeld programme is a joint initiative of the Faculties of Biology, Physics and Mathematics as well as the Faculty of Engineering. The language of instruction is German.

The modular structure of the *Genome-Based Systems Biology* programme

The *Genome-Based Systems Biology* programme breaks down into modules (Tab. 1). In their first year of study, students receive a basic introduction to mathematical methods in systems biology as well as instruction in applied bioinformatics (Fig. 1). In these modules they acquire the mathematical knowledge to design models of biological processes and provide experimental measuring data for testing them, as well as expanding on their previous knowledge of the life sciences. Bacteria, the model plant *Arabidopsis thaliana*, biotechnologically relevant CHO cell cultures, and human stem cells are used as model systems. The module "Methods and Examples of Functional Genome Research" then enables students to gain active experience with modern "omics" technologies. In laboratory experiments, starting with genome sequencing, students learn transcription analysis with microarrays, protein and metabolite analysis using mass spectrometry, and the basics of automated microscopy techniques. This knowledge is expanded and consolidated in the modules "Physiology and Genetics of Prokaryotic Cells", "Metabolic Competence of the Eukaryotic Cell", and "Regulatory Networks of the Eukaryotic Cell". Students follow a fixed programme of study in the first two semesters, whereas the subsequent two semesters offer a great deal of freedom to shape their own special areas of interest.

Table 1: Overview of modules of the Master's programme in *Genome-Based Systems Biology*

1st Semester	Master's module I Functional Genome Research	Master's module II Physiology and Genetics of the Prokaryotic Cell	Master's module III Mathematical Methods
2nd Semester	Master's module IV Metabolic Competence of the Eukaryotic Cell	Master's module V Regulatory Networks of the Eukaryotic Cell	Master's module VI Applied Bioinformatics
3rd Semester	Research module theory Systems Biology by Examples	Research module, Practice I or Practice II	Extension module
4th Semester	Master's thesis		

Fig. 1: Close mentoring of students on the *Genome-Based Systems Biology* course
The programme is designed for 12 students per year. This enables optimal mentoring of students, as here in Applied Bioinformatics (Photo: University of Bielefeld).

These can include practical handling of state-of-the-art laboratory equipment and developing new bioinformatics applications for systematic data analysis. The overriding goal of all projects, however, is to develop, using mathematical methods, models that reveal the functional interplay between genes, transcripts, proteins and metabolites. The individual projects are conducted by students largely on their own responsibility, within the framework of research and extension modules. Experience gained in these projects facilitates the preparation of a Master's thesis, the final component of the programme.

Unique resources for gathering experimental data and carrying out model-based analysis

Students on the *Genome-Based Systems Biology* programme have full access to the Bielefeld University infrastructure, in particular to the resources of the natural science faculties and the Center for Biotechnology (CeBiTec). At the CeBiTec, students mainly use the genomics and bioinformatics technology platforms (Fig. 2). This enables them to work directly with cutting-edge large-scale laboratory apparatus, such as the ultra-high-throughput sequencers Roche 454 Genome Sequencer FLX and Illumina Genome Analyzer IIx. They can later analyse the data obtained as part of their Master's theses, for example. For transcriptome experiments, the CeBiTec provides the opportunity to design and produce own microarrays in-house. RNA sequencing techniques are being developed to supplement this. For proteome analysis, students have access to mass spectrometry methods, such as identification of proteins by MALDI-TOF using a Bruker ultrafleXtreme mass spectrometer after the proteins have been separated by means of two-dimensional gel electrophoresis. Mass spectrometry methods are also a key tool in metabolomics. Here, via coupling with different chromatographic separation methods, such as liquid or gas chromatography, one can identify and quantify a wide range of different metabolites. This can be done, for example, with the combined GC-GC-TOF Leco Pegasus 4 system for two-dimensional gas chromatography with identification by mass spectrometry. The resources available in bioinformatics, however, are not limited to just optimal hardware, such as computers, storage and databases. CeBiTec software resources also provide many advantages, as they can greatly enhance the ease with which experimental data is analysed, for example when metabolic models are generated automatically in systems biology markup language (SBML) using CARMEN as a tool. Portable MacBooks, which are available to students, have proved very useful for independent consolidation of initial modelling and simulation experience. Students are also urged to participate in scientific conferences, such as the annual CeBiTec symposiums, each of which is devoted to a future-oriented topic. CeBiTec symposium findings are published in special issues of the *Journal of Biotechnology* (Tab. 2).

Table 2: Overview of the topics of previous CeBiTec symposia

Year	Topic and publication in the *Journal of Biotechnology*
2006	Molecular Systems Biology *J. Biotechnology Volume 129, Issue 2*
2007	The Future of Genome Research in the Light of Ultrafast Sequencing Technologies *J. Biotechnology Volume 136, Issues 1-2*
2008	Solar Bio-Fuels *J. Biotechnology Volume 142, Issue 1*
2009	bioIMAGING *J. Biotechnology Volume 149, Issue 4*
2010	New Frontiers in Microbial Genome Research

Fig. 2: Taking samples from a bioreactor
In the context of projects or the Master's thesis, students can work with complex technologies largely on their own responsibility in order to gather data for the verification of previously compiled models (Photo: University of Bielefeld).

Systems biology students from Bielefeld participate successfully in iGEM competition

Students on the Master's programme are not only taught cutting-edge science, but also have scope for their own projects. That was evident when students on the *Genome-Based Systems Biology* Master's programme, supported by students of molecular biotechnology, participated successfully in the iGEM competition for synthetic biology in summer 2010 (Fig. 3). iGEM, which stands for *International Genetically Engineered Machine*, is an international research competition for undergraduate students of the life sciences. It has been organised since 2003 by the Massachusetts Institute of Technology (MIT) in Boston, Massachusetts, USA. The competition honours innovative systems and ideas in the field of synthetic biology. The Bielefeld students designed their project independently and successfully advanced it in the laboratory within a very short time. The aim of the project, named MARSS (*Modulated Acetosyringone Receptor Sensor System*), was to construct a molecular receptor in *Escherichia coli* for spice hotness. The substance capsaicin is responsible for the hotness of chilli peppers and black pepper, and thus of many foods. The project was designed as follows: a receptor protein in the soil bacterium *Agrobacterium tumefaciens*, which detects the secondary plant metabolite acetosyringone, was modified in the laboratory so that it recognised capsaicin. Recognition triggered a signal transduction in the model organism *E. coli*, and this led to the emission of light. The project thus developed a biosensor that reflected the capsaicin concentration as brightness, easily and reliably recordable by measurement technology. The iGEM jury deemed the project highly successful, and the Bielefeld team was awarded a gold medal in this competition organised by MIT.

Fig. 3: Bielefeld students took part in the iGEM competition organised by MIT in 2010

Students from the Master's programme in *Genome-Based Systems Biology*, seen here in front of the Bunker Hill Monument in Boston, Massachusetts, successfully took part in the 2010 iGEM competition for synthetic biology and won a gold medal (Photo: Dr. Jörn Kalinowski, University of Bielefeld).

Outstanding prospects for graduates of the Master's programme in *Genome-Based Systems Biology*

All graduates from the Master's programme to date have retained their enthusiasm for science. The majority have opted to go on to complete a doctorate in Germany or abroad. Graduates of the *Genome-Based Systems Biology* Master's programme had a higher than average success rate with applications for doctorate scholarships, for example within the framework of the CLIB Graduate Cluster Industrial Biotechnology, a future-oriented initiative of the federal state of North Rhine-Westphalia in which the Universities of Bielefeld, Dortmund and Düsseldorf are partners.

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how to fight age-related blindness with a simple moss

Freiburg systems biologists develop a drug against age-related macula degeneration

by Ralf Reski, Eva Decker and Sabine Stebel

Macula degeneration, also known as age-related blindness, comprises a group of disorders associated with a loss of function of the area of visual acuity, also called "yellow spot". The central field of vision, crucial for focused view, is slowly lost. The most frequent form of macula degeneration is age-related (AMD) which, by definition, occurs past the age of 50. Due to the considerable shift in age distribution in industrial countries a growing part of the population is affected by this disorder, causing substantial costs to health insurance companies, as age-related macula degeneration is the main reason for blindness after the age of 50. Approximately 25 to 30 million people suffer from AMD, 2 million in Germany alone.

Fig. 1: Prof. Ralf Reski with cultures of *Physcomitrella patens*

Image: Thomas Kunz, PR Freiburg University

Complement factor H and age-related loss of vision

Scientists from the University of Freiburg have now managed to close a gap in the treatment of AMD. Eva Decker's and Ralf Reski's team (Fig. 1) are the first to be able to synthesise "complement factor H" (Fig. 2), which can be used in the treatment of AMD (Büttner-Mainick *et al.*, 2010). The decrease of "complement factor H" in patients' blood is one of the main reasons for the occurrence of AMD. "Complement factor H" is part of the so-called complement system, a system of proteins in blood plasma, activated by immune response. It is involved in fending off microorganisms (bacteria, fungi, parasites) with more than 30 proteins and part of the so-called hereditary immune system. Factor H is a large protein containing sugar molecules (glycoprotein). Its main purpose is to prevent the complement system from mistakenly attacking the patient's own tissue. A dysfunction of factor H can thus cause severe health problems. Apart from age-related macula degeneration, a mutation of factor H can lead to atypical haemolytic uremic syndrome (HUS), a rare disease affecting babies and infants which can cause life threatening kidney damage. This very vital protein, however, cannot yet be purchased since it is an "orphan drug", meaning the market is too small to justify the involvement of the pharmaceutical industry, since, due to substantial expenses for development and production, a sufficient financial return cannot be expected during the period of patent protection. However, this official "orphan drug" status means that the authorities specifically support development and licensing of such drugs.

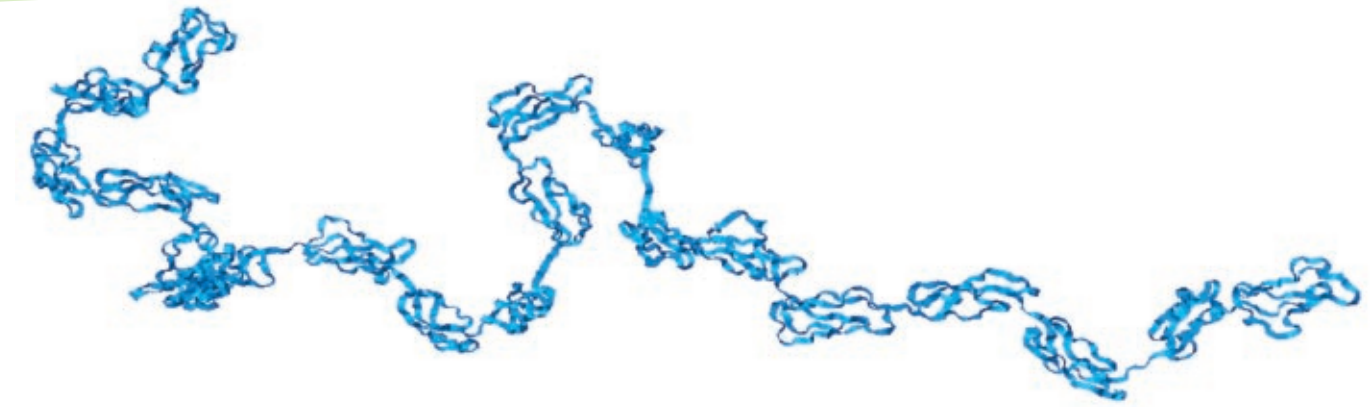


Fig. 2: Complement factor H
Factor H is found in human plasma in a concentration of 500-800 µg/ml. The protein's structure consists of 20 globular domains, the so-called "short-consensus repeats" (Source: 1haq.pdb, imaging provided by starbiochem programme. Photo: Sabine Stebel).

A moss comes to the rescue

In the development of this drug, the scientists from Freiburg focused on *Physcomitrella patens* (Fig. 3). In the early 1990s, BASF became aware of the potential of this little moss and invested a substantial amount of money in the exploration of this novel model organism. In 1999 Prof. Ralf Reski and colleagues founded the biotechnological company greenovation (**http://greenovation.com**) for the production of protein-based drugs in moss. Since then *Physcomitrella patens* has been established as a plant model system due to its very valuable specific features. In contrast to other plants it has a much simpler blueprint and only few cell types. Additionally, in moss, genes can be selectively removed with homologous recombination (exchange of DNA sections between two similar DNA molecules), which is not possible in any other plant. Thus, genes can be selectively inserted or deactivated. *Physcomitrella patens* possesses further advantages which other plants do not have: Its prevailing appearance is haploid, meaning it harbours just one single chromosome set, therefore mutations become visible immediately, since they are not compensated by a "genetic back-up copy" (Fig. 4). Furthermore, moss can grow in liquid medium in a bioreactor. Its growth can thus be closely monitored, at the same time preventing the genetically modified mosses escaping into the environment.

Drug production in moss

Therapeutic genes generated in bacteria, animals or plants are not new. This method is called "molecular pharming", a neologism composed of the terms "pharmaceutical" (for pharmaceutical drugs), "farming" (for agriculture), and "molecular" (based on the methods of molecular biology). Basic insulin, formerly extracted from meat offal, can be produced in bacteria. Some vaccines (e. g. hepatitis vaccine) are produced in genetically modified yeast cells. More

Fig. 3: *Physcomitrella patens*

Physcomitrella patens is a bryophyte and a member of the Funariaceae family. In biology it serves as a model organism for the research of evolution, development and physiology of plants. This species is found in Eurasia and Northern America where it grows on silty or clay ground, along dry riverbeds and drained ponds. The plants grow to a size of up to 5 mm in height. This form is very short-lived, completing its life cycle in just four weeks (Image: Plant Biotechnology Freiburg University, Prof. Reski).

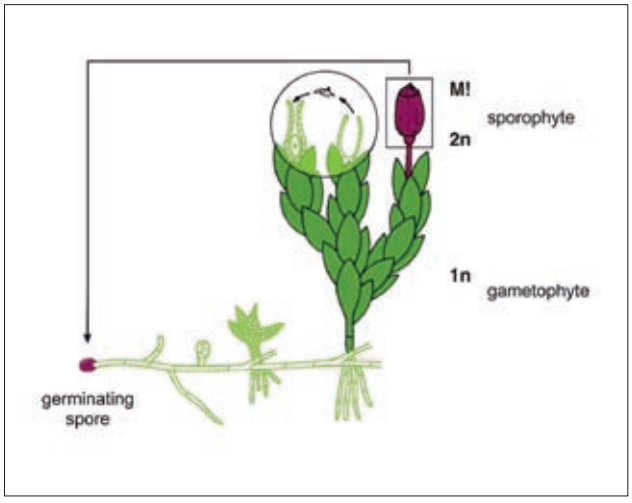
complex protein molecules, however, which are additionally decorated with sugar molecules, need more complex organisms for production. Complement factor C1-inhibitor which is used for the treatment of hereditary angiooedema (a rare hereditary disorder causing recurring swelling of the skin (oedema), the mucous tissue and internal organs that can be life-threatening) can be produced in rabbit milk. However, the milking of these animals is very laborious. The enzyme gastric lipase, needed for treatment of cystic fibrosis, can be produced in maize. This, however, is problematic, especially in Germany, where cultivation of genetically modified plants is highly controversial.

After genetic manipulation *Physcomitrella patens*, unlike other plants, is able to decorate the produced proteins with human sugar structures (Decker *et al.*, 2007). This is crucial since residual vegetal sugar can cause allergies (e. g. hay fever). The moss can be cultivated cleanly and safely in a bioreactor, needing just water, light, and carbon dioxide (Fig. 5).

To make things easier, the moss releases the produced drug ready for use into the culture medium. It is also rather undemanding regarding the Ph-value of its culture medium, which can be optimally adapted to the requirements of the protein to be produced. This allows drug production according to the strict GMP rules (Good Manufacturing Practice, a guideline for quality control in production of pharmaceutical products and agents) and, most importantly, *Phycomitrella patens* is able to produce the substances continually. Additionally, there is no danger of contaminating the environment with genetically manipulated organisms or their genomes. In contrast to production in animals, ethical objections are rare. Unlike in cell cultures of mammal origin, moss has the great advantage of not being susceptible to pathogen pollution, is cheaper, easier to maintain and almost odourless.

This research, not taken seriously when it began 20 years ago, has developed into a market, potentially worth millions, in particular due to the support of the BMBF (Federal Ministry of Education and Research) and FRISYS (Freiburg Initiative for Systems Biology). In the future, it may help older people to retain their eyesight and might spare sick children kidney transplantation.

Fig. 4: *Physcomitrella patens* life cycle



The *Physcomitrella patens* life cycle is characterised by two forms: the haploid (1n = one chromosome set per nucleus) gametophytes (produces gametes) and the diploid (2n = two genome sets per nucleus) sporophytes (produces spores).

Spores transform into filamentous protonema cells which form gametophores (small moss plants with its leaves) on buds. These gametophores develop two forms of sexual organs, female (archegonia) and male (antheridia). They cross-fertilise in the presence of water. After impregnation an embryo develops and grows into a sporophyte, respectively spore capsule generating the spores (Image: Plant Biotechnology Freiburg University, Prof. Reski).

Fig. 5: ***Physcomitrella patens*** **in photo-bioreactor** (Image: Plant Biotechnology Freiburg University, Prof. Reski).

The FRISYS project in brief:

FRISYS – Freiburg Initiative for Systems Biology (FöKz: 0313921), **www.FRISYS.de**.

FRISYS deals with the elucidation of regulatory mechanisms on the cellular and supra-cellular regulatory level. The FRISYS project focuses on regulatory processes that cause cell differentiation and coordinated mutations in cell populations.

FRISYS is part of FORSYS, comprising the research units of systems biology (**www.FORSYS.net**), also supported by the BMBF.

Partners:
Core Facility Proteomics, ZBSA,
Freiburg
www.zbsa.uni-freiburg.de.

Fig. 6: *Physcomitrella patens* in liquid medium

(Image: Plant Biotechnology Freiburg University, Prof. Reski)

Participating scientists
Annette Büttner-Mainik, Juliana Parsons, Hanna Jérôme, Andrea Hartmann, Stephanie Lamer, Andreas Schaaf, Andreas Schlosser, Peter F. Zipfel, Ralf Reski, Eva L. Decker.

References:

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GerontoSys

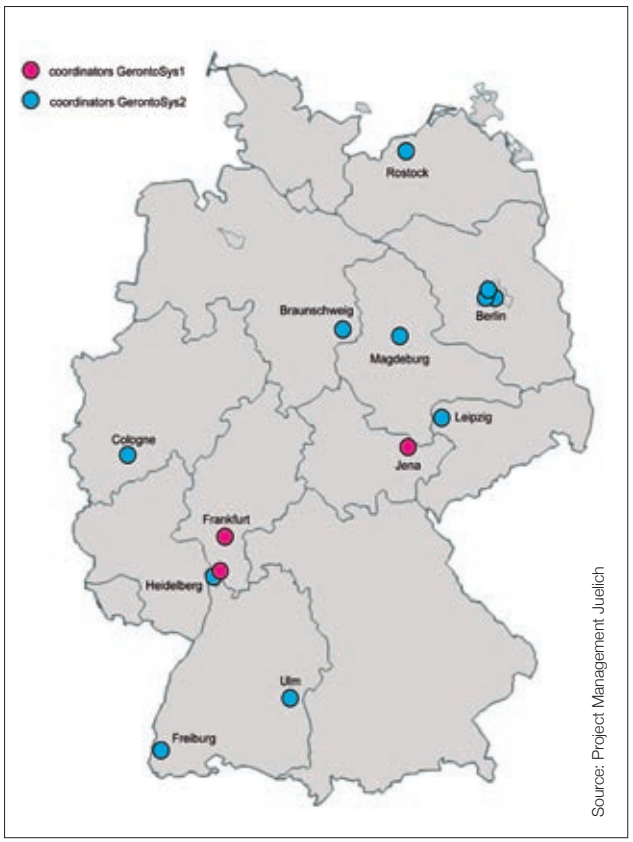
New approaches in research on aging

by Petra Boukamp, Jürgen Sühnel, Heinz D. Osiewacz and Björn Dreesen

Ageing - may sooner or later affect everybody and everyone can suffer under its consequences. That is especially significant in an increasingly mobile society, where an independent life style is a key issue for the old age. However, nowadays very little is known about the molecular causes that may lead to ageing associated diseases like dementia, metabolic disorders or cancer. In order to address this change in society, the German Federal Ministry of Education and Research (BMBF) has supported the efforts of the Systems Biology for Health in Aging – GerontoSys initiative since 2008. The significance that the Ministry sees in this field of research was further emphasized when, in December 2009, a follow up call was published. Both funding initiatives focus on systematic mapping of the complex interactions of age-related processes in humans and are expected to contribute toward the development of effective therapies for older patients.

In the first funding period, scientists with expertise in the fields of systems biology and age research were successfully brought together. A locally concentrated, interdisciplinary and collaboratively organized research core and two application-oriented cooperation projects will receive a total of nearly €11 million in funding to support their work over the next five and three years respectively. Within the second call, an international expert panel recommended funding of two more research cores, six joint projects and three young investigators groups. Doing so, the expert panel accentuated a forward-looking action and provided long-term strengthening of age-related systems biology research in Germany. By investing a further €32 million into this initiative, the Project Management Juelich agency (Projekttäger Jülich/PtJ), on behalf of the BMBF, will approve a total of €43 million in funding for research into aging in spring 2011 - thereby laying the foundation for an internationally competitive location. On September 26th and 27th, a joint BMBF event in Berlin will promote scientific exchange and networking among participants in a festive setting.

Distribution of GerontoSys research groups



Source: Project Management Juelich

Photo: Daniel Etzold – Fotolia.com

Projects arising from GerontoSys

The role of mild stress against the progression of ageing

This is the central topic of the research core at the **Jena Centre for the Systems Biology of Ageing – JenAge**. The usually favourable reaction of an organism to low-level stress, designated hormesis, has repeatedly been theorised to be the basic mechanism underlying the effects of a healthy diet or life-extending treatments. The JenAge Centre is now investigating this phenomenon from a new systems biology perspective. The aim is to identify conserved transcriptional or metabolic networks that are activated by mild stress. Researchers also want to clarify to which extent the functional integrity of these networks suffers with increasing age.

In addition to cell cultures and other animal models, such as threadworms as test subjects, the JenAge Centre also uses a new short-lived fish model developed at the Fritz Lipmann Institute for Age Research. In September 2010 three JenAge researchers, Cellerino, Englert and Platzer, received the Max Bürger Award from the German Society of Gerontology and Geriatrics for this model. Eventually, the mouse model may serve as a bridge to transfer the findings to humans where clinical patient samples are available.

Through the newly established JenAge Centre ageing research in Jena will be strengthened and already existing networking of expertise will be further developed. This is also demonstrated by the establishment of the Jena Centre for the Biology of Ageing (JCBA).

Project title:
Jena Centre for the Systems Biology of Ageing – JenAge: Systems biology of mild stress in healthy aging – a multi-species approach

Participating partners:
Coordinator Dr. J. Suehnel (Leibniz Institute for Age Research – Fritz Lipmann Institute e.V.), Prof. O. Witte (Jena University Hospital), Dr. R. Guthke (Leibniz Institute for Natural Product Research and Infection Biology e.V. – Hans Knoell Institute), Prof. S. Schuster (Friedrich Schiller University Jena)
Internet: www.jenage.de

Do the 'power stations' of cells influence aging?

Since November 2009, the **GerontoMitoSys** research network has been investigating the role of mitochondria, the 'power stations' of cells, during the aging process of biological systems. The project follows a systems biology approach by constructing mathematical models of experimental data and then validating these models in baker's yeast *Saccharomyces cerevisiae* and the fungus *Podospora anserina*.

To test the relevance of new findings from these two short-lived model organisms to ageing processes in mammals and to transfer the findings to humans as closely as possible, selective tests are being conducted in mice and rats. This allows researchers to gain new insights into the very complex network of molecular mechanisms leading to biological aging. The main focus in the first project phase was to define specific questions for mathematical modelling approaches and to develop experimental standards to generate reproducible qualitative as well as quantitative sets of data. A secure internet platform was established for data storage, analysis, and transfer between the project partners. An initial mathematical model of the cell´s defence against reactive oxygen species (ROS) was generated and will be further developed and fine-tuned in a second phase. Further modelling approaches are exploring the basis for the formation of ROS along the respiratory chain and the cellular mechanisms of mitochondrial quality control.

Project title:
The role of mitochondrial networks of signalling pathways in aging and lifespan control – a systems biology approach
Participating partners:
Coordinator Prof. H.D. Osiewacz (Goethe University Frankfurt), Prof. N.A. Dencher (Technische Universität Darmstadt), Prof. M. Roegner (Ruhr-Universität Bochum), Prof. E. Klipp (Humboldt-Universität zu Berlin), Prof. M. Meyer-Hermann (Helmholtz Centre for Infection Research, Braunschweig)
Internet: www.gerontomitosys.uni-frankfurt.de

Age(ing) gets under your skin

This is why the Gerontosys-consortium **Stromal Ageing** is looking at the cells in deeper skin layers, where the origin for skin ageing phenomena such as wrinkling or the weakening of connective tissue is suspected. Previous studies on ageing have performed the actual ageing process with cells in a Petri dish. Indeed, after a certain number of divisions, these cells stop growing and display various (morphological) changes. However, the real aging process is probably much more complex. Initial findings of the project show that, contrary to what had been suspected, human skin cells undergo far more changes than cells aged in a Petri dish and these changes are different. The consortium is therefore building up a cell and tissue bank of skin samples that is unique in the world. Skin samples from various places on the body that were exposed to or protected from light are taken from subjects between the ages of 20 and 70, to characterise them at the gene, RNA and protein level, using state-of-the-art scientific methods and imaging techniques. Cell cultures established from these samples make it possible to verify the relevance of certain findings in models, known as organotypic cultures, that are functionally comparable to skin.

For the first time the combination of cell bank and cell culture allows the use of a variety of systems biology methods in order to reconstruct the process of skin aging *in silico* in a computer. This will allow us to investigate which cellular processes change as the years pass by. However, skin ageing is not only manifested in such well-known characteristic features as wrinkling, but also in changes in the dynamic regeneration processes involving timescales of days and weeks, such as the slow-down healing of skin wounds. Now our cell bank makes it possible to study these processes in cells of different ages. Through analysis as well as from gene-regulating networks we can identify the crucial points of cellular control in mathematical models and compare how and where age-related changes take place in cells. This also lays the foundation for understanding the 'why' of ageing, or being able to identify, and possibly control, the signalling pathways that govern aging.

Project title:
Stromal aging
Participating partners:
Coordinator Prof. P. Boukamp (DKFZ Heidelberg), Dr. K. Stuehler (Ruhr-Universität Bochum), Prof. Dr. F. Theis (Helmholtz-Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt), Dr. H. Busch (Freiburg University), Dr. N. Grabe (Heidelberg University), Prof. G. Reifenberger (Heinrich Heine University Duesseldorf), Prof. J. Krutmann (Leibniz Research Institute for Environmental Medicine at Heinrich Heine University Duesseldorf)

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heidelberg and mannheim to be the mecca for systems biologists

12th International Conference on Systems Biology (ICSB)

August 28 - September 1, 2011

by Klaus-Peter Michel, Jan Eufinger, Ulrike Conrad, Angela Oberthür and Roland Eils

International congresses and meetings add extra spice to the daily routine of scientists. They offer a welcome opportunity to escape the routine of everyday lab work and experience science in a different and inspiring way once again. During reunions with colleagues and old friends, projects can be reflected and new contacts can be made. Participation in a conference also is a good time to critically evaluate your own scientific projects. Despite manifold electronic devices to communicate via e-mail, twitter, facebook and the like, quite often only the opportunities for intensive exchange with other scientists offered on the occasion of a conference, ring the bell for the beginning of new scientific projects and co-operations. In addition, a conference always provides the opportunity for an up-to-date overview on trends and the latest research findings in condensed form, and to brush up your own state of information.

So, the community of German systems biologists will be pleased to learn that a committee of renowned scientists around Prof. Roland Eils (DKFZ and University of Heidelberg) has the privilege of organising the International Conference on Systems Biology (ICSB) for a second time after 2004. The ICSB is the world's biggest and most outstanding meeting on systems biology and is organised once a year by the *International Society for Systems Biology (ISSB)*. The corner stone for this conference series was laid in 2000 with a meeting in Tokyo under the aegis of Prof. Hiroaki Kitano. Kitano is one of the founding fathers of today's systems biology research and one of its most acknowledged protagonists worldwide. During this year's ICSB, he will give his keynote lecture on August 31 to open up the Systems Biology Party Night.

In 2011, the main programme will take place at Conference Center Rosengarten in the heart of Mannheim from August 28 to September 1 (Fig. 1). The conference is supplemented by specific workshops and tutorials, held on the life sciences campus in Heidelberg at the university's systems biology center BioQuant and the German Cancer Research Center (DKFZ).

"We are very happy to welcome systems biologist from all over the world in Heidelberg and Mannheim", says chairman Prof. Roland Eils. "In 2004 systems biology still was in its infancy, and so the ICSB 2004 in a way gave a starting signal to the enormous

Fig. 1:

Left: Front view Rosengarten Congress Center. Right: Extension Rosengarten Congress Center (copyright: m:con-mannheim: congress GmbH).

Fig. 2: Foyer level 2 Rosengarten Congress Center (copyright: m:con-mannheim:congress GmbH).

progress of this line of research in Germany. So I am particularly proud to host the ICSB once again in Heidelberg/Mannheim, being able to present the achievements of recent years to a broader audience." The federal minister Professor Annette Schavan's conference patronage underscores the importance of systems biological research for Germany.

Numerous German and international scientists have been involved in planning the programme. Apart from Roland Eils, members of the planning committee are Peer Bork (EMBL Heidelberg), Thomas Höfer (DKFZ Heidelberg), Ursula Kummer (Heidelberg University), Ursula Klingmüller (DKFZ Heidelberg), and Peter Sorger (Harvard Medical School, Boston). This team is supported by more than 30 colleagues from the scientific committee and the offices of FORSYS (Klaus-Peter Michel, Ulrike Conrad), the Helmholtz Alliance on Systems Biology (Jan Eufinger) and BioQuant/ViroQuant (Angela Oberthür). Just like in Gothenburg 2008, in Stanford 2009 and in Edinburgh 2010, we are expecting more than 1,000 participants.

Congress chairman Prof. Roland Eils welcomes more than 1,000 scientists to the 12th International Conference on Systems Biology 2011

(Photo: DKFZ / Heidelberg University)

The scale of the 5-day main programme reflects the high profile of the ICSB. 21 plenary speakers have confirmed their participation, and around 100 speakers will give lectures on a large variety of topics of systems biology research in the parallel sessions.

"We are particularly looking forward to our two honorary speakers' lectures", says Eils. Nobel laureate Roger Y. Tsien from the University of California in San Diego will open the conference and talk about his revolutionary work to create molecules, which are able to "spy on" cellular processes. Prof. Tsien is a member of the American National Academy of Sciences and the British Royal Academy. In 2008 together with two of his colleagues, he was awarded the Nobel Prize for Chemistry for his work regarding the discovery and further development of the *Green Fluorescent Protein (GFP)*. GFP, related proteins and other fluorescent molecules help scientists to visualise and understand intracellular processes *in vivo*, i. e. in the living cell.

Dutch-born Alexander van Oudenaarden is another leading scientist who ICSB 2011 managed to get on board as honorary speaker. Prof. Oudenaarden holds a chair for systems biology at the renowned Massachusetts Institute of Technology (MIT), USA. In recent years, his work regarding the individual variation of gene expression and evolutionary processes in cell populations has been published in prestigious scientific journals such as *Nature, Science* and *Cell* and has made important contributions to the understanding of non-genetic variations between cells. Van Oudenaarden will speak on the final day of the conference.

Fig. 3: Entry Foyer Rosengarten Congress Center (copyright: m:con-mannheim:congress GmbH).

In addition to these two highlights, there will be a series of both interesting and top-class key-note lectures, held by internationally recognised systems biologists. Among the speakers you will find such well-known names as Judy Armitage (Great-Britain), Naama Barkai (Israel), Philippe Bastiaens (Germany), Gaudenz Danuser (USA), Sandro J. de Soza (Brazil), Trey Ideker (USA), Josef A. Käs (Germany), Markus Covert (USA), Andrew J. Millar (Great-Britain), Jean Peccaud (USA), Yitzhak Pilpel (Israel), Eytan Ruppin (Israel), Uwe Sauer (Switzerland), Rob de Boer (The Netherlands) and Kim Sneppen (Denmark). Each of these speakers is a distinguished expert in his/her field, extending from plant systems biology and various other topics to cancer research.

In addition to the scientific main programme, the ICSB will offer a series of other interesting topics. The industrial exhibition will present business ventures, illustrating the significance of systems biology for their individual research in a special Industry Session.

Due to the inevitable multitude of resources that are necessary, modern research in life sciences is no longer feasible "behind closed doors", but only in joint projects. Universities, non-commercial research institutions, science initiatives and other institutions will present themselves in the so-called Science Arena to demonstrate their specific expertise aiming to recruit young talents or forging new alliances. The Science Arena will be held in direct vicinity to the industrial exhibition in the light-flooded foyer of level 3 of Rosengarten Conference Center (Fig. 2).

Poster sessions in the spacious entry foyer of Rosengarten Congress Center are another essential item on the ICSB agenda (Fig. 3). Large-scale posters give junior scientists as well as well-established researchers the opportunity to present their own achievements, and to discuss these face-to-face with their colleagues.

Embedded into the scientific programme of ICSB 2011, the Bio:Fiction Science, Art & Filmfestival will be presented as spin off of the world's first synthetic biology film festival @ Vienna in May 2011. The BioFiction@ICSB2011-Mannheim event will show selected filmlets on synthetic biology followed by a panel discussion on the pros and cons of synthetic biology with Prof. Roland Eils (Scientist, DKFZ and Heidelberg University), Joe Davis (Artist and Scientist, MIT Boston), Sonja Bäumler (Biofiction Film Festival Award Winner), Prof. Ursula Damm (Artist und Scientist Bauhaus University Weimar), and Camillo Meinhart (Biofiction-Team).

"Of course, we also want to introduce our guests to the hospitality of Heidelberg and the neighbouring regions. The optional boat ride on the river Neckar, followed by the festive conference dinner at Heidelberg castle will certainly be long remembered", says Roland Eils. "I am particularly looking forward to the *Systems Biology Party Night* on the last evening of the meeting. After a special lecture of Hiroaki Kitano, all guests will have the chance to cheerfully celebrate the successful conference by dancing or listening to the live music of *The Wright Thing*". A wide variety of sightseeing activities for the conference participants and accompanying persons in and around Heidelberg and Mannheim will complete the ICSB agenda.

For details and information please see the conference homepage **www.icsb-2011.net**. In addition, the organisers provide a conference blog (**http://icsb2011.blogspot.com**) and a twitter feed (**http://twitter.com/icsb_2011**) to all interested in the latest information on events regarding the ICSB 2011.

news

e:Bio – Systems Biology Innovation Competition

The e:Bio – Systems Biology Innovation Competition funding measure is another decisive impulse the German Federal Ministry of Education and Research (BMBF) has provided for the development of systems biology in Germany. e:Bio is a platform for research that makes a direct and novel contribution towards solving societal problems with the aid of systems biology. Together with the "Health Research Framework Programme" and the "National Research Strategy BioEconomy 2030", it can address a wide range of applications in biomedicine and biotechnology.

The innovation competition combines four modules under one roof in which successful approaches can be taken forward or new aspects can be brought into play. In "Ideas Competition national" (I) new impulses, ideas and innovations are fostered, while in "Transfer" (II) basic research findings are included and developed further with possible applications in mind. Young scientists can establish themselves with their own systems biology projects in "Young Investigators" (III), while "Ideas Competition international" (IV) provides transnational joint projects with a platform for scientific cooperation. The first submission deadline for Modules I, II and III project outlines was May 2, 2011; the deadline for Module IV is to be published at a later date. The next submission deadline for Modules II and III is May 2, 2012.

Source: BMBF

Single gene defect triggers brain tumour

Scientists from the German Cancer Research Center (DKFZ) and Heidelberg University Hospital have demonstrated in mice that a defect in a single gene is all that is required to trigger a dangerous brain tumour.

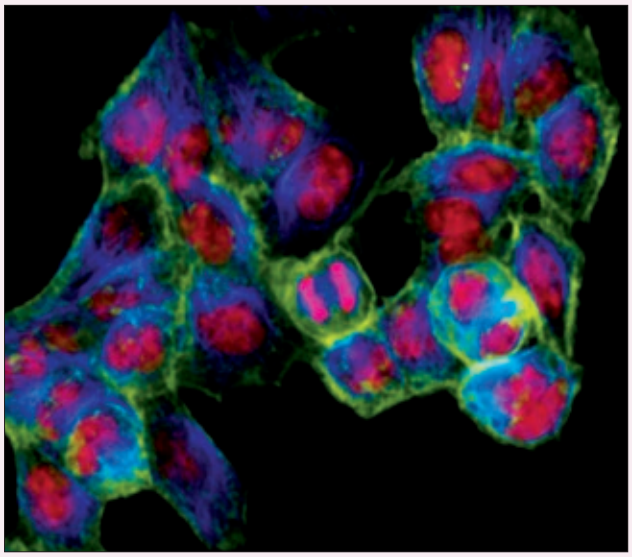
Pilocytic astrocytoma, the brain tumour that most frequently occurs in children, grows slowly and is usual benign. It grows diffusely, however, and often cannot be removed entirely by means of surgery. Follow-up therapies are therefore required. As chemotherapy or radiation treatment has little or no influence on these very slow-growing tumours, children who suffer from them urgently need new targeted treatments.

In the overwhelming majority of pilocytic astrocytoma cases, a defect in the BRAF gene leads to an important cellular signal pathway being permanently active; in healthy cells it is only activated in cases of acute need. Jan Gronych and colleagues added a defective BRAF gene to a virus and used the virus to smuggle the gene into the neural precursor cells of mice. In 91% of the laboratory animals treated in this way, tumours developed in the area where the injection had taken place that corresponded in their biology, growth properties and cell structure to pilocytic astrocytoma. The cells of these tumours all showed the typical symptom of a defective BRAF gene, leading to a permanent activation of the MAP kinase enzyme, which transmits continuous growth signals to the cancer cell. "This proves that a single gene defect is all that is required to trigger pilocytic astrocytoma," explains Prof. Lichter, summarising the results. As a new model system, the BRAF mice now open up opportunities to test newly developed kinase inhibitors or other drugs in a targeted manner for their efficacy in treating this kind of cancer.

Source: DKFZ press release

Facebook for genes: a new process shows how genes collaborate

The individual risk of suffering from a specific disease can be established nowadays by comparing the gene variants of sick and healthy people. As it frequently takes not just one gene, but rather interaction among several genes to trigger a disease, the method published in *Nature Methods* by Michael Boutros (DKFZ/Heidelberg University) and Wolfgang Huber (EMBL/EBI), which specifically reveals gene combination effects, represents a genuine scientific breakthrough. By using RNA interference to "silence" individual genes and all paired gene combinations, the scientists compile a systematic catalogue of all interactions between important signal molecules. For each gene, a list of interaction partners – comparable with a list of friends on Facebook – has been drawn up. "If two users of Facebook have the same friends, it can be assumed with a high degree of probability that they know each other, even if they themselves do not number among their respective Facebook friends," Boutros explains. "Applied to the genetic make-up, comparison of interaction can serve to predict which genes perform a common function."



Cell nuclei, dyed red, containing DNA (Image: DKFZ).

This method will thus help to identify new components in cancer-relevant signal cascades and possible new cancer treatment options. Horn, T. *et al.*, 2011; *Nature Methods*, 8, 341-346, March 2011. DOI: 10.1038/nmeth.1581.

Source: DKFZ press release

Treasure trove from the data jungle: DKFZ and IBM sign framework agreement on cancer genome analysis

The International Cancer Genome Consortium (ICGC) is analysing the genetic make-up of thousands of cancer patients. This undertaking involves enormous quantities of data. Intelligent IT systems are thus needed to identify the gene segments that are crucial for carcinogenesis and cancer treatment. The German Cancer Research Center (DKFZ), Heidelberg, and IBM accordingly signed a strategic framework agreement at CeBIT 2011. Its aim is to put the data obtained from gene sequencing to use for cancer medicine.

"In the years to come, sequencing of cancer genomes will generate considerable amounts of data. That will fundamentally improve diagnosis and treatment for cancer patients," says Prof. Otmar D. Wiestler, the DKFZ's Chairman and Scientific Director. "But if we are really to be able to make use of the findings from the enormous flood of data, we need intelligent information technology. The technology will help us to identify and utilise the really crucial segments. In IBM we have found an ideal partner for this huge task."

The framework agreement between the DKFZ and IBM covers various aspects of dealing with the enormous amounts of data that accumulate in the life sciences. They include strategies for effective sequence data compression, similar to using compressed mp3 files in the music industry, and solutions for the effective transfer of data in bulk between storage facilities and high-performance computers for analysis. Procedures are also to be developed for matching genome data and clinical parameters, such as the progress of the disease or its response to targeted drugs.

Prof. Otmar D. Wiestler, Chairman and Scientific Director of the German Cancer Research Center left, and Martin Jetter, CEO of IBM Deutschland GmbH at CeBIT 2011 (Image: DKFZ).

Data from the three German ICGC projects are being brought together by Prof. Roland Eils at the DKFZ. For this purpose, Prof. Eils is setting up one of the world's largest data storage facilities for the life sciences at Heidelberg University's BioQuant Center. Genome sequence data is expected to require a storage capacity of six petabytes.

Source: DKFZ press release

Helmholz Zentrum München clarifies molecular mechanism of the formation of neural connections

Scientists at the Helmholtz Zentrum München have determined how sensory and motor fibres interact during the formation of nerves in extremities: both types of nerve fibres can take this process forward. The researchers are thereby making an important contribution to understanding how neural networks take shape during embryo development and to finding a new approach to explaining neurodegenerative disorders.

Sensory and motor nerve fibres join forces in extremity nerve formation during embryo development. A team led by Dr. Andrea Huber Brösamle has now revealed how this collaboration works at the molecular level. The surface receptor Neuropilin-1 exists in both motor and sensory nerve fibres and controls their interaction to ensure correctly controlled growth.

It was observed in the study that both motor and sensory fibres can take the lead in forming the spinal nerves of arms and legs. This finding came as a surprise in that it was previously assumed that it is always the motor nerves that determine the correct right path. At the same time, the researchers have developed a model to understand structural adjustments after traumas and in neurodegenerative diseases in humans. "Our next target," Dr. Huber Brösamle says, "is to establish the extent to which Neuropilin-1 also influences nerve formation in the brain."

Original publication: Huettl R.E. *et al.* (2011). PLoS Biol 9(2): e1001020. doi:10.1371/journal.pbio.1001020

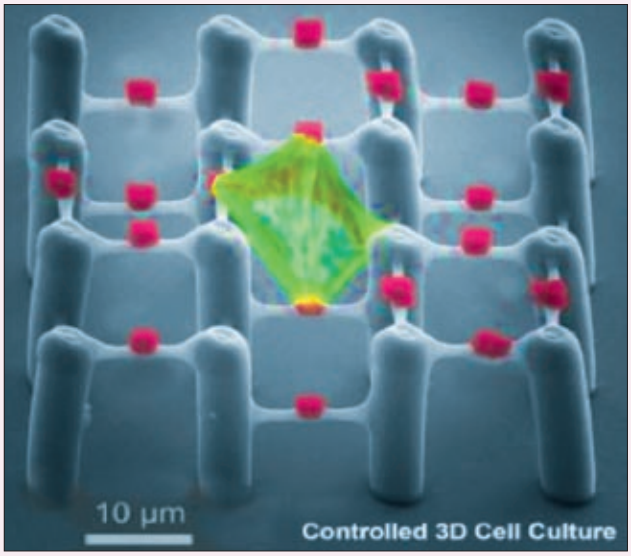
Source: Helmholtz Zentrum München

Third dimension realised in targeted cell cultivation

CFN scientists develop a two-component polymer backbone for controlled three-dimensional cell culture

Research scientists at the DFG (Deutsche Forschungsgemeinschaft) Center for Functional Nanostructures (CFN) at the Karlsruhe Institute of Technology (KIT) have succeeded in cultivating cells on three-dimensional structures in a targeted fashion. What is so fascinating about this is that the cells are offered tiny backbone "handles" only micrometres in size to which they can adhere. They can only do so at these points and not anywhere else along the backbone.

With these results, the team headed by Prof. Martin Bastmeyer has made significant progress in biomaterials engineering by precisely influencing adhesion, and with it the shape of a cell, in three dimensions for the first time. Using this technology, parameters such as cell shape, cell volume, intracellular strength development or cellular differentiation are determined systematically, dependent on the external geometry of the surroundings.



Cell in a two-component polymer backbone (Image: CFN).

These findings will be very useful in order to later develop, systematically and on a larger scale, three-dimensional growth environments for tissue cultures that are needed in, for example, regenerative medicine. The results are an important step towards gaining a general understanding of how the natural three-dimensional tissue surroundings influence cell behaviour.

For further information, visit
www.kit.edu/besuchen/pi_2011_6187.php

Source: KIT Karlsruhe press release

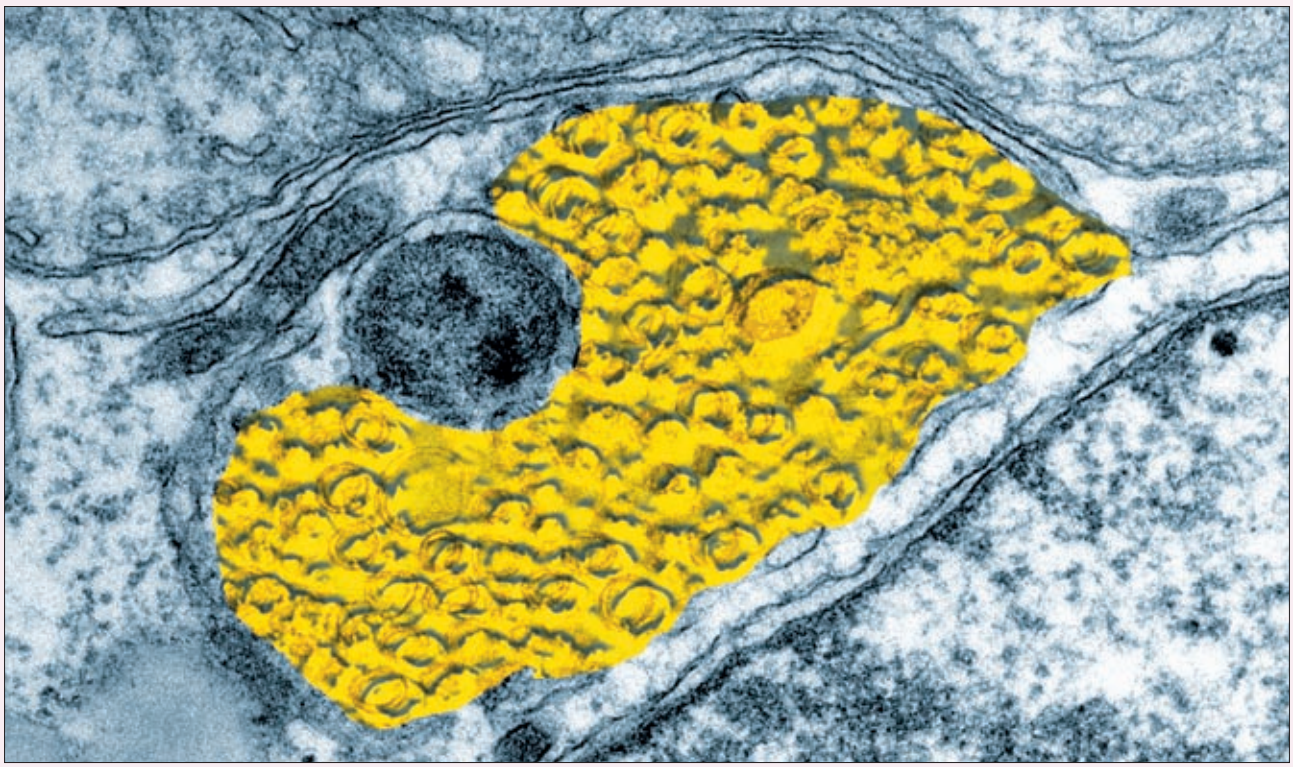
New target for hepatitis C therapy discovered

Over 170 million people around the world suffer from a chronic hepatitis C (HCV) viral infection. Hepatitis C can destroy the liver progressively and leads to cancer. Current therapies lead to a cure in only 50% of cases and are accompanied by numerous side effects. There are currently no indications of a vaccine to prevent HCV infection being developed in the near future.

Research scientists, led by Dr. Volker Lohmann and Prof. Dr. Ralf Bartenschlager of Heidelberg University Hospital, have succeeded as part of the BMBF-funded FORSYS-ViroQuant consortium in identifying a key cellular protein that forms the basis for new approaches to treating hepatitis C infections. Using a genome-wide screening assay, the enzyme phosphatidylinositol 4 kinase III alpha (Pi4KIII α) was identified as a critical host cell factor for successful replication of HCV in liver cells. The scientists also clarified how the virus makes use of the lipid kinase Pi4KIII α at the molecular level for its replication. The viral protein (NS5A) recruits the enzyme in the liver cell at the place where viral replication takes place and activates Pi4KIII α directly via binding. The enzyme Pi4KIII α thereupon produces large quantities of the phospholipid PI4P, which is essential for the functionality of the viral replication complex.

Original publication: Reiss S, Rebhan I, Backes P, Romero-Brey I, Erfle H, Matula P, Kaderali L, Poenisch M, Blankenburg H, Hiet MS, Longerich T, Diehl S, Ramirez F, Balla T, Rohr K, Kaul A, Bühler S, Pepperkok R, Lengauer T, Albrecht M, Eils R, Schirmacher P, Lohmann V, Bartenschlager R. (2011)
„Recruitment and Activation of a Lipid Kinase by Hepatitis C Virus NS5A Is Essential for Integrity of the Membranous Replication Compartment."
Cell Host & Microbe, Volume 9, Issue 1, 32-45, 20 January 2011.

Source: Heidelberg University Hospital press release



An electron microscope image of a liver cell that lacks the key protein PI4KIII α. Changes in structure presumably lead to the hepatitis C virus no longer being able to replicate itself (Image: Ralf Bartenschlager and Volker Lohmann, Heidelberg University Hospital).

New diagnostics for acute myeloid leukaemia

A team of scientists, led by Dr. Philipp Greif and Prof. Stefan Bohlander of the Pathogenesis of Acute Myeloid Leukemia (AML) Clinical Cooperation Group at the Helmholtz Zentrum München (HMGU) and the Medicinal Clinic III of the Ludwig-Maximilians-University Munich (LMU), have developed a method by which they study only the active genes in tumour cells for changes.

Using transcriptome sequencing, five previously unknown AML-specific gene mutations were discovered, of which two (RUNX1 and TLE4) are highly likely to jointly play a role in development of the disease. Dr. Tim Strom of the HMGU Institute of Human Genetics provided crucial support. "Without the latest generation of sequencing equipment and the evaluation expertise of the Institute of Human Genetics, the project would not have been possible," Dr. Greif comments. With the new diagnostics, for the first time specific genetic changes can be established for each patient, thereby making it possible to not only predict the individual course of the disease more precisely, but also monitor the response to therapy better, identify imminent recurrences at an early stage and to infer targets for specific therapy. In 2010, Dr. Greif received the Merit Award of the International Society of Oncology and Biomarkers and the research prize of the Anne-Liese Gaebel Foundation for the study's findings. The project is funded by the cancer charity Deutsche Krebshilfe, and its findings were recently published in the scientific journal *Leukemia.*

Source: Ludwig-Maximilians-University Munich press release

Controlling natural killer cells – modelling identifies switch for immune cells

The human immune system has numerous mechanisms to protect the body from damage by viruses or by abnormal own cells. So-called natural killer cells (NK cells) play a key role in this process. These immune cells can recognise tumour cells and cells infected by viruses and kill them systematically. Thus, they constitute a powerful weapon for the immune system. Scientists at Heidelberg University and the German Cancer Research Center (DKFZ) have investigated how these weapons are kept under control to prevent attacks on healthy cells. In a study published in the scientific journal *Science Signaling***, undertaken as part of a Helmholtz Alliance on Systems Biology SBCancer network project, groups led by Prof. Carsten Watzl (Heidelberg University, Institute for Immunology) and Prof. Roland Eils (BioQuant, Heidelberg University and DKFZ) demonstrated that the regulatory protein Vav1 plays a crucial role in the activation pro-cess.**

By means of a combination of mathematical modelling and experimental verification, the scientists were able to prove that Vav1 continuously receives signals from both activating and inhibiting receptors on the exterior of the NK cells, leading to phosphorylation or dephosphorylation of Vav1. The protein functions as a kind of switch and is regulated very swiftly via various mediator proteins (kinases and phosphatases). It thus plays a crucial role in the NK cells' killing activity. Using mathematical models, new insights were gained into the integration of positive and negative signals in this key process in NK cells. This new approach also has great potential for investigating similar decision-making processes in other immune cells.

Original publication : Mesecke, S., Urlaub, D., Busch, H., Eils, R., and Watzl, C. Integration of Activating and Inhibitory Receptor Signaling by Regulated Phosphorylation of Vav1 in Immune Cells. Science Signaling 4, ra36 (2011).

events

Stem Cells in Development and Disease

September 11-14, 2011, MDC Berlin-Buch, Germany

Stem cells have the remarkable property of being both permanently able to renew themselves by means of cell division and to differentiate themselves into specialised cell types. This ability has enormous potential for developmental biology research, for the development of new approaches to therapy for various diseases and for the further development of regenerative medicine. Although much research is still needed, some scientists see stem cells as the miracle cure of the future.

The conference to be held in Berlin at the Max Delbrück Center for Molecular Medicine in September 11–14, 2011 is aimed at students and research scientists interested in understanding the basic mechanisms by which stem cells are regulated during embryonic development and illnesses.

The conference will present latest results, developments and trends in stem cell biology and deal in detail with genetic and epigenetic reprogramming mechanisms and the retention of pluripotency and differentiation ability.

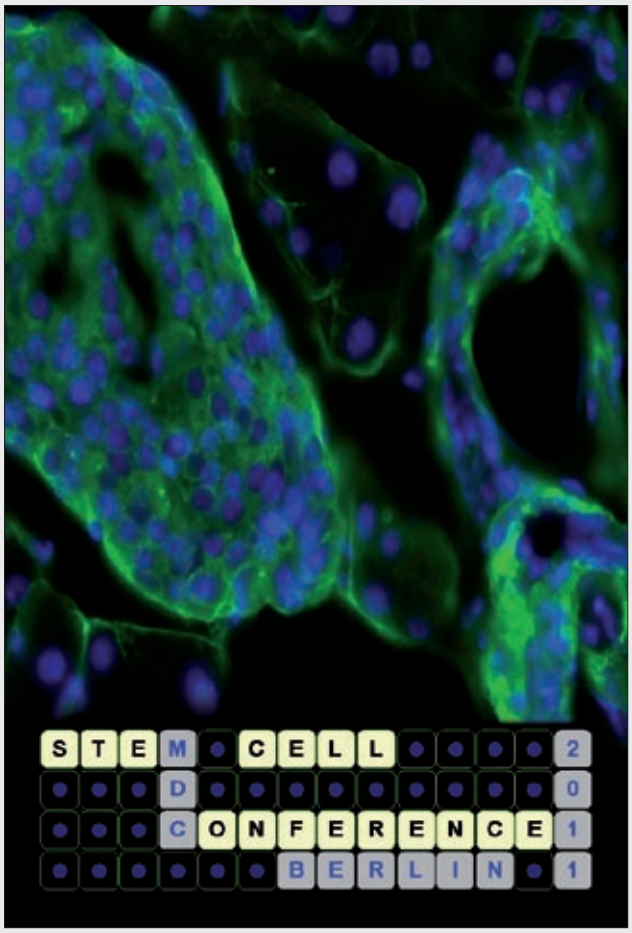
For further information, please visit:

www.stemcell2011-mdc-berlin.de/cms/default.asp?id=0

CMSB 2011 9th International Conference on Computational Methods in Systems Biology

September 21 – 23, 2011, Institut Henri Poincaré, Paris, France

The 9th International Conference on Computational Methods in Systems Biology (CMSB 2011) will be organized by INRIA (*Institut national de recherche en informatique et en automatique*) in Paris, France, on September 21-23, 2011. CMSB 2011 solicits



original research articles on the analysis of biological systems, networks, and data. The conference brings together computer scientists, biologists, mathematicians, engineers, and physicists interested in a system-level understanding of biological processes. Topics of interest include, but are not limited to: original paradigms for modelling biological processes; original models together with their application domains; frameworks and techniques for verifying, validating, analyzing, and simulating biological systems; high-performance computational systems biology and parallel implementations; inference from high-throughput experimental data; model integration from biological databases; model reduction methods; multi-scale models; control of biological systems. Contributions on modeling and analysis of relevant biological case studies are especially encouraged.

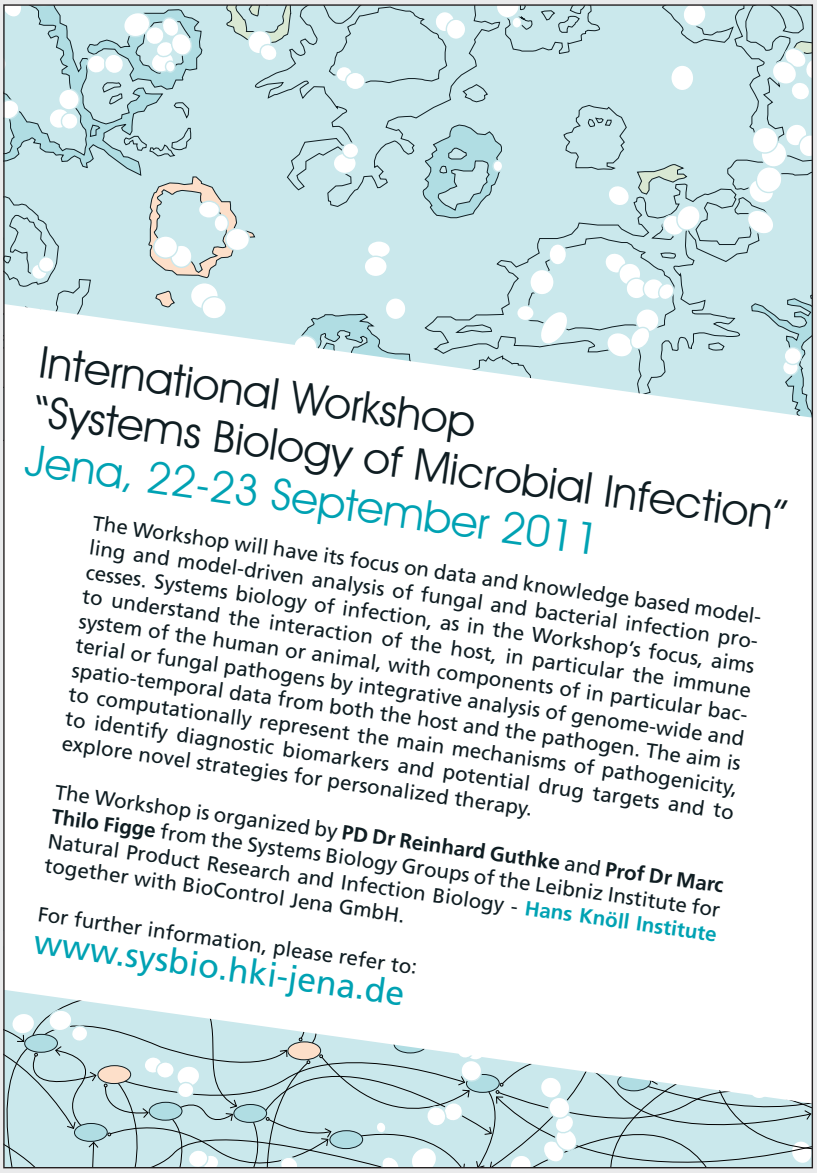
Please find more on:

http://contraintes.inria.fr/CMSB11/

International Workshop on Systems Biology of Microbial Infection

September 22-23, 2011, Jena, Germany

The International Workshop on Systems Biology of Microbial Infection is to be held for the ninth time in succession on September 22-23, 2011 in Jena by the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute. This year's workshop will focus on "Data- and Knowledge-based Modelling" and "Model-driven Analysis of Microbial Infection Processes" with a view to better understanding the interaction of the host and pathogen, especially the immune system of humans and animals, within the context of pathogenesis. The overriding objective is to describe host-pathogen interaction with the aid of a spatiotemporal modelling process in such detail



that diagnostic biomarkers and potential approaches to developing improved medication or therapy can be identified and novel strategies for personalised therapy can be facilitated or at least promoted.

For further information, please visit:

www.sysbio.hki-jena.de

7th Workshop on Molecular Interactions

October 5–7, 2011, Berlin, Germany

As in previous years, the organisers of this workshop are especially targeting young and future scientists in the life sciences. This year's workshop is entitled "*In vivo – in vitro – in silico*". The programme includes lectures by 32 renowned scientists from Germany, the UK, Spain, Brazil and Switzerland, who will report on latest developments in their fields of research. Lectures will cover the following areas: (i) RNA technologies, (ii) cellular structures, (iii) molecular medicine, (iv) cell systems, (v) regulatory networks, (vi) systems biology, (vii) technical biology, (viii) chemical biology, (ix) modelling and (x) technology transfer. In addition to the scientific lectures, the conference programme will include a career planning session.

For further information, please visit:

www.molecularinteractions.de

1st International SystemsX.ch Conference on Systems Biology

October 24 – 26, 2011, Basel, Switzerland

The 1st International SystemsX.ch Conference on Systems Biology will be held at the Congress Center Basel on October 24-26. This conference is intended to provide an international venue for bringing together top systems biology researchers from Switzerland and worldwide. The organizers are pleased to present an impressive speaker panel whose talks will cover topics like modeling of complex systems, novel technologies in systems biology, synthetic biology and medically relevant systems biology.

Please find more on:

http://conference.systemsx.ch/

International Conference on the Systems Biology of Human Disease – SBHD 2012

May 2-4, 2012, Heidelberg, Germany

The International Conference on Systems Biology of Human Disease (SBHD) is a transatlantic event and communication platform for scientific exchange between the United States and Germany. It takes place alternately between the two countries. SBHD 2012 is continuing a conference series launched by Peter Sorger and colleagues from Harvard Medical School some years ago. The conference focus is on mammalian systems biology, particularly as it applies to human disease and therapy. In 2012, the conference is organised by the two largest systems biology initiatives in Germany, FORSYS-Research Units for Systems Biology (www.forsys.net) and the Helmholtz Alliance on Systems Biology (www.helmholtz.de/systemsbiology) headed by Roland Eils, together with the Council for Systems Biology CSB² (www.csb2.org) and the Harvard Medical School in Boston. SBHD 2012 will take place in Heidelberg for the first time on May 2-4, 2012 at the German Cancer Research Center (DKFZ).

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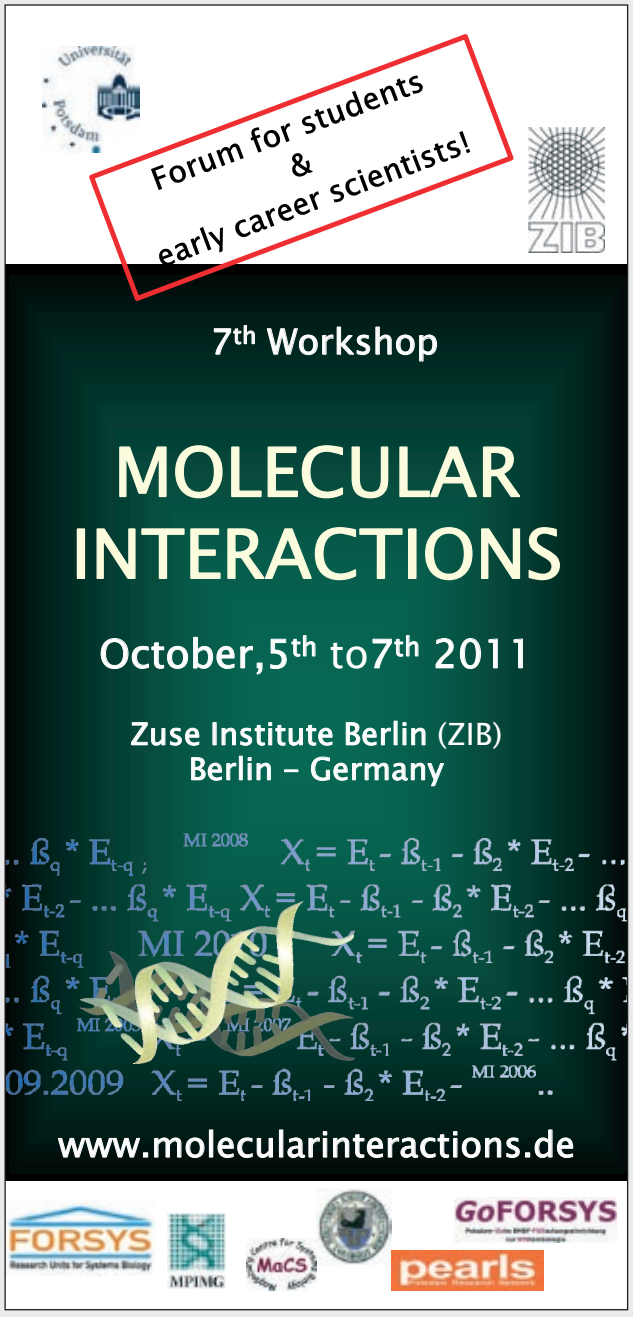

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Presenting the systembiologie.de editorial team

systembiologie.de would like to make the successes of German systems biology accessible to a wider public in an illustrative way. The magazine, which will be published twice a year in German, is produced jointly by the offices of the nationwide systems biology networks Helmholtz Alliance on Systems Biology, FORSYS –Research Units for Systems Biology, and the Virtual Liver Network. It is financed by the Helmholtz Alliance on Systems Biology, which receives funding from the Helmholtz Association's Initiative and Networking Fund, and by the German Federal Ministry of Education and Research (BMBF).

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