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From Scientists to Scientists in 65 Countries 2.14

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XXIV. ICORS Jena 2014

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XXIV. International Conference On Raman Spectroscopy

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GLASS

Overcoming limits

In order to meet the challenges resulting from an increasingly ageing population, new diagnostic and therapeutic methods and technologies are called for. The goal here is to diagnose medical conditions even before the first warning signals appear in the body and the person becomes sick. Biophotonic research uses light in order to detect pathological changes on the molecular level at the earliest possible stage. Light is a unique tool. Without direct contact, it can quickly and accurately register the condition of a cell without doing any damage. In view of the exceptional properties of light, photonic solutions – like e.g. Raman spectroscopy – are highly promising approaches for medical diagnostics – especially when classic microbiology is reaching its limits. Raman spectroscopy facilitates non-contact marker-free in vivo measurements, without any lengthy preparation of samples. Very low sample volume is sufficient to yield a Raman spectrum in high spatial (i.e. submicron) resolution. Raman spectra can be recorded in just a few seconds, and represent a molecular fingerprint of the investigated specimen like e.g. biological cell or tissue sample.

As long ago as 1928 the Indian physicist Sir C.V. Raman discovered the effect that a small portion of light scattered by molecules is frequency shifted i.e. has a different wavelength than the incoming light. For the discovery of this effect, named after him, he was awarded the Nobel Prize for Physics in 1930. As the result of numerous innovations in the fields of lasers, spectrometers, detectors, optical filters, data evaluation, automation and so on, Raman spectrocopy has gradually come to be accepted as an important method, with a particularly significant part to play in medical diagnostics.

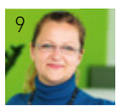
In partnership with technology experts, physicians and users, the Leibniz-Institut für Photonische Technologien [Leibniz Institute of Photonic Technology] researches Raman-based solutions for improved medical diagnostics – for example, so as to diagnose sepsis at an earlier stage, to detect single pathogens and judge their resistance potential, to draw a clear line between diseased and healthy tissue or to determine the chemical composition of plaque in an artery. In my article “Swift and non-invasive illumination” (see page 16 ff) I describe the potential and the limits of procedures based on Raman spectroscopy for medical diagnostics. Raman spectroscopy is not only used for biomedical diagnosis, moreover – it also plays an important role in life sciences, pharmaceutical applications, in the geosciences or in art history and research.

At the Twenty-fourth International Conference on Raman Spectroscopy, all the important current trends and developments in the wide research area of Raman spectroscopy will be presented and discussed. Established scientists, scientists of the younger generation and representatives of industry will be coming together in Jena from 10 to 15 August for this major industrial event.

We hope you enjoy reading this issue!

→ **Professor Jürgen Popp**
Scientific Director,
Leibniz-Institut für Photonische Technologien (IPHT)
[Leibniz Institute of Photonic Technology]
Director, Institut für Physikalische Chemie
[Institute of Physical Chemistry],
Friedrich Schiller University of Jena

content



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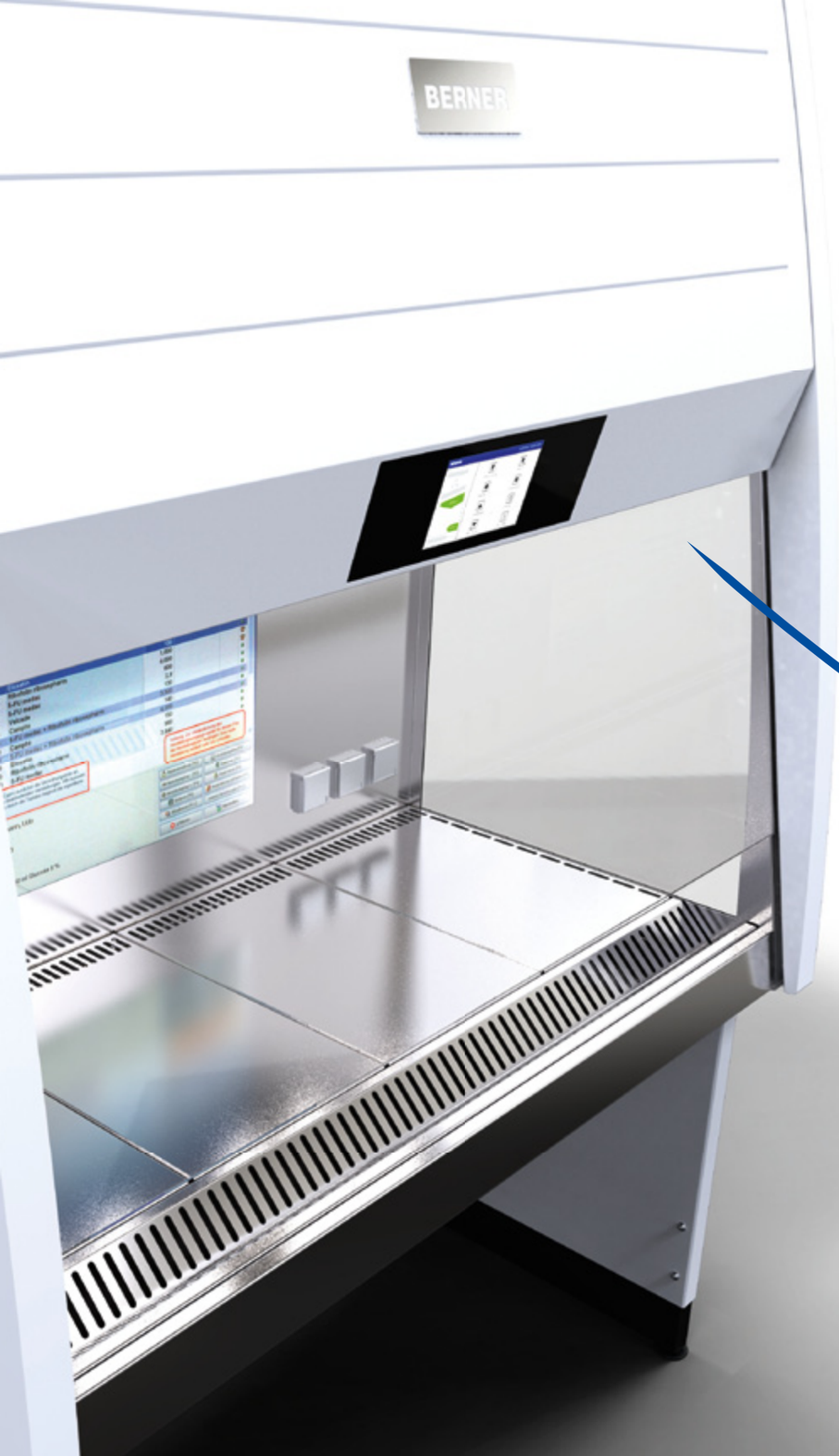

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market view

Agilent Technologies introduces new SureDirect Blood PCR Kit

Agilent Technologies Inc. has introduced the new SureDirect Blood PCR Kit for amplification of DNA directly from blood and blood derivatives, without the need for nucleic acid extraction and isolation. This kit simplifies the DNA amplification workflow and decreases turnaround time by enabling PCR (polymerase chain reaction) directly from fresh, frozen and dried blood, as well as plasma and serum from human and animal samples. The Agilent SureDirect Blood PCR Kit includes the company's latest novel inhibitor-resistant polymerase, DMSO, and positive control template and primers, providing the ability to overcome anti-coagulant and blood PCR inhibition and allowing for robust DNA target amplification even in high blood concentrations. Consistent amplification of GC-rich DNA targets and long amplicons are also achieved, giving researchers greater flexibility in experimental design.

→ **www.agilent.com**

Roche and Hitachi renew alliance in diagnostics

Roche and Hitachi High-Technologies Corporation today announced the signing of a renewed 10-year contract for the joint development and manufacture of the next generation of instruments and workflow automation solutions for medical laboratories. The renewed alliance marks a significant milestone towards new platform solutions in Roche's immunochemistry and clinical chemistry business that will help laboratories meet future needs. The agreement follows a successful 36-year partnership that yielded a number of industry-first innovations in modular designed analyser platforms and workflow automation instruments for the laboratory's serum work area. This resulted in more than 55,000 installations in immunodiagnostics and clinical chemistry worldwide.

→ **www.roche.com**

Bio-Rad Acquires GnuBIO and DNA Sequencing Technology

Bio-Rad Laboratories, Inc., a global manufacturer and distributor of life science research and clinical diagnostic products, announced that it has purchased GnuBIO, Inc. Based in Cambridge, Massachusetts, GnuBIO is developing a faster and fully integrated droplet-based DNA sequencing technology that incorporates all the functions of DNA sequencing into a single, integrated workflow for medical diagnostics as well as research markets. While other sequencing technologies require separate workflows for target selection, DNA amplification, DNA sequencing and analysis, GnuBIO's technology will integrate the entire workflow into a single, low-cost solution that produces results within hours rather than days.

→ **www.bio-rad.com**

Abbott acquires CFR Pharmaceuticals

Abbott announced a definitive agreement to acquire Latin American pharmaceutical company CFR Pharmaceuticals, more than doubling its Latin American branded generics pharmaceutical presence and further expanding Abbott's presence in fast-growing markets. Under the terms of the agreement, Abbott will acquire the holding company that indirectly owns approximately 73 percent of CFR Pharmaceuticals and will conduct a public cash tender offer for all of the outstanding shares of CFR. Assuming all publicly-held shares are tendered, the total purchase price would be approximately $ 2.9 billion, plus the assumption of net debt of approximately $ 430 million.

→ **www.abbott.com**

Complix appoints Dr Yvonne McGrath as Chief Scientific Officer

Complix, a biopharmaceutical company focused on the discovery and development of Cell Penetrating Alphabodies™ (CPABs), a unique class of protein therapeutics that are active against intracellular disease targets, today announces that it has appointed Yvonne McGrath as Chief Scientific Officer (CSO). As CSO of Complix, Dr McGrath will be responsible for the use of the Company's Alphabody platform to identify new drug candidates and take these forward into development, either internally or with partners. This is part of Complix´ wider goal to build a pipeline of transformative Alphabody protein therapeutics. Dr Ignace Lasters, previously CSO and co-founder of Complix, will assume the role of Chief Technology Officer (CTO), and will be responsible for the further development and expansion of the Alphabody technology platform.

→ **www.complix.com**

Gecko Biomedical receives RadTech's Emerging Technology Award

Gecko Biomedical announced that it has received the prestigious "RadTech's Emerging Technology Award" at RadTech 2014, RadTech's annual technology conference. Gecko Biomedical is developing a platform of biocompatible, biodegradable adhesive solutions specifically designed for minimally invasive procedures. Its platform leverages advances in cross-functional fields ranging from biomimicry, biocompatible polymers and tissue regeneration to UV light activation. Gecko Biomedical's adhesives are based on the combination of safe, naturally occurring compounds to form a biocompatible pre-polymer with tunable adhesive and mechanical properties.

→ **www.geckobiomedical.com**

Bruker introduces Beta-Lactamase Testing for MALDI Biotyper

At the 24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Bruker introduces new and expanded research-use only (RUO) capabilities for the MALDI BiotyperTM platform. The MALDI Biotyper (MBT) is the market-leading system for microbial identification based on MALDI-TOF mass spectrometry. It is widely used in industrial microbiology, animal health and food safety, and its IVD and FDA-cleared versions are changing the paradigm in clinical microbiology. With nearly 1,200 customer systems, the MBT platform has become an accepted laboratory standard for next-generation microbial identification and emerging further high-value functional assays. The MALDI Biotyper RUO and IVD-CE versions cover a broad range of about 2,300 species with more than 5,600 well-characterised microbial isolates from gram-negative bacteria, gram-positive bacteria, yeasts, multicellular fungi and mycobacteria. Microbial identification with the MALDI Biotyper is done using proteomic fingerprinting, where unique species-specific patterns are automatically compared with reference spectra in the MALDI Biotyper library.

→ **www.bruker.com**

Taros Chemicals becomes a partner of the OPTIBIOCAT project

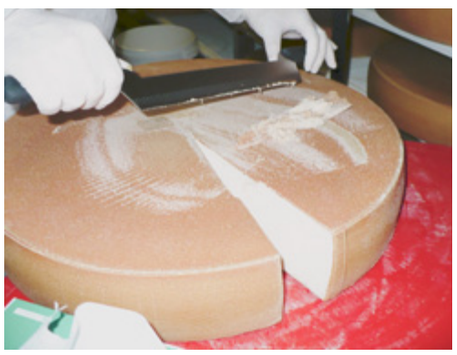
Taros Chemicals is an independent, owner-managed contract chemical research company, active in various business sectors including biotechnology, pharmaceuticals, plant conservation and materials. It has just joined a new EU-financed research project known as OPTIBIOCAT – 'Optimised Esterase Biocatalysts for Cost-Effective Industrial Production'. The project is dedicated to the goal of developing new effective ingredients and biocatalysts for the cosmetics industry. OPTIBIOCAT is a challenging research project involving multidisciplinary teams of highly qualified experts coming from 16 organisations and 8 different European countries. Taros will contribute to the synthesis of a top-quality library of effective ingredients from the enzymes developed by the project. The biocatalysts being used are feruloyl esterase (FAE) and glucorynol esterase (GE). With the help of these biocatalysts, it is hoped that phenolic fatty acid esters and sugar esters with antioxidant properties can be produced for the cosmetics industry. The FAE and GE being used in the project have been synthesised by members of the consortium.

→ **www.taros.de**

researched

Food microbiology

Cheese as a living environment



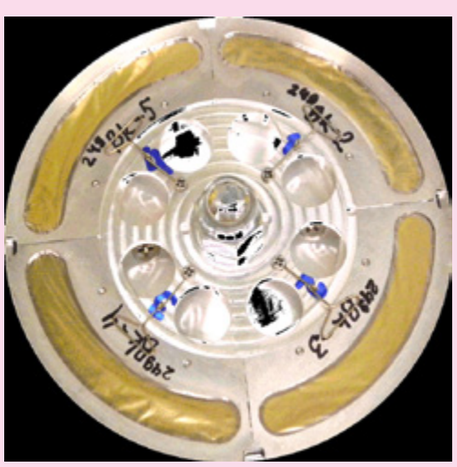
In order to take samples, the rind of the cheese was shaved off.
Picture: Vetmeduni Vienna / Elisa Schornsteiner

Bacteria and funguses make a crucial contribution to the maturing and the flavour of various kinds of cheese. The microbiome of Vorarlberg mountain cheese has hardly ever been studied in the past. Now scientists of the Institute for Milk Hygiene at Vetmeduni Vienna, in collaboration with the Vorarlberg Chamber of Agriculture, have made it the object of their research. Their genetic analysis makes it possible for the first time to present the complete spectrum of microorganisms living in Vorarlberg mountain cheese. One finding was particularly important. The bacterium halomonas, a halophilic germ probably having its origin in the sea, proved to be the most commonly found microorganism in the cheese. It is particularly in evidence on young cheese rinds. As the process of maturing causes the concentration of salt in the cheese rind to decrease, the researchers accordingly found fewer traces of halomonas in older rinds.

Source: www.vetmeduni.ac.at
Original publication: Schornsteiner, E. et al. (2014) International Journal of Food Microbiology (180), 88–97

Heavy ion research

Superheavy element 117 found



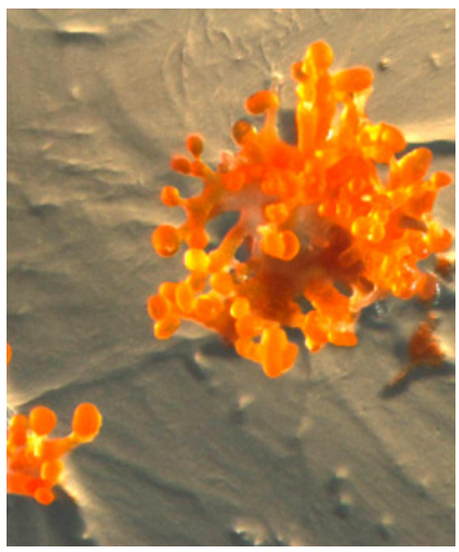
Target wheel with Berkelium-249, synthesised at the Institute for Nuclear Chemistry of the Johannes Gutenberg University of Mainz (JGU).
Picture: © GSI Helmholtz Centre for Heavy Ion Research

An international research team led by Professor Christoph Düllmann, working on the accelerator of the GSI Helmholtz Centre for Heavy Ion Research in Darmstadt, has produced several atoms of the superheavy element with the atomic number 117 and demonstrated its identity. The properties of element 117 that have been measured are in agreement with earlier findings from the Dubna Research Centre in Russia, and so support the official recognition of the new element 117. The experiment also resulted in the creation of atoms of the elements Dubnium and Lawrencium with exceptionally long life. Demonstrating their existence was only possible with the help of an extremely refined method of measurement, representing an important step in the direction of the 'island of stability' where superheavy elements with a particularly long life are expected to be found. Elements with an atomic number greater than 104 are classified as superheavy elements. No superheavy elements have been discovered in nature hitherto, but they can be produced artificially. Element 117 was isolated and demonstrated by means of the TASCA magnetic separator (TransActinide Separator and Chemistry Apparatus).

Source: www.uni-mainz.de
Original publication: DOI: 10.1103/PhysRevLett.112.172501

Biotechnology

Unusual lipids from soil bacteria



Fruiting bodies of the myxobacterium Stigmatella aurantiaca on an agar plate.
Picture: Dr Roland Garcia and Professor Rolf Müller, Helmholtz Institute for Pharmaceutical Research, Saarbrücken.

An unusual class of lipids are the ether lipids, which play an important role in human blood clotting. Bacteria are also capable of producing these lipids, but only recently has a working group under Professor Helge Bode succeeded in describing ether lipids derived from myxobacteria, identifying the gene for their formation and characterising the enzymes responsible. Myxobacteria are found everywhere in the soil. They can develop fruiting bodies with a height of up to 0.3 mm, similar to those of fungus. These bacteria thus represent a simple model for multicellular organisms. It appears that ether lipids help the bacteria to communicate. Professor Bode is the Merck Foundation Professor of Molecular Biotechnology at the Goethe University of Frankfurt. His research is not without practical application. If he succeeds in future in producing large quantities of ether lipids in bacteria by biotechnological methods, this would be a 'shark-friendlier' and so more sustainable approach towards making them available to the cosmetics and pharmaceutical industries.

Source: www.muk.uni-frankfurt.de
Original publication: DOI: 10.1038/nchembio.1526

Cancer research

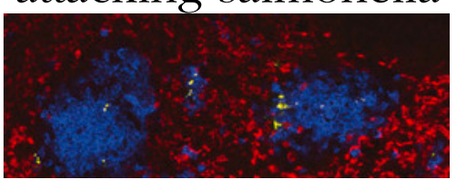
Blocking brain tumour stem cells increases survival chances of mice

Scientists at the German Cancer Research Centre investigating malignant brain tumours in mice have identified the molecule that gives the tumour stem cells their dangerous potential. When they switched off this stem cell marker, the mice with cancer survived for longer. The stem cell experts at the Cancer Research Centre identified the protein Tlx as the key molecule in the cancerous brain cells of the mice. When they switched off the Tlx, the cancerous stem cells became incapable of replicating themselves and the sick animals survived for longer. Human brain tumour stem cells likewise lose the ability to replicate themselves when the stem cell marker is switched off. So blocking the marker could also check the growth of aggressive tumours in human beings. In the eyes of the scientists, the Tlx molecule has other properties as well which make it a promising target structure for new approaches to therapy. The protein only occurs in cells of the central nervous system, so that no serious side-effects are to be feared. In addition, nuclear receptors can be effectively blocked by a careful choice of effective ingredients.

Source: www.dkfz.de
Original publication: DOI: 10.1016/j.stem.2014.04.007

Biology

Protein sharpens attacking salmonella



Section of a spleen infected with salmonella (yellow); red = red blood corpuscles, blue = neutrophils.
Picture: University of Basel, Biocentrum

Minute injections on the nanoscopic scale are the weapons wielded by salmonella bacteria. With these they inject molecular ingredients into their host cells, and so manipulate them to their own advantage. Researchers working under Professor Dirk Bumann at the Biocentrum of the University of Basel have now shown for the first time that the bacterial protein EIIAGlc is involved in activating these injections, and so is essential to the proliferation and dissemination of the salmonella throughout the body. It thus plays a central role in the colonisation of the host organism. The attention of the researchers was however drawn to the fact that the ability of the salmonella to multiply on an intracellular basis and spread through the organism was completely lost when the EIIAGlc was defective. EIIAGlc attaches itself to the injection apparatus of the bacterium, stabilises it and so activates the release of the effectors. By inhibiting the EIIAGlc protein it would be possible deliberately to put the secretion mechanism out of action. In this way infections could be checked effectively and systematically, without any deleterious effects on the natural intestinal flora..

Source: www.unibas.ch
Original publication: DOI: 10.1016/j.celrep.2014.04.022

Commentary

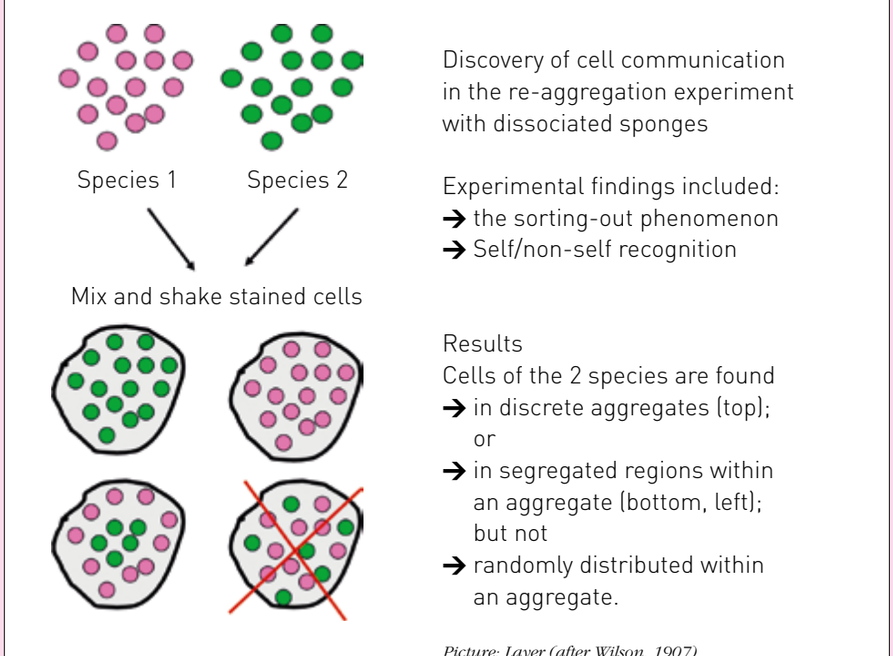
From 2D to 3D

A technology comes of age

Prof. Dr Paul G. Layer

Not so very long ago, applications to the German Research Foundation (DFG) for research projects involving three-dimensional cell cultures (3D cultures) would go straight to the bottom of the pile. The likely reason was that the appraisers simply didn't understand the technology's fundamental significance and its potential applications: the author penning these lines knows what he's talking about, after three decades of innovative production of retinal spheroids. But there has been a pretty abrupt about-face since the emergence of stem cell biology and tissue engineering, i.e. of technologies for artificial tissue culture as a starting-point for regenerative medicine. Indeed, 3D cell cultivation is truly the flavour of the month right now.

And yet 3D cell cultures have had a long history: in truth, 3D cell cultures actually lie at the very heart of cell biology itself (see e.g. Layer et al., 2001). As the twentieth century began, embryologists were in fact all working with dissociated cells from sponges, sea urchins, newts and other sorts of animals. Particularly revealing and historically relevant were the experiments by Wilson (Wilson, 1907; see fig. below), for example, where he separated sponges into their constituent cells before then placing them in salt water in a glass dish to observe their behaviour. The cells reformed themselves into complete sponges. When Wilson then altered the experiment to use cells from two related sponge species (differently coloured), he found that the various cells either reformed into discrete "re-aggregates" or separated out into discrete regions within a single aggregate. One finding from this experiment was the "sorting-out" phenomenon, i.e. cell-cell recognition. One can view this as the beginning of cell biology itself. Noteworthy names include Holtfreter, then Moscona



The re-aggregation of isolated cells from sponges marks the advent of cell biology (see discussion in article).

and Steinberg, and later my group on retinal re-aggregation, as well as Gierer with experiments on hydra, etc. (see Layer et al., 2001). With their various cell models, they have all put decades of work into advancing the technology. These researchers have also viewed their discoveries as steps towards in vitro tissue generation and have certainly made some key contributions to the success of modern tissue engineering. For a long time, however, 3D cultures remained the preserve of a select few. Research work was time-consuming (and hence expensive) – and its analysis and documentation even more so. Hardly surprisingly, cell biologists much preferred to work with 2D cultures. That these cultures now had little in common with the natural tissue environment was largely overlooked, however. It was the stem cell biologists, looking for ways to rapidly propagate their cells in vitro, who rediscovered the advantages of re-aggregation (and spheroid) technology and turned it into the red-hot research topic it's become today. Recently, for example, the culturing of mini-brains in Jürgen Knoblich's lab sent shockwaves around the world following its publication in Nature (Lancaster et al., 2013). So 3D cultures, it appears, are finally back in fashion.

In the 1980s, a growing body of research was showing how cells in a cell layer are strongly dependent on their specific extracellular matrix (ECM). This was the era of discoveries such as cell adhesion molecules (NCAM, the IGSF family, cadherins, etc.), giant intercellular proteins such as laminin and fibronectin, and of all sorts of transmembrane receptor/signal transduction proteins such as the integrins. And perhaps worth mentioning once again: the first cell adhesion molecules were also discovered with re-aggregation techniques. Many of these ECM materials contain sugar groups (polysaccharides), i.e. they are glycoproteins or glycans that, by prodigious water retention – and especially their mechanical properties such as elasticity, rigidity, etc. – make a major contribution to the volume of the tissue in question. Accordingly, it's not surprising that today's "tissue engineers", looking to manufacture tissue artificially with stem cells, are reliant on suitable polymeric materials in order to replace the properties of natural ECM components either partially or wholly in vitro. Work on discovering biocompatible materials that are suitable for use not only with 3D tissue cultures but – naturally – as matrices in a tissue implant within patients is now an important field of research in its own right, and one that will be fundamental to the success of regenerative medicine. The following article offers a highly promising example of one such development in biomaterials research, while also demonstrating that the potency of 3D cell cultures has now also been recognised here in Germany.

→ **layer@bio.tu-darmstadt.de**

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Lancaster, M.A. et al. (2013), Cerebral organoids model human brain development and microcephaly, Nature 501, 373–381.

Dessert for organ regeneration

Development of the next generation of synthetic extracellular matrices for 3D cell culture

Prof. Dr V. Prasad Shastri, Aurélien Forget
Institute for Macromolecular Chemistry and Excellence Cluster,
Albert-Ludwigs-University Freiburg

Imagine that you are dining at a Japanese restaurant and at the end of the meal, the waiter serves you this beautifully laid out and decorated dessert. Besides being brightly colored and simply delicious, some of these desserts might remind you of what is called "pudding" in western cuisine. Believe it or not, these Far Eastern sweet deserts are made of agarose, a polysaccharide extracted from red algae, which has been used as a cooking ingredient for more than 400 years. In Germany however, agarose has only recently been introduced into the vegetarian kitchen as a substitute for gelatine in cakes.

Besides, being found on German bakery racks, walk into a biology laboratory and you will find a bottle of agarose neatly tucked away among other laboratory supplies, ready to be dissolved in water and poured into slabs for various biological assays. Agarose has become an indispensable tool for the life scientist for analyzing and purifying DNA and proteins. This polysaccharide can form a hydrogel, which is a polymeric water container. This enables it to replicate in vitro the environment found in human tissue and as a result lends itself ideally to the growing field of regenerative medicine, i.e. engineering of tissue or organs, to restore normal function [1]. Since the mechanical properties of agarose recreate the natural surroundings of chon-drocyte cells, its use was confined to only mimicking cartilage tissue. However, a team in our laboratory at the University of Freiburg recently succeeded in extending agarose applications by replicating in vitro the whole range of mechanical properties found in mammalian tissues. This innovation offers completely new perspectives for the development of human tissue replicates in research laboratories [2].

The 2D cell culture model and its limitations

Cell cultures in the laboratory are usually performed on gamma-irradiated polystyrene (tissue culture plastic). The properties of the material allow the deposition of protein on its surface. Cells deposited on the protein field attach, spread and multiply. This technique has been in use for decades in every cell biology laboratory around the world because of its convenience, ease of use and affordability. In short: it represents a user-friendly product. Unfortunately, the petri dish technique is not representative of the environment mammalian cells encounter in tissues. Indeed, while cells on tissue culture plastic (TCP) are organized in a 2D monolayer, cells in tissues organize around a 3D scaffold called the extracellular matrix (ECM), Figure 1. Made of different components such as macro proteins and polysaccharides, the ECM provides the nutriment to the cells, presents signaling proteins responsible for intercellular communication, and last but not least provides mechanical support. On a 2D monolayer, while the delivery of nutriment and proteins replicates quite accurately what is found in mammalian tissues, the mechanical support provided by the petri dish is completely different from the natural cell surroundings. Indeed, on 2D surfaces cells organize in a different way than in vivo: on TCP, cells that form the inside of blood vessels – endothelial cells – remain single and adopt an elongated shape, Figure 2A, whereas endothelial cells in the kidney collaborate to form a blood vessel, Figure 2D. In addition to the differences in shape, it has been demonstrated that the genetic information of 2D cells diverges from the same cell type in mammalian tissue [3,4].

Fig. 1 In their natural environment, the cells (green) use specific markers (pink) to bind to a mechanical support matrix of polysaccharides (yellow) and fibrous proteins (blue). Dissolved proteins such as growth factors (purple) enable communication between the cells and matrix-degrading enzymes (black), thus remodelling the matrix.

tissue engineering

Prasad Shastri, born in 1967, is a Full Professor at the University of Freiburg, Germany and the Director of the Institute for Macromolecular Chemistry. He is one of the core faculties at the BIOSS Center for Biological Signaling Studies, which is one of the national clusters of Excellence in Germany. He received his Ph.D. from Rensselaer Polytechnic Institute (Troy, NY) in 1995 and carried out his post-doctoral work with Robert Langer at MIT. He has published over 100 peer-reviewed papers, proceedings, extended abstracts and book chapters and authored over 30 issued and pending patents in polymers, biomaterials, pharmaceuticals and regenerative medicine, many of which are licensed to pharma- and biotech companies. He has pioneered several technologies in biomaterials, drug delivery, and nanotechnology, including the In Vivo Bioreactor, a ground-breaking approach for autologous engineering of bone and cartilage. His laboratory is actively involved in the development of biomaterials for controlling cellular microenvironments, in vivo engineering of tissue, intracellular delivery, cancer therapeutics and functional imaging.

Aurelien Forget, born in 1984, studied chemistry at the Université Pierre et Marie Curie in Paris, ultimately obtaining a master's in polymer chemistry. Since 2009, he has worked in Prof. Shastri's lab at the University of Freiburg as part of his doctoral research into developing new 3D cell culture materials.

The tissue engineering paradigm

In order to reduce the gap between in vivo/in vitro experimental results, one of the envisioned solutions is to reproduce the natural tridimensional environment found in the different organs. This idea was first put forth by Steinberg in the early 60s [5] and evolved into the tissue-engineering paradigm in the mid-1980's by Langer and Vacanti [6]. So as to recapitulate the cell environment one has to be able to (1) deliver the soluble signals, (2) provide a mechanical support, (3) present the specific and dedicated attachment motifs to the mechanical support and (4) give the possibility for the cell to degrade the support, thus allowing the remodeling and evolution of the tissue [7], Figure 3. Pursuing the goal toward the recapitulation of these features, many systems have been developed offering an alternative to the 2D models on TCP.

Tissue mechanics as a biological signal

Concomitant to the engineering of 3D substrates, advances in matrix biology have enabled the identification of specific amino acid sequences recognized by the cells as an anchor to the matrix. The study of the impact of matrix topography on the shape and fate of the cells has revealed that the matrix also carries biological cues [8]. Therefore, projects aiming at understanding the biological signals involved in inter- and intra-cellular communication, e.g. research at the cluster of excellence BIOSS Centre for Biological Signalling Studies from the University of Freiburg, need also to take into account the biological implications of physical cues. In this regard, our group at the Institut für Makromolekulare Chemie investigates the interaction of cells with their environment, and how physical information such as surface topography and substrate stiffness can affect the organization and function of cells. This knowledge is expected to aid one day in recreating fully functional tissues in the laboratory.

Challenges in engineering a synthetic ECM (SynECM)

3D cell culture systems developed to date unfortunately suffer from several drawbacks, key among them being: cost, need to have a customized material for each cell system, complexity of set up and use, and finally, difficulty with translation to the clinic due to the recourse of complex chemistry or materials originating from animals. These characteristics have slowed down the adaptation of 3D scaffolds over the use of 2D TCP in many laboratories for "everyday" experiments, and have limited their use to specialized laboratories having the "know-how"

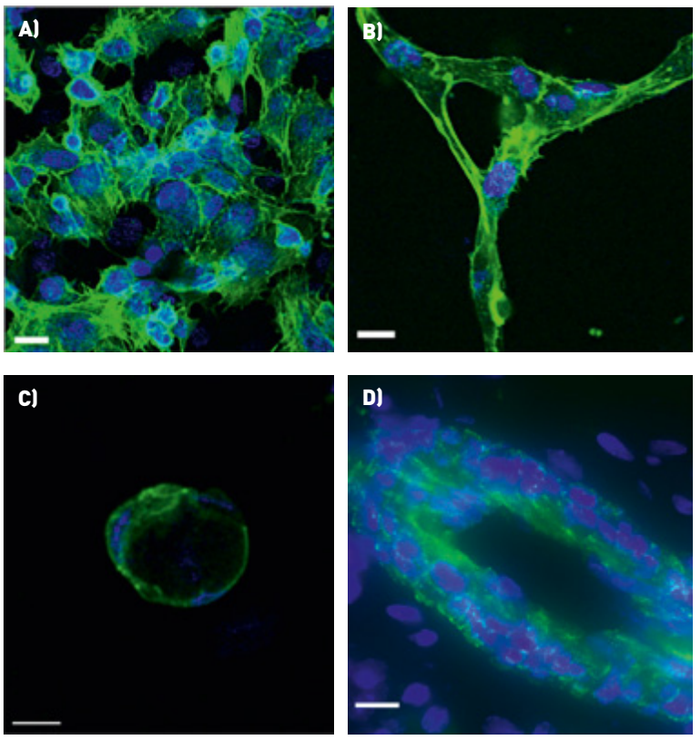

Fig. 2 The substrate on which the cells grow influences their shape and organisation. Fluorescence microscopy image of human umbilical vein endothelial cells (HUVECs) on separate substrates. The nucleus is blue, the actin filaments are stained green. **A**: On a 2D plastic tissue culture, the cells grow separately and have a flat shape. **B**: On Matrigel®, an animal extracellular matrix (ECM), the cells organise themselves into a network of capillaries. **C**: On the next-generation synthetic ECM, the cells form multicellular structures similar to blood vessels. **D**: Mouse kidney blood vessel; the cell nuclei are blue and the CAV1 protein specific to endothelial cells is stained green. Bars, 10 µm.

in both material science and cell biology. In order to compete with the user-friendly TCP 2D model, one has to provide innovative solutions offering a universal platform that can be used by any cell biologist without prior knowledge of material science. To fulfill this user need, we have developed an innovative synECM platform that overcomes the limitations of current 3D models by offering characteristics such as: (1) affordability, (2) translatability, (3) versatility and (4) ease of use, Figure 4.

A new generation of synECM

(1) As a matter of fact, we have based our system on an abundantly naturally-occurring polysaccharide used to prepare Japanese dessert. Apart from being edible, agarose is an inert biocompatible material with a history of use as a matrix for in vitro and in vivo applications. Since agarose does not exhibit any biological information, the biological signals presented in the synECM only originates from the soluble or immobilized protein on the backbone introduced by the user, thus resulting in what is referred to by scientist as a low biological background noise system.

(2) Chemical modification of agarose enables physical hydrogels, which exhibit a sol-gel transition around room temperature and can be

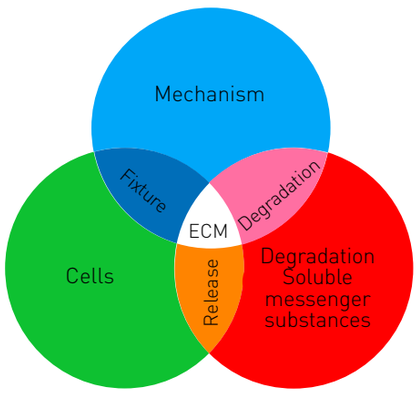

Fig. 3 The tissue engineering paradigm. A synthetic extracellular matrix (ECM) must copy the essential characteristics of tissue, i.e. it must offer mechanical support to which cells can bind, it must convey dissolved signalling substances to the cell, and it must permit its own degradation and remodelling by cell enzymes. (Aurelien Forget, doctoral dissertation (2013)).

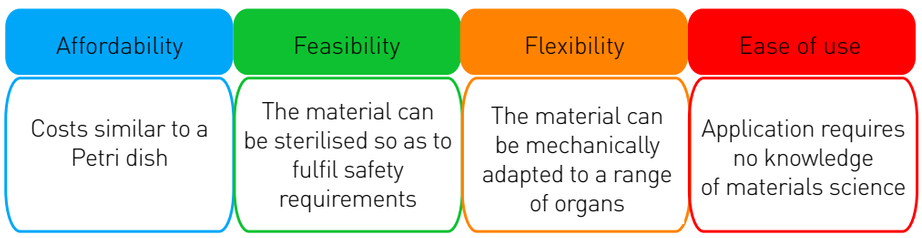

Fig. 4 Characteristics required to develop the next generation of synthetic extracellular matrices

formed in a body cavity. This characteristic makes agarose a material with good translational potential as it can be injected as a solution that will gel instantly in the tissue without resorting to chemical crosslinkers.

(3) Current synthetic ECM systems are designed for the recapitulation of a single tissue environment. Therefore, we have chemically modified agarose backbone in such a way that the hydrogel mechanical properties can match the one of many tissue environments ranging from cartilage to brain tissue. This unique and versatile property permits the use of our system for the design of environments for various cells.

(4) Finally, by characterizing the modified agarose at the fundamental level, a material system that can be reconstituted to present precise mechanical properties and cell-interaction peptides has been realized.

The agarose-based synthetic ECM recapitulates the different components that constitute natural tissues. In doing so, the system provides a unique environment in which endothelial cells organize in vitro (Figure 2C), in a similar manner to what is observed in vivo (Figure 2D), and which does not occur on 2D TCP platforms and most commonly used animal extract matrix – Matrigel (Figure 2B).

The future of synthetic ECM

SynECMs are expected to play a vital role on many fronts in the burgeoning arena of biotechnology. In addition to providing a reliable alternative to biologically-derived products such as systems made of decellularized animal tissue as research platforms, synECMs also provide an attractive option in the development of automated high throughput screens that are based on organ and organoid cultures. Such organ culture-based screening platforms can aid in the more rapid discovery and development of drugs candidates, which can not only reduce the lead time for the translation of a potential drug from the lab to phase-I clinical trials but also help in reducing animal testing. In order for synECMs to be integrated in high-throughput technology, the cost and ease of manipulation are vital. Ability to impose highly-defined cellular microenvironments both at the physical and biological level may help in unraveling (deciphering) new clues in disease development, detection and treatment. A synECM essentially adds a long-sought third dimension to what is typically a 2D screening platform. The "one pot" synthesis of highly-defined synECMs can further assist this transition from 2D to 3D. With this goal in mind, our agarose platform provides the first step toward a 3D scaffold system in which the physical attributes and biologically active motifs can be assembled by the final user through simple mixing of the different components. Such technology could be developed by leveraging emerging concepts in peptide-protein interactions.

Under the auspices of the cluster of excellence BIOSS Centre for Biological Signalling Studies, our efforts in engineering a naturally-occurring poly-saccharide to possess diverse physicochemical characteristics has accelerated the transition of routine laboratory cell biology studies from a 2D to 3D system. Our focus on the end user has enabled us to identify and implement key properties such as affordability and ease of use to ensure maximum adoption of the agarose-based synECMs. So, next time you visit a Japanese restaurant please don't forget to order the dessert. You never know – it may save your life one day.

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Quick, comfortable diagnosis

The potential and limits of Raman spectroscopy methods in medical diagnostics

Dr Thomas Mayerhöfer [2], Dr Christoph Krafft[2], Dr Ute Neugebauer[2,3], Dr Sebastian Dochow [1,2], Dr Christian Matthäus[2], Prof. Dr Matthias Kirsch[4], Prof. Dr Gabriele Schackert[4], Dr Joachim H. Clement[5], Prof. Dr Michael Bauer[3], Prof. Dr Bernhard R. Brehm[6], Prof. Dr Jürgen Popp [1,2,3]

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As the proportion of elderly people in the population continues to rise, we are facing the growing challenge of providing affordable and sustainable healthcare systems. An impending collapse of these systems can be avoided only by developing new methods and equipment that enable disease to be detected and tackled as early as possible. Ideally, this would be no later than the occurrence of initial disease-driven changes at a molecular level.

On account of the special properties of light, photonic solutions offer highly promising results – and none more so than Raman spectroscopy and its various techniques. As one example, Raman spectroscopy differs from several other photonic solutions as it enables contactless measurement that requires no exogenous labels. This is particularly beneficial since such labels face the same regulatory hurdles as medicines. In addition, the fact that Raman spectroscopy is comparatively fast and precise means it offers a diagnostic approach that is especially comfortable and direct for the patient. Its qualities as an imaging method mean that it offers high specificity while simultaneously remaining low- or non-invasive when combined with other optical methods and chemometrical methods in particular. Other advantages of Raman spectroscopy include its high spatial resolution, low effort for sample preparation and the weak Raman spectrum given by water, a property offering the possibility of working with aqueous samples.

This article will present some examples of possible applications, selected for their relevance to medicine and clinical practice.

Cell diagnostics

In pathogen diagnostics, the gold standard is the incubation and analysis of a bacterial culture. This requires both time – as much as a week, in some cases – and experienced lab personnel. In the example of a patient with sepsis, however, this quantity of time is simply unavailable. Not least because the rate of survival after the onset of septic shock drops to less than 1 in 5 after merely twelve hours without specific treatment [1]. Raman spectroscopy has the potentially to help identify the pathogen responsible within just a few hours. Every species of bacteria possesses its own personal Raman signature, and the spectrum from just a single bacterium can be sufficient for identification [2]. Since spectral differences between species are often subtle, the application of chemometrical methods is imperative to ensure their proper assignment.

A bacterial spectrum is made up of spectral signatures from its constituent substances, such as water, proteins, fats, nucleic acids, carbohydrates, etc. (fig. 1-I). Accordingly, subtle differences can even be present in spectra from members of the same bacterial species, as a result of differences in age, nutritional condition and environmental factors. By utilising databases that store spectra from bacteria in different physical conditions, chemometrical methods are then able to assign the Raman spectra to the corresponding species. Effectively, the process involves splitting a spectrum into especially data-rich areas, which are then compared with the corresponding areas from spectra held in the

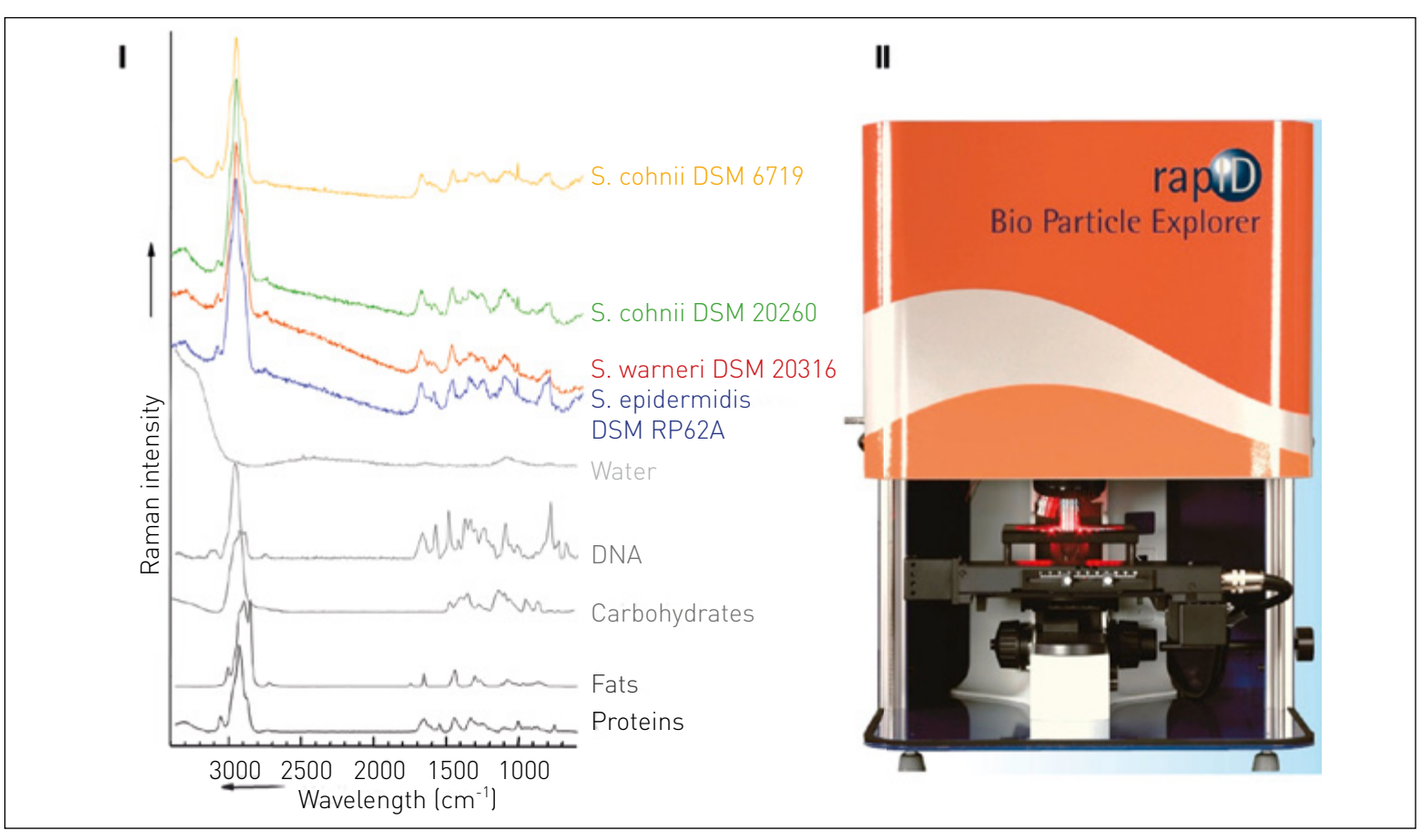

Fig. 1 **I**) Raman spectra of the biological constituents of bacteria: water, proteins, nucleic acids (DNA), carbohydrates and fats. In the examples of Raman spectra given, separate Staphylococcus strains can be identified from the various bands produced by individual constituents. **II**) Bio Particle Explorer (a rapID product).

database. The process enables almost 99% of bacteria to be assigned to the correct species [2]. For identifying bacteria in less complex matrices, e.g. in cleanroom air, a corresponding solution is already commercially available (RapID Bio Particle Explorer, fig. 1-II). This approach uses fluorescence spectroscopy to distinguish between non-living particles and bacteria. The latter are then targeted for identification by Raman spectroscopy. In more complex media, however, such as saliva, urine or (especially) blood, bacteria must first be separated from these media, since the medium's own Raman signature otherwise makes identification difficult or impossible. This separation stage can be performed by microfluidic chips, which may apply the principle of dielectrophoresis, for example, so as to trap and hold bacteria, and thus make them available for measurement [3]. This obviates the need for actual physical separation of the medium: instead, the bacteria can also be measured directly in solution. Apart from merely identifying the bacteria, the latter step also provides data on bacterial susceptibility or resistance to antibiotics. This involves using Raman spectroscopy to measure bacterial growth curves under the influence of antibiotics. Within two hours, the presence of resistance can be determined with a sensitivity and specificity each of 90%.

Alongside bacterial detection, diagnosis of tumour cells in blood also has a major role to play. Such cells can be shed by cancerous tissue, enter the bloodstream and then cause metastases. In the bloodstream, isolated tumour cells are fairly easily accessible. A procedure using Raman spectroscopy can be used to detect them in a way similar to flow cytometry. The blood specimen first passes through a microfluidic chip. Within this chip, single cells are held by optical traps, and are then analysed and classified with the aid of Raman spectroscopy. Based on this classification, the cells are then sorted for further processing. Due to vibrational spectroscopic characterisation, Raman spectroscopy permits considerably more precise diagnostic results to be achieved at the level of the single cell, compared to flow cytometry. The process does require more time, however, resulting in a considerably reduced flow (5–6 cells/minute). Yet technical improvements to equipment will no doubt lead to considerably higher rates of flow in the future. Figure 2 shows a microfluidic chip of this kind made from quartz [4]. The Raman spectra recorded still exhibit artefacts from the spectral properties of the optical filter, the trapping laser and the substrate material. Accordingly, these still need to be eliminated in order to ensure successful classification, and render visible the spectral fingerprints of white blood cells (green) and tumour cells (orange, brown, blue).

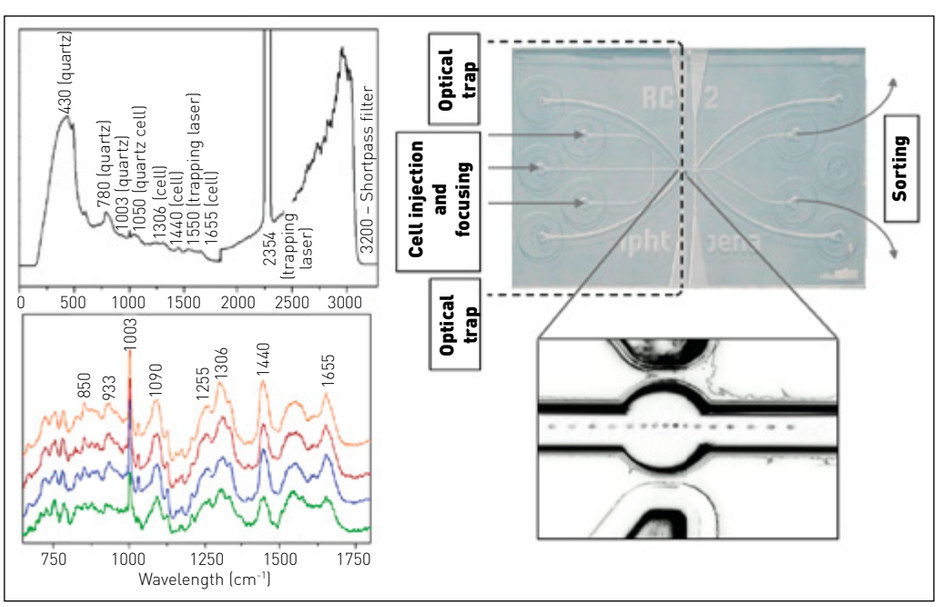

Fig. 2 Microfluidic chip for Raman-activated cell sorting

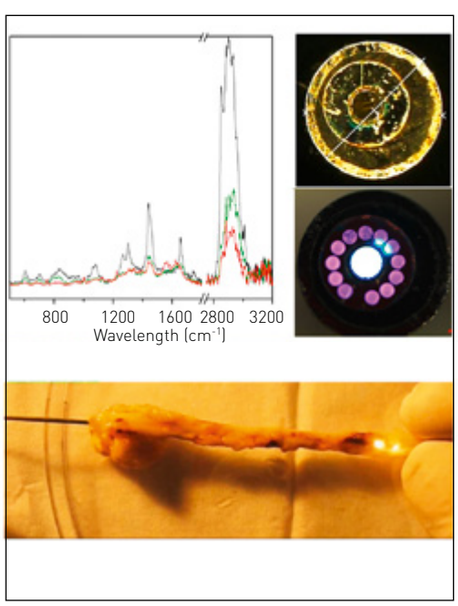

Fig. 4 In vivo investigation of rabbit arteries using Raman endoscopy.

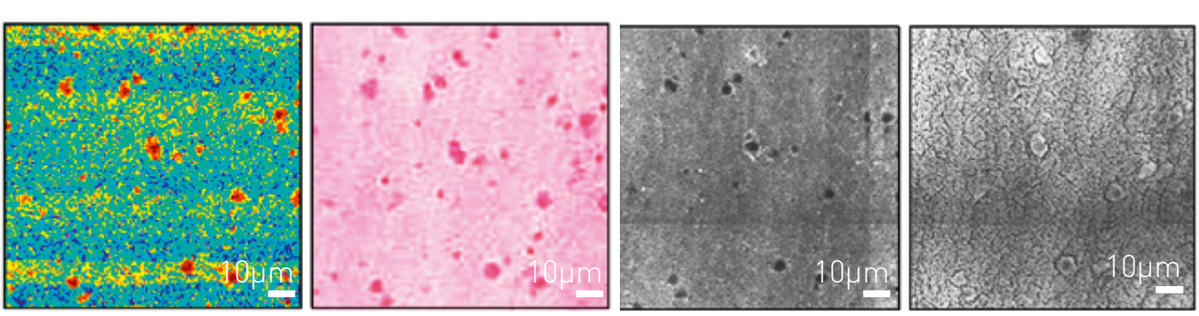

Fig. 3 Comparison of TPEF, CARS and Raman microscope images of an undyed brain tumour thin-section with a light microscope image of the prepared specimen subsequently dyed with haemotoxylin and eosin (from left to right: Raman, light microscope, TPEF, CARS).

Jürgen Popp, born in 1966, studied chemistry at the universities of Erlangen and Würzburg. After receiving his doctorate in chemistry, he completed post-doctoral work at Yale University before returning to the University of Würzburg, where he completed his habilitation in 2002. He was appointed Professor for Physical Chemistry at Jena University's Institute of Physical Chemistry in 2002. Since 2006, he has also held the position of Scientific Director at the Leibniz Institute of Photonic Technology (Jena). His research interests lie primarily within the field of biophotonics. He has published more than 400 journal articles and is a Fellow of SAS and SPIE. In 2012, he received an honorary doctorate from Babeș-Bolyai University, Cluj-Napoca, Romania.

anti-Stokes Raman scattering (CARS), two-photon excited fluorescence (TPEF) while using endogenous markers and second harmonic generation (SHG) [5]. With Raman spectroscopy, all (Raman-active) vibrational modes are excited simultaneously, whereas in CARS, the overlaying of three separate, spatially oriented light pulses causes a chosen vibration to be isolated and coherently excited, resulting in the generation of a fourth, spatially aligned and coherent light pulse. In contrast to Raman imaging, the strongly enhanced scattering cross-section enables the recording of an image in a much shorter time frame (factor of 10^4). While Raman and CARS provide us with chemical data, SHG and TPEF augment these to include morphological details. SHG emphasises highly ordered, non-centrosymmetric structures such as collagen, while TPEF is particularly amenable to endogenously fluorescent substances such as NAD(P)H, flavins, elastins, etc. Figure 3 presents a comparison of TPEF, CARS and Raman microscope images of an undyed brain tumour thin-section with a light microscope image of the prepared specimen subsequently dyed with haemotoxylin and eosin. The cell nuclei, which are resolved by all methods, are particularly important for the histopathological evaluation. By combining morphological and functional information, this approach has the potential not only to detect and classify tumours at an early stage, but also to localise tumour boundaries with an adequate level of precision and reliability.

Tissue diagnostics

For some tumours, radical removal may not be desirable or even possible. A brain tumour is just one example. The aim here is to remove as little healthy tissue as possible: a difficult task, since tumour and healthy tissue are often not clearly differentiated. In general terms, an objective method would be desirable, which could then be used directly in the operating theatre. The ideal instrument would be an operating microscope, able to image tumour boundaries directly. To implement such a microscope, it would be advantageous to combine multiple imaging techniques that increase contrast while largely avoiding labels. Our experience has shown us that especially promising techniques here include combining Raman with coherent

Organ diagnostics

For the endoscopic investigation of arterial plaque, a morphological evaluation alone is not sufficient, since this is unable to assess whether deposits are harmless or whether these may detach themselves from the vessel wall, producing obstructions that are capable of causing heart failure or a stroke. Since the Raman spectra of calcium phosphate, connective tissue, triglycerides and cholesterol are characteristic, endoscopic Raman spectroscopy could be used to determine plaque composition and thus the hazard that these plaques represent. The value of this diagnostic approach has already been confirmed in animal experiments. Figure 4 shows how a probe 1 mm in diameter was combined with a central excitation fibre and 12 detection fibres for use in ex vivo measurements in rabbits [6]. Measurement conditions were chosen to accurately simulate in vivo conditions. In terms of their intensity and spectral position, the signals from plaque deposits are clearly distinguishable from arterial wall lipids with bands of collagen and from blood with bands of red blood cells. The combination of chemical and morphological data should be equally advantageous in this scenario. We are therefore planning to combine Raman spectroscopy with optical coherence tomography and/or ultrasound. Miniaturisation also offers the possibility of making even finely-structured arteries accessible to measurement.

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Research laboratories and offices for young companies in the life sciences sector

Bayer HealthCare opens new 'CoLaborator' building in Berlin

In the picture (from left to right): Professor Stefan Jaroch (GDD), Sonja Jost (CEO of DexLeChem GmbH), Professor Andreas Busch (Head of GDD), Klaus Wowereit (Governing Mayor of Berlin) and Andreas Fibig (Head of Bayer HealthCare Pharmaceuticals).
Picture: Bayer Healthcare AG.

Entrance of the new CoLaborator building.
Picture: Bayer Healthcare AG.

Under the name of 'CoLaborator', Bayer HealthCare has just officially dedicated its second research incubator for young companies working in the life sciences at its base in Berlin. The solemn opening took place in the presence of Governing Mayor Klaus Wowereit.

The object of Bayer's global CoLaborator scheme is to offer a suitable laboratory and office infrastructure to young companies in the fields of chemistry and biology in the immediate vicinity of its own research facilities. The CoLaborator centre thus creates the ideal conditions for encouraging research and innovation.

The recently opened CoLaborator in Berlin will make it possible for young life science companies to rent completely furnished research laboratories and office premises. It is hoped that the field of activity of the young companies will be in keeping with the wide range of research interests at Bayer, so that young businesses and pharmaceutical expertise can work to their mutual benefit. The CoLaborator has sufficient space to accommodate up to nine small firms. It is an independent building with a surface area of around 800 square metres, some 420 square metres being dedicated to laboratories. The new CoLaborator is situated on the Bayer site at Müllerstrasse 178, 13353 Berlin.

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Korean biotechnological associations and the Free State of Saxony launch cooperative partnership

The South Korean cluster Wonju Medical Industry Techno Valley Foundation (WMIT) and biosaxony e.V., the Biotechnology / Life Sciences Association of the Free State of Saxony, have resolved to embark on a close partnership. On the occasion of the state visit of the South Korean President to Dresden, the President of the Wonju Medical Industry Techno Valley Foundation, Wonbok Lee, and biosaxony Managing Director André Hofmann signed a Memorandum of Understanding to this effect. The ceremony was also attended by the South Korean Minister for Trade, Industry and Energy, Yoon Sang Jick, and Saxony's Economic Minister Sven Morlock.

The principal objective of the partnership is close cooperation and mutual support in the medical technology sector. The aim is to encourage the sustainable development of medical technology firms, research institutes and universities both in Saxony and in Korea's Gangwon Province. With a view to speedy commercial exploitation, joint research programmes will receive support and conferences will be held on a regular basis, enabling companies to stay in contact with one another and providing assistance for cooperative ventures. Another objective specified in the Memorandum of Understanding relates to the planning and support of the marketing activities of the companies involved, both in Europe and in Asia.

→ **www.biosaxony.com**

Source: biosaxony e.V.

After signature of the Memorandum of Understanding, from left to right: André Hofmann, Minister of Economics Sven Morlok, Minister Yoon Sang Jick, Wonbok Lee.
Picture: biosaxony Management GmbH

Photons on duty for health

Raman spectroscopy for biologists and clinicians

Dr Rainer Gangnus[1], Dr Paulius Grigaravicius[2], Prof. Dr Karl-Friedrich Becker[3], Dr Karin Schütze[1]

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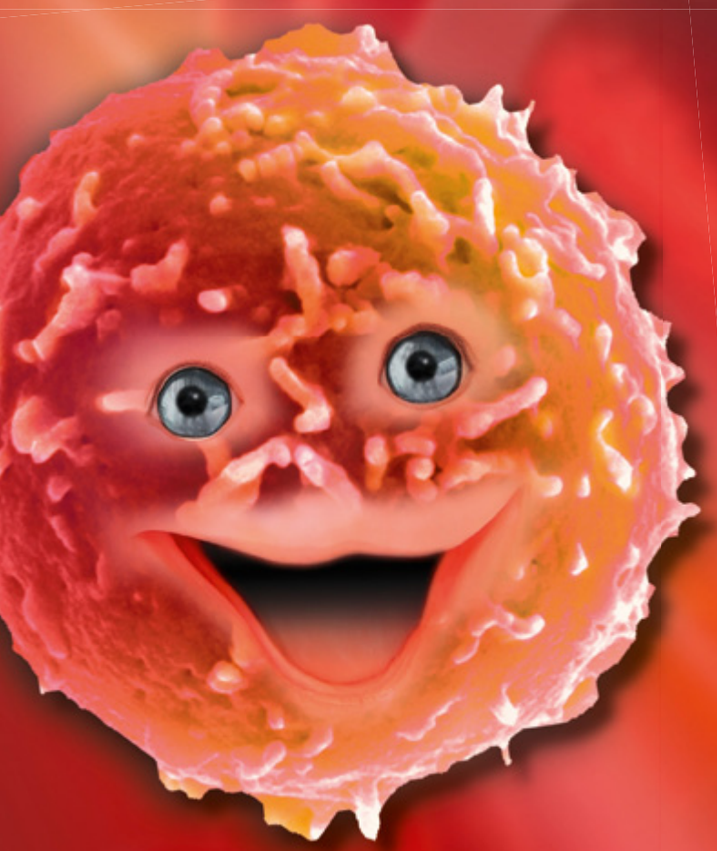
[2] Clinical Cooperation Unit Neuropathology at German Cancer Research Center, Heidelberg
[3] Technische Universität München, Pathologie, München, Germany



Spectroscopy the bugbear for medical students and technicians: Asked by biologists or clinicians what exactly we are doing at CellTool® we originally answered, that we are developing a unique spectroscopic microscope system that enables easy cell analysis based on Raman spectroscopy. The two "non-words" in the world of biomedical scientists namely spectroscopy and Raman immediately caused to raise hackles and one could see how interest vanished. We gradually realized that this was mainly due to the fact that most of us biologists and physician have heard or had to learn about spectroscopy during our studies. However, those abstract zig-zag graphs reminded us more of a mountain scenery than of a method that could give valuable information about matter or even about biological samples.

Amazingly Raman microscopy can indeed identify and analyze individual cells – alive or fixed – providing detailed information about the actual metabolic status of the cell. We only have to learn how to read and interpret the "zig-zag" graphs of those complex Raman spectra, where all molecules within the laser focal spot contribute and yield a specific sum spectra as characteristic as a "fingerprint". Most important Raman microscopy measures in a complete non-invasive manner keeping cells unaffected for further investigations or cell therapeutic applications.

Actually there are several high sophisticated pure physical methods that finally made their way into the daily routine of clinical diagnosis and therapy – such as ultrasound, x-ray examination, magnetic resonance tomography (MTR), or positron emission tomography (PET) as well as various laser treatments such as laser lithotripsy or laser treatment of the eye (laser shaping of the cornea).

Raman spectroscopy could be another one of those emerging highly physical technologies that most probably once will be commonly used in the hospital to support the pathologist at appraisal of tissue sections [1], to find patient-specific most effective anticancer drugs or to assist the surgeon during operation for minimal tumor tissue extraction [2]. Raman microscopy can also identify bacteria as each has a distinct Raman spectrum [3]. This could speed-up pathogen identification and may provide great impact on sepsis treatment [4].

For decades Raman spectroscopy was the domain of physicist, chemists as well as pharmacists, who used Raman spectroscopy to investigate or to proof quality of alloys, metals, pure substances and compounds. They also measured spectra of almost any-kind of isolated and purified biomolecules. It was never expected that Raman spectroscopy could also characterize biological cells, which are composed of thousands of molecules of various kinds and concentrations undergoing a permanent changeover.

The advantage of Raman spectroscopy is its sensitivity as well as the feasibility to work within fluidics. This provides a wide range of possibilities in biological research and medical applications. In particular, Raman microscopy enables simultaneous investigation of cellular components and gives insight into the cells metabolism in a completely non-destructive way. Thus, changes in molecular composition and activation can be measured coming along with cell cycle, cell differentiation or induced by drug exposure or environmental impact. Raman microscopy identifies and analyzes cells with a high degree of precision and specificity – without the need of biochemical markers, fluorescent labels, antibodies or beads. Most importantly Raman microscopy works within liquids and thus living cells can be investigated under culture conditions leaving them unaffected and preserved for further use.

Facts and potential

In recent years numerous publications appeared that clearly demonstrated the feasibility of Raman microscopy to identify and examine cells. In most cases a physicist having a Raman system available collaborated with a biologist or clinician who provided the samples. In spite of promising results Raman spectroscopy did not yet make its way into routine cell analysis and clinical diagnostics. This is mainly due to extra preparation that is required to mount the cells onto special substrates that have high spectroscopic quality but are not appropriate for routine cell analysis work. Cells do not necessarily like to seed on those substrates, simultaneous microscopic observation might be hindered or these substrates are simply too expensive to be suited as consumables. Furthermore, special expertise from a physicist is required to operate the complex Raman systems and expert knowledge is necessary to process and interpret the spectra for meaningful conclusions.

Unmet need for daily routine

A "bio-compatible" Raman system is required to fill the gap between laboratory bench-top studies and clinical diagnosis. The biologists and technicians need a tool that can be integrated in routine lab work. A straight forward and easy to use device that match the requirements of cell culture and cell biological work as well as tumor diagnostics would be of great interest. Cells cultured in common culture ware or on routine glass slides should be able to be investigated by Raman microscopy without further preparation steps.

The solution – a Raman Microscope for biologists and physicians

That was our intension to develop the BioRam® (CellTool GmbH, Bernried, Germany), a Raman trapping microscope system, where all the complex Raman related issues are running in the background. There is no need for the user to interfere with this "black-box". Thus, laser based Raman microscopy is as easy as fluorescence or laser scanning microscopy. The applied 785nm laser wavelength is well tolerated by living cells. Various inserts have been designed to hold culture dishes, multiwell-slides, microtiter-plates or up to three glass slides to analyze serial tissue sections. The inverted microscope platform allows safe and comfortable working with living cells cultured in media.

Specimen are simply placed on the microscope table and observed using bright field illumination. Cells adherently growing

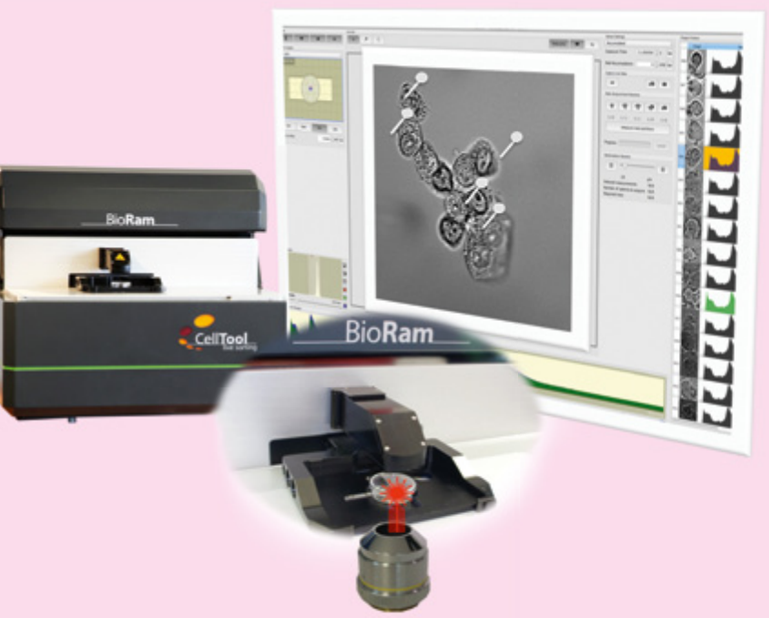


Fig.1 BioRam® – the cell and user friendly Raman microscope system (CellTool, Bernried, Germany) consists of an inverted microscope platform with a motorized sample holder and integrated 785 nm Raman spectroscope. The Raman laser is focused through the objective to a spot size of about 1 µm in diameter. Application specific software facilitates spectra retrieval and supports data processing.

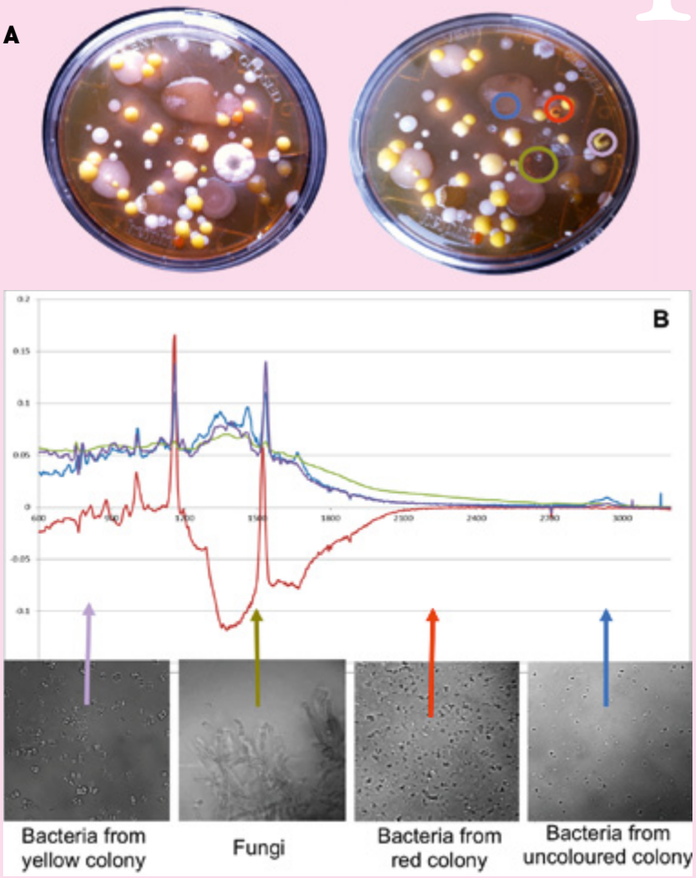


Fig. 2 Raman spectra from airborne microorganism. Colonies were scraped off the Agar plate **A** and resolved in buffer. **B**: Raman spectra were taken from individual living bacteria and fungi utilizing the simultaneous trapping effect of the Raman Laser configuration. Raman spectra of colored colonies differ at wavenumbers corresponding to carotenoids.

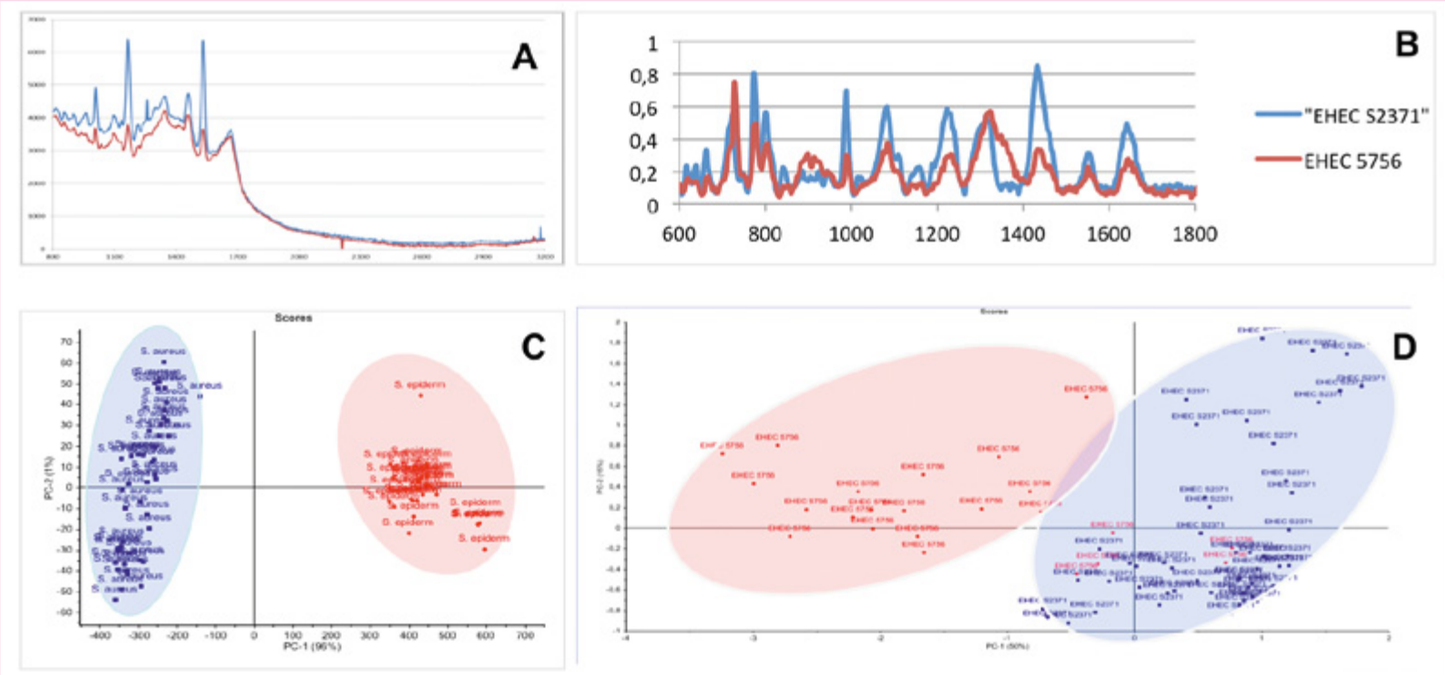


Fig. 3 Mean Spectra of Staphylococcus aureus (blue) and epidermidis (red) as well as of EHEC strains EHEC S2371 (blue) and EHEC 5756 (red) with the corresponding Principal Component Analysis, respectively (C and D).

can be pin-pointed prior to automated spectra retrieval. The microscope table moves with high precision from cell to cell. Focusing of the Raman laser beam through the objective generates a focal spot of about 1 µm in diameter, depending on the objective magnification. This enables to distinguish between cytoplasm or nucleus – depending on the experiment and questions to be answered (Fig. 1). On the other hand, focusing of the laser beam generates an electromagnetic gradient along which microorganism or cells in suspension are aligned and driven towards the laser focus, where they are trapped during Raman spectra retrieval. User defined cell ore tissue areas can also automatically be screened.

The application-oriented operation and data processing software allows quick access to the required spectral information of cell state and fate. As a special requirement in biomedical application we have to deal with routine glass slides and the corresponding fluorescence. Therefore, an algorithm was developed to subtract the glass background from the spectral data. For spectra interpretation data are processed applying customized data processing software. Data analysis is performed with the Unscrambler® statistical software (Camo, Norway) using "principal component analyses" (PCA), the most common statistic tool for the analysis of spectral data.

Living microorganism

As proof of concept we measured Raman spectra of living airborne microorganism grown on an Agar plate [Fig.2A] that was opened for about 2 minutes and incubated at room temperature for five days. Some of the sprouted colonies [Fig. 2a] were scraped off and diluted in 0.9% NaCl buffer. Raman spectra of individual single specimen were taken from exploiting the trapping effect to hold them within the laser focus during spectra retrieval. Spectra from the examined samples clearly differ. Especially bacteria from the colored colonies show prominent peaks at wavenumbers (i.e. wavelength^{-1}) corresponding to carotenoids. Here of course the samples as well as the related spectra are so different that they can be discriminated by eye.

But also similar specimen such as bacterial strains staphylococci aureus and epidermidis [5] as well as subpopulation of EHEC bacteria could be discriminated using Raman microscopy. In Fig. 3 mean Raman spectra and the related Principal Component Analysis (PCA) are demonstrated. PCA-plots visualize each individual measured spectrum as a dot transferred from n-dimensional data room towards a 2-dimensional graph. When spectra cluster within distinct areas this means that the investigated populations differ from each other. One significant difference between Staphylococcus strains again is due to carotenoids.

In summary the BioRam® measures living bacteria quick and easy and might become a valuable tool in fast pathogen identification and resistance testing providing specific information without special preparation.

Cancer research and tumor diagnosis

Cancer is still one of the most common causes of death and only little is known about its origin. Meanwhile strong effort is

Karin Schütze, born in 1956, studied biology and sports in Heidelberg to become a teacher. She did her PhD work in the Institute for Applied Physical Chemistry in Heidelberg under supervision of Prof. Horst Ludwig and K.O. Greulich. As a postdoc at the University of California in Berkeley in Prof. Manfred Schliwa's lab she assembled her first Optical Trap with the support of Dr Art Ashkin. She also collaborated with Dr Rangaswamy Srinivasan appling UV-lasers for cell ablation. In 1993 she and her husband Raimund Schütze founded the former PALM company. They were developing and marketing Optical Tweezers and laser microdissection systems for biomedical application. In 2004 PALM was successfully sold to Carl ZEISS. At Zeiss she worked as Innovation Officer until 2008 when she and her husband founded CellTool GmbH. Here they focus on engineering Raman systems for biomedical applications and established a service lab for research collaborations and contract research. In 2006 both received the "Berthold Leibinger Innovationpreis" and were nominated for the 'Deutscher Zukunftspreis' of Germany's Federal President. She has published 80 articles. Her expertise is non-contact cell handling and enrichment based on innovative photonic technologies with focus on developing complex photonic systems into easy-to-handle tools. Her specialty is cell research using biophotonic methods and the development of dedicated application protocols.

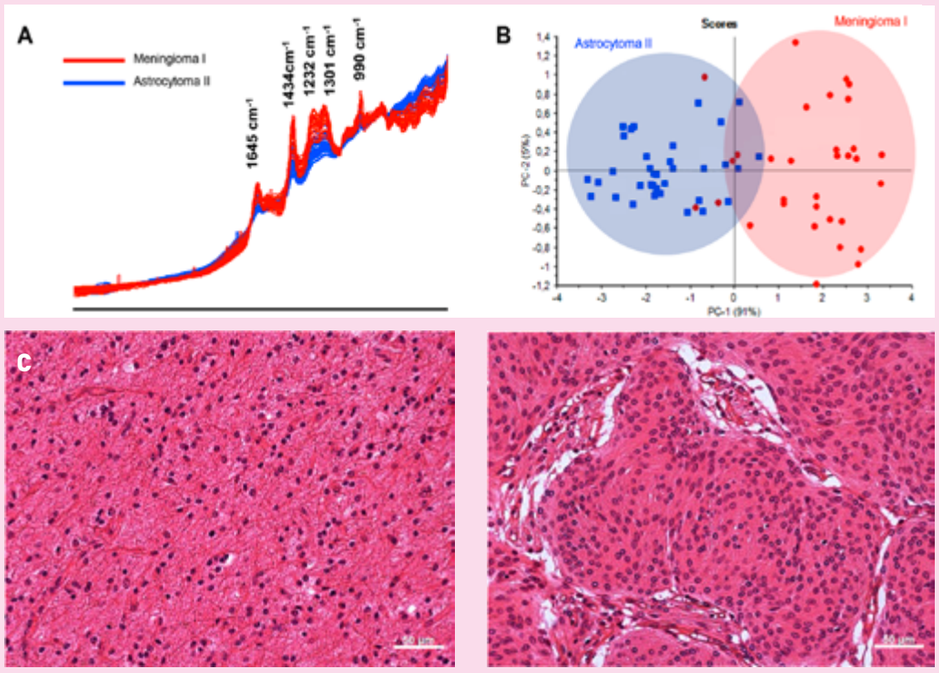


Fig 4. **A** – Comparison of Raman spectra recorded from 40 different cells of paraffin embedded and deparaffinized sections of Astrocytoma grade II (blue) and Meningioma Grade I (red) tumors. **B** – Principle Component Analysis (PCA) of Raman spectra depicts obvious separation of the two populations **C** – Corresponding tissue sections of the tumors stained with Hematoxylin and Eosin (HE).

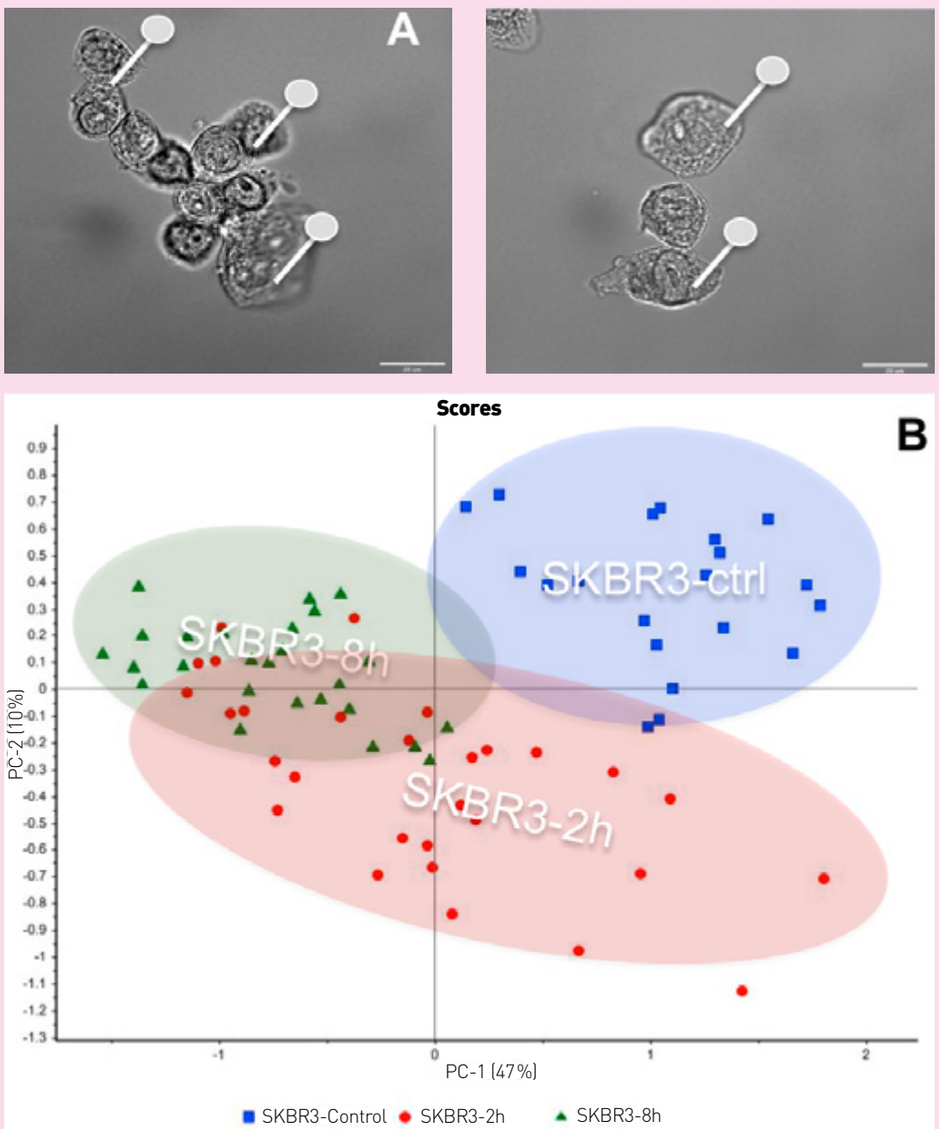


Fig. 5 Monitoring of Herceptin uptake – **A**: bright field images of SKBR3 tumor cells. Pins demonstrate site of Raman measurement. **B**: PCA-Plot of untreated control (blue) and two time points at 2 (red) and 8 (green) hours of incubation, respectively. Distinct clusters show that cells react on Herceptin treatment resulting in change of molecular features.

spend on early diagnosis of diseased cells to avoid tumor development. The potential of Raman microscopy in clinical oncology was emphasize by Fenn et al. [6] who summarized recent research work on different cancer types. Chen et al. [7] identified Raman microscopy as promising clinical analytical technique for rapid and non-destructive diagnosis of human diseases. However, for clinical use a dedicated Raman system is required to enable routine access to Raman spectra.

We used the BioRam® system to investigate cell stages such as apoptosis and necrosis and to analyze lymphoma lines derived from Hodgkin lymphoma and to compare them to non-Hodgkin lymphoma cells. [8,9]. Clear discrimination of cell state and fate and even tumor subpopulations could be demonstrated. Raman spectrosocpy also could follow glioblastoma cells invading into engineered neural tissue to study aggressiveness of the brain tumor [8].

Brain Tumors

Primary brain tumors constitute approximately 5% of human neoplasia and roughly half of these tumors are malignant. The variation among brain tumors is very high with currently approximately 100 variants being recognized. We compared Raman spectra from two very different brain tumor entities, namely meningioma and astrocytoma. In brief, routinely processed paraffin embedded samples from meningioma and astrocytoma each were cut into 5 µm slices, deparaffinized and submitted to Raman microscopy. At least 40 Raman spectra were analyzed from each tissue sample. We detected clear differences between the spectra of meningioma and astrocytoma (Figure 4 A and B). These findings together with the reports of other scientists [10] point towards the potential of Raman microscopy to recognize individual brain tumor types and warrants further exploration. Especially the power of Raman spectroscopy may have great potential for intraoperative decisions. This needs to be tested on fresh frozen cryostat sections.

Follow drug effectiveness

Furthermore, we wanted to test the sensitivity of Raman microscopy to follow the uptake of drugs in cancer cells. Therefore, cells of Herceptin-positive breast cancer

cell line SKBR3 were exposed to the anticancer drug Herceptin (20 µg/ml). Raman spectra of different time points yield distinct clusters as shown in Fig 5B. Analyzing the most prominent differences in Raman spectra yield three wavenumber areas corresponding to Amid I (at wavenumber 1660 cm^{-1}), lipids and proteins (at wavenumber 1450 cm^{-1}) as well as Phenylalanine (at wavenumber 1003 cm^{-1}). These results demonstrate the feasibility of Raman microscopy to follow internal changes of the metabolism of cells as reaction on drug application and to even allocate the molecules responsible for the change.

Potential for clinical practice

These investigations show that Raman spectroscopy has huge potential to support clinical diagnostics, assist minimal invasive surgery and to facilitate patient specific therapy. Using this label-free and non-invasive spectroscopic method cancer cells might be detected earlier and even unknown types of tumor cells might be discovered, not possible so far due to missing labels and markers. Further investigation need to be done to prove reliability and potential of this promising technique. It need to be investigated how early Raman can detect tumor cells and how fine tumor staging and grading will be possible. The existence of a dedicated "cell- and user-friendly" BioRam® system as well the availability of a service lab for Raman analysis of cells and tissue may greatly facilitate the introduction of this ubiquitous technology into clinical routine.

Happy cells – healthy people

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Picture: © CellTool GmbH

food profiling

Fine flavours

The utility of the chloroplast genome in verifying food authenticity: a case study looking at Ecuadorian fine/flavour cocoa

Luise Herrmann, Maike Blauhut, Nadine Barz,
Dr Ilka Haase, Tim Hünniger and Prof. Dr Markus Fischer
Institute for Food Chemistry, University of Hamburg

Chocolate and other cocoa-based products have long been popular foodstuffs, associated as they are with pleasure and enjoyment of the finer things in life. If we think for a minute about the product design, the choice of packaging – and also the price – of some dark chocolate brands, we can see how these are promoted as premium products, just as is the case for other foods such as wine or coffee. Accordingly, consumers often find that cocoa-based products include details of the place of origin for the beans used, as a sign of the product's quality. Since consumer demand for these kinds of foods continues to rise, production of fine/flavour (i.e. premium) cocoa varieties (currently about 5 % of total cocoa production) must also increase to match this demand.

The popularity of fine/flavour cocoa products continues to rise

Arriba is probably the most important fine/flavour cocoa variety. Making up around half of total fine/flavour cocoa production, the variety is cultivated primarily in Ecuador and is known for its unique and full-bodied flavour. Alongside Arriba cocoa, another variety has also been cultivated in Ecuador since the 1970s – the bulk grade cocoa CCN 51 [1, 2]. A clonal variety that offers high yields and is more robust and resistant, CCN 51 nonetheless fails to match the flavour of the more fragile Arriba [3, 4]. Since cultivation of CCN 51 carries less risk of a poor harvest, this variety is grown preferentially by Ecuadorian cocoa farmers. Yet increased production of bulk cocoa is a trend that runs counter to the growing consumer demand for fine/flavour beans.

It's reasonable to assume, therefore, that the two Ecuadorian cocoa varieties become mixed together, ultimately resulting in a poorer-quality end product. This intermixing could be deliberate, so as to boost revenue by selling what are only apparently fine/flavour cocoa beans. Yet lower-grade beans could also become mixed with the premium beans inadvertently, too, since both varieties are often cultivated and processed alongside one another. For both of these reasons, there is an abiding interest in identifying a method capable of distinguishing between the two cocoa varieties (fig. 1).

DNA: a biomarker candidate

Of all the biomarkers that are available within a plant cell, DNA – unlike proteins

or metabolic end products, for example – has the unusual property of being specific to an individual organism. DNA is also unchanging, i.e. it remains impervious to external factors for the period of time that is crucial for food analysis work. Alongside the nuclear genome, each plant cell also has a chloroplast genome (i.e. plastid DNA). Since CCN 51 and Arriba are organisms that are very strongly interrelated, conducting the search for sequence differences in plastid DNA rather than nuclear DNA is a more viable approach, since we know that chloroplast genomes exhibit a greater level of variability than the nuclear genome [5].

The first task is to carry out comprehensive sequencing work. Ordinarily, sequencing the complete chloroplast genome would first require its isolation from the nuclear genome. Happily, Kane *et al.* (2012) have demonstrated how to avoid time-consuming steps in separating the two genomes. For each cell, a single copy of nuclear DNA is available. This self-same cell also contains about three to ten chloroplasts, each of which possesses around 70 copies of its DNA. While the chloroplast genome is about 2,000 times smaller than the nuclear genome, it's far more numerous within each cell. This fact is exploited by the method referred to as "low coverage whole genome shotgun sequencing", as developed by Kane et al. As a result of this low coverage during sequencing, the high copy fractions are recorded. Even with nuclear DNA present, this method can be used to sequence the plastid DNA [6].

SNPs in the chloroplast genome

A comparison of the chloroplast genome from the two cocoa varieties CCN 51 and Arriba shows, as expected, only minimal deviation (about 0.03%). This deviation merely involves single base substitutions (single nucleotide polymorphisms, SNPs), which make applying straightforward PCR (polymerase chain reaction) more difficult. Since a few of these are located in the recognition sequence for a restriction enzyme, however, these SNPs can actually be utilised as a means of cocoa variety differentiation [7]. The first step is to conduct variety-unspecific PCR, whereby the primers flank the SNP at a distance. For both cocoa varieties, this generates a PCR product that, in a subsequent stage – termed "restriction fragment length polymorphism" (RFLP) – is then digested by a restriction enzyme. Since these are high-affinity endonucleases, they can cut only the PCR product containing the predefined sequence. In a final stage, detection is performed using AGE (agarose gel electrophoresis), CGE (capillary gel electrophoresis) or dHPLC (denaturing HPLC). If digestion is successful – due to a correct recognition sequence – then two fragments of a smaller size will be observed. If the amplicon does not contain the exact sequence, digestion does not occur and the undigested PCR product is detected instead of the two fragments. In accordance with the stated objectives of the project AiF/FEI 16796 N in relation to the mixing of Ecuadorian fine/flavour Arriba cocoa with the lower-cost bulk CCN 51 cocoa, the PCR-RFLP procedure can be designed to enable the detection of the CCN 51 variety in the final stage following enzymatic digestion. Accordingly, the presence of CCN 51 will result in the presence of two fragments, since the bulk cocoa sequence contains the correct recognition sequence for the restriction enzyme (fig. 2).

A further deviation in the chloroplast genome sequence is exhibited by the IRR (inverted repeat region), following its partial sequencing with the aid of 27 primer pairs by Dhingra *et al.* (2005) [8]. The IRR is a sequence that, when reversed, has a complement sequence located in the same genome. In the chloroplast genome under consideration, this contains the five-base sequence (TAAAG)n (repeat), whose rate of repetition differs between the two varieties (fig. 3).

Since the issue is not one of qualitative sequence differences but simply the difference in the number of repeat sequences in the two cocoa varieties, it is not a problem that can be resolved by designing a PCR method with variety-specific primers. On the other hand, the difference in repetition frequency can once again be used for a detection method. For both cocoa varieties, PCR products were generated, whereby the PCR region flanked the IRR at a distance. In a subsequent step, the PCR products obtained were detected using AGE, CGE or dHPLC. Following this step, it was possible to observe variety-specific amplicons that differed from one another by 40 bp, since the five-base sequence is repeated 6 times in one variety $(TAAAG)_6$ and 14 times in the other $(TAAAG)_{14}$ (fig. 4).

Alternative methods

The sequence differences between CCN 51 and Arriba are restricted to SNPs or a variation in repeat repetition in the chloroplast genome. By using these deviations as a starting point, it was possible to design PCR methods capable of detecting an intermingling of fine/flavour cocoa with bulk cocoa. Alternatively, other methods could be developed on the basis of the sequence differences mentioned.

If a SNP is present, then LPA (ligation-dependent probe amplification) can be utilised. Two primer probes hybridise immediately adjacent to one another to a target sequence. The next stage involves the covalent ligation of these two varieties by a high-affinity ligase. Ligation can only take place, however, if hybridisation completed fully at the target sequence – which does not happen in the case of a SNP. PCR then follows, whereby the necessary primers are already hybridised to the probes. The gene-

Fig. 1 Mature cocoa pods from CCN 51 (left) and Arriba (right). While these pods are visually distinct, this is no longer true of the processed cocoa products.

ration of a PCR product is assured only in the case of ligated probes, however: if a SNP is present, then neither ligation nor PCR occurs. No PCR product can then be detected [9].

Work on differentiation could also utilise a comparison of microsatellites. Microsatellites are short, non-coding genome sequences that repeat themselves at an identical locus, as already described in the case of the IRR in the cocoa chloroplast genome. As a rule, these repetitions occur with variable frequency. Microsatellites also occur in nuclear DNA [10, 11, 12]: After analysis and detection of the PCR products obtained from CCN 51 and Arriba, acquired from the nuclear genome in accordance with the pub-

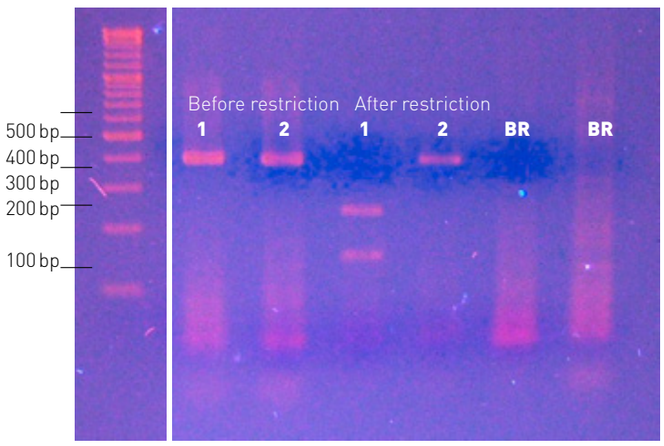

Fig. 2 Detection following PCR-RFLP via AGE and CGE. Following PCR, PCR products are obtained (1 and 2, before restriction, AGE). Amplicon 1 contains the recognition sequence of the restriction enzyme deployed and is digested: two fragments of around 170 bp and 260 bp are detected (1, after restriction, AGE and CGE). Amplicon 2 does not contain the recognition sequence, due to a SNP, and is not digested: instead of two fragments, the unfragmented PCR product is once again detected at around 420 bp even after restriction (2, after restriction, AGE and CGE). BE = blank reading.

Luise Herrmann graduated in food chemistry from the University of Hamburg in 2010. Her Diplom thesis investigated the use of protein patterns to differentiate between wheat and spelt samples. Following graduation, she divided work in her practical year between Nantes (France) and Hamburg. Since May 2011, she has been studying for her doctoral degree at the University of Hamburg's Institute for Food Chemistry. Her work focuses on verifying the authenticity of cocoa varieties, and designing DNA-based methods to differentiate between the fine/flavour cocoa Arriba and the bulk cocoa CCN 51.

Markus Fischer studied food chemistry at Munich Technical University (TUM), receiving his doctoral degree in 1997 in molecular biology/protein chemistry. In 2003, he completed his habilitation in the departments of food chemistry and biochemistry. Director of the Institute for Food Chemistry at the University of Hamburg since 2006, he is the founder (2011) and Director of the Hamburg School for Food Science (HSFS). Markus Fischer engages actively in many research organisations, examples including his positions on the Federal Institute for Risk Assessment (BfR) Scientific Advisory Board and Research Association of the German Food Industry (FEI) Scientific Committee and his role as German delegate to the European Food Chemistry Division.

lished research, this could also produce corresponding conclusions about the varieties.

Summary

Verifying the authenticity of plant-derived foodstuffs constitutes an interesting challenge within the field of food analysis. One can expect to see further innovations and techniques developed within sequencing. Since these are also less time-consuming and costly than they were some years ago, it has now become possible to sequence entire genomes in a short space of time. This has been accompanied by rapid advances in the development of methods, with whose help the problem of foodstuff authenticity can be viewed in an entirely new light. The procedure sketched out in relation to cocoa is merely one example: we may assume that a similar strategy can be adopted for a wide range of other raw materials and thus, in turn, for foodstuffs.

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Fig. 3 Section of alignment following sequencing of the IRR.
The repeat $(TAAAG)_n$ occurs 6x in one cocoa variety and 14x in the other variety.

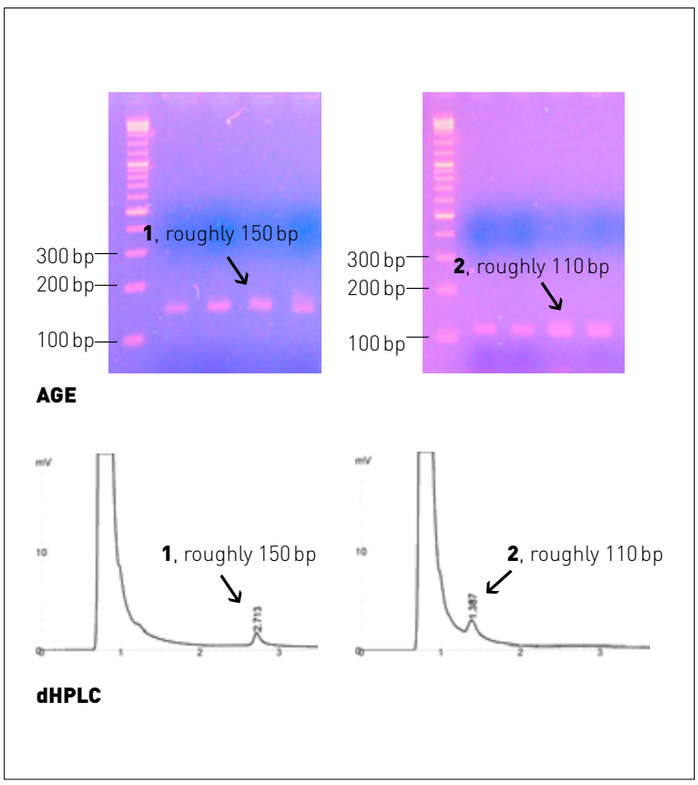

Fig. 4 Detection of the PCR product following a variety-unspecific PCR, in which the primers flank the (TAAAG)n repeat. Since the repetition frequency of the repeat differs between the varieties (1:$(TAAAG)_{14}$ and 2:$(TAAAG)_6$), PCR products are obtained that differ by 40 bp (1: roughly 150 bp and 2: roughly 110 bp).

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Good vibrations

Near-infrared spectroscopy in pharmaceutical analysis

Dr Alfred Steinbach and Stephanie Kappes, Metrohm AG, Switzerland

Near-infrared spectroscopy (NIRS) is based on the absorption of radiation by matter. Not only does the versatile technique allow the parallel identification of substances (active agents, excipients, contaminants), it is also suited to monitor processes such as blending, granulation, and drying.

NIRS – interaction of light and matter

Molecular vibrations are induced in the near-infrared region of the magnetic spectrum (800–2500 nm) – i.e., from the end of the visible to the mid-infrared (MIR) range. The main absorption bands of the functional groups of chemical substances are located in the MIR range and are very strong. The absorption bands of the harmonics, and the combination of the fundamental molecular vibrations, however, are in the NIR spectral region. They are significantly weaker and enable direct measurement without sample preparation, while at the same time offering deep insights into the chemical and physical properties of the sample. The strongest overtone absorptions in the NIR range are displayed by compounds with OH, CH, NH, and SH bonds. Because the NIR spectrum represents the result of numerous overlapping absorption bands, it is normally evaluated with multivariate chemometric methods.

Many parameters in a single analysis

NIRS offers numerous advantages over many wet-chemical analytical methods. A diverse range of parameters can be determined simultaneously. NIRS is economical and fast enabling qualitative and quantitative analyses that are noninvasive and nondestructive.

NIRS is an indispensable analysis technique that can be used along the entire production chain – from incoming materials to processing to the quality control of finished products (Fig. 1). NIRS meets the requirements of numerous international pharmacopoeias, e.g., USP, Ph. Eur., and JP.

Analyses of diverse matrices

Near-infrared spectroscopy requires no sample preparation and can handle any sample matrix, whether it is powders or granulates, tablets or capsules, creams or gels, solutions or suspensions, polymer films, or freeze-dried samples.

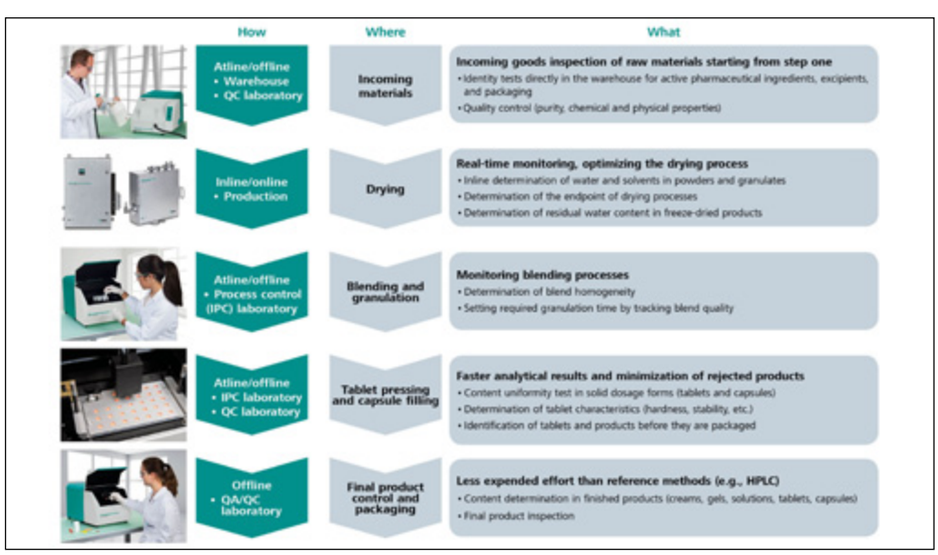

Fig. 1 Applications of NIRS along the production chain.

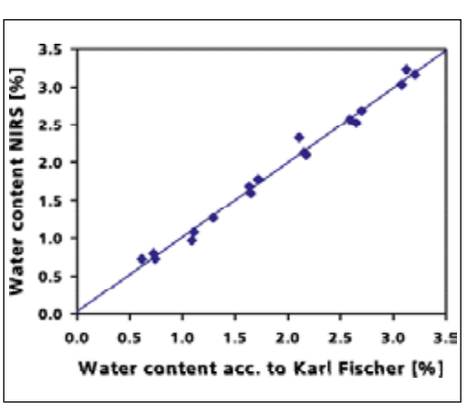

Fig. 2 Calibration model for quantitative determination of water content in powders. Karl Fischer titration is used as reference method.

Screening through packaged materials

NIRS can even perform determinations on contents sealed in transparent packaging such as glass and films. This is particularly appealing for incoming goods inspections and packaged end products. Handling is so easy that NIRS can be used directly in pharmacies and customs offices.

Nondestructive analysis

NIRS has long been one of the most important and versatile analytical techniques in the pharmaceutical industry – and not just because everybody in the pharma industry is talking about process analytical technologies (PAT) and Quality by Design (QbD). The decisive benefit of NIRS is the possibility of obtaining reliable analysis results in just seconds without altering the material under investigation and without any sample preparation or reagents whatsoever.

PAT and QbD – in search of the best of all methods

Drug manufacturing is subject to strong changes. The FDA's stated goal is to cut development time for new drugs while at the same time significantly improving quality. This requirement can only be fulfilled with analytical techniques that monitor the entire process – from incoming raw materials to the final product. To achieve that, perfect PAT sensors are needed that enable "live" tracking of the manufacturing process. NIRS is the technique that makes this possible. An inline sensor monitors product quality in real time. This prevents charges related to rejected products and reduces overall costs.

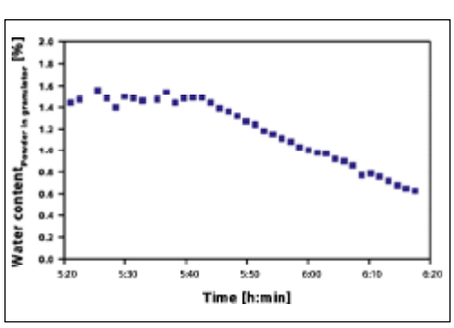

Fig. 3 Reduction of water content in a pharmaceutical powder over time. Rapid and nondestructive NIR analysis makes it possible to determine the optimal moment for further processing in real time. The sensor is installed directly in the granulator.

An example: granulation and drying

A key manufacturing process in the pharmaceutical industry is the granulation and drying process for powders that precedes tablet manufacturing. This process makes it possible to press powders into tablets in the first place. NIRS is the method of choice for determining the reaction endpoint when pressability is at the optimal point. Probes in the drier or granulator make it possible to track the process in real time. That reduces the process duration and thus increases the drying and granulation capacity of the system. At the same time, it minimizes the deviation of the required setpoint values. Figure 2 shows a calibration model for water determination that correlates NIRS to Karl Fischer titration which is the reference method. The progressive diminution of the water content during the granulation process, measured by real-time NIRS, can be seen in figure 3.

In accordance with international pharmacopoeias

As a secondary test method, NIRS is recommended in all of the key pharmacopoeias – from the European (Ph. Eur. 2.2.40) to the American (USP<1119>) to the Japanese pharmacopoeia. Metrohm NIRSystems offers instruments that meet the standards for wavelength precision, reproducibility, and photometric noise. Numerous reference standards and the user-friendly software make it easy to verify the instrument's compliance with requirements specified in the pharmacopoeias. The pharmaceutical version of the Vision software is fully validated and compliant with 21 CFR Part 11. Metrohm NIRSystems also offers complete IQ/OQ documentation and instrument performance certification (IPC). Documented parameters guarantee that the instrument performs properly.

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Stephanie Kappes is scientific technical writer in the Marketing Department of Metrohm. She obtained her Bachelor degree in Nanosciences from the University of Basel (Switzerland). During her Master's studies of Molecular and Cellular Life Sciences which she completed at Utrecht University (The Netherlands) and several internships, she focused on the public communication of science.

Alfred Steinbach is scientific technical writer in the Marketing Department of Metrohm AG, based in Herisau, Switzerland. He obtained his Masters in nuclear chemistry from the University of Cologne, Germany, and his PhD in environmental and analytical biogeochemistry from the University of Hamburg, Germany. Before obtaining his PhD and joining Metrohm AG in 2006, he carried out research in nuclear, analytical and environmental chemistry and worked as production manager at BASF Venezolana S.A in South America.

Scanning electron microscope image of the structure of Chromolith®.
Photo: Merck KG aA

Innovating with the customer

Efficiency gains in day-to-day lab work

At this year's analytica, Merck Millipore's exhibition was all about the customer. The life sciences division at Merck KGaA positions itself as a strategic partner, dedicates its work as one of the three leading R&D investors for products in the life sciences industry to furthering progress in this highly forward-looking sector.

Dr Oliver Mell, Head of Marketing & Product Management Lab Essentials at Merck Millipore, spoke to labor&more's Claudia Schiller about the company's latest products and services, and industry opportunities and trends.

labor&more: *Dr Mell, this year's exhibitors can look forward to a highly successful fair with a record number of visitors. What's your personal experience of analytica, and what relevance does the fair have for your company?*

Dr Oliver Mell: We're exceptionally pleased with our results at analytica. Customer feedback about our fair programme, the innovations we're presenting and our supporting activities is all very positive indeed. We're also very happy that our sales talks

are going into such depth: this level of interest shows us that we've struck the right nerve with our customers.

At Merck Millipore, you're responsible for marketing and product management for the Lab Essentials segment in the Lab Solutions business unit. What are the competencies for your segment?

Within Merck Millipore's overall lab programme, we cover a wide range of analytics solutions. The Lab Essentials portfolio covers products such as inorganic basic reagents, solutions for instrumental analysis, solvents, chromatography columns and thin-film chromatography, photometric tests, as well as dye solutions for microscopy and building blocks for organic synthesis.

This means we cover the full range of applications not only in instrumental analysis and photometry but also for in-process control and organic synthesis – primarily for the pharmaceutical/food industry but also for general industrial applications.

One aspect of your development work on new solutions is the close involvement of end users. Can you offer an example of a situation where customer feedback was used as an input for the product development process?

One example is our new, rugged and portable Fotometer Move 100 that we're exhibiting at analytica. It has an impressively long list of methods: of these, 100 are pre-programmed and 35 are user-specific. This answers our customer needs. Alongside the usual market research, our product managers also survey customer needs via regular face-to-face contact with clients.

In your opinion, what are the latest trends driving customer requirements in the pharmaceutical/biotech industry?

One trend is especially prominent at this year's analytica: customers are looking for help to make efficiency gains in day-to-day lab work. One goal here is thus to achieve higher rates of sample throughput. Our Chromolith High Resolution range is just one approach we took to meeting this need: this new generation of monolithic HPLC columns accelerates the results of analysis even for complex samples.

In addition, customers are also looking to expand automation and simplify sample measurement. We've satisfied this requirement with the MOVE 100 I mentioned above. This system uses pre-programmed methods to maximise measurement simplicity and help accelerate results, even when used for mobile applications.

Many of Merck's products represent milestones in the history of applied chromatography. HPLC analysis is taking on an increasingly central role in research, quality control and food/environmental analysis. What solutions can you offer to meet the current requirements in these areas?

In chromatography, it's all about selectivity. Here, our Chromolith HR offers a next-generation monolithic column that ensures a significantly higher plate number and peak symmetry even for complex samples and low-pressure columns. We're also exhibiting a wide range of new columns with surface modifications for particulate column applications and from our ZIC-HILIC range. The icing on the cake is the next generation of our Chromebook app for tablet computers: the app offers users all of the latest details on typical applications for Merck Millipore columns.

What other innovations have you been showcasing and what kinds of products is Lab Essentials focusing on at the moment?

Alongside the new column types, we're also presenting further innovations at analytica, covering three main areas. The first group comprises our innovations for instrumental analysis, e.g. the chromatography columns described above, ISO Guide 34-accredited standards and HPLC solvents. Second, we're exhibiting products for point-of-use analysis. Key products here are our MOVE 100, plus a range of test sets such as measuring COD with high chloride concentrations.

We also cover lab productivity with products covering solvents, organic synthesis and inorganic basic reagents. Here, we're offering new kinds of packaging and solutions designed to make lab work more efficient – and more sustainable.

This year's analytica has seen particularly strong international growth. What's the input of international markets for your industry – and where do you see potential?

Merck Millipore has always had a strong presence internationally. A significant portion of our supply chain serves global customers, including those in the emerging economies. These are our prime candidates for growth, while European and US demand is likely to increase only moderately. Often, customers relocate production or development work to a country where we're already established. Here, we can then offer our customers the expected level of service. In Europe and the US, we expect to see rising demand for products that help customers' analytical work become faster, more efficient and deliver more precise results.

Dr Mell, thank you very much for the interview.

Oliver Mell studied chemistry at the Philipps University of Marburg and completed his doctorate in biochemistry there in 1993. This was followed by a period at Abbott Deutschland GmbH and B. Braun Melsungen AG, where he worked in marketing and sales/distribution. Moving to Merck in 2000, he was appointed Head of Marketing and Product Management Laboratory Chemicals for the Americas region in 2004. In his current position, he is the Head of Marketing & Product Management at Lab Essentials, a business segment within Merck Millipore's Lab Solutions business unit.

High Precision and Speed

JULABO Temperature Control Solutions

JULABO presents an extensive program of temperature control solutions ranging from -95 to +400 °C. Since JULABO's foundation in 1967 the company has pioneered the development of technology for liquid temperature control. JULABO equipment is used successfully around the world providing application solutions for science, laboratories, technology centers and process industries. During every step of development and production JULABO equipment has to meet high quality criteria and its "made in Germany" technology reliably fulfills customers' requirements.

Sophisticated design, user-friendly operation

The current program features heating and refrigerated circulators, highly dynamic temperature control systems, recirculating coolers, water baths and additional special equipment. All JULABO products impress with sophisticated design and user-friendly operating concept. Their integrated highly precise control technology is unique. It guarantees highly accurate temperatures and rapid reaction to temperature changes. Another special feature of JULABO units: no side vents. Operation of JULABO instruments is intuitive. All important information is displayed intelligibly and easy to read. The parameters for any application are set quickly using only a few keys. The EasyTEMP and WirelessTEMP by JULABO products facilitate innovative control and software solutions for remote operation, control, monitoring and documentation of applications – powerful tools for simplifying workflow automation.

From routine to high performance temperature applications

JULABO's product range includes temperature control systems for a variety of applications. Heating and refrigerated circulators accomplish almost every temperature control application with temperatures ranging from -95 to +400 °C and are available in many variants. JULABO water baths are high-quality and durable products, with a working temperature range from 20 to 99.9 °C, equipped with the latest microprocessor technology. In laboratories they are ideal for temperature control of samples, incubations, material testing, corrosion tests, cell cultivation or testing of food and beverages. State-of-the-art heating and refrigerated circulators by JULABO rapid and precise temperature control for a wide range of applications. Devices such as jacketed reactor vessels can be controlled within a temperature range from -92 to +250 °C. JULABO recirculating coolers are used for a variety of cooling applications in laboratories and industry. Users may choose from a large range of air or water cooled models with cooling capacities from 0.3 to 20 kW. JULABO also offers specialized equipment for individual applications and an extensive list of accessories for all products.

Comprehensive service and on-site support

JULABO takes pride in offering customers expert advice for pairing the proper JULABO temperature control solution to their specific application. JULABO service and support options include installation and calibration, equipment qualification documentation and application training. These invaluable services ensure customer confidence in the operation and maintenance of their JULABO unit.

Quality "made in Germany"

JULABO units function reliably providing years of operation with quality at your fingertips. Every unit meets strict internal requirements and conforms to the highest national, European and international standards. Each JULABO instrument must pass numerous tests in every production phase. A sophisticated quality management program in accordance with DIN EN ISO 9001 guidelines guarantees that only technologically superior products are shipped from our facility. Find comprehensive information on the JULABO program in the current catalog.

Contact JULABO by phone or via internet for your free catalog.

→ **www.julabo.de**

R-Biopharm offers different test systems for the analysis of antibiotic residues in food and feed.

While Premi®Test allows a fast and easy qualitative screening for a broad spectrum of antibiotics; RIDASCREEN® ELISAs enable quantitative and specific screening of foodstuff for antibiotic residues. Additionally, EASI-EXTRACT® and RIDA® immunoaffinity columns allow sample clean-up of chloramphenicol from complex matrices such as honey, wine, enzymes and seafood prior to analysis with ELISA or LC/MS.

www.r-biopharm.de

Christ presents its new corporate design

First-class style for an international presence: Christ has completely revamped its brand design. The company is a leading global supplier of freeze-dryers. This premium quality is what makes the new brand design of Christ even more visible – from the website to the various brochures. Grand premiere: Christ presented the new design of the company for the first time at the Analytica in Munich. In doing so, the company is gearing up for the future. The new design reflects the reliability, targets, and quality of the company in a modern way to the outside. In addition, it underlines the connection between Christ and its sister company Sigma Laborzentrifugen GmbH. The new website will lead visitors to the website with the new design. The website welcomes visitors with an extensive, photo-filled slideshow with a news character and well-structured menu navigation. The responsive design enables the suitable display of the website on all mobile devices.

www.martinchrist.de

Eppendorf's new Mastercycler Nexus X2

Eppendorf has launched the latest offering in its range of molecular biology instruments. The new Mastercycler nexus X2 is ideal for researchers looking to carry out two runs of PCR simultaneously, without any compromise on the number of samples. The instrument comprises of two separate blocks, consisting of 64 and 32 wells, which can be programmed and run completely independently, enabling two separate PCR protocols to be run in parallel. With reduced noise emission (<40 dB), low power consumption and a small, well-designed footprint, the Mastercycler nexus X2 is perfectly suited for use in busy academic laboratories, or any institution with multiple users or large research groups. In comparison to other dual block cyclers, it provides an elegant solution for users wishing to run procedures using a large number of samples, without taking up a large amount of bench space. As the latest addition to the popular Mastercycler range, the Mastercycler nexus X2 continues Eppendorf's legacy of exceptional design and ease of use combined with efficiency and accuracy.

www.eppendorf.com

Smallest spectrometer in the world

Hamamatsu Photonics K.K. has developed the world's smallest "fingertip size" micro-spectrometer, an ultra-compact, lightweight and low-cost device which, according to Hamamatsu's research, provides nearly the same performance as Hamamatsu's MS series mini-spectrometer while being roughly half its size. The new C12666MA micro-spectrometer offers measurement in the visible wavelength range, and can be used for applications such as colour sensing in printing machines and LED lighting, point-of-care testing connected to smartphones, and other types of portable measurement. Samples of the C12666MA will be available to manufacturers from the 25th December 2013.

www.hamamatsu.de

Shelf life testing of dairy products in China

Ina Falkner, Journalist Rednitzhembach/Germany

China has discovered milk. In order to meet the demand of more than 1.3 billion people, companies such as Bright Dairy & Food build state-of-the-art production facilities. In this report, we pursued the question of why the Chinese have started drinking milk a long time after Europeans; a visit was also paid to the Bright Dairy & Food quality assurance lab. In the laboratory, a Memmert Peltier cooled incubator is used for microbiological testing and shelf life testing.

China encourages people to drink milk

For the most part, the Chinese traditionally bred cattle for meat consumption. Due to calcium deficiency in children, schools in China started to hand out free milk in the 1990ies. Food from abroad found its way into the supermarkets and this is how dairy products became an integral part of the Chinese diet.

Product quality is top priority

In 2011, Bright Food, the Shanghai-based parent company of Bright Dairy & Food that goes back more than 50 years, was the second largest food company in China. In addition to increasing its production capacities for milk, yogurt, ice cream, cheese and other dairy products, excellent product quality is the company's top priority. In research and development, as well as in production, the main focus is put on safety, freshness and nutrient content of Bright foods, as well as on consumer health. The Chinese government is accelerating the implementation of strict standards such as batch traceability or an unbroken cold chain during transport. For this reason, it goes without saying that the quality assurance facilities at Bright Dairy & Food use state-of-the-art technology.

Since 2012, the food safety team at Bright Dairy & Food has been using a Memmert Peltier cooled incubator IPP for microbiological testing and shelf life testing. Microbiological testing on fungal colonies is done at 20 °C for a duration of 3 to 5 days. During shelf life testing, the microbiological status of a product is, however, continuously tested over the entire shelf life period. In this case, test duration and temperature in the cooled incubator vary from sample to sample. To obtain valid results, an exact temperature distribution and the smallest possible temperature deviations in the interior are essential. In addition to the simple user interface, long-term stability, energy efficiency as well as environmental friendliness were the reasons why the Memmert Peltier cooled incubator IPP was chosen.

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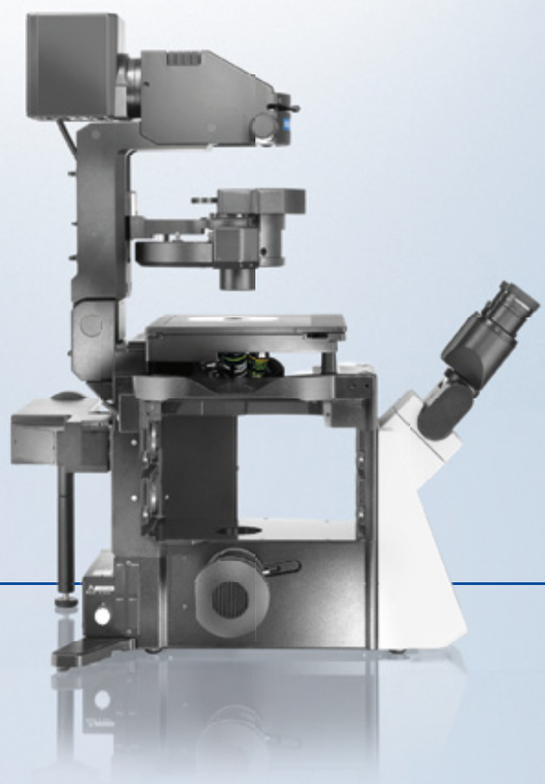

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