2007

Issue
02

RESEARCH NEWSLETTER – EUROPEAN EDITION

reSOLUTION

04 **EXPLORING THE SECRETS OF CELL LOGISTICS**
TIRF Microscopy Visualises Intracellular Transport Paths

06 **IN THE FOOTSTEPS OF LINNAEUS**
Microscopic Imaging in Marine Biodiversity Research

10 **CHARACTERISATION OF PHOTOSYNTHETIC BIOFILMS FROM ROMAN CATACOMBS**
via 3-D Imaging and Subcellular Identification of Pigments

Cover Picture

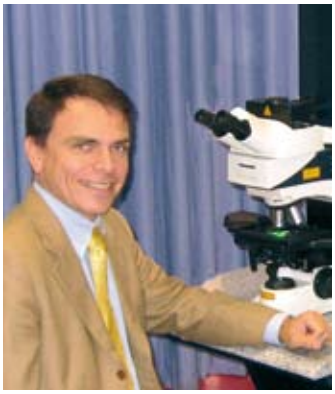
Fascinating Sea World

Sepietta sp. is a so-called bobtail squid that lives in the open water of the East Atlantic and can be found in the Mediterranean as well as in the cold waters of Scandinavia. The animal grows to about five centimeters in length and belongs to the group Cephalopoda which means "head foot" animals. Cephalopods are found in all oceans on our planet, anywhere between the warm water of the tropics and the near freezing water at the poles. They can also reach down to the darkest parts of our oceans – the deep sea. Today there are around 800 species of cephalopods known, but many new species have very recently been discovered and there are still many that need to be described. This and many other marine organisms are studied at Kristineberg Marine Research Station on the West coast of Sweden. In order to advance the study of minute marine animals Kristineberg and Leica Microsystems have assembled an image centre with the purpose to acquire and analyze microscopic images of these organisms. The image centre currently runs with a number of stereo- and compound microscopes as well as a confocal laser scanning microscope. Many of the images produced here are not only of high scientific value; they are also very well suitable to communicate modern marine research to the public.

Please see page 6 for further details.

Photo: Frederik Pleijel, Kristineberg Marine Research Station

EDITORIAL



Going deep

Dear Reader,

Whether we go up into space to observe life without the force of gravity or down into the depths of the underwater world, microscopy always has the capacity to take our breath away with stunning pictures. This edition features extraordinary images illustrating the biodiversity in the sea world. The opportunity is given by the 300th anniversary of Carl von Linné, Swedish scientist pioneer in designing a universal system for classifying living organisms in the early 18th century, which was celebrated with an open exhibition at the Kristineberg Marine Research Station in collaboration with Leica Microsystems.

For scuba divers (like myself in my leisure time) "Going Deep" is synonymous with discovering undersea life, but when it comes to imaging in science, looking below the surface does not apply exclusively to water. The introduction of a new exclusive technique like the Fibre Confocal allows scientists to go deep and get real *in situ* imaging in living animals like never before, as unveiled in the article "Deep Brain Imaging".

Of course, for those who do not want to get wet, the surface remains a topic full of interest, like the observation of the cell membrane through MultiColor TIRF technique where depth of penetration is always under control. Alternatively one can go underground, as we did when visiting the Roman Catacombs, and ask how historical monuments can be protected from uncontrolled growth of photosynthetic biofilms, better known as "maladie verte".

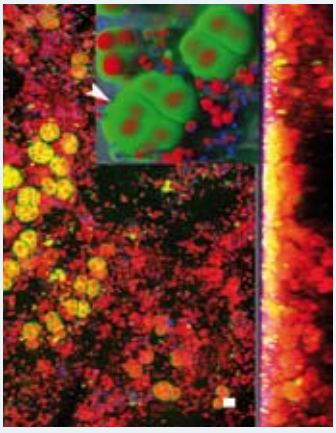
Curious? Then let's dive together into this new issue of the European Research Newsletter!

Didier Goor
European Marketing Manager Research

Photosynthetic Biofilms

Bacteria Damage Roman Hypogean Monuments

Roman catacombs are hypogean monuments that form part of a tourist route. Visited areas suffer intense changes caused by the visitors themselves and/or by the conditioning of the galleries for visits [5], the latter of which implies the use of artificial illumination. With the aim of preventing biodeterioration or aesthetic damage in Roman hypogean monuments, a confocal technique was used to analyse fluorescent pigments from a single cell, based on spectrofluorometric methods.



The study allows the establishment of a simultaneous relationship among in vivo pigment identification, organism morphology and 3D localisation of cells that have particular fluorescence signatures within the biofilms. This technique permitted a comparison of the effects of illuminating photosynthetic biofilms with green light and with white light. The results show retarded biofilm growth in the case of green light, suggesting their utility for the illumination of cultural patrimony sites.

Please see page 10 for further details.

CONTENTS

COVER STORY

In the Footsteps of Linnaeus – Microscopic Imaging in Marine Biodiversity Research 06

APPLICATION REPORTS

Exploring the Secrets of Cell Logistics – TIRF Microscopy Visualises Intracellular Transport Paths 04

Characterisation of Photosynthetic Biofilms from Roman Catacombs via 3-D Imaging and Subcellular Identification of Pigments 10

Identification of Candidate Genes – Laser Microdissection for Studies in Alzheimer's Disease 16

TECHNOLOGY

Deep Brain Imaging 20
Watch Life as it Happens with Leica FCM1000 Endoscopic Confocal Microscope

PRODUCT NEWS

Leica M165 C and M205 C – Human Brain Takes On the Job of the Optics 19

Leica AM TIRF MC – Excellent Flexibility for Multi-Color Cell Membrane Studies 22

CONTEST 18
Your Opinion is Valuable!

EUROPEAN RESEARCH EVENTS 23

IMPRINT 23

TIRF Microscopy Visualises Intracellular Transport Paths

Exploring the Secrets of Cell Logistics

Dipl.-Phys. Bernd Schneider and Prof. Dr. Ralf Jacob, Philipps-University Marburg, Germany

Epithelial cells, which control the exchange of substances between the organism and the outside world, are equipped with highly specific mechanisms for selective transport of substances to the apical or basolateral membrane. Using TIRF microscopy, scientists have been able to take a closer look at intracellular transport processes with the example of the galactose-binding protein Galectin-3, which has been identified as a potential apical sorting receptor.

As polarised cells, epithelial cells have clearly separated functional domains for selectively regulating the exchange of substances with the outside world and, at the same time, protecting the organism from harmful influences: the apical (facing the lumen) and the basolateral membrane. The enterocytes of the small intestine also have a highly complex system of sorting and transport mechanisms which are used, for example, to channel digestive enzymes out of the cell and nutrients into the cell via the apical membrane. Many details of polarised protein transport are still unclear. Malfunctioning of these processes may lead to intolerances or metabolic disorders. Cystic fibrosis, for example, is caused in some cases by a genetic defect in the apical transport of osmotically effective chloride ions from epithelial cells. The consequence is the secretion of mucus in the lungs, pancreas and small intestine, among other places.

The cell's sorting machinery

The starting point of the intracellular transport is the trans-Golgi network (TGN), which supplies functional glycoproteins (e.g. hormones, digestive enzymes) in vesicles. The apical protein transport probably works in two main ways: through the association with specific membrane microdomains called lipid rafts, and via a raft-independent form of transport. Sorting signals in the protein structure and corresponding receptor proteins could make sure, on the one hand, that vesicles are charged with apical proteins and, on the other, that the secretory vesicles are channeled into the right transport path. Professor Ralf Jacob and his study group at the Institute of Cytobiology and Cytopathology of the Philipps University in Marburg, Germany, have identified the galactose-binding protein Galectin-3 (Gal-3), which could act as sorting receptor (1, 2). It seems to be im-

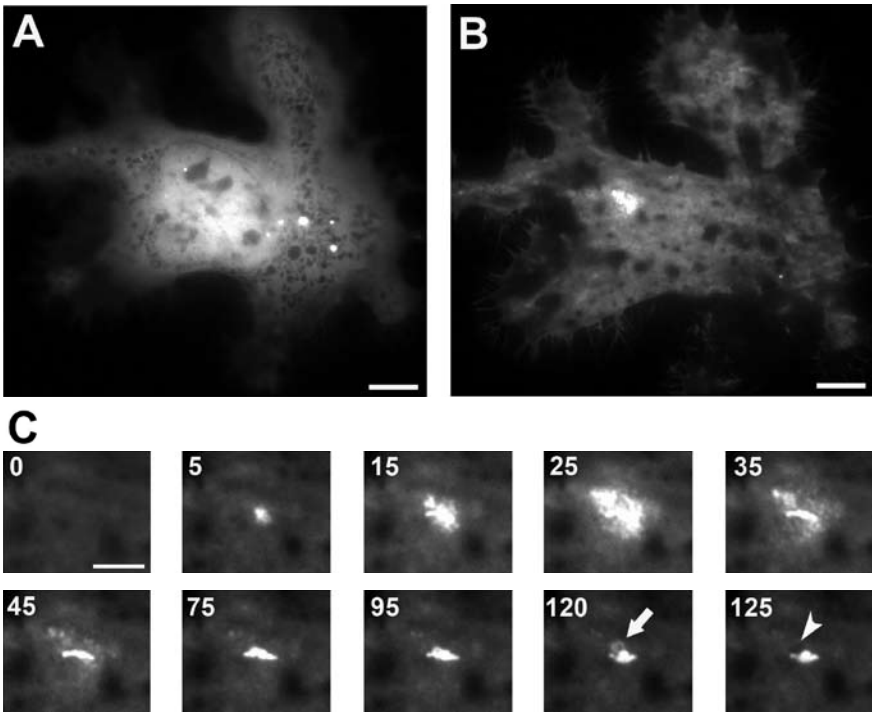
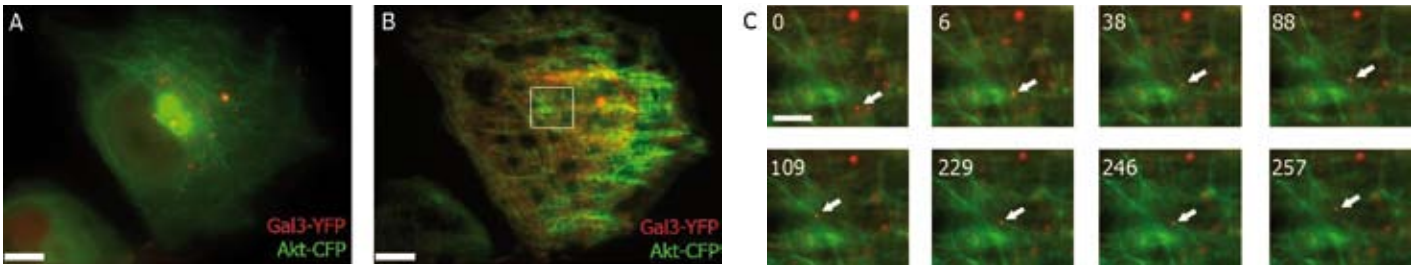


Fig. 1: Possible fusion of a Gal-3 vesicle with the plasma membrane
A – Overview image with epifluorescence
B – Overview image with TIRF, 25 s after the beginning of the event
C – Time sequence of section of TIRF image; time given in seconds
A Gal3-YFP vesicle fuses with the membrane and discharges its contents. Further event at 120 s (normal arrow). After the event, a Gal3-YFP-free space is left (curved arrow at 125 s).
Scale of overview images: 20 µm; image section: 5 µm; penetration depth TIRF: 110 nm.



portant for apical transport that Gal-3 forms clusters of high molecular weight with apical-sorted proteins – packing its cargo into the largest parcels possible, so to speak. A current study recently examined COS cells to explore the intracellular localisation of Gal-3 in the secretion pathway and the structures involved in its exo- and endocytosis.

Visualising transport processes in real time with TIRF

To visualise these processes, the Marburg scientists use TIRF microscopy (Total Internal Reflection Fluorescence). The combination of high z resolution and fast frame rate offered by TIRF microscopy enables a far better image to be obtained of dynamic processes in or near to the cell membrane than with epifluorescence (Figs. 1, 2). TIRF is able to selectively excite fluorophores in an aqueous or cellular environment at a depth between 70–300 nm below the surface. The quality of the results depends on the precise setting of the TIRF angle and the exact positioning of the laser beam. Leica Microsystems' scanner-sensor technology automatically matches the TIRF angle to the desired penetration depth at all wavelengths and positions the laser in such a way that the TIRF penetration depth automatically remains constant even when the wavelength is changed. Selected settings can be reproduced at the press of a button.

The intracellular transport of Galectin-3

All in all, the results of the Gal-3 studies in COS cells paint a largely coherent picture of intracellular transport (Fig. 3). The first contact between Gal-3 and the glycoproteins it sorts presumably takes place in a still unidentified post-Golgi compartment. By manipulating the pH, it was possible for the first time to localise YFP-labeled Gal-3 in endosomal compartments which are probably EEs (early endosomes) or REs (recycling endosomes) and which are discussed as the possible location of post-Golgi sorting. The vesicles formed by budding could then be transported via microtubules and then switch to actin microfilaments (Fig. 2) in the vicinity of the membrane. After fusion of the vesicles with the membrane, the apical glycoproteins sorted by Gal-3 have reached their destination and are distributed together with Gal-3 in the membrane. However, a different mechanism seems to be responsible for transporting Gal-3 into the inside the cell interior. Whereas Gal-3 is important for the apical transport of non-raft-associated proteins, the endocytosis of Gal-3 is probably raft-dependent. Gal-3 could then be transported into the inside of the cell via actin filaments in order to rejoin the raft-independent transport.

References

1. Delacour, D., Greb, C., Koch, A., Salomonsson, E., Leffler, H. , le Bivic, A., Jacob, R.: Apical sorting by galectin-3-dependent glycoprotein clustering. Traffic 8 (4): 379–88 (2007).
2. Delacour, D., Cramm-Behrens, C. I., Drobecq, H., le Bivic, A., Naim, H. Y., Jacob, R.: Requirement for galectin-3 in apical protein sorting." Curr Biol 16 (4): 408–14 (2006).
3. Schneider, Dominik: Analyse der zellulären Transportwege des Galactose-bindenden Lectins Galectin-3, Diplomarbeit zur Erlangung des Grades eines Diplom-Humanbiologen, Philipps-University, Marburg, Germany (2007).

This article was published in BIOforum Europe, 09/2007
GIT VERLAG GmbH & Co. KG, Rößlerstraße 90, D-64293 Darmstadt, Germany
Contact: Dr. Martin Friedrich, Phone: +49 (0)6151-8090-171, Fax: -176, m.friedrich@gitverlag.com,
www.imaging-git.com/order/IMRegistration.pdf

More information on intracellular transport of Galectin-3: Jacob@staff.uni-marburg.de

Fig. 2: Transport of Gal-3 vesicles close to the membrane along actin filaments
A – Overview image with epifluorescence
B – Overview image with TIRF, labeled section is shown in C
C – Time sequence of section of TIRF; time given in seconds
A Gal3-YFP vesicle close to the membrane (arrow) is first transported along an actin filament (from the bottom upwards), switches to another filament (88 s), moves to the left (109 s), is transported to the right again, switches filament again and is then transported upwards (246 s).
YFP – red; CFP – green; scale of overview images: 20 µm; section: 6 µm; penetration depth TIRF: 110 nm.

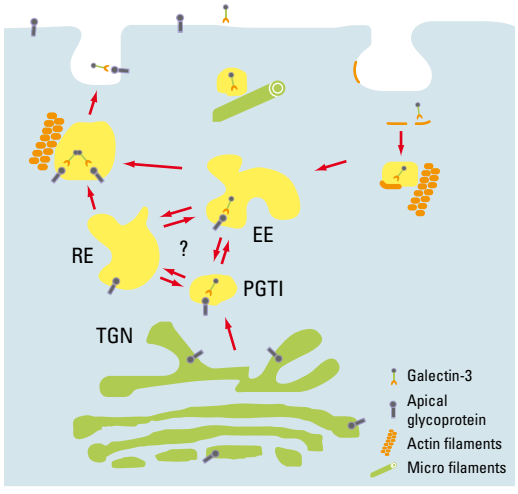


Fig. 3: Diagram of the intracellular transport of Gal-3
RE – recycling endosome; EE – early endosome; PGTI – Post-Golgi-Transport-Intermediate; TGN – Trans-Golgi Network. Red parts of membrane are lipid rafts.

In the Footsteps of Linnaeus

Microscopic Imaging in Marine Biodiversity Research

Matthias Obst, Sam Dupont, Mike Thorndyke, Kristineberg Marine Research Station, Sweden

Kristineberg Marine Research Station is at the forefront of marine research. The laboratory is one of the oldest marine research facilities in Europe and was founded in 1877 by the Royal Swedish Academy of Sciences. It is optimally located on the Swedish West coast at the transition region between the North Sea and the Baltic and at the entrance to the only true Fjord in Sweden. The unique marine archipelago of the rocky coast begins a short distance from the station and offers a vast variety of habitats and faunal communities, from sandy beaches to deep water habitats. These conditions make Kristineberg one of the leading laboratories in Europe for marine research. For the study of minute marine organisms such as free swimming larvae or bottom dwelling invertebrates it is a great advantage to have modern microscopic equipment in close proximity to the natural habitat. This allows the acquisition of images from organisms under virtually natural conditions. Leica Microsystems and Kristineberg have now engaged in a strong partnership at the Kristineberg Marine Image Centre, a facility to acquire and analyse microscopic images of marine invertebrate animals. The image centre currently runs with a number of high performance stereo- and compound microscopes as well as a confocal laser scanning microscope, e.g. a stereomicroscope Leica MZ16, a confocal laser scanning microscope Leica TCS SP5, and several compound microscopes. This summer the centre launched its first exhibition featuring microcosmic images from the marine environment.

Fig. 1 (above): Carl von Linné 1707–1778. Courtesy of the Royal Swedish Academy.

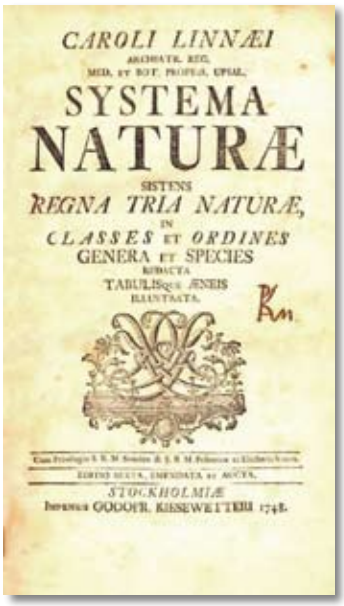


Fig. 2 (at top of page): Systema naturae, Linné's revolutionary contribution to the scientific world was published for the first time in 1735. Courtesy of the Royal Swedish Academy.

Father of biological systematics

This year Sweden celebrates the 300th birthday of Carl von Linné (Carolus Linnaeus, 1707–1778), one of the key figures in the history of natural sciences. Linné's work is an incredible contribution to our understanding of the living world. His great achievement as a scientist was the design of a universal system for classifying living organisms. In this respect, Linné is often regarded as the father of taxonomy and biological systematics. In 1735 Linné published his most famous work "Systema naturae" in which he described a system that divided plants from animals and minerals. The paper was barely twelve pages long, but nevertheless was an outstanding contribution to science. Before Linnaeus, classifying plants and animals was a rather chaotic enterprise. Naturalists ordered organisms in all

Fig. 3: Bringing order into nature. A typical drawing of Linné that accompanied his systematic classifications. Courtesy of the Royal Swedish Academy.

http://www.leica-microsystems.com/products/SP5 | http:// www.leica-microsystems.com/products/MZ16

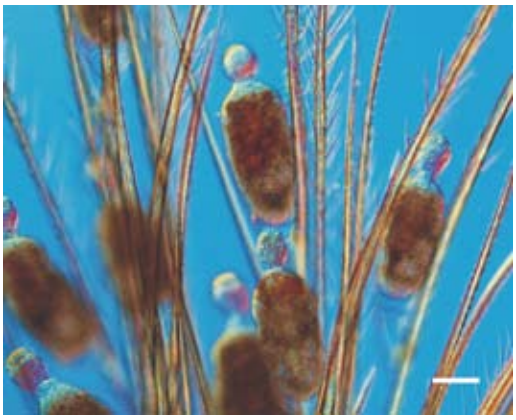


Fig. 4: A yet undescribed species of the enigmatic animal phylum *Cycliophora* living on the mouthparts of the European lobster. This species is currently described at Kristineberg. Scale bar = 50 µm. Photo: Matthias Obst.

Fig. 5: Eulalia sp. – the green leaf worm is often found in crevices, shell gravel or mussel beds and on kelp holdfasts along the coast of North Europe. The animal can grow longer than a centimeter but its larva is tiny.
Photo: Frederik Pleijel

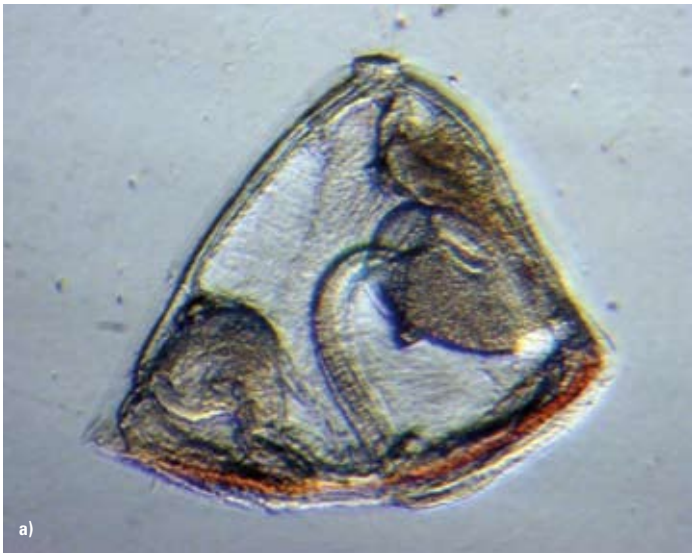
kinds of ways, and that was obviously done for practical reasons. For example, plants were often separated in edible and poisonous flora. The method of naming plants and animals was equally chaotic. At the time it was usual to describe a particular species simply by assigning a series of adjectives to them. In this way the same species could end up being described in entirely different ways by two or more people, which made it nearly impossible to know for sure whether the same organism was being identified. This made communication and scientific work so difficult that Linné used to say (Linnaeus 1737, Critica botanica): "If you don't know their names, knowledge about things is useless."

Systema naturae

Linné invented a universal system to describe, identify, and classify organisms, which we know today as the binomial nomenclature. He allocated to each species a first name that defines the genus, and a second name referring to the particular species. For example *Homarus gammarus* identifies the European lobster. Given this name biologists all over the world will know which animal is described. This system became established very quickly and it made scientific work much easier so contemporaries often said that "God created the world, but Linné has put it in order". It was Linnaeus that coined the name *Homo sapiens* and grouped humans with apes as early as 1766, almost hundred years before Charles Darwin presented his ideas of evolution. However, like most contemporaries, Linnaeus believed that all species including humans were created by God at some point and since then have not changed.

Linnaeus' work continues

With his *Systema naturae* Linnaeus started something wonderful – the discovery of biodiversity. Dur-



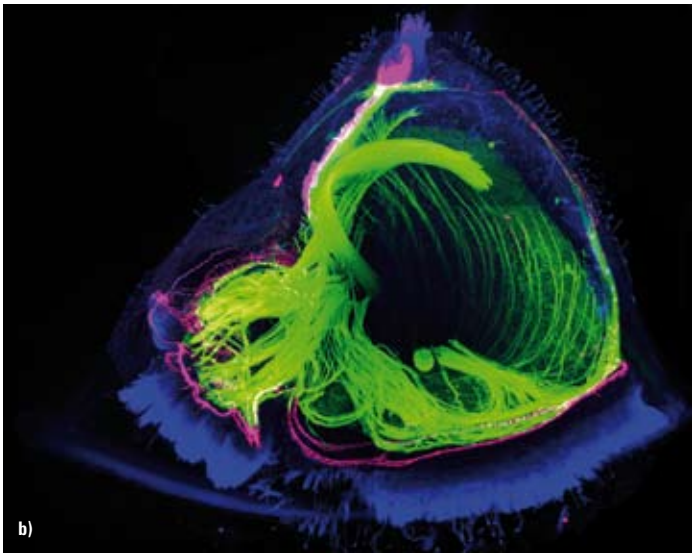


Fig. 6: Light microscopy (a) and CLSM (b) of the microscopic larva of the marine bryozoan *Membranipora membranacea* (size ~500 µm). The triple staining shows three organs in one image. Cells of the nervous system are indicated in pink, ciliated organs in blue, muscular structures in green. Photo: Judith Fuchs (a) and Katrine Worsaae (b).

ing his lifetime he recognised about 8,000 plants and 6,000 animal species. Today this number has risen up to approximately 1.75 million named species and it surely would make Linnaeus happy to see his legacy growing with such tremendous progress over the past 250 years. However his work is far from being finished and the discovery of biodiversity is still in its infancy. The estimated number of all species on the planet is at least 10 million and scientists at the Global Biodiversity Information Facility in Copenhagen have calculated that it will take us at least another 1,000 years to describe all species if we continue at the same rate as in the past. This confronts us with a serious problem because at the same time biodiversity declines rapidly all over the world due to the destructive influence of human activity, causing species to disappear forever. These are species that possess a unique and valuable potential for our own technological advancement, species from which we can learn and extract drugs or biomaterials, and perhaps most importantly species on which other organisms critically depend. As a consequence of this dilemma, taxonomy, the science of describing and classifying organisms, has been revitalised over the last decade as one of the leading scientific disciplines in modern biological research.

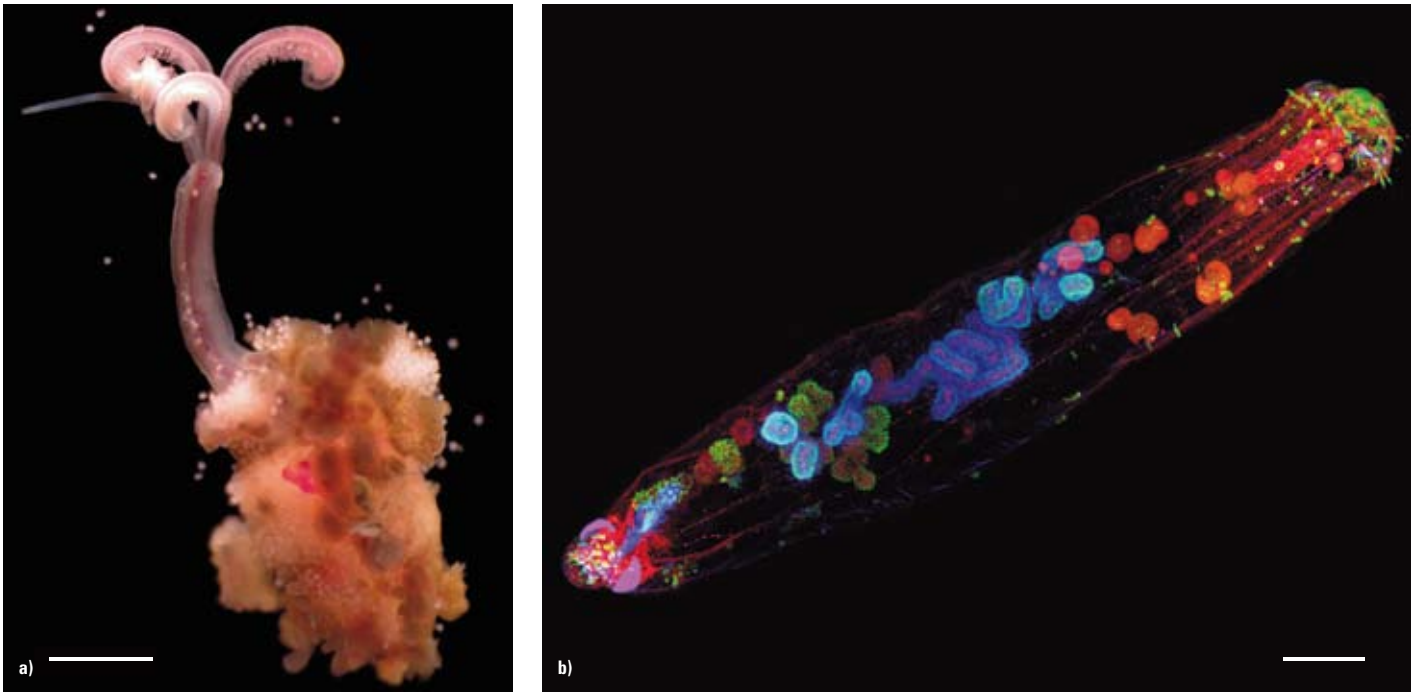
The Swedish Taxonomy Initiative

There is still a lot to be discovered, especially in the microscopic world. Most of the organisms in the sea are microscopic with body sizes of less of than a millimetre, and although they play a central role in marine ecosystem function, their biology is largely unstudied. The Swedish government has recently launched a large scale project with the goal of describing the complete flora and fauna in of its country. The so-called Swedish Taxonomy Initiative (www.artdata.slu.se) is a 20 year assignment and can be regarded as a birthday present to Linnaeus by his mother land. More importantly perhaps is the exemplary role of such a survey, as this project makes Sweden the first country worldwide that attempts to carry out an inventory of its entire multicellular life.

The Kristineberg Marine Image Centre

Most of the species recognised in Linné's time had a reasonable size and could be studied with the naked eye or simple magnifiers. However, today we are able to advance into a world where sand grains are gigantic. This world is inhabited by a bewildering number of small organisms, like flatworms, foraminifers, roundworms, copepods, rotifers, tardigrades, loiciferans, and many other creatures with extraordinary shapes and abilities. It is also home to the minute larvae of most of the larger marine invertebrate animals. By using the modern methods which are available nowadays it is possible to obtain concise and detailed anatomical reconstructions of such organisms. This provides us with essential information in ecological and taxonomic studies that was difficult to obtain before. For example the effects of pollution on minute organisms can now easily be recorded by 3-dimensional reconstructions

Fig. 7: Stereo microscopic image (a) of the female of the bone-eating worm *Osedax mucofloris*. The worm lives exclusively in whale carcasses on the bottom of the deep sea but was recently discovered at the Swedish West coast. The dwarf male (b) shown by CLSM is much smaller than the female and parasitises in large numbers the uterus of the female. Photo: Thomas Dahlgren (a) and Katrine Worsaae (b). Scale bar = 1 mm (a), 100 µm (b).



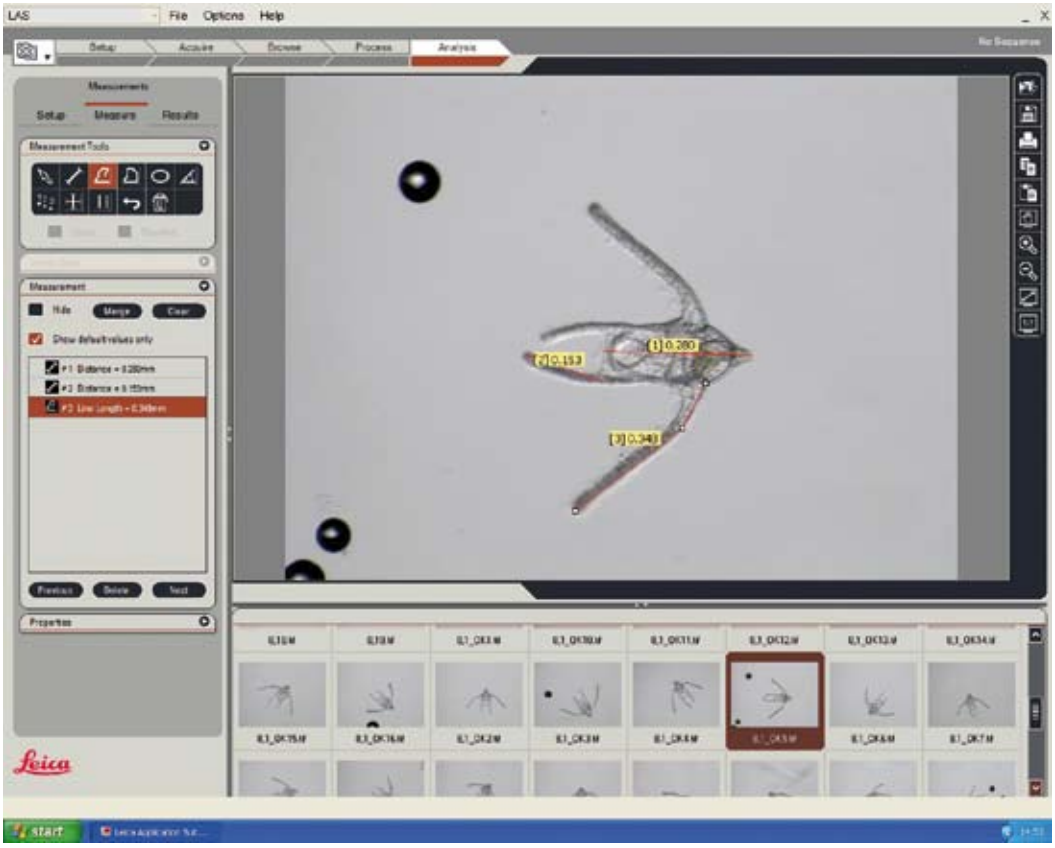


Fig. 8: Morphometric comparision using Leica application suite on the Leica MZ16 stereomicroscope. In Kristineberg scientists are currently studying the effects of ocean acidification and pollution on microscopic invertebrate larvae. The image shows a Pluteus larvae from the common brittle star *Amphiura filiformis*. Photo: Samuel Dupont.

of the major internal organ systems in the confocal laser scanning microscope.

Such methods are equally helpful when describing new organisms. In traditional practice species descriptions contain hand drawings that assemble all features observed in the microscope. Such drawings take time and even today can be very subjective. Using multiple immunocytochemical staining methods in combination with confocal laser scanning microscopy is a relatively fast and objective process that can assemble information from a number of internal features in just one single image. Likewise, external features are relatively easy be to combine in one image using montage software packages in the stereo microscope. The microcosm is full of mysteries and new imaging methods are powerful tools to unravel these. There is no doubt that Linné would find much to admire in today's technical possibilities to observe tiny organisms. Let's hope we are able to study them before they disappear.

More information on Kristineberg Marine Research Station: Matthias.Obst@kmf.gu.se

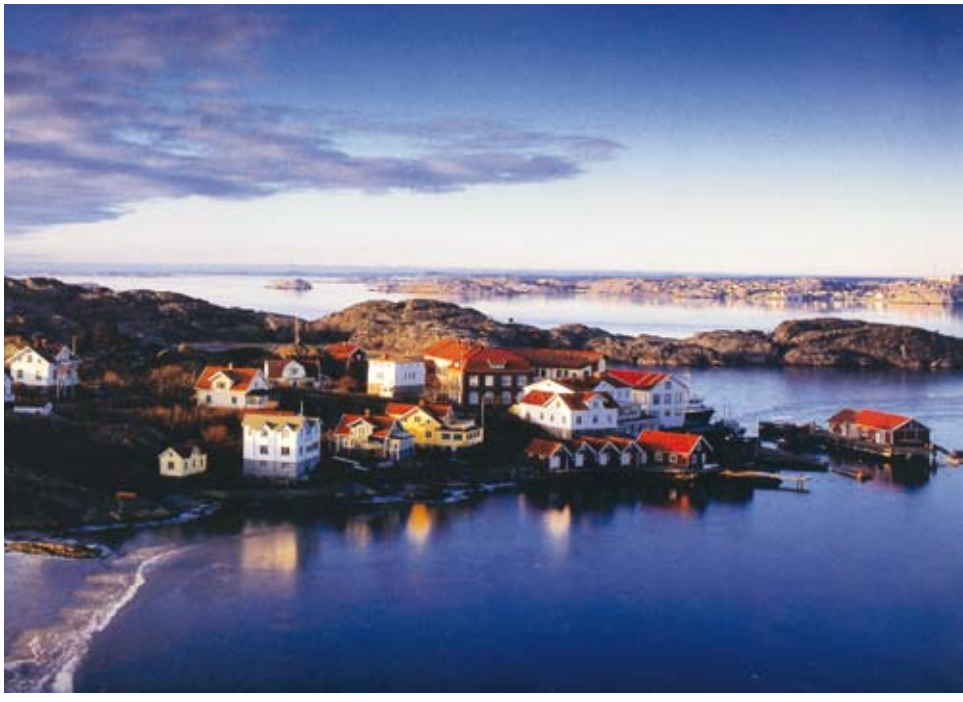


Fig. 9: Kristineberg Marine Research Station. Photo: S. Gren

References

Blunt, W.: Linnaeus. Frances Lincoln Publishers (2004). ISBN-10: 0711223629.

Miller, G.: Linnaeus's legacy carries on. Science 307: 1038–39 (2005).

Characterisation of Photosynthetic Biofilms from Roman Catacombs

via 3-D Imaging and Subcellular Identification of Pigments

Monica Roldán[1,2], M. Hernández-Mariné[2]

Artificial illumination can harm hypogean monuments by inducing the uncontrolled growth of photosynthetic biofilms (green sickness or maladie verte). With the aim of preventing biodeterioration or aesthetic damage in Roman hypogean monuments (the St. Callistus and St. Domitilla catacombs, Rome, Italy), a confocal technique is used for the analysis of fluorescent pigments from a single cell, based on spectrofluorometric methods. The study allows the establishment of a simultaneous relationship among in vivo pigment identification, organism morphology and 3D localisation of cells that have particular fluorescence signatures within the biofilms. This technique permitted a comparison of the effects of illuminating photosynthetic biofilms with green light (GL) and with white light (WL). The results show retarded biofilm growth in the case of GL, suggesting their utility for the illumination of cultural patrimony sites.

[1] Servei de Microscòpia, Universitat Autònoma de Barcelona, Edifici C, Facultat de Ciències, 08193 Bellaterra, Spain.
[2] Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain.

The vital activities of epilithic microorganisms contribute to undesirable changes in works of art. The organisms responsible for biodeterioration build complex structured communities, called biofilms, detracting from the aesthetic impression and eventually inducing chemical and physical damage [1]. Qualitative and quantitative characterisation of biodeterioration demands an understanding of the substratum, organisms and abiotic factors implicated [2]. Such information may help administrators to choose the appropriate preventive and eradication methods [3] for cultural patrimony at risk [4].

Roman catacombs are hypogean monuments that form part of a tourist route. Visited areas suffer intense changes caused by the visitors themselves and/or by the conditioning of the galleries for visits [5], the latter of which implies the use of artificial illumination. We ultimately aimed to produce innovative, non-destructive technologies to control and prevent uncontrolled growth of photosynthetic biofilms on rock surfaces.

Light quality is a determining factor in photosynthetic biofilm development, as light is fundamental to the metabolism and development of phototrophic microorganisms [6]. Owing to the artificial illumination installed for visitors, hypogean areas earmarked for tourism suffer from more phototrophic activity – and hence, more biofilm growth – than non-tourist sights. Spectral confocal microscopy equipped with a lambda scan function has been used for 3D localisation and *in vivo* discrimination by fluorescence signatures of individual organisms from different phylogenetic groups (*e.g.* chlorophyta, bacillariophyta, and phycoerythrin- and non-phycoerythrin-containing cyanobacteria) in complex communities [7].

In this research work, we also evaluated the effects of monochromatic green light (GL), nearly unused by photosynthetic organisms, on the adaptation and viability of phototrophic organisms, the structure of biofilms, and ultimately, on its efficacy in preventing biofilm growth in hypogean environments that are normally illuminated with artificial white light (WL). We chose monochromatic green light because it provides maximum sensitivity for human vision, thereby preserving the details and tonal values of cultural attractions.

Material and methods

Biofilms and lighting system

Natural biofilm samples were obtained from artificially illuminated surfaces in hypogean monuments (St. Callistus and Domitilla catacombs, Rome, Italy). The samples were maintained on a 2 mm layer of BG11 medium [8] at a nutrient concentration of 10% and solidified with 1% agar (Merck). Artificial biofilms were constructed by inoculating sterilised calcareous slabs, deposited on the aforementioned medium, with 1g of a mixture of axenic cultures of *Gloeothece membranacea* (Rabenhorst) Bornet CCAP1430/3 (Pasteur Culture Collection, Paris, France) and green alga *Chlorella sorokiniana* Shih. & Krauss from the Culture Collection of Instituto Isla Cartuja (CSIC) (Sevilla, Spain). The slabs were placed in Petri dishes and kept at 19–22 °C under continuous green light (GL) (Narva LT 18 W/017 green

TT, Narva, Czech republic) or white light (WL) (Chiyoda F15S daylight, Chiyoda Corporation, Japan) at a constant photon flux density of 20 µmol · m^{-2} · s^{-1} for 60 days. The emission spectrum of each lamp was measured with a LICOR Li-1800 (Lincoln, NE, USA) spectroradiometer.

Visualisation of biofilm structure

Confocal Scanning Laser Microscopy (CSLM) was performed with a Leica TCS SP2 in fluorescence and reflection modes. The reflection mode (excitation at 488 nm, and emission at 480 to 490 nm) allowed recording of reflective signals originating from inorganic solid material. Autofluorescence from photosynthetic pigments was viewed in the red channel using the 543 and 633 nm lines of an Ar/HeNe laser in the emission range of 590 to 800 nm. Extracellular polysaccharides (EPS) were labeled with the lectin Concanavalin-A-Alexa Fluor 488 (Molecular Probes, Inc., Eugene, OR, USA) (0.8 mM final concentration) and observed in the green channel (excitation at 488 nm line, and emission at 490 to 530 nm). Nucleic acids were specifically stained with the DNA-selective dye Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA) and viewed in the blue channel (excitation at 351 and 364 nm and emission at 400 to 480 nm).
We acquired optical sections in both x-y planes obtained at different intervals (z step) along the optical axis. Images were presented as multichannel and 3D projections using Imaris software (Bitplane, Zürich, Switzerland). The projections were used to describe the spatial distribution of organisms within the biofilms as well as differences in biofilm thickness and architecture.

Pigment fluorescence analysis: lambda scans

Single cell pigment identification was performed according to Roldán *et al.* (2004b) [7]. Wavelength scans were performed using the 488 nm line of an Ar laser. Each image sequence (wavelength scan) was obtained by scanning the same x-y optical section using a bandwidth of 20 nm for the emission. The x-y-λ data set was acquired at the z position having maximum fluorescence. Gains and offsets were the same for each field and remained constant throughout the scanning process. The variation in intensity of a particular spectral component was represented on the screen using a false-colour scale (warm colours represent maximum intensities, whereas cool colours show low intensities). Mean Fluorescence Intensity (MFI) of the x-y-λ data sets was measured using Leica Confocal Software, version 2.0. The region of interest (ROI) function of the software was used to determine the spectral signature of a selected area from the scanned image. For the fluorescence analysis, ROIs of 1 µm² taken from the thylakoid region inside the cell were set in each x-y-λ stack of images. The mean and standard errors for all the ROIs were calculated.

Results

Organisms and biofilm structure

Biofilms from Roman catacombs

In naturally or artificially illuminated areas, biofilm-forming phototrophic microorganisms developed profusely on surfaces composed of plaster, fresco, tufa, brick or mortar (Fig. 1a, 1b). The biofilms (Fig. 2) primarily comprised coccal and filamentous cyanobacteria and mosses, but also included diatoms, fungi hyphae and actinobacteria [9]. The biofilms were generally porous and very heterogeneous in thickness (from 80 to 550 µm), as determined by the three dimensional projections from autofluorescence and EPS (Fig. 2). For the majority of biofilms, the EPS were most abundant at the upper layer (Fig. 3a). Interestingly, the reaction of morphologically similar phototrophic cells to Con-A varied with their relative position inside the biofilm.

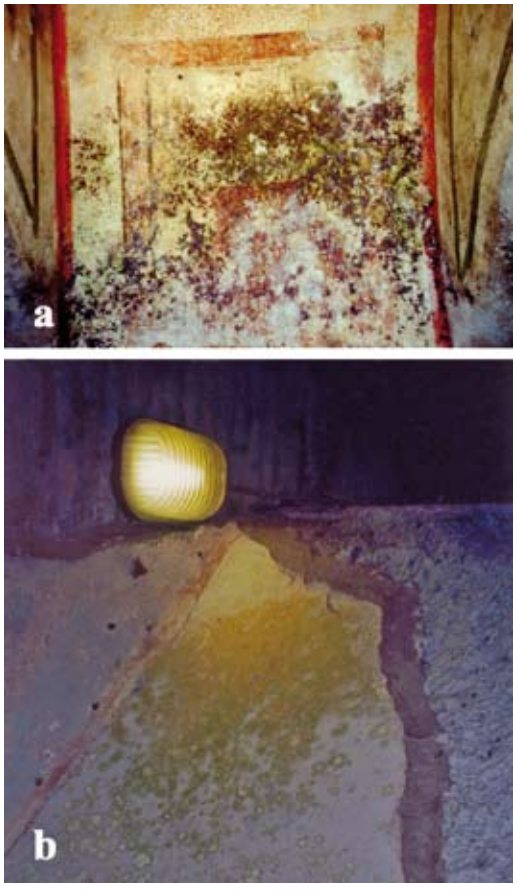


Fig. 1. Two sampling sites at the Roman catacombs. **a.** Macroscopic image of the biofilm colonisation on frescoes, inside the *Cubicolo di Oceano* of the St. Callistus catacomb. **b.** Photosynthetic biofilms thriving near the lamp on a corridor wall in St. Domitilla catacomb.

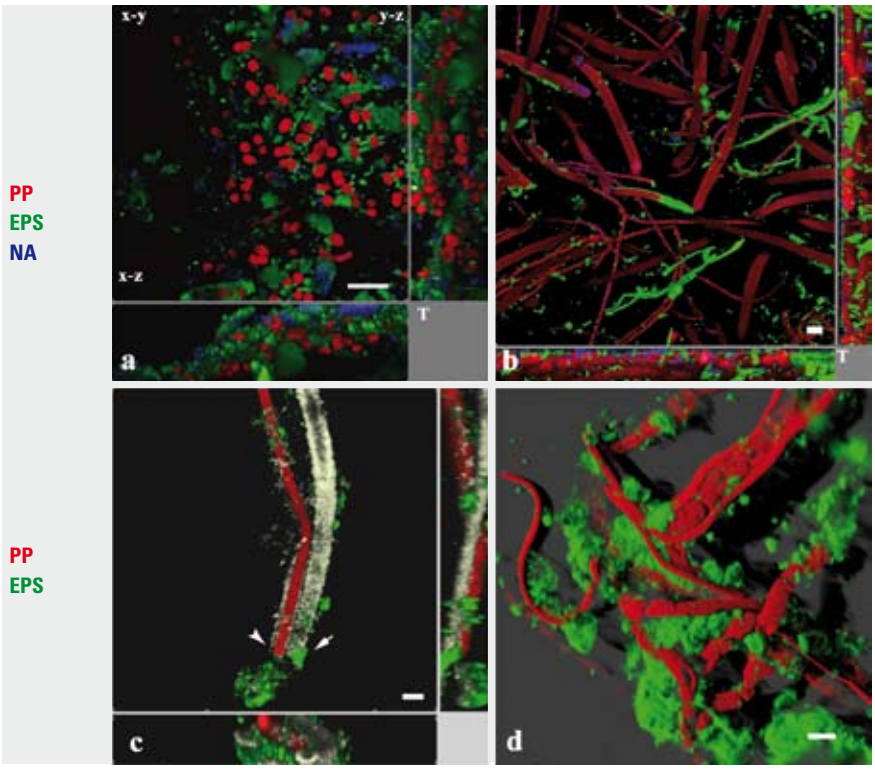


Fig. 2. Confocal projections of biofilm-forming microorganisms at the St. Callistus and St. Domitilla Catacombs (Rome). Colour key: photosynthetic pigments (PP), red; EPS, green; nucleic acids (NA), blue; and reflection (REF) from inorganic materials, grey. **a.** Biofilm dominated by *Gloeocapsopsis magma*. Pigment fluorescence shows the thylakoid arrangement inside the cells. Thickness biofilm = 33.5 µm. **b.** Biofilm dominated by *Scytonema julianum* and *Leptolyngbya sp*. **c.** *Scytonema julianum* characterised by the presence of carbonate needles on the polysaccharide sheaths that surround the trichomes. EPS was recovered substrata and greyish calcified sheaths (arrow). Biofilm thickness = 45 µm. **d.** Biofilm formed by moss protonemata and *Leptolynbya sp.* embedded in EPS. T= Biofilm surface. Scale bar = 10 µm.

Living phototrophic and the heterotrophic microorganisms were distinguished by coupling the results from Hoechst 33258 DNA staining with those from pigment autofluorescence studies. The nucleic acid stain did not show an extended heterotrophic bacterial community. However, these communities were highly developed in zones having weak photosynthetic pigment fluorescence. The reflection channel revealed the presence and thickness of inorganic hard substrates, such as calcified sheaths (Fig. 2c); coupled to autofluorescence it discriminates empty calcified sheaths from calcified live filaments.

Artificial biofilms

Changes in the structure and composition of artificial biofilms submitted to either GL or WL were evaluated using three-dimensional images, whereby photosynthetic pigments (PP) fluoresce red, and polysaccharide-bound Con-A fluoresces green (Fig. 4). Both GL and WL biofilms were stratified (Fig. 4).

GL: Slabs maintained under GL contained clusters of two to four cells of *Gloeothece membranacea* surrounded by a relatively thick sheath, only labeled for Con-A to the oldest external layers (Fig. 4a). Vacuolised thylakoids of *G. membranacea* were seen as empty spaces inside the cells. *C. sorokiniana* was nearly absent, and the few remaining cells had weak pigment fluorescence.

WL: Slabs kept under WL displayed clusters of *G. membranacea* mixed with sub-spherical cells of *C. sorokiniana.* Both organisms had higher respective pigment fluorescence than in the case of GL. There were dense clusters of 8 to 16 cells of *G. membranacea,* which were sometimes further aggregated and surrounded by an apparently compact sheath that was strongly labeled (Fig. 4b). Biofilms grown in WL (Fig. 4b) were thinner (PP thickness = 3.7 ± 1.53 µm) than those grown in GL (PP thickness = 4.65 ± 0.9 µm), and included a compact bottom layer of small, irregularly dense colonies of *G. membranacea* while *C. sorokiniana* grew close to the surface.

Pigments

Biofilms from Roman catacombs

Extended focus images of stratified biofilms showed the differential distribution in depth of the biofilm microorganisms (Fig. 5). For each biofilm, the corresponding emission spectra at 488 nm excitation wavelength are shown on the right (Fig. 5b).

Biofilm BF1: Thin filaments of *Leptolyngya sp.* were horizontally oriented on top of wide *Scytonema julianum* (Fig. 5a). Both cyanobacteria had a broad emission peak at 658.4 ± 3 nm from the overlap of Chlorophyll *a* and phycobiliproteins, phycocyanin (PC) and allophycocyanin (APC) (Fig. 5b). Additionally, *Leptolyngybya sp.,* but not *S. julianum,* presented an emission peak (579.7 ± 3.8 nm) attributable to the presence of phycoerythrin (PE) (Fig. 5b). We did not observe changes in the emission peak of particular specimens from the same taxa (e.g. *Scytonema*

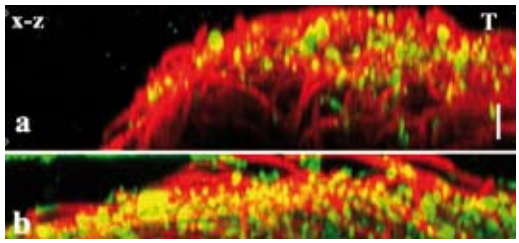


Fig. 3. Sagittal projections of biofilms formed by *Leptolyngbya sp.* were very heterogeneous in thickness. Colour key: photosynthetic pigments, red; EPS, green. Along the xy-axis, the distribution of the microorganisms was irregular, and along the xz-axis, the biofilm molded the substrata. **a.** The EPS was distributed mostly in the upper layer. Biofilm thickness = 48 µm. **b.** Compact and thin (21.17 µm thickness) biofilm with heterogeneously distributed EPS. T= Biofilm surface. Scale bar = 10 µm.

julianum when covered by thick sheaths, EPS or calcareous investments).

Biofilm BF2: CSLM revealed two layers. *Diadesmis gallica* – Bacillariophyta – was mainly concentrated on the top of the biofilm (Fig. 5a). Their highest maximum, at 676.2 ± 5 nm (Fig. 5b), did not coincide with the emission peak of the other groups due to the presence of Chlorophyll *c*.

The unidentified Chroococcal formed a discontinuous bottom layer (Fig. 5a) and presented the same spectral shape as *Leptolyngybya sp.* (BF1). The cyanobacteria presented higher mean fluorescence intensity (MFI) in the range from 640 to 740 nm than did Bacillariophyta (Fig. 5b). The cyanobacteria also presented high MFI at 577 to 580 nm (Fig. 5), due to PE.

Artificial biofilms

MFI and the half-band width of the spectra from both species, at 488 nm excitation wavelength, were different for GL than for WL whereas the spectrum shape was identical (Fig. 6).

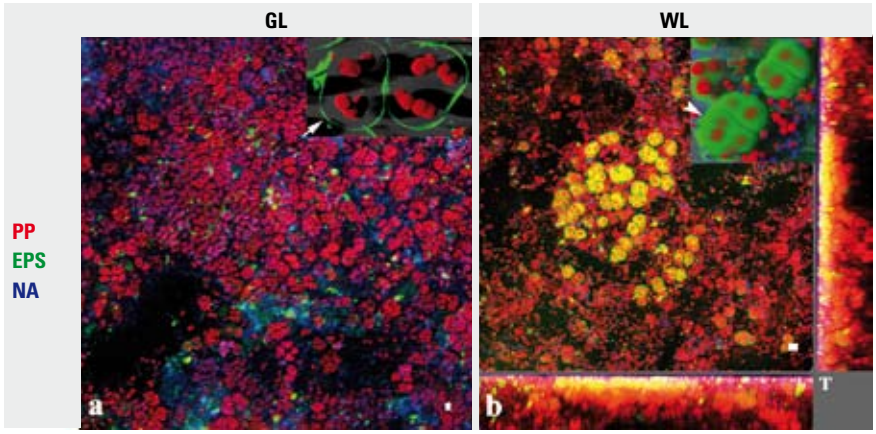


Fig. 4. Confocal projections of cyanobacterial biofilms grown under GL and WL. Colour key: PP, red; EPS, green; NA, blue. **a.** *Gloeothece membranacea* colonies grown in GL. The sheath was not compact, and only the external layer was labeled by Con-A lectin (arrow). **b.** *G. membranacea* colonies grown in WL. The cells were grouped in dense clusters of up to sixteen cells and surrounded by a compact sheath (arrowhead). *Chlorella sorokiniana* was found mostly in the upper layer. T= Biofilm surface. Scale bar = 10 µm.

Gloeothece membranacea: For the GL sample, the highest maximum corresponded to Chl *a* (ca. 670.7 nm), whereas the emission bands for WL samples were considerably higher (659.3 to 666.4 nm), corresponding to the phycobiliproteins PC and APC (Fig. 6b). In both GL and WL, *G. membranacea* showed PE fluorescence at an emission wavelength of ca. 580 nm when excited at 488 nm. In GL, the peak

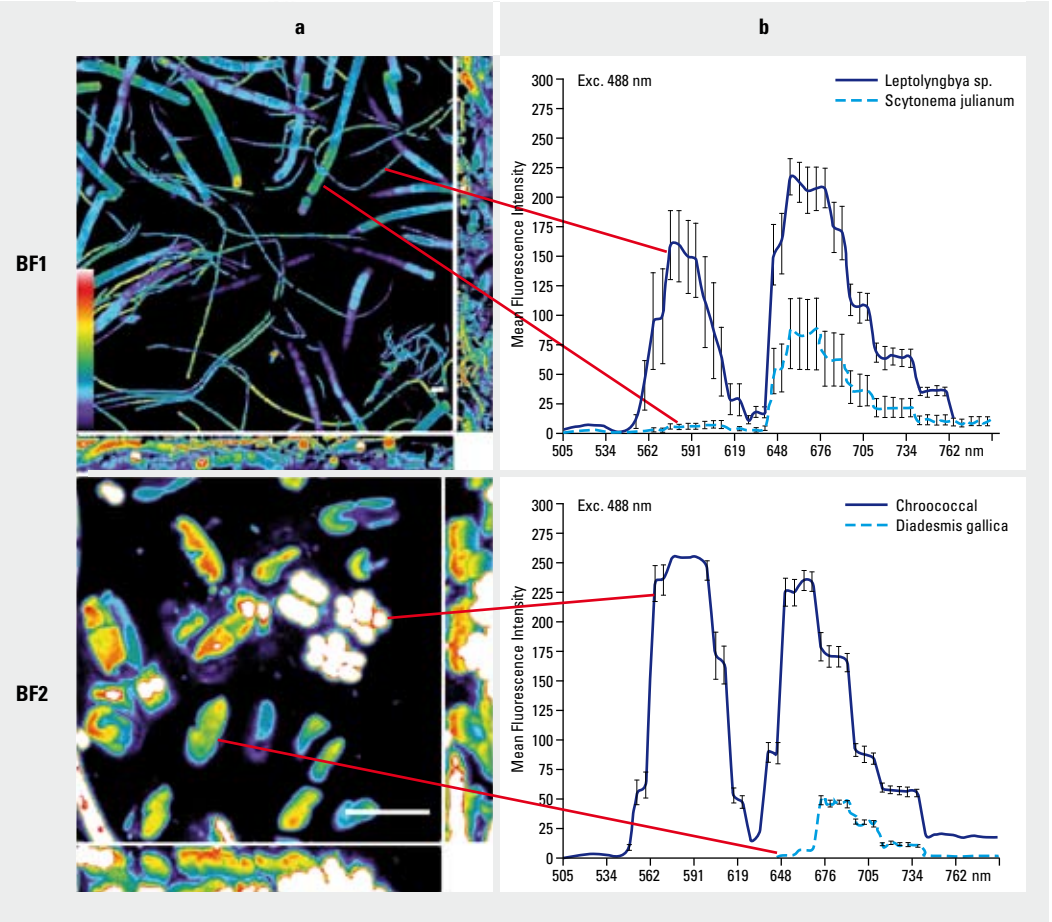


Fig. 5. CSLM projections and fluorescence properties of two aerophytic biofilms from Roman catacombs. **a.** Each image represents the autofluorescence emitted in the range 590–775 nm (Excitation wavelength = 543 nm). **BF1.** Biofilm formed by *Scytonema julianum* and *Leptolyngbya sp.* **BF2.** Stratified biofilm, consisting of two strata, the upper epilithic layer composed of colonies of *Diadesmis gallica,* and a lower layer formed by Chroococcal colonies. **b.** Spectral profile from single cells of each species from a lambda scan (excitation wavelength = 488 nm, steps = 50). The differences among the emission profiles of the biofilms indicate the presence of different groups of algae and cyanobacteria. T= Biofilm surface. Scale bar = 10 µm (published in: Applied Environmental Microbiology 70: 3745–3750, 2004).

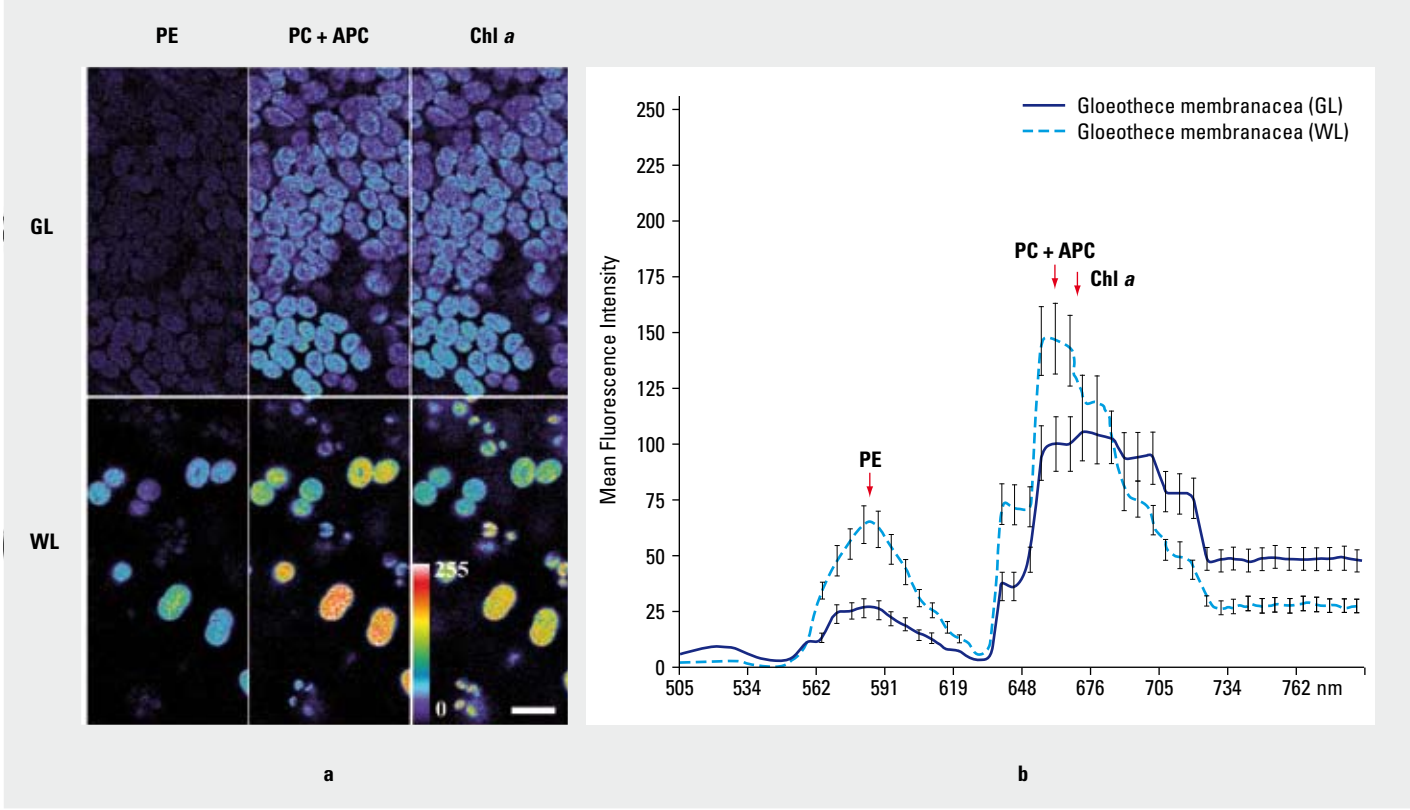


Fig. 6. Fluorescence properties from *Gloeothece membranacea* single cells after illumination with different light qualities (GL and WL). **a.** Optical sections taken from a lambda scan (excitation wavelength = 488 nm, steps = 50) corresponding to the emission peak of Chl *a* and phycobiliproteins (PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin). **b.** Spectral profile representing the mean fluorescence intensity spectra for *G. membranacea* under GL and under WL. Scale bar = 10 µm (published in: Applied Environmental Microbiology 72: 3026–3031, 2006).

position closely matches the main photosystem II (PSII) emission peak at 685 nm, with a secondary maximum near 730 nm that corresponds to photosystem I (PSI) emission bands (Fig. 6b). Significant differences between the MFI of GL and WL treated samples were found for the emission range of 573.6 to 590.7 nm, in which PE emits, and 653.6 to 659.3 nm, in which PC and APC emit. We can assume that GL influences fluorescence properties by decreasing the net rate of fluorescence.

Chlorella sorokiniana: At 488 nm excitation wavelength, significant differences in the Chl *a* maximum were not observed for samples of either treatment (Fig. 7).

Discussion

Organisms and biofilm structure

CSLM is an excellent technique for the study of the distribution of microorganisms and their biodeteriorative effects [10]. It has been employed for imaging phototrophic biofilms and mats in various habitats, catacombs [9] and other dimly lit aerophytic environments such as sinkholes and caves [11]. For biofilm samples, the use of multiple excitation and detection wavelengths at different focal depths enabled us to three-dimensionally visualise target-specific elements such as molecules (*e.g.* DNA and pigments), structures (e.g. surfaces, matrices, sheaths and filaments) and properties (*e.g.* stage of cell division, growth and senescence).

Pigments

To date, CSLM with the lambda-scan function has only been used to determine the optimal detection and separation of emission spectra for either new or known fluorochromes. In this study, we used CSLM with lambda scan to create a powerful tool with a broad scope of applications. Fluorescence detection allows the description of a complex community in terms of physiological state [12] and quantification of biomass [13]. Chlorophyll *a* is a photosynthetic pigment found in most plants, algae and cyanobacteria. In addition, most cyanobacteria use phycobiliproteins (phycoerythrin, phycocyanin and allophycocyanin) to capture light energy. Using lambda scan function, species belonging to one phylogenetic group showed spectrophotometric profiles distinguishable in shape and pigment emission peak from other phylogenetic groups [7]. The main features of the technique are: (i) analysis for both global and single fluorescent pixels, providing their three dimensional localisation *in vivo;* (ii) direct anal-

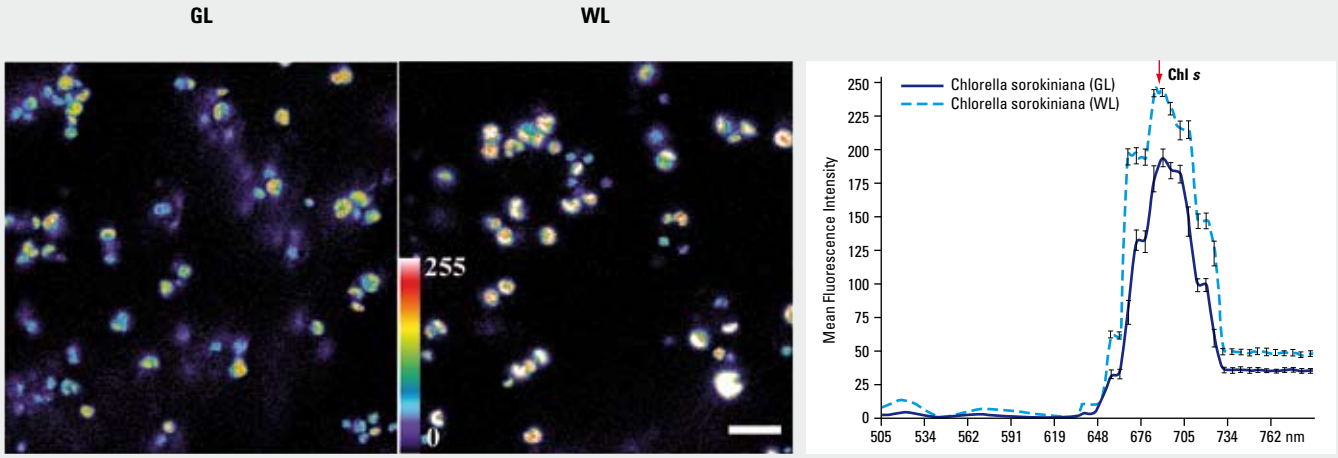


Fig. 7. Fluorescence properties from *Chlorella sorokiniana* single cells after illumination with different light qualities (GL and WL). **a.** Optical sections taken from a lambda scan (excitation wavelength = 488 nm, steps = 50) corresponding to the emission peak of chlorophylls. **b.** Spectral profile representing the mean fluorescence intensity spectra for *Chlorella sorokiniana* under GL and under WL. Note the decrease in mean fluorescence intensity of the pigments from GL treatment relative to the WL control. Scale bar = 10 µm (published in: Applied Environmental Microbiology 72: 3026–3031, 2006).

ysis of fluorescent pigments from a single cell *in situ* in thick samples without isolation; (iii) establishing a simultaneous relationship between fluorescence properties, morphology and position inside complex microbial assemblies; and (iv) discrimination of cells with particular fluorescence signatures within the colony, and correlation with individual cell states.

Methods for preventing biodeterioration

The elimination of microbial colonisation from works of art is not trivial. Most of the treatments to remove phototrophic biofilms consist of biocide applications which often have undesirable effects. Hence there is a pressing need for alternative, low-risk methods [14]. We think that the key to controlling biodeterioration is the use of measures that prevent favourable growth conditions for microflora. Our results suggest that the use of monochromatic GL, which is poorly absorbed by the majority of photosynthetic organisms, is useful for preventing green sickness in hypogean environments. Only some cyanobacteria are able to harvest sufficient light energy to survive in extremely dim ambient light or in GL [6] although they do not thrive [15]. This indicates that illuminating hypogean environments with GL instead of WL can reduce the diversity of moss and algae species.

There is a need to further develop techniques to monitor the natural conditions in which phototrophic biofilms grow, and to evaluate the qualitative and quantitative changes that preventive or eradicative treatments have on biofilms. CSLM is advantageous for use in protecting cultural patrimony sites because it allows the *in vivo* exploration of very small samples (*e.g.* samples from precious works of art), and provides information in real time and space. This method combined with a new technique such as white light laser or fluorescence lifetime imaging microscopy (FLIM) [16] creates the potential for a broad range of new experiments regarding photosynthetic microorganisms. Moreover, spectral CSLM has enabled us to identify the pigments used by photosynthetic microorganisms at specific wavelengths [15, 17]. We are currently exploiting this information to develop illumination alternatives for threatened sites of cultural patrimony.

Acknowledgements

We thank Dr. Oliva for his contributions to statistical calculations. This work was partially supported by the EU Energy, Environment and Sustainable Development Program within the framework of the CATS project, contract EVK4-CT-2000-00028.

References and more information on photosynthetic biofilms: Monica.Roldan@uab.es

Laser Microdissection for Studies in Alzheimer's Disease

Identification of Candidate Genes

In recent years, laser microdissection has increasingly become established in the biological sciences as a means of acquiring homogeneous specimen material. To better understand the cellular and molecular basis of Alzheimer's disease, Professor Thomas Deller (Johann Wolfgang Goethe-University Frankfurt am Main, Germany) and his working group study regulatory molecules in the brains of mice after acute and chronic injury, as well as those in the brains of mice with Alzheimer's. To do so, the working group uses a laser microdissection system from Leica Microsystems, combining this method with quantitative RT-PCR. Unlike traditional methods, this allows gene expression changes to be quantified on a cellular level.

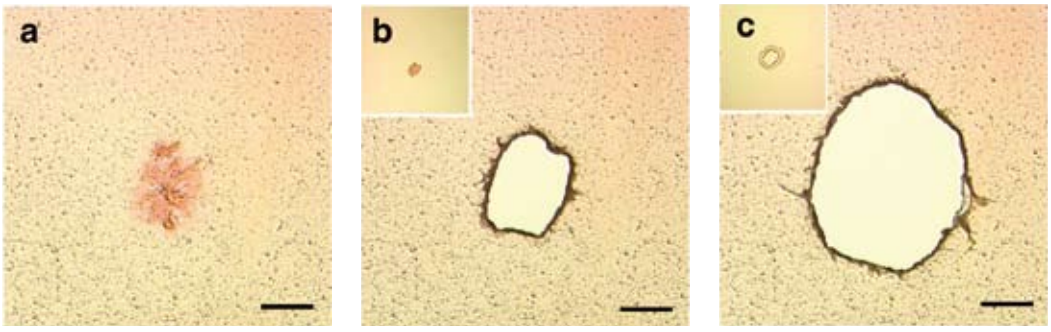
Neurodegenerative diseases such as Alzheimer's disease lead to the loss of synapses and neurons in the CNS. The brain reacts to these changes with complex regulation mechanisms, known as lesion-induced neuronal plasticity. An important process in this regard is the growth of new nerve fibers from surviving cells. This axonal sprouting results in partial reinnervation and new synapses and may contribute to a partial restoration of function. In Alzheimer's disease, however, there are indications that axonal sprouting can also take on an aberrant form. In the immediate vicinity of the amyloid plaques that characterize this disease, pathological sprouting processes have been observed which could worsen cognitive deficits associated with Alzheimer's disease. If the effort to explain the functional significance of axon sprouting, identify regulatory molecules and ultimately intervene in the sprouting processes succeeds, new therapeutic intervention strategies may be developed for treating neurodegenerative diseases and acute brain injuries.

Neuronal plasticity after injury

At the Johann Wolfgang Goethe-Universität in Frankfurt, Germany, Dr. Thomas Deller, Professor of Anatomy and Director of the Institute of Clinical Neuroanatomy, and his working group are particularly involved in researching the cellular and molecular basis of neuronal plasticity after brain lesion. Their focus is on the molecular regulation of axonal sprouting and its significance for Alzheimer's disease. To analyze the changes in the brain, APP23 transgenic mice were used, which, after six months, develop plaques in the brain that are similar to those caused by Alzheimer's disease [1].

Research focuses on astrocyte response

One of Deller's research approaches is concerned with the response of the astrocytes [2], which play an important role in how the brain responds to an injury. These cells multiply in the affected brain regions and change their gene expression, particularly that of the GFAP (glial fibrillary acidic protein) molecule. The expression of GFAP mRNA is determined by the quantitative reverse transcriptase polymerase chain reaction (qPCR). Earlier studies with brain tissue, however, used tissue homogenates that contained various cell types. This meant that the mRNA could not be definitively traced to one source. Therefore, Deller's workgroup uses laser microdissection, which allows the isolation of defined cell populations.



http://www.leica-microsystems.com/products/LMD6000

Using the Leica Microsystems laser microdissection system, small areas can be isolated from a tissue segment. To do so, a pulsed laser beam is focused on the specimen using an objective.

The high energy of the light destroys the tissue in focus. Many pulses are arrayed in sequence to obtain the desired section cut. The laser hits the specimen for very short intervals of less than one second. This completely eliminates any heat transfer outside the focus area. A powerful diode laser enables fast, highly precise cutting, even of individual cells. With an objective with 63x magnification, a section width of approximately 1 µm is attained; with a 150x dry objective, the width is even less than 1 µm. The area that is cut out falls into a collection container along with the carrier membrane. In the Leica Microsystems microdissection system, the precise cut guidance is controlled by prisms and increases in direct proportion to the objective magnification.

An important consideration when combining laser microdissection and qPCR is maintaining the integrity of the mRNA from the time the specimen is obtained until the time of the qPCR. Deller and colleagues Dr. Guido J. Burbach, Charlotte Nolte-Uhl and Stefanie Frank developed a rapid immunofluorescence protocol that permits minimal staining and incubation periods and rapid further processing before dissection, while at the same time ensuring high mRNA quality [3]. To obtain sufficient mRNA for an analysis, 60 astrocytes were dissected. After isolation and transcription into cDNA, the specimens were analysed using qPCR.

Astrocytes respond to lesions in a differentiated manner

The results show clear differences in the height of the measured gene expression between dissected individual cells and dissected tissue areas. While an 18-fold increase in the GAFP mRNA over the control specimens was measured in the astrocytes cut out of the immediate plaque environment, the GAFP-mRNA in the dissected tissue increased only fivefold (Fig. 2). Deller's workgroup was able for the first time to describe and quantify GAFP mRNA expression in isolated astrocytes for acute and chronic brain injuries and in Alzheimer's mice. The researchers were able to infer from the results that the type and severity of the damage is an important factor for the strength of the postlesional astrocyte reaction in the brain [2].

Unwanted axonal sprouting due to neurotrophic factors

The workgroup used a comparable combination of methods when analysing axonal sprouting in Alzheimer's mice [4, 5]. The BDNF (brain-derived neurotrophic factor) can trigger axonal growth and also changes in Alzheimer's disease. Plaque tissues were dissected to study the gene expression of BDNF mRNA in Alzheimer's mice. In addition, a 30 µm wide ring of tissue surrounding the plaques was cut out. Dissected specimens from plaque-free tissue areas served as the control (Fig. 1, a-d). The quantitative PCR analysis identified a gradient of BDNF mRNA (Fig. 1, e, f) in the direction of the plaques. These results, along with the immunohistochemical findings, indicate that in the immediate vicinity of amyloid plaques, glial cells are activated that synthesise and release BDNF. BDNF – probably along with other factors – could have a stimulating effect on axonal growth. Axons could grow along the BDNF gradient to the plaque. The inflammatory and toxic environment of the plaques could damage the growing axons and cause disturbances in synaptic connections.

High sensitivity of the methodology confirmed

The experiments are an impressive demonstration of the advantages of laser microdissection when used for cell-specific and molecule-specific analyses. Deller and his colleagues praise the precision

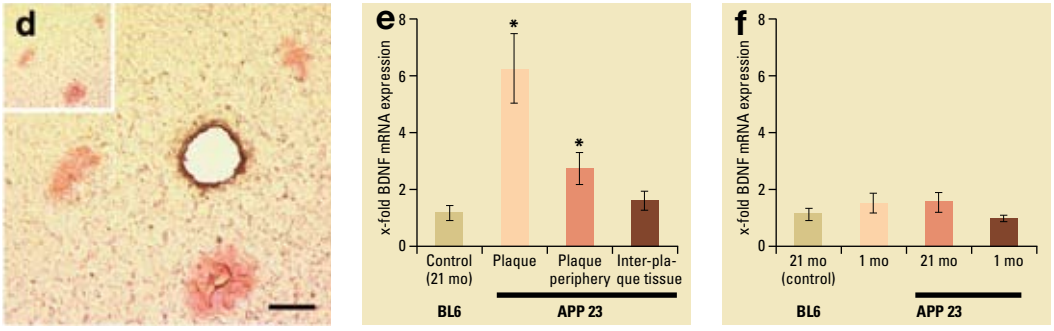


Fig. 1: Increasing BDNF mRNA expression in the direction of amyloid plaques. Cortex tissue of 21-month-old APP23 transgenic mice stained with Congo Red. a: Plaques in APP23 mice. b: Dissection of the plaques; c: dissection of a 30 µm wide ring of tissue. d: Dissection of tissue between the plaques. e: Results of the qPCR showed increasing BDNF mRNA expression in the direction of the plaques. f: Comparatively low BDNF mRNA expression in the tissue between the plaques. P ≤0.05; size of scale bar = 50 µm in a–d.

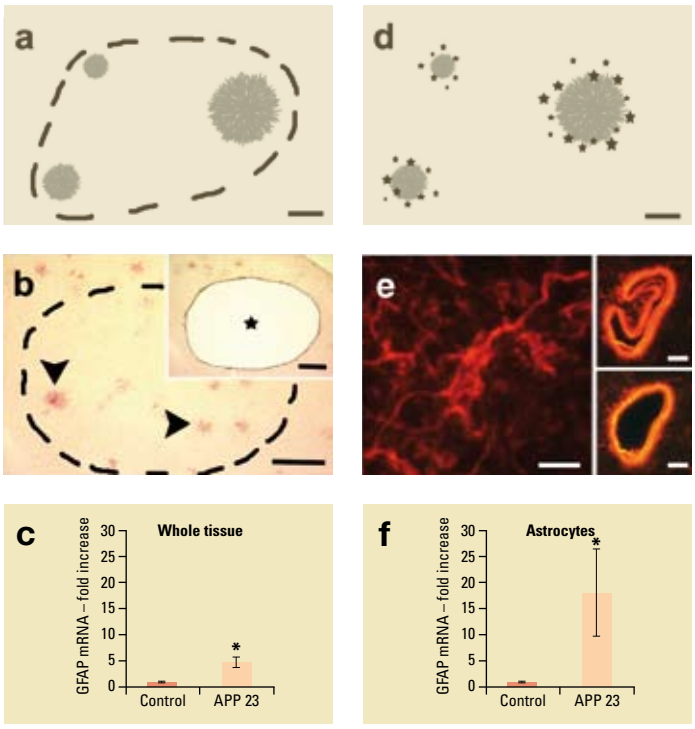


Fig. 2: Increased expression of GFAP mRNA in the vicinity of amyloid plaques.
a, b: Plaques in the cortex of APP23 mice (a: schematic, b: cortex tissue stained with Congo Red, before and after dissection).
c: GFAP mRNA in tissue containing plaque is five times greater than that in control specimens.
d, e: GFAP-marked astrocytes in immediate vicinity of plaque (d: schematic, e: fluorescence photograph before, during (top right) and after dissection (bottom right, with autofluorescent residual tissue).
f: GFAP mRNA in astrocytes is 18 times greater than that in control specimens.
$P \leq 0.05$; size of scale bar = 50 µm in a, d; 200 or 250 µm in b; 10 or 5 µm in e.

and ease of use of the Leica Microsystems laser microdissection system as well as its wide variety of applications – and foresee even more interesting possibilities in the future. If the technology can be further refined and new upstream and downstream methods that are better matched to the systems become available, laser microdissection could also become established in proteomics research.

References

1. Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K. H., Mistl, C., Torhacjer, S., Ledermann, B., Burki, K., Frey, P., Paganetti, P. A., Waridel, C., Calhoun, M. E., Jucker, M., Probst, A., Staufenbiel, M., Sommer, B. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc Natl Acad Sci USA 94: 13287–13292 (1997).
2. Burbach, G., Dehn, D., del Turco, D., Staufenbiel, M., Deller, T. Laser microdissection reveals regional and cellular differences in GFAP mRNA upregulation following brain injury, axonal denervation, and amyloid plaque deposition. GLIA 48: 76–84 (2004).
3. Burbach, G., Dehn, D., Nagel, B., del Turco, D., Deller, T. Laser microdissection of immunolabeled astrocytes allows quantification of astrocytic gene expression, J Neurosci Methods 138: 141–8 (2004).
4. Burbach, G., Hellweg, R., Haas, C. A., del Turco, D., Deicke, U., Abramowski, D., Jucker, M., Staufenbiel, M., Deller, T. Induction of brain-derived neurotrophic factor in plaque- associated glial cells of aged APP23 transgenic mice. J Neurosci 24: 2421–30 (2004).
5. Burbach, G., Dehn, D., del Turco, D., Deller, T. Quantification of layer-specific gene expression in the hippocampus: effective use of laser microdissection in combination with quantitative RT-PCR. J Neurosci Methods 131: 83–91 (2003).

This article was published in LaborPraxis, 03/2007
Vogel Industrie Medien GmbH & Co. KG, Würzburg, Germany
Contact: Marc Platthaus, Phone +49 931 418-2352, Fax -2750, marc_platthaus@laborpraxis.de, www.laborpraxis.de

http://www.med.uni-frankfurt.de/institut/zmorph/index.html

The winner of our last contest: Prof. Nikolaj Scharff from the Zoological Museum, University of Copenhagen, Denmark, won the Apple iPod nano Red Special Edition.

Contest

Your Opinion is Valuable!

Win a **Leica EZ4 stereomicroscope**
or one of 50 Leica poster carriers.

Dear Reader,

Please give us your comments on this European Edition of research reSOLUTION newsletter. Send us your complete name and address of the institute where you work, along with your comments by going to the following link by 29 February, 2008:

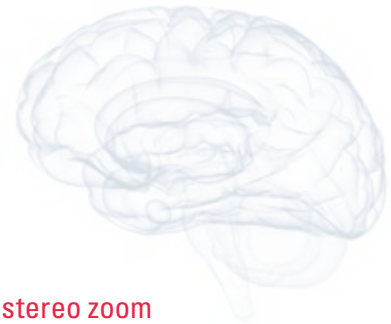
www.leica-microsystems.com/EU-research

Winners will be drawn from all completed entries.

http://www.leica-microsystems.com/products/LMD6000

Leica M165 C and M205 C

Human Brain Takes On the Jobs of the Optics



With the Leica M165 C and M205 C stereomicroscopes, Leica Microsystems offers the new, revolutionary M series. Especially in natural sciences, such as embryology, genetics, microfluidics and earth sciences, the new devices allow applications which were, until now, impossible in traditional stereomicroscopy.

A fascinating phenomenon

With this series, Leica Microsystems has made advantageous use of a neurological phenomenon by implementing the new Leica FusionOptics™ (patent applied for): with FusionOptics™, the left beam path produces great depth of field, while the right beam path provides a high-resolution image. The human brain itself then combines the best information from both channels, using it to compose an image whose resolution and depth of field have never been achieved in any stereomicroscope before.

World's best stereo zoom

The Leica M205 C is the world's first stereomicroscope with a fully apochromatically corrected 20.5:1 zoom. In the zoom range from 0.78x to 16x, the resolution increases continuously up to 1050 lp/mm (planapochromat objective 2x). Due to the natural laws of optics, this performance class has never been seen before in stereomicroscopes. Up until now, optical attachments could only achieve a maximum zoom range of 16:1 or a magnification increase without an increase in resolution (empty magnification).

The exceptional performance of the Leica Fusion Optics™ in the Leica M205 C is evident in its impressive maximum magnification of 1280x. This new generation of objectives brings with it the absolute largest working distances (61.5 mm with planapochromat objective 1x) in stereomicroscopy.

With the Leica M165 C, the classic design principles for stereomicroscopes were stretched to the very limits of optics. With a zoom range of 16.5:1 and a maximum resolution of 906 lp/mm (planapochromat objective 2x), this is the most powerful stereomicroscope in its class.

Fully integrated system

Leica Microsystems has consistently pushed ahead with system integration. The optical components are encoded and communicate directly with each other via the Leica Application Suite software (LAS). All data are coordinated through the central interface where they are bundled and then synchronised with the computer via USB. These adjustments are stored and can subsequently be called up with a click of the mouse. Error sources are thus eliminated reliably and experiment conditions are fully traceable at all times.

Deep Brain Imaging

Watch Life as it Happens with Leica FCM1000 Endoscopic Confocal Microscope

As bio-medical research progresses by applying new methods, the emphasis on *in vivo* imaging and analysis constantly grows. Today, observing life as it happens is the dream of most researchers. From whole body imagers to intra-vital microscopy, many solutions have been developed to approach this goal. However, they always have to struggle with the trade-off between high resolution and low invasiveness. Leica Microsystems answers this challenge with the Leica FCM1000, the very first imaging solution developed for – and fully adapted to – *in vivo* and *in situ* small animal imaging.

Leica FM Microprobes:
- Optical head diameter 0.3–4.2 mm
- Length 2 m (6 m for MRI-compatible microprobes)
- Slender and flexible
- Resolution 1.8–3.9 µm
- Field of view ∅ 240–600 µm
- Working distance 0–170 µm
- Optical section 10–70 µm
- Plug and play – immediate connection to laser scanning unit, no alignment
- Robust and handy

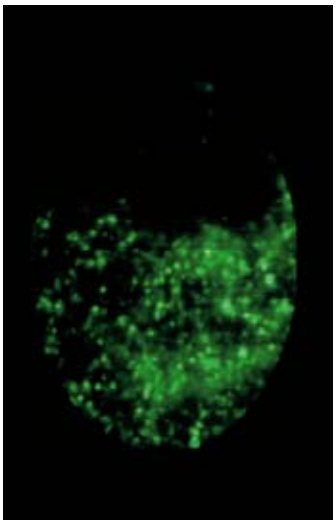
Real-time imaging anywhere in the living animal

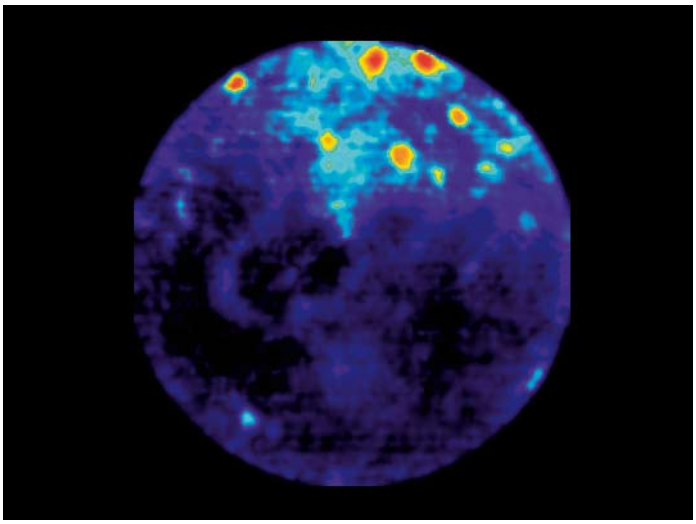
In vivo observation of live processes requires a high degree of miniaturisation for minimally invasive access as well as an ultra-high frame rate for real-time dynamic recording. With its fibered microprobes the Leica FCM1000 is the only solution for imaging cells anywhere in the brain of living animals with minimal disturbance. It offers a revolutionary approach to brain imaging with *in situ* observations. By combining a minimally invasive access to any structure of interest and an exceptionally high frame rate, the instrument enables real-time recording of live processes as they happen, where they happen. A simple contact with the tissue of interest is enough to generate high-speed recordings of cellular or vascular events.

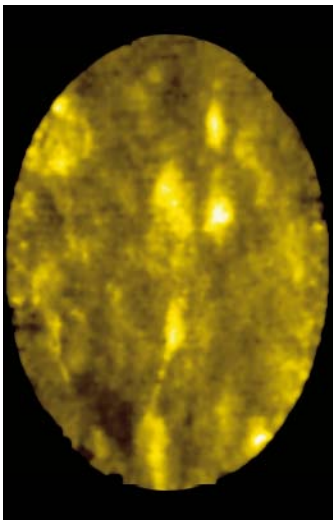
Immediate, intuitive, easy

Special emphasis has been put on software simplicity, resulting in a highly intuitive user interface. From instrument setup to image acquisition and data analysis, tools have been designed to be as easy to use as possible. Complement current imaging practices with true *in vivo* imaging. The Leica FCM1000 is a complete solution. It has been specifically developed for *in situ* observations of cellular or vascular events.

http://www.leica-microsystems.com/products/FCM1000







From left to right:

GFP stem cells injected and imaged in living mouse brain, FOV: 300 x 400 µm.

Neurons loaded with Oregon Green BAPTA 1 for calcium imaging, FOV: ∅ 300 µm.

Neurons of transgenic mouse expressing Clomeleo, FOV: 300 x 400 µm.

Leica FM – fibered and miniaturised

With fibered Leica FM Microprobes featuring diameters as small 0.3 mm, the Leica FCM1000 enables in situ imaging at any location in the brain of lab animals. Leica FM Microprobes are readily compatible with a stereotaxic approach and can be attached to most micro-pipette holders. The shaped tip allows minimal disturbance to the tissue and a good tissue-to-microprobe contact.

A unique tool for *in situ* brain imaging

The Leica FCM1000 is a complete solution which combines high performances with very limited invasiveness and user friendly environment. From instrument set up to image acquisition and data analysis, every step has been made easy. Moreover, the system can be combined and synchronised to other modalities such as fMRI, EEG, and electrode recording. Applications of the instrument to brain research cover a wide range of observations of cells or micro-vessels in the brain or olfactory bulb:

- Recording of neuronal activity
- Monitoring of reporter gene expression
- Migration and fate of stem cells and neuron precursors
- Functional changes in micro-vascularisation
- Blood/brain barrier studies

Integrated image and data extraction tools offer qualitative and quantitative analysis of experiments.

References

Vincent, P., Maskos, U., Charvet, I., Bourgeais, L., Stoppini, L., Leresche, N., Changeux, J.-P., Lambert, R., Meda, P., Paupardin-Tritsch, D.: Live imaging of neural structure and function by fibered fluorescence microscopy. EMBO Reports 7 (11): 1154–1161 (2006).

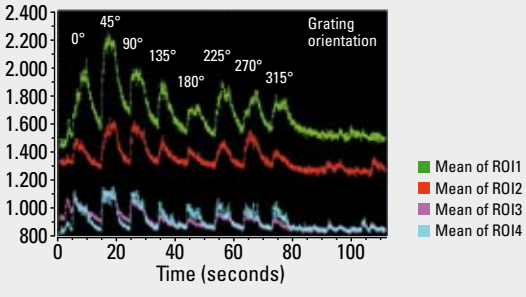
Bharali, D. J., Klejbor, I., Stachowiak, E. K., Dutta, P., Roy, I., Kaur, N., Bergey, E. J., Prasad, P. N., Stachowiak, M. K.: Organically modified silica nanoparticles: a nonviral vector for in vivo gene delivery and expression in the brain. PNAS 102 (32): 11539–11544 (2005).

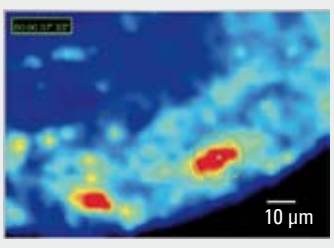
Lledo, P. M., Saghatelyan, A.: Integrating new neurons into the adult olfactory bulb: joining the network, life/death decisions, and the effects of sensory experience. Trends in Neuroscience 28 (5): 248–254 (2005).

Davenne, M., Custody, C., Charneau, P., Lledo, P. M.: In vivo imaging of migrating neurons in the mammalian forebrain. Chemical Senses 30: 115–116 (2005).

Courtesy

Images courtesy of T. Kuner, G. Feng, G. Augustine, MPI, Heidelberg, Germany; P. Vincent, R. Lambert, L. Bourgeais, ERT 1059, Pierre et Marie Curie University, Paris, France; Dr. Cheng, Taichung Veteran's General Hospital, Taichung, Taiwan ROC. Prof. Haydon, School of Medecine, University of Pennsylvania.





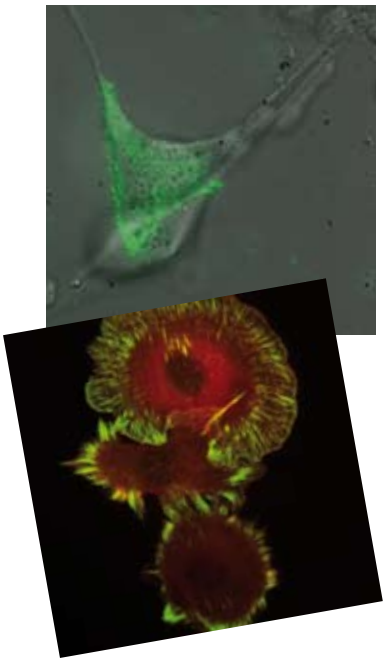
Neuron activity recording with calcium sensitive dyes: Recording of neuron activation in the cat visual cortex with fibered fluorescence microscopy. Neuron orientation specificity was checked by presenting to the anesthetized animal stripes with changing orientations, in cycles of five seconds. Courtesy: Aaron Kerlin, Kenichi Ohki, Clay Reid, Harvard Medical School, Boston, MA.

http://www.leica-microsystems.com/products/FCM1000

Leica AM TIRF MC

Excellent Flexibility for Multi-Color Cell Membrane Studies

The new Leica AM TIRF MC (MultiColor, Total Internal Reflection Fluorescence) allows excitation in the entire range of the fluorescence spectrum to now be used for real-time visualisation of live cell dynamics. Its unique fast frame rate and fast wavelength switching without changing penetration depth offers live cell researchers leading-edge technology that reliably provides reproducible scientific results.



The Leica AM TIRF MC integrates four solid-state lasers for the excitation of fluorophores at wavelengths of 405, 488, 561, and 632 nm. The system features extremely short switching times and an ultra-high synchronised frame rate. Individual laser lines are paired and controlled quickly and precisely via AOTF (Acousto-Optical Tunable Filters) during a switching time of only 1 ms. The system is particularly useful for exploring molecular interaction at cell membranes and interaction of proteins and receptors involved in transport mechanisms. The scientist can examine single molecules near the cell membrane, study co-localisation and vesicle transport, and combine TIRF and fast FRET analysis.

Automatically the right TIRF angle

Leica Microsystems's unique scanner-sensor technology automatically matches the TIRF angle at all wavelengths to the required penetration depth, and positions the laser so that the TIRF penetration depth remains constant even when the wavelength is changed. Researchers using Leica's TIRF technology benefit from a unique level of convenience as well as from reproducible scientific results.

Dynamic processes in real time

The newly designed SyncBoard controls and synchronises all components with the result of a fast frame rate of up to 30 fps. This high temporal resolution for the excitation of multiple fluorophores, combined with the high spatial TIRF resolution of 70–300 nm in the z-axis, are key prerequisites for visualising transport processes in cell membranes or protein interactions in real time. With an excellent signal-to-noise ratio, the entire dynamic range of the high-sensitivity EMCCD camera can be used. Leica's high-performance camera takes top quality pictures even of weak fluorescence signals.

Compact, safe and compatible

The compact system has a small footprint and is a safety class 1 laser unit that uses a quiet air-cooling system. All four laser lines are guided via a single fibre optic. The TIRF module can be connected to Leica's inverted microscopes as well as to the high-end Leica TCS SP5 confocal system.

http://www.leica-microsystems.com/products/AM_TIRF_MC

European Research Events

Please also visit our website on www.leica-microsystems.com/events for further information on Leica Research workshops in Europe.

Events 2007

SBCF Congress (Société de Biologie Cellulaire de France)
October 28–31
Grenoble, France
www.sbcf.free.fr/

IX Curso Leica para el nuevo técnico-especialista en Microscopía Confocal Espectral
November 20–23
Bellaterra, Spain

Leica Scientific Forum
Prof. Roger Tsien
November 13
German Cancer Research Center (DKFZ), Heidelberg, Germany

Medica 2007
November 14–17
Düsseldorf, Germany
www.medica.de

Leica Scientific Forum
Prof. Roger Tsien
November 19
Institute Pasteur
Paris, France

Journée Utilisateurs Microscopie Confocale Leica 2007
November 12
Paris, France

Events 2008

German Society for Cell Biology
March 12–15
Marburg, Germany
www.zellbiologie2008.de

Laborama 2008
March 13–14
Brussels, Belgium
www.laborama.be

Zebrafish 2008
March. 14–15
Veysonnaz, Switzerland
www.swisszebra.ch

Analytica 2008
April 1–4
Munich, Germany
www.analytica.de

Advances in Multidimensional Optical Fluorescence Microscopy towards Nanoscopy
April 17–30
Erice, Italy
www.ccsem.infn.it/

LENS, New Frontiers in Micro and Nano Photonics
April 23–26
Florence, Italy
www.lens.unifi.it/fmnp

ESHG, European Society of Human Genetics
May 31 – June 3
Barcelona, Spain
www.eshg.org/

Het Instrument
May 20–23
Utrecht, The Netherlands
www.hetinstrument.nl/2008/en/

ELMI meeting
May 27–30
Davos, Switzerland
http://elmi08.unibas.ch/

Microscience 2008
June 23–26
London, United Kingdom
www.microscience2008.org.uk

2nd International Congress on Stem Cells
July 6–9
Dresden, Germany
www.stemcellcongress-dresden.de/

FENS, 6th Forum of European Neurosciences
July 12–16
Geneva, Switzerland
http://fens2008.neurosciences.asso.fr/

ELSO
August 30 – September 2
Nice, France
www.elso.org

EMC2008, 14th European Microscopy Congress
September 1–5
Aachen, Germany
www.emc2008.de/

Heidelberg – Paris – London

Leica Scientific Forum

Just two years after Leica Microsystems opened the first Leica Scientific Forum in December 2005, "Advances in Life Sciences," in Heidelberg, the feedback from the series remains very positive. In the midst of one of Germany's largest research centres, the Leica Forum offers a top-class platform for scientific lectures that promote international communication on the latest discoveries in life science, as well as exchanges between the academic world and industry. Initiator and organiser Dr. Thomas Zapf, Director Scientific Relations, has broadened the successful concept this year at research centers in France (Paris, Marseille, Bordeaux) and UK (London, Oxford, Cambridge, Liverpool).

Right from the beginning Leica Microsystems was able to recruit top-ranking scientists to present the latest research projects at the Leica Scientific Forum. The speakers and topics resonate with the interested public, which includes institute directors, doctoral candidates, academic employees, as well as interested students, customers, and representatives of companies in the life sciences. The Leica Forum has proven to be a fertile platform for new ideas and cooperative seeds. Leica Microsystems plans to establish the Leica Scientific Forum next year in the US, too.

More information on Leica Scientific Forum: Thomas.Zapf@leica-microsystems.com

Imprint

This reSOLUTION Edition is the magazine for Leica Microsystems research customers

Publisher
Leica Microsystems GmbH
Ernst-Leitz-Straße 17–37
D-35578 Wetzlar (Germany)
www.leica-microsystems.com

Editors in Chief
Anja Schué, Corporate Communications, Anja.Schue@leica-microsystems.com

Didier Goor, European Marketing, Didier.Goor@leica-microsystems.com

Contributing Editors
Thomas Deller
Oliver Garner
Petra Kienle
Matthias Obst
Monica Roldán
Bernd Schneider

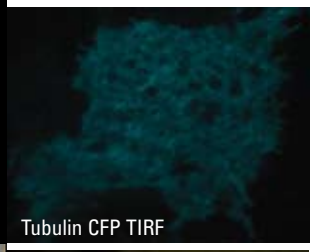
Layout
Heinz Flick

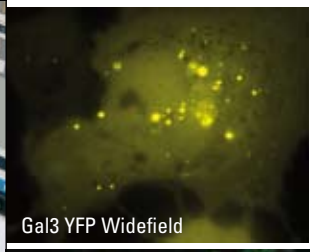
Cover Picture
Frederik Pleijel,
Kristineberg Marine Research Station

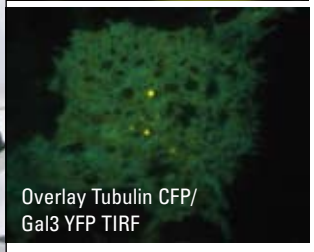
Production
Uwe Neumann, Central Marketing

Printing Date
November 2, 2007


Tubulin CFP Widefield


Tubulin CFP TIRF


Gal3 YFP Widefield


Overlay Tubulin CFP/
Gal3 YFP TIRF

Courtesy of: Prof. Dr. R. Jacob,
University Marburg, Germany

Visualize Life's Secrets – Leica AM TIRF MC

Leica's new, innovative MultiColor TIRF system features a

- **dynamic scanner** that can be used to
- **precisely position** the laser beam and determine the
- **exact and reproducible penetration depth** of the evanescent field
- **with fast changing wavelengths**

The powerful Leica AF6000 fluorescence software offers full control of all TIRF system functions.

www.leica-microsystems.com/products/AM_TIRF_MC

Leica
MICROSYSTEMS

Order no.: 11924109