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In December 2008 we celebrated our 10th anniversary and are proud and happy to look back on 10 successful years which were accompanied by remarkably good friends, colleagues and partners. In the beginning of 2009, things continued to be exciting. By launching Imaging & Microscopy’s new online-blog in January, GIT VERLAG made an important step into the age of cross-media works. This means, a new feature to extend the information added to our portfolio. The latest news are now accessible online on our blog http://blog.imaging-git.com, providing you with important information from the microscopy and imaging community. Breaking news from associations, academic research, industry as well as event announcements and reports are presented there short and concise. Subsequently in summer 2009 GIT VERLAG will provide you with another comprehensive web site, creating a place for professionals in the field of imaging to exchange their expertise and to establish an active and agile web-community. On the new portal the interchange of up to date issues will be possible in various discussion forums. Besides a strong scientific content, several service tools will be available to ease daily laboratory work. By breaking into this new sphere, Imaging & Microscopy wants to connect professionals from all over the world. We are very excited and curious what 2009 will furthermore bring. We are looking forward to meeting you online, soon!

Enjoy reading this issue and visit us at: http://blog.imaging-git.com

Martin Friedrich, Editor-in-Chief

Thomas Matzelle, Scientific Editor

Anja Szerdi, Online Editor
EDITORIAL
Dr. M. Friedrich, Dr. T. Matzelle, A. Szerdi, GIT VERLAG, Germany

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MICROSCOPE IMAGING
C. Keogh, Nikon Instruments, United Kingdom

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THE RMS VICE PRESIDENT’S FUND
A. Winton, Royal Microscopical Society, United Kingdom
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Embyronic Development Imaged by Scanned Light Sheet Microscopy

P.J. Keller and colleagues extended the approach of Selective Plane Illumination Microscopy (SPIM) into Digital Scanned Light Sheet Microscopy (DLSM) in which the illumination light sheet can be rapidly moved through the sample by a scanning mirror. This allows very fast acquisition of volume data and thus can be used to generate highly resolved 4D representations of dynamic 3D samples, e.g. developing embryos. This approach is demonstrated by following the movements of nuclei in developing zebrafish embryos during the first 24 hours of development.

> Science, 322, pp. 1065–1069

Photoswitching of Common Fluorophores for Super-resolution Imaging

J.S. Biteen and her colleagues [1], as well as J. Fölling and co-workers [2] show in two separate works that super-resolution imaging techniques can be applied to fluorophores that are commonly used in fluorescence microscopy. Biteen use the long-known photoswitching effect found in EYFP for photoactivated localization microscopy (PALM) to image bacterial structures at a resolution below 40 nm. Fölling and colleagues extend the PALM concept by performing fluorescence nanoscopy through ground state depletion. This does not require a photoactivating property of the molecule, but utilizes the standard dark-state transitions generally found in fluorophores (e.g. the triplet state).


Genetically Timed Multicolor Neuronal Activity Imaging

Using retrograde transsynaptic viruses as vectors, Z. Boldogkői, K. Balint and colleagues extended the rainbow approach to labelling neurons inside a tissue with different color mixtures. They demonstrate the possibility to additionally monitor Ca²⁺ activity as well as to determine the time since infection of the neurons, as later stages of infection cause damage to the neurons and are not suitable for functional studies.

> Nature Methods, 6, pp. 127–130

Multiplexed FRET through Fluorescence Lifetime Imaging

D.M. Grant and co-workers described a combined ratiometric imaging and Fluorescence Lifetime Imaging Microscopy (FLIM) approach to simultaneously image two FRET sensors in the with minimal cross-talk. By using the possibility to only use information of the donor fluorophore for the calculation of FRET, the relatively weak signal of mPlum as acceptor of the second sensor could be ignored, thus it was possible to clearly separate the ratiometric information provided by an ECFP/Venus Calcium sensor from the FLIM-FRET signal coming from a TagFP/mPlum Ras sensor.

> Biophys. J., 95, pp. L69–L71

Theoretical Limits in Localizing Switchable Fluorophores

Exploring the limitations for factors that are relevant for super-resolution imaging techniques like PALM and STORM, A.R. Small develops a formalism that derives a relationship between error rates, image acquisition rates and the image processing performed on the data.

> Biophys. J., 96, pp. 16–pl18
> Dr. Timo Zimmermann, Centre for Genomic Regulation (CRG), Barcelona, Spain

New Dimensions to Laser-scanning Fluorescence Microscopy

A. K. De and D. Goswami from the Indian Institute of Technology Kanpur, India, described a novel method of optical imaging by exploiting simple ideas borrowed from pulsed optics. They show that the use of ultrafast pulsed one-photon excitation in laser-scanning fluorescence microscopy brings together several advantages offered by two widely used present day microscopic techniques, confocal and multi-photon fluorescence microscopy. The method appears as a novel tool in the context of laser-scanning fluorescence microscopy by having a “built-in” 3D spatial resolution.

> J Microscopy 233 (2) 320–325
DOI:10.1111/j.1365–2818.2009.03122.x

Accuracy Assessment of Elastic Strain Measurement by EBSD

S. Villert and co-workers from the Ecole Nationale Superieure des Mines, SMS-UMR CNRS 5146, Saint Etienne, France, presented a detailed accuracy analysis of electron backscatter diffraction (EBSD) elastic strain measurement using both simulated and experimental patterns. Strains are determined by measuring shifts between two EBSD patterns (one being the reference) over regions of interest (ROI) using an iterative cross-correlation algorithm. An original minimization procedure over 20 regions of interests gives a unique solution for the eight independent components of the deviatoric displacement gradient tensor. This method leads to strain measurements on simulated patterns with accuracy better than 10⁻⁴. The method is illustrated by examples using four-point bending of a silicon single crystal and Si₃N₄ layers on a Si substrate. Experimental results are compared with finite-element simulations.

> J Microscopy 233 (2) 290–301 (DOI) 10.1111/j.1365–2818.2009.03120.x

Laser-scanning Confocal Microscope with Tunable Amplitude, Phase, and Polarization

B. R. Boruah and M. A. A. Neil from the Gauhati University, India described a new system allowing the control of the amplitude, phase, and polarization of the laser beam used in the microscope in real time, with the help of a liquid crystal spatial light modulator, acting as a computer generated hologram, in conjunction with a polarization beam splitter and two right angled prisms assembly. Two scan mirrors, an on-axis fast moving scan mirror for line scanning and an off-axis slow moving scan mirror for frame scanning, minimize the movement of the scanned beam over the pupil plane of the microscope objective, and form the X,Y scan unit. Efficiency of the system is demonstrated by generating a bottle structured focal volume, resulting from the overlap of two cross polarized beams, and improve both the lateral and axial resolutions if used as the de-excitation beam in a stimulated emission deexcitation confocal microscope.

> Rev. Sci. Instrum. 80, 013705 (2009);
DOI:10.1063/1.3072663

Thermally Actuated Tapping Mode in AFM with Polymer Microcantilevers

B. Mitra and A. Gaitas from Picocal Inc. Michigan, USA, presented a thermally actuated tapping mode AFM with a polymer cantilever. The cantilever (350×250×3 µm³) is made from polyimide and includes an embedded resistive heater for thermal actuation. The oscillation of the cantilever is due to the stress gradient caused by alternating heating and cooling from the periodic ac excitation of the heater. The tip oscillation amplitude is 5–10 nm in air. The oscillation occurs at 2π and is a linear function of the applied voltage. The maximum oscillation amplitude is seen at 0.8 Hz with a 3dB frequency of 26 Hz. The damping of the oscillation due to tip-sample interaction is used to image the sample without any optomechanical feedback. Scans with a 200 nm tall grating indicate a resolution comparable to deflection signal from the AFM in contact mode.

> Rev. Sci. Instrum. 80, 023703 (2009);
DOI:10.1063/1.3078010
> Dr. Stéphane Portha, Free University of Brussels ULB, Brussels, Belgium
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<td>MSM XVI</td>
<td>March 17–20</td>
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<td>Krakow, Poland</td>
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<td>MRS Spring Meeting</td>
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<td>Control</td>
<td>May 5–8</td>
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<td><a href="http://www.control-messe.de/de/control">www.control-messe.de/de/control</a></td>
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<td>EMAS 2009, 11th European Workshop on Modern Developments and Applications in Microbeam Analysis</td>
<td>May 10–14</td>
<td>Gdansk, Poland</td>
<td><a href="http://www.emas-web.net">www.emas-web.net</a></td>
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<tr>
<td>12th Ceramics, Cells and Tissues</td>
<td>May 19–22</td>
<td>Faenza, Italy</td>
<td><a href="http://www.iom3.org/events">www.iom3.org/events</a></td>
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<td>Latest developments in Scanning Probe Microscopy</td>
<td>June 24–25</td>
<td></td>
<td><a href="http://www.spl.co.uk/ukspm">www.spl.co.uk/ukspm</a></td>
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<td>Scandem 2009</td>
<td>June 8–10</td>
<td>Reykjavik, Iceland</td>
<td><a href="http://scandem2009.hi.is">http://scandem2009.hi.is</a></td>
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<tr>
<td>European Light Microscopy Initiative Meeting 2009</td>
<td>June 8–12</td>
<td>Glasgow, UK</td>
<td><a href="http://www.elmi2009.org">www.elmi2009.org</a></td>
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<td>June 16–19</td>
<td>Segovia , Spain</td>
<td><a href="http://www.microscopia.org/iberm2009">www.microscopia.org/iberm2009</a></td>
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<td>Advanced Electron Microscopy Summer School</td>
<td>June 20–July 4</td>
<td>Copenhagen, Denmark and Gotenborg, Sweden</td>
<td><a href="http://www.fei.com/events/Microscopy/School/overview.aspx">www.fei.com/events/Microscopy/School/overview.aspx</a></td>
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<td>UK SPM 2009, Two-day conference exploring the latest developments in Scanning Probe Microscopy</td>
<td>June 24–25</td>
<td>Teddington, Middlesex, UK</td>
<td><a href="http://www.npl.co.uk/ukspm">www.npl.co.uk/ukspm</a></td>
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<td>The 10th European Congress of Stereology and Image Analysis</td>
<td>June 26–29</td>
<td>University of Milan, Italy</td>
<td><a href="http://ecs10.mat.unimi.it">http://ecs10.mat.unimi.it</a></td>
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<td>June 27–July 2</td>
<td>Sant Feliu de Guixols, Spain</td>
<td><a href="http://www.esf.org/conferences/09290">http://www.esf.org/conferences/09290</a></td>
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<td>Learning from Single Molecules</td>
<td>June 29–July 1</td>
<td>Bonn, Germany</td>
<td><a href="http://www.th.ch.uni-bonn.de/microscopy">www.th.ch.uni-bonn.de/microscopy</a></td>
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<td>Light Microscopy Summer School 2009</td>
<td>July 6–8</td>
<td>University of York, UK</td>
<td><a href="http://www.rms.org.uk/event_imschool06.shtml">www.rms.org.uk/event_imschool06.shtml</a></td>
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<td>School on Electron Precession</td>
<td>July 6–10</td>
<td>Bouvines (Lille), France</td>
<td><a href="http://fpes.univ-lille1.fr/formacion">http://fpes.univ-lille1.fr/formacion</a> Electron Precession</td>
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<td>Getting the most from your Confocal</td>
<td>July 9–10</td>
<td>York, UK</td>
<td><a href="http://www.rms.org.uk/event_Confocal.shtml">www.rms.org.uk/event_Confocal.shtml</a></td>
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Crossword Puzzle Closed

In the fourth issue of 2008, Imaging & Microscopy was celebrating its 10th anniversary and announced its Anniversary Crossword Puzzle. The bidding is now closed. The winner of the Olympus E-1 Digital SLR camera will be drawn and announced in our next issue.
Kleindiek Nanopositioning Products Available from Agar Scientific

Agar Scientific announced their appointment as distributors for Kleindiek Nanotechnik’s products to the UK and Irish markets. With an innovative driving concept, Kleindiek is entering new fields for micro- and nano-positioning. Due to miniaturization in semiconductor technology, optics, micro-mechanics, medicine, gene- and bio-technology, highly precise positioning techniques are becoming increasingly important. The products meet and often exceed customer requirements, offering a new level of precision.

www.agarscientific.com

Witec Academy Established

Witec has established the Witec Academy as a new forum for advanced instrument and software operation training. It offers a variety of basic and expert level courses covering the entire range of the Company’s products and potential applications. Experienced and new users can both benefit from a graded course structure with seminars and extensive hands-on training sessions. The full day courses are held frequently throughout the year in order to accommodate the attendees’ schedules. Small group sizes for each course guarantee individual mentoring for the most thorough instruction. Participants will receive training materials and a certificate of attendance.

www.witec.de

JPK Wins Prism Award for Photonics Innovation

JPK Instruments has won the international Prism Award for Photonics Innovation in the USA. The Berlin-based company managed to beat its direct competitors following a review and ranking of more than 135 products and applications by a panel of international judges. The Company’s Nanotracker was voted the world’s most innovative product in “Life Sciences”, one of nine categories of the competition.

www.jpk.com

Working of Cells Visualized

GE Healthcare has announced the winners of the In Cell Image Competition at the HCA Conference in San Francisco. All 30 short-listed images can be viewed at www.gelifesciences.com/incellcompetition. This year’s competition saw over 80 images generated by scientists from around the world using the IN Cell Analyzer system. An expert scientific panel short-listed 30 entries, which then went on to the public vote. Delegates at the High Content Analysis conference in San Francisco also had the opportunity to vote for their favorite image. This year’s competition was supported by Biotechniques.

www.gelifescience.com/incellcompetition

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Asylum Research
Young Investigator Award

On January 2009, SPIE organized a special symposium about „Biomedical Spectroscopy, Microscopy, and Imaging” as part of North America’s Largest Photonics Event („Photonics West”). Special attention was paid to the presentations of Young Investigators, which were honored with an award sponsored by PicoQuant. The winners are Sigrun Henkenjohann (University Bielefeld), Jonas Fölling (MPI Göttingen) and Nathan P. Wells (Los Alamos Nat. Labs). One of the offers is a registration fee waiver for the leading European Meeting on “Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences”.

The next conference will be held in San Francisco in January 23–28, 2010.

Contact
Anika Vöge
mkt@picoquant.com

Andor Strong in Japan and China

Andor Technology has strengthened its position in Japan. The Company began its Japanese operations in August 2000 by opening an office in Tokyo. Since then the company has grown in the region, where a strong team of expert staff has developed a customer list made up of almost every leading research institute and university. With the opening of a new office last year in Osaka, Japan’s second largest city, the Company has consolidated its position as a supplier of choice with sales in the country increasing by 22.5% for the financial year ending September 30, 2008. Just recently the Company has won the Rising Star category of the 2008 Cathay Pacific Business Awards. These annual awards are designed to acknowledge and celebrate UK business dynamism and success in Hong Kong and China. The Company was selected from a pool of entries from a range of sectors including design, manufacturing, professional services and marketing.
Leica Has Acquired Surgipath Medical

Leica Microsystems has reached an agreement to acquire Surgipath Medical Industries, a provider of consumables and medical device accessories for clinical histology and research laboratories. Surgipath’s range of products includes processing reagents, storage and specimen transport devices, cytology accessories and safety products. These products will serve to complement and expand Leica Microsystems’ broad histology product range. Anne De Greef-Safft, President of Leica Microsystems’ Biosystems Division, said “this acquisition is another significant step towards enabling the pathologist to achieve a more reliable diagnosis”.

www.leica-microsystems.com

Interim CEO for Carl Zeiss Vision International

Carl Zeiss Vision International announced a change in its management board. For personal reasons, Dr. Norbert Gorny, CEO of Carl Zeiss Vision, has decided to leave the Company. In his role as CEO, he was also responsible for the integration of Sola International which Carl Zeiss Vision acquired in 2004. Arne Frank, chairman of the advisory board of Carl Zeiss Vision will act as interim CEO and lead the company together with the executive board members Klaus Leinnmüller and Dr. Raymund Heinen, until a new long term CEO has been appointed. Frank is a senior industrial advisor of the private equity company EQT and in this role chairman of the advisory board of the Company, which is equally owned by EQT and Carl Zeiss.

www.vision.zeiss.com

Agar Scientific: Management Changes

Agar Scientific has appointed Steve Woodland as Managing Director with current MD, Dr. Lynne Joyce moving to a new role as Director of Market Development where she will focus on technology, product development and customer relationships. This strengthening of the management team will focus on business development as the Company moves forward with increased products and services. Steve Woodland brings a wealth of experience in general management, sales & marketing, catalog distribution through working with a number of UK and international companies. These have included positions at Lancaster Synthesis, Haarman & Reimer and Sigma Aldrich. Most recently, he has run a consultancy company assisting a number of international organizations providing specialist R&D products.

www.agarscientific.com

Carl Zeiss: Scanning Electron Microscope Installed

Carl Zeiss SMT officially put into service the 1,500th Gemini-class scanning electron microscope. The customer is the “Center for Non-Destructive Nano Evaluation Nanoeva” in Dresden, a joint facility of the Electronics Packaging Laboratory (German abbreviation: IAVT) and the Fraunhofer Institute for Non-Destructive Testing (IZFP-D) through its Dresden-based department. One of the key applications will be research on the packaging of electronic and microtechnical modules for system integration. The involved mix of materials, ranging from silicon to plastics, requires the development of tailor-made methods of analysis.

www.smt.zeiss.com

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www.jpk.com/nanowizard
The RMS Vice President’s Fund

...Funding for Worthy Microscopy Projects

Positioned at the forefront of new ideas and development in microscopy and imaging, the RMS has set up a funding channel for backing projects that will further develop the field of microscopy. Founded in January 2008, the Vice President’s Fund was set up to support worthy projects that use microscopy to contribute to the public understanding of science or be of benefit to the Developing World. This new fund will support such projects that are in line with the objectives of the society up to a maximum of £5000 for each award. Dr. Paul Monaghan, RMS Vice President and International Secretary commented, “The Society receives a number of requests for financial support each year, most of which are very interesting and worthy of support. However, previously there were no channels through which they could be funded. This exciting new development has enabled us to remedy this and play an important role in the advancement of microscopy”.

The first batch of applications were considered when the Council met in October last year. “The standard of applications was very high, which was extremely pleasing. The Council members were delighted as they were just the sort of projects that we envisaged when the fund was established” said Dr. Monaghan.

After much deliberation, it was agreed that the fund would be awarded to support three different projects.

- Lead applicant Don Henderson has been awarded funding for “Facing Science in Health and Life”. This will feature at the 6th Annual Schools Science Conference as part of National Science and Engineering week, a ten day celebration running from the 6–15 March, 2009.

This unique award has proven to be a novel way in which the Society supports worthy causes. The high level of quality applications received demonstrates the requirement for such an award within the microscopy sector.

2009 Applications

If you are seeking support for a project that will enhance the public understanding of science, or will benefit the developing world, then full details of the Vice President’s Fund can be found on the Society’s website (www.rms.org.uk). The deadline for the next round of submissions is May 1, 2009, and applications will be considered by the Council at their July meeting. Applicants will be informed of the outcome shortly after the meeting and reports on all successful applicants will appear on the RMS website. Application forms are available on the Society’s website.

Getting more out of 2009

The RMS is dedicated to advancing science, developing careers and supporting a wider understanding of science and microscopy. Therefore, the RMS has gained an excellent reputation for providing high-quality training with excellent tutors. As a result, uptake of places on training courses can be very fast. If you are interested in
any of the courses listed below, then book now to avoid disappointment!

**Summer School in Electron Microscopy, University of Leeds, June 22–26, 2009**

This popular school will be held in three streams: Biological EM (SEM and TEM), general SEM and TEM for physical science and advanced TEM. There will be practical demonstrations as well as lectures and attendees are encouraged to bring their own specimens for discussion.

**Light Microscopy Summer School, University of York, July 6–8, 2009**

This annual residential course runs over three days and covers the principles, as well as training the participants in the practical issues surrounding of light microscopy. This course will be taught predominantly through hands-on practical sessions.

**Getting the most from your confocal, University of Leeds, June 22–26, 2009**

Participants will develop their confocal microscopy background as well as get to try FRAP, FRET and spectral unmixing. The two days will consist of short tutorials followed by hands-on practical sessions.

**Cell Imaging Techniques, Oxford Brookes University, September 7–11, 2009**

This course is aimed at biology and biomedical research and technical staff who wish to learn a range of techniques for imaging cells. It covers a wide range of applications in pathology, cell biology and plant science.

**Flow Cytometry, University of York, September 7–11, 2009**

This course is constructed as a set of three modules, where participants can choose which modules they wish to attend. Module one covers the basics, and no prior knowledge is required. Module two covers the clinical applications of flow cytometry, and module three focuses on the applications of flow cytometry in cell biology.

Full course details, fees and booking forms, as well as details of all other RMS Events can be located on the RMS website.

**RMS Learning Zone – at SEB**

Also in line with the Society’s dedication to supporting understanding in microscopy, the highly acclaimed RMS Learning Zone (normally only featured at Microscience) will be in the exhibition area at the Society for Experimental Biology’s Annual Main Meeting in Glasgow, June 28 – July 1. Operating as a free “turn-up and learn” facility, visitors can meet renowned RMS experts and discuss the microscopical challenges that they may face, as well as experiment with a wide range of different light microscopes that will be on display. This provides an excellent free resource, fulfilling a number of training needs.

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Email: sales@agarscientific.com

Images supplied courtesy of: David McCarthy, John Runions, Chris Hawes and Spike Walker
Dear EMS member,

It is with great sadness that we announce the death of Prof. Leo Ginsel, Treasurer of the EMS, who passed away on January 7, 2009. He will be greatly missed by his many friends and colleagues throughout the microscopy community. This month’s EMS Newsletter is dedicated to Leo Ginsel in a tribute to his work as a scientist, teacher and first Treasurer of EMS.

Leo Ginsel was born on July 24, 1947 in Leiden and died on January 7, 2009 in Mook, both in The Netherlands. In Leiden he was educated at the Christian Lyceum after which he chose to study biology at Leiden University. He finished his studies in 1973 and his ambition was to obtain a research position at the Laboratory for Electron Microscopy. His scientific work focused on the structure and function of intestinal cells, for which he used the EM and associated techniques. His diligent laboratory work accumulated results which culminated in a PhD thesis in 1979. The title was called: “Lysosomes and storage diseases, a morphological, cytochemical, and autoradiographical study on the function of lysosomes in the absorptive cells of the human intestine in relation to the transport and secretion of cell-coat material”: a long title for a nice story about the network of interactions between cell coat, surface organelles, lysosomes, uptake and degradation in intestinal epithelial cells. After his thesis, Leo managed to get a permanent position at the EM lab, where he ultimately became Head of the Department in 1987. In 1991 he moved to Nijmegen and became full professor of Cell Biology and Histology. Teaching was not, or almost not an issue for him in Leiden, but he accepted a full teaching load in Nijmegen. We have seen Leo as a hardworking, sympathetic biologist engaging himself in many different aspects of life. Apart from scientific work itself, it became evident that scientific organizations also attracted his attention. He was not only chairman of the Dutch Society for Microscopy (NVvM) in the period 1996 – 2003, but also a member of the Executive Board of the European Microscopy Society (EMS), where he accepted the role of Treasurer by the end of the year 2000 soon after the birth of this new society. Initially, the cash-box did not contain an appreciable amount of money but, thanks to the adoption of new rules, such as the en-bloc membership, and particularly Leo’s careful management, the revenues grew with the result that the EMS is now on quite firm financial grounds. It was in connection with the European Microscopy Society that his human qualities shone out most brighty for those of us who did not know him as a scientist. His ability to deal with the different styles and attitudes of the presidents, secretaries and treasurers of the many national and regional microscopy societies throughout Europe was most impressive – even those most reluctant to pay their membership fees finally succumbed to his courteous pressure! Leo published many articles, at first concerning his work on intestinal cells; later his studies also included monocytes, macrophages, granulocytes and finally the diaphragm. Many of his articles are written in collaboration with colleagues from other universities, indicating his ability to cooperate successfully with investigators outside his home institute. These articles were published in high-ranking international journals. Leo was also a member of many learned societies.

In Nijmegen, he found himself teaching medical and biomedical students at different levels, including both practical and theoretical training. He was actively involved in lecturing, but he was also a moving force in many committees concerned with the maintenance and development of education. From outside Nijmegen, it is difficult to describe these aspects of Leo’s professional life, but we can say with confidence that every single person involved in education must have appreciated his human and practical attitude. In recent years Leo was strongly involved in the production of the histology textbook “Functionele Histologie” from the eighth to the eleventh edition (2000–2007). The publication profited greatly from Leo’s experience of what students need and appreciate during the process of increasing their knowledge. During many other occasions, all of us also appreciated Leo’s stories about the long and distant travels he made to different countries and the way he enjoyed meeting and talking to people, often in support when needed, apparently another expression of his interest in the human soul.

The shocking news of his early death brought many people to his funeral at which the strong appreciation for his person was expressed by the many flowers and touching speeches. We sincerely hope that Marija, his spouse, and his children Bastiaan and Dorien felt supported by the expression of our appreciation for Leo. We will miss his kindness, his professional support and his great humanity very much, so it is with the deepest respect that we would like to co-sign this in memoriam for Leo who has been a meticulous professional and caring person who will leave a strong and lasting impression on all of us.

Wolfgang Baumeister, Last President CEMES, Martinsried, Germany; Eddie Wisse, First secretary EMS, Keerbergen, Belgium; Peter Hawkes, Founder-President EMS, Toulouse, France; José Carrascosa, First President EMS, Madrid, Spain; Ueli Aebi, Former President EMS, Basel, Switzerland; Paul Midgley, President EMS, Cambridge, UK; Nick Schryvers, for all former and present members of the Executive Board of EMS, Secretray EMS, Antwerp, Belgium

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Biological Low-Voltage Scanning Electron Microscopy

Biological Low-Voltage Scanning Electron Microscopy is an essential guide for researchers using Scanning Electron Microscopy at low beam voltage (LVSEM) for imaging complex biological surfaces at high resolution.

The book comes with 11 chapters, each well written by a different author, covering both an optimal usage of LVSEM instrumentation and thorough biological specimen preparation.

A description of the early development of SEM (McMullan) is given prior to the most substantial chapter dealing with LVSEM for Biology (J.B. Pawley). Four chapters are emphasising instrumental aspects with focus on aberration-correction, noise effects, beam-tilt stereo for viewing live-time experiments and microanalysis (chapters 3, 4, 7, 11, Joy, Boyde and Newbury). Chapter 5 deals with the applicability of High-Resolution, Low Voltage, Field-Emission Scanning Electron Microscopy (HR-LVFESEM) in Cell Biology (Schatten). Chapter 6 is devoted to molecular labeling for correlative microscopy (Albrecht and Meyer), chapter 8 to Cryo-SEM of animal cells (Erlandsen), chapter 9 to LVSEM of plant cells (Cox, et al.), and chapter 10 to Cryo-SEM of biological samples (Walther).

These chapters, written by leading experts, provide a thorough understanding of both theory and practice of LVSEM of biological specimens. The book fills a definite need also for advanced users optimising their LVSEM instrumentation and technique to obtain better images of biological samples.

Thomas Matzelle

Reference:
Schatten H., Pawley J.B. (Eds.): Biological Low-Voltage Scanning Electron Microscopy
Springer 2007, 318 p. 13 illus. in colour, Hardcover, € 106,95
ISBN: 978-0-387-72970-1
Photonics West 2009 – Good bye San Jose…

… hello San Francisco! This year’s Photonics West ended on January 29, 2009 successfully. While observing several shrinking international photonic trade shows, the Photonics West seems to be the rising star. With 17,903 attendees (about 500 attendees more against last year) the number of visitors slightly increased, against all current economic trends. This effort is mainly accounted to the exhibition attendees. Photonics West established to “the place to be” to inform about the latest innovations, products and applications in the field of photonics, beside its excellent conferences.

To get a brief picture: Jenoptik’s presented its new high power laser bars at 808 nm and 100 W cw. It features high laser power, high efficiency, long lifetime, high reliability as well as excellent beam characteristics for pumping of solid-state and fiber lasers, materials processing and several medical applications.

Coherent presented a new member of its Matrix family. They offered a new ultraviolet model optimized to produce 1.5 Watts at a high repetition rate of 70 kHz, in order to support high-throughput applications. Its performance ratio is specifically targeted at industrial processing applications.

Toptica showed its newest fiber laser product named iChrome for fast laser scanning microscopy. It enables scientists to select a wavelength between 488 nm and 640 nm. The highly repetitive laser pulses exhibit 3 nm bandwidth and 3.5 ps pulse duration with an average fibre-coupled output power of more than 1.5 mW.

Texas Instruments showed their latest DLP pico projector development kit in action. With more than 20 million sold chips, the DLP technology is already well established in projectors. The new DLP development kit enables engineers and scientists finding new applications ranging from virtual cosmetics, augmented reality, measurement applications to wavelength-selection for spectroscopy. Much more is imaginable. The pico projector kit includes the DLP chip, an universal power supply (operates from 110–220 V), a HDMI cable as well as a light source consisting of a green, red and blue LED for full color video output at seven lumens. With US-$ 149 it is a budget way to explore the possibilities of the DLP technology.

Osram OS is another step closer to extremely miniaturized projectors. At Photonics West they presented the smallest blue laser diode (3.2 mm height) to date. Integrated with a green and red diode into mobile devices like cell phones, notebooks or cameras razor sharp projections could be possible. The blue one has a wavelength of 450 nm and an output of 50 mW. Its voltage is 5.5 V, and the slope efficiency 0.9 W/A. The small size and high efficiency make it ideal suited for mobile projection applications. The development of the green laser diode is scheduled for the end of 2009.

A lot more established and new companies presented their latest photonic innovations – doing their best to fight the economic downturn. Companies with diversified target markets have clear advantages compared to companies focusing on industrial materials processing.

A wide collection of 46 German enterprises were combined into the German Pavilion, organized by OptecNet Germany, Spectaris, IEC Berlin and the BMWi. More than ever presented their innovations at the Photonics West although the exhibition space this year was limited versus the previous years. For example: OptecNet’s planning for Photonics West 2010 already began. Actually more than two third of the enterprises assured their participation.

The next Photonics West will take place from January 23–28, 2010 in the San Francisco Moscone Center which offers more space, more hotel rooms in walking range and a better international access to meet the needs of both – exhibitors and attendees.

http://spie.org/x2584.xml

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The 9th international ELMI meeting on advanced light microscopy will be held from June 9–12 on the grounds of Caledonian University in Glasgow.

ELMI: A Unique Network

The European Light Microscopy Initiative (ELMI) is a partnership between the users and producers of advanced light microscopy equipment. A large proportion of the academic members are microscopy facility managers, who form the interface between the equipment manufacturers and a diverse range of users. ELMI meetings showcase the latest developments in imaging applications and technology. The meeting comprises both academic presentations and commercially driven workshops. The workshops are substantial opportunities for users to test the latest equipment, and for companies to learn more about the needs of users and emerging applications. They provide the chance to compare many high-end imaging systems back to back.

The meeting travels around Europe, and is typically hosted by an imaging facility. Last year’s successful meeting in Davos was hosted by a consortium of imaging facilities from the Friedrich Miescher Institute, the Swiss Institute of Technology (EPFL), the ETH Zurich, and the Center for Microscopy at the University of Basel. This year’s meeting in Glasgow will be hosted by Gail McConnell from the Institute for Biophotonics at the University of Strathclyde and Kurt I. Anderson from the Beatson Institute for Cancer Research. The meeting will be sponsored by the Royal Microscopical Society (RMS), who also sponsored the ELMI meeting in York two years ago. Next year the meeting will return to the EMBL for its 10th anniversary.

A Small Symposium on the Cytoskeleton

This year’s meeting will be dedicated in part to the work of J. Victor Small, a founding member of ELMI and a pioneer in the use of light and correlated electron microscopy to study living cells. Speakers will include past and present collaborators such as Yu-Li Wang (Carnegie Mellon), Benny Geiger (Weizmann Institute), and Alan Hall (Sloan-Kettering), in addition to many former lab members including Rob Cross (Marie Curie Cancer Research Institute), Dieter Fürst (University of Bonn), and Irina Kaverina (Vanderbilt University). This “Small Symposium” will emphasize the use of imaging techniques in the study of cytoskeleton dynamics. For more information visit www.elmi2009.org or www.rms.org.uk.

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Learning from Single Molecules
June 29 – July 1, 2009

In the last 15 years single molecule fluorescence has become a powerful research tool in the biosciences. A team of internationally renowned researchers will teach a course on current experimental approaches for detection and imaging of single molecules, and related data analysis methods.

The workshop “Learning from Single Molecules” is intended to provide an expert introduction into the still expanding toolbox of single molecule fluorescence techniques and its application. This microscopy meeting will take place from June 29 – July 1, 2009, near Bonn in Bad Honnef, a small city beautifully situated in the Rhine valley.

The meeting addresses researchers being interested in gaining insight into the current methodology and exemplary applications of single molecule fluorescence in life science research. The workshop preferentially aims at advanced graduate students and postdocs, but also experienced scientists from other disciplines that want to expand their knowledge of this innovative and fast growing field would certainly benefit from the meeting.

We will focus on fluorescence techniques, because these represent currently the major tools to study and apply single molecule features in the life sciences. The workshop shall provide the participants with a solid background in the existing state-of-the-art of single molecule research, as well as the chance to meet prominent European contributors to the field. The program comprises detailed lectures on topics such as single molecule observation in living cells, fluorescence correlation spectroscopy, particle tracking, membrane receptor mobility, microscopy image processing, and so forth.

This workshop is organized within the framework of the EU Marie Curie Research Training Network “Biocontrol” and the SFB 624 “Templates – Functional chemical matrices” of the Department of Chemistry of the Rheinische Friedrich-Wilhelms University Bonn.

We kindly invite you to attend “Learning from Single Molecules”, and look forward to meet you in Bad Honnef this summer. The conference venue is the Katholisch-Soziale Institut, a comfortable and modern meeting location.

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The 12th Ceramics, Cells and Tissues (12 CCT) seminar-meeting will focus on the Science and Technology of Biomaterials in Medicine, with particular reference to Surface Reactive Bioceramics for tissue engineering scaffolds and biomedical coatings. This is a multidisciplinary research field which can make use of surface engineering, nanotechnology, biomolecular tools and materials science approaches for optimization of the surface chemical reactivity and topography of bioceramics for their application in tissue engineering scaffoldsands coatings of biomedical devices. A special symposium is planned, to show how “natural technologies” can be used to develop modern technologies for health.

Main Topics:
- Tissue engineering scaffolds and biological inspired materials
- Surface chemical reactivity and nanotechnology
- Inert and bioactive coatings
- Genetic engineering
- Giacobbe Project: human body metamorphosis towards aging
- Bio-hybrid composites, cements standardization
- Natural nanotechnology for health: the chemical secrets of the medical clays
- Biomaterials Societies Forum
- Social events (7th CCT prize for young researchers and prizes for international artistic competitors)

Location:
Congress Hall of Banca di Romagna
Via P. Costa, 3 — Faenza — Italy

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World of Photonics

In June 2009, two international events for optical technologies in Europe will once again take place in Munich, Germany. From June 15–18, the New Munich Trade Fair Centre will host the exhibition “LASER World of PHOTONICS” and from June 14–19 the “World of Photonics Congress” will be held in the International Congress Centre Munich. Both events address representatives from research, the laser and optoelectronics industries, professionals involved in medicine and technology as well as physicists, chemists, doctors, engineers, system designers, retail companies and service-providers. This year, the trade show and the congress have expanded their offerings for biophotonics and life sciences. For the first time, the trade show is setting up an exhibition focus area dedicated to the subject of biophotonics. Research institutes, developers and manufacturers of optical and photonic methods and processes will provide insights into their biophotonic technologies. The areas of application include dentistry, dermatology, urology, molecular diagnostics, drug-delivery technologies, the monitoring of bodies of water and the processing of drinking water. The World of Photonics Congress will present the field of biophotonics at the “Medical Laser Applications” conference. Experts in the field of lasers in medicine, optical diagnostics, high-end endoscopy and minimally invasive surgery will give practically orientated presentations.

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Neccesity for Standardization in Fluorescence Pattern Analysis

Fluorescent pattern analysis is used in cellular and molecular biology as well as in medicine, agriculture or other applications. To make it feasible in daily practice, standardization is necessary to obtain authentically and reproducible results. Standardization has many aspects (fig. 1). It has to do with sample preparation, imaging techniques, knowledge acquisition, and image interpretation. It is an iterative process and cannot be solved from scratch.

For research purposes, only a few images are usually studied in order to establish the imaging method. In this case it is feasible to manually do the image-interpretation. The resulting image descriptions make up a data base that can be used as a basis for the development of automatic image-interpretation system. Methodically collected image catalogues of examples based on commonly excepted image vocabulary/ontology are necessary for standardization.

After an imaging method has passed qualification and goes into industrial applications, it should work on-line in a process. The huge amount of data created in these processes cannot be handled manually. They require automatic image interpretation systems.

An automatic image interpretation system should produce results that are reproducible and objective. An automatic system will always crede from the same image the same output. It does not matter if the image is processed today or later. Due to anthropogenic aspects pictures can be evaluated differently. If features are not calculated from the image by an automatic image processing procedure, results are qualitative and not quantitative. Therefore, an automatic image interpretation system will always be a big step towards standardization of the desired image inspection tasks.

A necessity for good image interpretation is good image quality. Protocols for sample preparation as well as robust and solid-working imaging devices are important to ensure, objects get imaged with high contrast and constant brightness. This is another step towards Standardization in fluorescent pattern analysis. Researcher in the image processing field are therefore more and more engaged with the development of methods for accessing the image quality to select the best image during the imaging process.

The problem related to the automatic processing of multimedia content resulted in the MPG-3 standard, grouping conventional image processing methods to visual symbolic low-level terms. This is to provide the right image processing method to obtain the desired information for image retrieval or other purposes.

In defect classification, medical image interpretation, and non-destructive testing, the visual terms are usually more complex and cannot be described by a single low-level term. The recent developments in multimedia processing are therefore not sufficient for many new arising visual image interpretation tasks and a further develop of new methods is needed. This should be done in a more systematic way. Here a categorization of the tasks in the application area of fluorescent pattern analysis is needed.

The observation of prototypical pattern or scenes is done empirically and is usually a time consuming process. Much more it would be preferable to discover the prototypical appearance of patterns automatically. New data mining methods (conceptual clustering, case-based reasoning, etc.) and preferable incremental methods are necessary to automatically discover the final information needed for the respective process.

We like to establish a new forum for standardization in fluorescent pattern analysis.

One step in this direction is our new conference on Mass Data Analysis of Images and Signs in Medicine, Biotechnology, Chemistry and Food Industries, MDA (www.mda-signals.de).

With this article we like to encourage you to contribute to our new forum and send us your opinion about standardization. The aim of our new forum is to improve communication between experts and practitioners from different fields to share their views on Standardization in order to pave the way for automatic fluorescence pattern analysis.

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Nonlinear Spectral Imaging Microscopy

Noninvasive Characterization of Living Tissues

The deep tissue penetration of nonlinear microscopy and the high detection efficiency of a spectograph are utilized to record spectral images of the intrinsic emission of living mouse skin tissues. Visualization of the spectral images by wavelength-to-RGB color conversion allowed identification and discrimination of tissue structures. Nonlinear spectral imaging microscopy (NSIM) can provide a wealth of information not easily obtainable with present conventional nonlinear imaging systems.

Particularly interesting is the application of 2P excited fluorescence microscopy in autofluorescence imaging of cells and thick tissues [1–4].

In addition to morphological imaging of tissue, spectroscopy provides complementary. Tissue autofluorescence spectroscopy has the potential to identify endogenous fluorophores. The relative intrinsic fluorescence of biological tissues originates from endogenous fluorophores that are primarily derived from the UV-emitting aromatic amino acids and vitamin derivatives. These include tryptophan, tyrosine and phenylalanine, riboflavin, the nicotinamide ring of reduced pyridine nucleotides NAD(P)H derived from niacin, and the pyridoxine-based pyridolamine cross-links found in elastin and collagen. The autofluorescence of proteins is extensively used in biochemical research to study their structure, functions and dynamics whereas intracellular autofluorescence is widely used to study cellular energy metabolism. However, in situ applications have been limited due to photobleaching, photodamage and considerable light scattering and absorption in turbid tissue environments. These difficulties can be overcome by using nonlinear microscopy. Nonlinear microscopy is a collective term used for various imaging microscopy techniques that include two-photon (2P) excitation, three-photon (3P) excitation, second-harmonic generation (SHG), and third-harmonic generation (THG). Its advantages include intrinsic three-dimensional resolution, negligible out-of-focus photobleaching, reduced light scattering and photodamage, and improved fluorescence collection efficiency.

Autofluorescence Spectroscopy and Microscopy

In addition to morphological imaging of tissue, spectroscopy provides complementary. Tissue auto fluorescence spectroscopy has the potential to identify endogenous fluorophores. The relative

**Keywords:**
nonlinear spectral imaging microscopy, in vivo imaging, second harmonic generation, auto fluorescence, multiphoton microscopy introduction

**Fig. 1:** Schematic diagram of the nonlinear spectral imaging microscopy (NSIM) setup
concentration of these fluorophores is related to tissue physiological and pathological states. Nonlinear spectral imaging microscopy (NSIM) is an emerging technique that allows simultaneous recording of spectra and images and it has been applied to fluorescence microscopy and FRET measurements. However, its application to tissue autofluorescence has been limited primarily due to the difficulty in detecting the inherently weak intensity of autofluorescence and the low sensitivity of commercially available detectors in the visible to ultraviolet (UV) spectral region [2].

In this study, we coupled a highly sensitive prism-based spectrograph to a custom-built nonlinear microscope as shown in figure 1 [2]. Stacks of depth-resolved nonlinear-excited spectral images of in vivo mouse skin tissue samples were acquired. A simple method of converting the spectral image data into RGB images enabled us to distinguish different structures at different depths within the skin tissue specimens.

**In vivo Mouse Skin NSIM**

Figure 2 shows real-color RGB representation of the nonlinear spectral images from living mouse tissue at different relative depths from 5- to 40-µm below the skin surface. Individual cells are distinctly observed in the stratum spinosum at about 5 µm below the surface of the stratum corneum (fig. 2(a)). The blue fluorescence of these cells mainly originates from the cytoplasm. In previous studies, it has been suggested that the main source of intracellular autofluorescence is NAD(P)H. It is fluorescent only when reduced and has a characteristic blue fluorescence peak at around 460 nm. Another source of redox related autofluorescence comes from cellular flavins. In contrast to NAD(P)H, these molecules are fluorescent in their oxidized state and has a characteristic yellow fluorescence peak at around 535 nm. In between the cells, green-fluorescing structures are observed which we assume to be tonofilaments or keratin filaments.

A deeper section (10 µm) in the skin shows higher number of cells in the same image area (fig. 2(b)). The cells appear smaller in cross-section than the cells in figure 2(a). This is because these cells are more columnar in shape while the cells near the stratum corneum are flattened out. Hair follicles appear as round green-fluorescing structures. These hair follicles, mostly inactive in these hairless mice, become more apparent at a depth of about 15 µm (fig. 2(c)). Here, the purple color in the image increased in intensity. This purple color refers to the second-harmonic signal generated by collagen. This is a clear indication that the cells in figure 2(e) are mostly basal cells of the epidermis near the dermal-epidermal junction.

At about 20 µm deep, the fiber structure of the collagen becomes more distinct (fig. 2(d)). Some basal cells are still present in this section. Rings of cells surround the hair follicles and are still visible at 30 µm deep within the tissue (fig. 2(e)). At this depth, the collagen fibers are thicker and bright blue-fluorescing fiber-like structures are observed along the fibers. These blue fiber-like structures are likely to be elastin. At a depth of about 40 µm (fig. 2(f)), the collagen and elastin fibers are more distinct.

**UV-exposed Mouse Skin**

It is well known that ultraviolet (UV) radiation can give rise to cellular damage by direct excitation of DNA and indirect mechanisms that involve the excitation of cellular chromophores or endogenous photosensitizers. We used nonlinear spectral imaging microscopy (NSIM) to investigate both the morphological alterations and...
biochemical changes following UV irradiation of mouse skin in vivo [4].

Shown in figure 3 (left panel) are real-color spectral images of mouse skin tissues at the stratum spinosum. Morphological differences between the control and the UV irradiated skin tissues are clearly depicted. In comparison to the control skin, the UV irradiated skin appears less organized and the tissue layers less distinct. The control sample shows keratinocytes that are well-defined while irregularly-shaped structures characterize the irradiated skin tissues. This is a clear indication that the irradiated skin tissues have undergone morphological alteration.

To characterize the biochemical alterations resulting from chronic exposure to light, the emission spectra from the spectral images were obtained and analyzed. Shown in figure 3 (middle panel) are normalized average emission spectra obtained from the spectral images of the control and UV irradiated mouse skin acquired at the stratum spinosum. The average emission spectra of the irradiated specimens show clear difference compared to the average emission spectra of the control mouse skin.

To elucidate the variation between the emission spectra of the control and the irradiated mouse skin, linear spectral unmixing was applied to the average emission spectra. Pixel-averaged emission spectra (n = 6) were obtained from different regions of interest of the spectral images of each skin sample type and relevant tissue layer. In selecting the regions of interest, morphological features that best represent the tissue layer were considered. After plotting the spectra in wavenumbers, the spectra were fitted globally (i.e. fitted with the same center wavenumber and full-width at half-maximum (FWHM) values) with Gaussian models that are associated with the fluorescence emission of known fluorophores in the skin. The resulting fit coefficients represent the relative contributions in which these fluorophores contribute to the overall autofluorescence signal.

Shown in figure 3 (right panel) are the relative contributions (mean ± SD) of the keratin, NAD(P)H and FAD spectral component for each skin sample type. The relative contributions of keratin and FAD fluorescence for the UV-irradiated skin emission spectrum are found to be significantly lower (p < 0.001) and higher (p < 0.001), respectively, compared to the contributions for the control emission spectrum (fig. 3, right panel, keratin and FAD). The observed higher relative contribution of the component attributed to FAD for the irradiated skin tissues indicates a cellular redox shift to the oxidized direction, noting also that flavin cofactors are fluorescent only in its oxidized state. This is in agreement with previous studies that have reported shifts in the overall cellular redox state to a more oxidized direction following visible light irradiation.

In summary, nonlinear spectral imaging microscopy (NSIM) represents an effective imaging tool in characterizing living tissues non-invasively. It has excellent potential in assessing alterations in skin morphology and biochemistry.

References:

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Microscope Imaging
Completing the Picture

Since the development of the first optical microscopes in the 1600s, microscope manufacturers have worked to reveal more details of the ‘micro’ world, not previously visible to the naked eye. Today, optical microscopy is a sophisticated and powerful imaging tool that is fundamental to scientific discovery, biomedicine, materials, and inspection environments. Manufacturers continue to improve microscope design, not only to increase optical capabilities but also to make imaging more versatile, easier, and more accessible to scientists wishing to extend their research. Nikon, as one of the leading developers of optical technologies, has broadened its portfolio in recent years to meet the growing needs of scientists through in-house innovation and strategic collaboration with selected partners. Nikon now not only offers micro-scale imaging capabilities but has also extended its scope to include nano-scale and macro-scale imaging. It also offers a broad range of supportive technologies allowing researchers to meet their imaging needs from one central supplier.

A Long Tradition in Microscopic Imaging

The story of Nikon’s microscopic imaging started in 1917 when three Japanese optical manufacturers merged to form a company known as Nippon Kogaku KK (“Japan Optics”) producing precision optical glass. The first microscope was launched in 1925 and was followed rapidly by polarizing and stereo microscopes as well as instruments for industrial metrology and inspection. Nikon continues to manufacture its own glass optical components for a continuously evolving product range, which includes routine and research upright, inverted and stereo microscopes; entry level to high-end research confocal systems; inspection instruments; metrology tools; Nexiv video measuring systems; and innovative interdisciplinary products, such as the AZ100 multizoom and LV-Universal Design microscopes. Alongside developments in microscopes, Nikon’s digital imaging technologies and software solutions support all microscopy and imaging functions, image management and analysis.

In-house expertise in optical technologies has been instrumental in differentiating Nikon from its competitors. The development of the CFI60 optical system, for example, was a radical departure from traditional microscope design. CFI60 optics offer advantages for both industrial and biological applications as lenses generally have higher N.A.s and longer working distances than comparable lenses and provide increased resolution, greater light-gathering capability and high performance in confocal imaging. The infinity optical system also has the benefit that the distance between the objective and the eyepiece tube is not fixed, allowing a variety of imaging modules to be inserted into the light path without any compromise in optical quality. This has allowed microscopes to be transformed into versatile imaging workstations that provide users with instant access to several imaging methods. The Eclipse Ti series of inverted microscopes, for example, allows the simultaneous mounting of confocal, TIRF module and multiple stacked epi-fluorescence filter turrets to make this microscope the ideal live cell imaging platform. Users can switch easily between brightfield, darkfield, phase contrast, Nomarski DIC, Hoffman modulation contrast, and widefield fluorescence and confocal imaging to enable techniques such as TIRF, FRET, FRAP, FLIP and photoactivation studies.

The ability to mount more than one camera can also simplify cellular imaging. Dual cameras installed and registered perfectly on the microscope’s back and side ports, can capture two different wavelengths of light simultaneously. This removes the need for filter switching, which may limit sequential image acquisition speed and lose valuable information. Simultaneous dual camera imaging is ideal for FRET and the capture of rapid dynamic cellular events. The Eclipse Ti also has in-built PFS (Perfect Focus System), which overcomes focus drift in prolonged imaging and/or time lapse observations. PFS corrects focus drift instantaneously. Even with high magnification, high N.A. objectives and techniques like TIRF, images are always in sharp focus, avoiding wasted resources in having to repeat experiments.

From Micro to Nano

The resolution of optical microscopes is generally limited to the micro-scale (tab. 1). To meet an increasing need for nanoscale imaging across all imaging environments, Nikon has joined forces with JEOL to offer a versatile benchtop scanning electron microscope (SEM) suitable for users with little or no previous SEM experience. The NeoScope benchtop SEM makes it easy to obtain high magnifica-
tion images (10X-20,000X) with exceptionally high resolution and a large depth of field both unmatched by optical microscopes. This high depth of focus makes it possible to correlate areas of interest with optical microscopy images to provide continuity and to aid in the interpretation of results.

Samples prepared for optical microscopy do not require further treatment and can be loaded and imaged on the NeoScope in less than three minutes. The NeoScope operates in both low and high vacuum modes and for example has three kV filament voltage settings suitable for a variety of applications, all of which can be programmed in special pre-stored recipe files. With automatic settings, basic operation via the GUI, SEM is as simple as using a ‘point and shoot’ camera. Full manual control is also available.

From Micro to Macro

While there is an emphasis in microscopy to resolve increasing detail in a specimen, there is often a need to step back and review microscopic information in the context of gross specimen images. This is particularly important in pathology where imaging of gross specimens before cut up can be extremely useful in providing a permanent record for case histories and in aiding interpretation of subsequent microscopic tissue sections. The Mi macro imaging station has been designed for use in pathology laboratories before and during the cut up process. With hygienic wipe-clean user interfaces to reduce the risk of contamination, a dedicated top quality Nikon DS-Fi1 digital camera, integral LED illumination, “push button” operation, and pathology-focussed software everything is provided to capture, annotate and measure images of unfixed and fixed tissues. Mi software, in addition, can be integrated with Nikon’s NIS-Elements Documentation (D) software to bring all aspects of the digital pathology imaging environment together.

Increasing Microscopy Resources

Nikon is the official European distributor of SLEE’s pathology specimen preparation equipment including microtomes, cryostats, embedders, tissue processors, stainers and consumables. By offering these products alongside Nikon’s microscopes and digital cameras, Nikon is able to offer clinical markets a complete pathology solution.

Pathology laboratories also benefit from Nikon’s collaboration with Aperio, manufacturers of digital slide scanners. Digital slides are often used by the clinical laboratory for education and training, multidisciplinary team meetings, external quality assurance, and telepathology applications. Slide scanners may also be used in research environments although greater specimen throughput and the need to evaluate multiple parameters may demand more powerful scanners, image analysis and reporting capabilities. Aperio’s slide scanning system for tissue microarrays (TMALab), for example, manages all TMA processes from specimen selection to designing array blocks, scanning, analyzing digital array slides, and correlating analytical and clinical data.

Continuing Innovation

The needs of microscope users continue to push microscope imaging in new directions. Through continuing innovation in optical and digital technologies together with strategic alliances with partnering companies, Nikon continues to hold a leading position in microscope imaging solutions.

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The ability to measure polished and strongly reflective surfaces with steep flanks in true color information has been mandatory for the Christian Doppler Laboratory for Localized Corrosion when it came to purchasing a new measurement device to perform meaningful corrosive investigations. Today, the institute uses high resolution 3D measurement device for the evaluation of individual corrosive mechanics, leading to the development of new measures to avoid corrosive attacks.

Irrespective of the type of corrosion, attacked surfaces usually show steep flanks and strongly varying reflection properties. Targeting the aim of strengthening corrosion resistance, the laboratory was looking for an instrument that meets clearly specified requirements. Joachim Haberl from the scientific staff about the demands: “We were looking for an instrument that is able to measure polished and literally glassy surfaces and flanks of more than 80°. We wanted a system that offers true color visualization to make sure that surfaces are measured effectively. Also, we expected a system providing high resolution measurements on mechanically polished surfaces with a roughness Ra > 50nm. Also, the measurement of large measurement fields had to be provided.”

Corrosion yearly causes a remarkable loss of the gross domestic product. Besides various rather predictable forms of corrosion that cause general corrosive attack, industry also faces several hardly predictable corrosive forms that affect components locally. As corrosive attacks can lead to sudden damage and breakdowns of buildings, industrial facilities, plants, transport systems etc., high emphasize has to be placed on risk avoidance which is based on the understanding of corrosive mechanics.

Measuring of Corrodes Surfaces with a Vertical Resolution of up to 10 nm

Today, the laboratory works with high resolution optical 3D measurement device. Based on the principle of Focus-Variation, the instrument meets all requirements of the scientific staff. Measurements reach a vertical resolution of up to 10nm even at steep flanks and strongly varying reflection properties. Additionally, the entire surface topographic information is captured in combination with its true color information. Both, the topographic and color information are registered to the 3D data file.

The 3D system is mainly used to analyze mechanically influenced corrosion, e.g. stress corrosion cracking, corrosion fatigue cracking or erosion corrosion. Pitting corrosion, crevice corrosion and selective corrosion are further fields of in-
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Focus Variation. A New Technology Delivers Depth Measurement and True Color Information of Corroded Surfaces

The operating principle of Focus-Variation, developed by Alicona, combines the small depth of field of an optical system with vertical scanning to provide topographical and colour information from the variation of focus. Depending on the topography of a surface the information from the variation of focus is analyzed in relation to the distance to the optics. Using conventional optical measurement techniques a high vertical resolution can only be reached with a small vertical scanning range whereas the use of InfiniteFocus yields a high vertical resolution over the entire scanning range, allowing a dynamic of 1:430000. Additionally, a quality measure is determined for each measurement point.

The precision optics consists of various lens systems and can be equipped with different objectives, allowing measurements with different resolution. With a beam splitting mirror, light emerging from a white light source is inserted into the optical path of the system and focused onto the specimen via the objective. Depending on the topography of the specimen the light is reflected into several directions as soon as it hits the specimen. If the topography includes diffuse reflective properties, the light is reflected equally strong into each direction. In case of specular reflections, the light is reflected mainly into one direction. All rays emerging from the specimen and hitting the objective are bundled in the optics and gathered by a light sensitive sensor behind the beam splitting mirror. Due to the small depth of field of the optics only small regions of the object are sharply imaged. To allow a complete detection of the surface with full depth of field, the precision optic is moved vertically along the optical axis. This means that each region of the object is sharply focused. A sensor captures a series of 2D datasets during this scanning process. Thereby, all sensor parameters are optimized at each vertical position according to the reflective properties of the surface. After the scanning process, the 2D datasets are evaluated to generate 3D information as well as an image with full depth of field. This is achieved by analyzing the variation of focus along the vertical axis. Due to the large amount of data mechanical restrictions can be eliminated, allowing measurement results with a high resolution. Once all height measurements are determined, an image with full depth of field is computed.

The technique of Focus-Variation has been accepted in the draft of the new ISO standard 25178, which is a recently developed standard for the classification of topographical measurement techniques.

Summary: Corrosion Becomes Measurable and Visible in 3D

Focus-Variation is used for the analysis of different forms of corrosion, corrosive data and clarification of individual corrosive mechanics. The knowledge about circumstances causing erosive and corrosive attack of material surfaces and protective layers of components leads to the establishment of new guidelines to develop more resistant material. In particular, profile and area measurements are used for further mathematical computations of degradation rates. Also, measurement data and 3D visualizations of fractured surfaces are used for further research and development activities.

The technology is a well established technique to characterize surfaces with complex geometries. Typical surface features coming along with corrosive specimens are steep flanks and highly reflective properties. The vertical resolution up to 10nm can also be achieved at very inhomogeneous and rough geometries.

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Nanomanipulation with Optical Tweezers
From Single Molecules to Cells

In recent years, the manipulation of nanostructures using optical tweezers developed from proof-of-principle experiments to an established quantitative technique in fields ranging from (bio)physics to cell biology. With optical tweezers, microscopically small objects can not only be held and manipulated, but also tracked to the nanometer. At the same time, forces exerted on trapped objects can be accurately measured with a resolution well below a picoNewton.

Introducing Optical Tweezers

The physical principle underlying the technique is the radiation pressure exerted by light when colliding with matter. Whereas in the macroscopic world this pressure is way too small to have any measurable effect, it can have considerable effects for objects of microscopic dimensions. The tight focus of a laser beam formed by a microscope objective can be used to capture and hold small objects in 3D: an optical trap or ‘optical tweezers’.

Optical tweezers have been used extensively not only to manipulate colloids, biomolecules and cells, but also to directly and accurately measure the minute forces involved.

JPK Instruments recently introduced a new platform, the NanoTracker (fig. 1). This first quantitative optical tweezers system available on the market allows the controlled trapping and accurate tracking of particles of sizes from microns down to a few tens of nanometers.

Fig. 1: The NanoTracker – JPK’s new optical tweezers platform

Keywords:
Optical trap, force spectroscopy, cell particle interaction

PicoNewtons, Nanometers

Using the trapping light scattered by the object, one can measure the external forces (i.e., other than the trapping force) acting upon the trapped object. These external forces tend to push or pull the object from the center of the trap. The refractive object, in turn, acts like a tiny lens that refracts the rays passing through it. As illustrated in figure 2a, the far-field interference of the laser light with that scattered by the trapped particle can be used to get a sensitive measure for the 3D displacements of the particle from the focus. This interference signal is typically recorded using position-sensitive or segmented photodiodes, in much the same way as cantilever deflection is measured in atomic force microscopy (AFM). The detector signals can be accurately calibrated either into nanometer displacements or picoNewton.
The elucidation of this phenomenon. Optical tweezers have been key in gaining almost a factor of 2 in DNA can undergo an "overstretching transition", in gaining almost a factor of 2 in length. Optical tweezers have been key in the elucidation of this phenomenon.

Fig. 2: (A) Detection principle of optical tweezers. Displacements of a particle from the trap center cause transmitted light to deflect, which is readily picked up by a quadrant photodiode. After the spring stiffness of the trap is calibrated, displacement measurements are readily converted to forces. (B) Screenshot of the JPK NanoTracker software showing a live microscope image, various control and status panels and the online calibration manager to accurately calibrate detector signals.

Fig. 3: Typical force spectroscopy on a single DNA molecule using the NanoTracker optical-tweezers platform. When extended beyond its full length, double-stranded DNA can undergo an "overstretching transition", in gaining almost a factor of 2 in length. Optical tweezers have been key in the elucidation of this phenomenon.

forces. Figure 2b shows a screenshot of the "calibration manager", that is integrated in the NanoTracker software.

By feeding the measured signals back to the trap position using fast optics steering, one can keep a constant force during the experiment, or, with a softer trap, track a particle undergoing diffusive motion. As such, optical tweezers can be employed as a high-resolution, non-invasive 3D tracking tool.

With its 3D detection system, the JPK NanoTracker (fig. 1) allows the recording of particle displacements in the two traps with nanometer precision simultaneously. Moreover, dynamic forces acting on the trapped particles (e.g., exerted by bound motor proteins) can be measured with better than picoNewton resolution. With the NanoTracker, optical tweezers finally transcend from the labs of self-building scientists who helped the technique mature to a user-friendly system able to serve a much wider community of life science researchers.

Applications
Optical tweezers have so far found their most prominent quantitative applications in the field of single-molecule biophysics. They have been used to unravel the complex elasticity of biopolymers – DNA, RNA, proteins – complementing measurements performed with other single-molecule techniques such as AFM (fig. 3). Moreover, they have contributed a great deal to the detailed understanding of how "motor proteins" work. Typically, the biomolecules of interest in such measurements are manipulated through trapped functionalised microspheres. Milestone papers in the motor protein field owe their first direct mechanical insights to optical tweezers [1]. Many "mechano-enzymes" have been studied since then, including many involved in DNA transactions. The number of such studies keeps growing, as a lot of open biological and biophysical questions have not even been touched. These efforts helped in maturing quantitative optical tweezers technology to its present-day level: the power of optical tweezers to measure picoNewton forces and nanometer displacements exactly in the realm where many such biological motors are active, has secured a central place for this cornerstone technique.

More recently, optical tweezers transcended single-molecule applications to provide a tool for studying questions in cell biology. Starting out mainly as a tool to manipulate and sort in a qualitative manner, today more and more reports appear where processes in or around live cells are analysed using quantitative tweezers measurements, for example for the unraveling of phagocytosis mechanisms [2]. Apart from cell-particle interaction studies through the cell membrane (fig. 4), microrheology and tracking measurements inside live cells have been reported. Such applications of optical tweezers have great potential to yield new insights in fields like nanotoxicity.

With the NanoTracker, compact and off-the-shelf quantitative optical tweezers become available as a turn-key experimental life science platform that lives up to the standards of the single-molecule biophysics field. At the same time, the instrument is user-friendly enough to allow easy operation by those researchers not used to working with lasers and optics. This renders the NanoTracker a platform for a broad range of quantitative nanomanipulation needs.

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Near Field Emission SEM

Localised Electron Excitation Imaging via SPM

Recent developments in electron optics enable “extreme” high-resolution Scanning Electron Microscopes (SEM) to attain subnanometer resolution using landing energies as low as 200 eV, which is essential to prevent damaging of the investigated object and minimise the interaction volume. We have also implemented low beam energies in a simplified SEM where the electron source, remote in standard SEMs, is brought within tens of nanometers to the object. This microscope, which we call the “near field emission scanning electron microscope” (NFESEM), is capable of imaging conducting surfaces with nanometer resolution using beam energies less than 60eV.

Description of the Method

E. Ruska was awarded the Nobel Prize in 1986 for his work toward the development of microscopes based on a primary beam consisting of electrons instead of light. In the same year, H. Rohrer and G. Binnig were also awarded the Nobel Prize for their discovery of an alternative method to perform ultra-high resolution microscopy: the STM (Scanning Tunneling Microscopy). This technique is based on the quantum mechanical tunneling effect, which limits the interaction of a tip (brought at sub-nanometer distances from a surface) to the surface atom residing nearest the tip; thus achieving the elusive atomic resolution. We have combined the fundamental concepts of these two types of microscopy, creating a new device (fig. 1). This innovative instrument is quite compact and can easily be integrated with pre-existing systems. The close proximity between the source and the object provides a means of overcoming the limitations of the conventional SEM and it opens the possibility to use lower primary beam energies, leading to other contrast mechanisms [1]. Although these experiments conceptually resemble the Binnig and Rohrer – STM, they generate images similar to an SEM without the need of a remote electron gun. However it is important to note that typically in both STM and remote SEM the tip is scanned in constant current (CC) mode, in stark contrast to our device.

Principle of NFESEM

Cold field emission (FE) occurs when a metallic tip is submerged in strong electric fields that alter the potential barrier at the tip surface permitting electrons near the Fermi level to tunnel through it, in accordance with Fowler-Nordheim FE [2]. These electrons act as the primary beam, which in conventional SEM originates from a remote source. They impinge on a well-defined area on the surface localised by the tip and excite secondary electrons (SE) that are subsequently collected and counted by an electron multiplier. The variations in the electron intensity from the multiplier signal as a function of scanning position, displayed in figure 1a, shows the surface topography. R.D. Young et al. [3] were in

Fig. 1: Schematic diagram of the NFESEM. In this instrument electrons are emitted from the sample surface after undergoing interactions with a primary beam of electrons field-emitted from a sharp Tungsten tip, positioned very close to the surface. The extraction optics collects all the emitted secondary electrons and accelerates them to a detector. The zoom shows micrographs of a W (110) surface (240 nm x 145 nm, primary beam energy: 33.2eV and initial emission current: 50nA). In a) the image is produced by the secondary electron intensity – as described in the figure. Alternatively in b) an image is generated simultaneously by measuring the field emission current.
The primary beam energy was 28.8 eV with a field emission current of 50 nA. (b) After imaging in field emission mode, the tip-sample separation was decreased for STM imaging in constant current mode, with a tunnel current of 0.2 nA and a bias of 200 mV.

Fig. 4: (a) This 400 nm x 400 nm micrograph of a W (110) surface shows the periodicity of the steps. The primary beam energy was 28.8 eV with a field emission current of 50 nA. (b) After imaging in field emission mode, the tip-sample separation was decreased for STM imaging in constant current mode, with a tunnel current of 0.2 nA and a bias of 200 mV.

Fig. 3: This NFESEM micrograph of a W (110) step-edge on the left shows a line scan, indicated by the blue line in the center. The measured profile of the step-edge on the right, where the z-component is given as the signal increase on the detector, is differentiated in the inset. A Gaussian fit (−) gives a lateral resolution of 1.5 nm.

Fig. 2: 300 nm x 160 nm micrographs of a single crystal W (110) surface. (a) Topographic image from the electron intensity of the secondary electrons. The primary beam energy was 28.8 eV with a field emission current of 50 nA. (b) After imaging in field emission mode, the tip-sample separation was decreased for STM imaging in constant current mode, with a tunnel current of 0.2 nA and a bias of 200 mV.

Comparison with STM

Apart from the lateral resolution, NFESEM has the ability to image terraces and monoatomic steps on a single crystalline substrate, a vertical resolution not yet attained with a conventional remote electron gun SEM (fig. 2a). Subsequent STM imaging of the W (110) surface in CC mode confirms sharp step edges with single atom height (fig. 2b) and demonstrates the close correlation between NFESEM and STM despite their evidently different physical origin. The line scan in figure 3 establishes “STM-like” lateral resolution and indicates that the actual theoretically expected resolution limit for the FE-based imaging has been achieved [5].

The capability of resolving surface steps, which are only one atom high, is an important tool in surface physics, as it allows one to image real surfaces displaying complicated topographies, e.g. the step-bunched W (110) surface shown in figure 4a. The surface reveals a periodic step array with a mean terrace width of 12.5 nm, as observed in the 2D Fast Fourier Transform (FFT) (fig. 4b). Such substrate phases provide ideal templates for the assembly of synthetic systems, such as a 1D mono-atomic magnetic or organic chain [6].

Conclusion

The NFESEM is a viable technique to measure a wide range of surface properties with high spatial resolution. We anticipate that scientists and/or engineers working with sub-micron surface structures will have use for this microscope, since the SE contain topographic, magnetic, and chemical information about the surface. The possibilities for application range from, but are not limited to, magnetic recording media, microelectronics, biotechnology, medical studies, magnetic sensors, surface and coating technologies.

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Fluorapatite-Gelatine Nanocomposites

Intrinsic Electric Dipole Field driven Biomimetic Mineralization

Fluorapatite-gelatine nanocomposites serve as model system for mineralization steps of teeth and bone. The biomimetic composites show a hierarchical structural shape development starting from a hexagonal prismatic seed via dumbbell states and ending up with slightly notched spheres. This complex morphogenesis is caused by protein fibrils which are integrated into the nanocomposite superstructure. As evidenced by electron holography an intrinsic electric dipole field is generated by parallel alignment of nanocomposite subunits. The developing intrinsic electric field is then responsible for the formation and integration of the fibril pattern.

 Teeth and bone are natural composites occurring in vertebrates and playing an essential role as endo skeleton or as grinding tool. They consist of hydroxyl apatite as inorganic component and collagen as bio-organic part. These natural systems are produced by cell activities in a complex biological environment in *vitro*. In order to mimic this biomineralization process *in vitro*, we reduced the level of complexity by excluding cells participation and replacing the highly cross-linked and therefore inactive biopolymer collagen by gelatine. Gelatine as denatured collagen exhibits a lot of free functional side groups. The most important feature is the presence of the monomeric unit of collagen the so-called triple-helix, which shows a well defined geometric and chemical unit with a typical length of about 280 nm and a width of about 1.4 nm consisting of three single α-chains. Main components are the amino acids such as glycine, proline and hydroxyproline. Further on, we replace hydroxyapatite by fluorapatite (FAP) (fig. 1, left) because of different reasons: fluorapatite forms a more simple crystal habit with strong resemblance to human dental enamel and it does not give rise to intrinsic piezoelectricity as is discussed for hydroxyl apatite. For the *in vitro* synthesis we create a 10 weight-% gelatine gel plug. This relatively high concentration of the protein may give rise to the formation of triple helical molecular arrangements and their subsequent aggregation to fibril bundles which serve as cross linking areas within the gel (fig. 1, right). This gel plug in a glass tube takes a centered position and from both sides inorganic salt solutions (CaCl₂ and Na₂HPO₄, NaF) are added. The set-up is known as the double diffusion technique and it assures a slow and diffusion controlled formation of the composite aggregates. After 3 weeks reaction time fluorapatite-gelatine composites are generated with a protein content of about 2.3 weight percent thus bearing strong resemblance to the chemical composition of human dental enamel [1]. After a cleaning process by washing with water a variety of morphologies of composite aggregates are found in the micron sized products.

 The different morphologies correspond to different growth states of the composites starting with a hexagonal prismatic seed followed by growing dumbbell aggregates and finally resulting in slightly notched spheres (fig. 2). As the aggregates start develop at different times and different places all kinds of growth states are formed when growth procedure is stopped.

 The formation of dumbbells is a widely occurring phenomenon in biology observed e.g. as precipitation product of bacteria, kidney stones or as biomimetic product of precipitation of inorganic compounds in the presence of organic molecules [2]. Astonishingly, dumbbell formation of inor-
ganic mineral phases also happen in the absence of organics explained by defect formation and supersaturation effects leading to nucleation of new crystals on the surface of the initial crystal [3]. This classical interpretation also holds for fluorapatite aggregates grown in a gelatine matrix where Calcium impregnation leads to rigid linear shaped dumbbells [4] and also for fluorapatite dumbbells grown in the absence of organics [5]. However, in this article we describe the case of phosphate impregnation of gelatine where highly symmetric and regular dumbbells are generated. This is a second kind of mechanism of dumbbell formation which is directed by intrinsic electric dipole fields.

**The Initial State: Hexagonal Prismatic Seeds**

The initial state is represented by a perfect hexagonal prismatic seed with a length of 3–7 μm and a width of 1–2 μm. As shown by synchrotron X-ray analysis and electron diffraction the seeds are single crystalline concerning the inorganic component described as Calcium deficient fluorapatite with about 2.3 weight percent built-in gelatine [6]. We could show by transmission electron microscopy (TEM) on focussed ion beam (FIB) cuts that the inner structure of the young seeds already bears the potential for proceeding branching and to form out-growth areas at both ends [7].

![Fig. 2: SEM images of composite aggregates: The initial state is represented by a perfect hexagonal prism (left top) followed by different dumbbell states (centre top) ending up in notched spheres (right top). The growth sequence is summarized in the superimposed image at the bottom. (Print permission kindly provided by “eye of science”, Nicole Ottawa, Oliver Meckes).](image)

**Fig. 3: (a) TEM image of a FIB cut of a perfect hexagonal seed. The orientation of the gelatine nano fibrils is indicated by yellow lines. Three different regions can be distinguished labelled 1,2,3. In region 1 the protein fibrils are oriented parallel to the long axis of the seed thus contributing to the intrinsic electric dipole field. Region 2 shows bent nano fibrils. In area 3 the dipole field is not strong enough therefore the fibrils are oriented normal to the long axis of the composite aggregate. (b) Scanning ion microscopy micrograph of composite seed before the cut. (c) Scheme of gelatine fibril (orange) orientation within the seed.**

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The nanofibril formation divides the seed in three parts labelled 1, 2 and 3 (fig. 3a). In region 1 the nanofibrils are aligned parallel to the long axis of the seed. Region 2 contains strongly bent fibrils whereas in region 3 all fibrils finally are oriented normal to the long axis. This finding was quite astonishing since from the morphology and the x-ray synchrotron investigations it was shown that the seed behaves like a single crystal. The hierarchical fibril arrangement within the seed cannot be caused by the inorganic compound of the composite but should have its origin in the built-in biopolymer.

In a first approximation, we assume that the fibril pattern is evoked by the electric dipole fields of parallel aligned mineralized triple-helical building blocs (fig. 4, left and centre). By electron holography we could evidence the suspected mesoscopic electric dipole stray field around the “virgin” seed as shown in figure 4, right [8, 9]. On the right bottom of the reconstructed electron hologram (phase image) the rectangular projection of the seed is situated whereas on the top of the seed (centre of image) the strongly bend electric field lines are visible leaving the right end entering on left at the front face of the seed.

The simulation of the mesoscopic electric dipole field is shown in figure 5a assuming a protein fibril dipole assembly (small arrows) in a parallel arrangement along the long axis of the seed. Assuming an effective dipole moment of $1/8 e \times 300$ nm for every triple helix, we constructed an energy profile which displays the local preferred position and orientation of triple helices. The energy difference concerning the orientation and the alignment within the seed is given by means of isopotential lines. Most of the triple helices in the surrounding gel prefer a different orientation than those within the seed mostly pronounced at the prism faces in the vicinity of the basal plane. The energy difference between the orientation parallel to [001] and the preferred new arrangement amounts to at least $4 \text{kJmol}^{-1}$ (fig. 5c).

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In a continuative work demonstrated by simulations that bend fibrils even enhance the intrinsic electric field. The dipoles being parallel to the long axis of the composite seed (α-dipoles) produce the intrinsic electric dipole field. Under the influence of this field some dipoles start to orient along the electric field lines (β-dipoles) and appear as arc-like structures [10]. Additional simulations on atomistic level help us to understand the biomineralization process of triple helical molecules with fluorapatite to elaborate new insights into the interplay governing the formation of apatite-collagen composites [11].

Conclusions

The biomimetic growth of fluorapatite-gelatine nanocomposites is a highly sophisticated model system for studying the biomineralization process in vitro. The formation of dumbbells and branching of seed aggregates is observed which can be explained by assuming an intrinsic electric dipole field within and around the initial seed. This field is evoked by the integration of parallel aligned triple-helical protein fibrils giving rise to a superstructure of 5–15 nm as evidenced by HR-TEM. During the growth process a second level of hierarchy becomes evident formed by the protein fibrils following the frozen electric field lines showing a characteristic bend arc-like pattern within the aggregate. Thus already within the perfect single crystal seed the pre-stage for bifurcation is highlighted leading to typical dumbbell structures although not perceptible from the habitus and morphology.

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Acknowledgements

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Electron microscopy
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In situ transmission electron microscopy was used to investigate the ageing behaviour of Si-based solar cells. The structural and compositional changes of the NiSi layer of such cells were determined.

Introduction

Solar cell industry is still a fast growing market, especially due to rising prices for fossil fuels. Efficiency improvement of solar cells as well as optimisation of the various fabrication steps are current topics of research and development [1]. Furthermore analysing the ageing behaviour of photovoltaic panels is a field of great importance. Since modern panels are released with proposed lifetimes in a range of 25 years, reliable information about thermal and radiation stress induced processes modifying the function of a solar cell are crucial. Accelerated aging experiments are a fast and effective possibility to obtain reliable data about the ageing behaviour [2] of a device.

In modern high efficient solar cells a silicide layer is applied between the bulk silicon and the metallic contact grid. Nickel monosilicide (NiSi) is a promising material to for this layer. In contrast to other silicides, NiSi can be cost efficiently applied using galvanic deposition followed by rapid thermal processing [3]. Due to thermal ageing the NiSi-layer can diffuse into the adjacent n-doped Si-layer. For correct function of a solar cell the thickness of the NiSi-layer may not exceed this n-doped Si-layer.

The aim of this study was a preliminary test if in situ transmission electron microscopy (TEM) is an appropriate way analysing the accelerated ageing behaviour of solar cells. Due to the limitations of TEM in analysing large regions, the described method cannot analyse the aging behaviour of a complete solar panel. Here the focus was set to thermal induced thickness and compositional changes of a solar cells NiSi-layer.

Method

All samples used for this investigation were supplied by NB technology. In situ transmission electron microscopy at elevated temperatures was used to analyse the ageing behaviour of the investigated solar cells. Prior to the TEM experiments, TEM lamellae with a thickness of about 70 nm were prepared using a focused ion beam milling device (Zeiss XB1540 Cross beam FIB). Elemental Analysis was done using energy dispersive X-Ray spectroscopy (EDS) prior and after the heating periods using a Zeiss Libra200 (CRISP) TEM with attached scanning unit. All heating experiments were performed in a 200 kV LE0922A TEM equipped with a LaB₆ emitter and a heating holder (Gatan, model 652). Heating temperature was 300°C with a maximum duration of 120 min.

Results

Figure 1A shows a bright field (BF) TEM image at t = 0 of the heating experiment. The nickel monosilicide (NiSi) layer is clearly visible and has a mean thickness of 160 nm. The adjacent platinum layer was applied during sample preparation and is not part of the solar cell. Heating of the sample was performed in the TEM using a furnace integrated into the sample holder. The temperature range of this heating sample holder is between 25 and 1,000°C. For the present study a temperature of 300°C was used. This temperature was chosen in accordance to MIL standard ageing tests. Figures 1B-D show the changes of the sample after t = 15, 60 and 120 min. Already after 15 min an additional layer is formed and grows permanently over the heating period. The mean thickness of the newly formed layer was about 40 nm while the origi-
nal NiSi-layer thickness did not change at all. The sample area in figure 1B–D varies slightly from the original position; strong carbon contamination occurred on the original area during an EDX-scan and influenced image contrast badly. The area imaged in figure 1B–D is adjacent to the original position. An originally planned in situ EDX investigation failed due to sample drift. Instead of life scanning, EDX-analyses were performed before and after the heating experiment. Figure 1E and F show the HAADF images corresponding to these EDX scans. Care was taken to measure the same sample area as imaged in figure 1B–D. The Z-contrast of the HAADF images in figure 1E and F indicate a chemical composition with lower Ni content compared to the original layer. Figure 2 shows the compositional analysis of the NiSi layer before and after the heating experiment. The bars represent the atomic ratio of Ni (blue) and Si (purple). The Pt-protection layer was measured as reference but not taken into account for the analysis. The original layer turns out as NiSi with good accuracy and remains its composition after the heating process. The newly formed layer is Si rich. An accurate phase determination was not possible within the accuracy of the EDX analysis.

Conclusion

In situ transmission electron microscopy is a versatile tool for investigation of structural and compositional changes on nanometer and micrometer scale. Life imaging of the structural changes is possible. Testing the growth of the NiSi layer in solar cells during ageing is important. Si-based solar cells with such layers are commercialised for about 10 years and the knowledge of the ageing behaviour of the NiSi layers is poor. Furthermore no standards for testing Si based solar cells are available at the moment.

For a more quantitative approach more temperatures and longer exposure times have to be analysed.

References


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The ITRS requires the integration of dielectric materials with effective dielectric constant (k) lower than 2.8. This is achieved using porous SiOCH. Unfortunately during integration in the devices, damages are introduced in the low-k layer by CMP. The impact of these damages on the microstructure and the electronic properties are studied using HAADF imaging and Valence Electron Energy Loss Spectroscopy in TEM environment. Results are compared to low-k capped with an etch stop layer.

The physical properties of the materials involved in semiconductor devices are pushed to their limits for each new technology node. One critical example is the requirements on the properties of the insulator filling the shrinking gap between metal lines. The dielectric constant needs to be as small as possible, but the insulator should still be compatible with chip fabrication steps like chemical mechanical polishing (CMP), etching or annealing. Ultra low-k materials based on porous Si-COH compounds are reported as promising candidates to replace the dense SiO₂ [1]. In bulk form a reduction of the dielectric constant from 4 to 2.8 is obtained for such materials. The main reasons for achieving lower dielectric constant is the lower polarizability of Si-C or Si-CH₃ bonds than Si-O bond. Unfortunately, during standard fabrication processes like CMP, ULK material is susceptible to be damaged, leading to the degradation of the dielectric properties. To minimize these damages an alternative consists in the deposition of a denser oxide layer on the top of the ULK before the CMP treatment. In this article, we compare the microstructure and the insulating properties of CMP and capped ULK at 7 Metal Level in a finished device.

**Experiments**

Microstructure and electronic properties of ULK are determined by the combination of HAADF imaging (High Angle Annular Dark Field) and EELS analysis (Electron Energy Loss Spectroscopy) in a TEM environment i.e. FEI TECNAI G2 [2]. Both specimens are prepared by focused ion beam (FIB) milling. First, cross-sections were cut out of a 300 mm wafer using the in-situ lift-out method. Then, the final thinning is performed using low kV gallium at 30 kV, 5 and 2 kV successively, to reduce surface amorphization. The insulator layer sequence is either SiCN/ULK/ capping material/SiCN or SiCN/ULK/SiCN for capped and CMP samples respectively (fig. 1). The relative thicknesses, calculated from low loss spectra recorded in the middle of the two SiCN layers, located be-
low and above of the low-k layer, are found to be equal. This indicates that the TEM foil thickness across the low-k layer is constant, as expected from FIB thinning. Since contrast variations in HAADF images are proportional to the product of the density, thickness (t) and atomic number \((Z^{3/2})\), HAADF imaging contrasts can be thus only attributed to chemical or density effects. Regarding EELS analysis, all experiments are performed in the low energy domain \(<100 \text{ eV}\), in line-scan mode across the stack. Spectra are recorded with short exposure time to prevent contamination and irradiation effects and are summed to get a final spectrum with a high signal to noise ratio. In this energy range, EELS signal results from individual and collective valence electrons excitations and gives information on chemical as well as physical properties. The zero-loss peak recorded simultaneously to the low-loss spectrum informs about the experimental resolution and the material density. The calculated single scattering distribution (SSD) spectrum is proportional to the product of the matrix element between VB and CB and the joint density of states (JDOS) [3]. Hence, almost each structure in the SSD Spectrum low energy region \(<10 \text{ eV}\) is related to an electronic transition. In particular, the first intensity jump can be attributed to the band gap.

Microstructure of Capped and CMP ULk

Figure 1 displays HAADF images of capped and CMP sample. In both cases, the interfaces between the different deposited layers can be observed by contrast variations, showing the ability of HAADF imaging to be an accurate metrology tool. In HAADF images the contrasts are reversed in comparison to bright field TEM imaging, heavy materials appear white and light element or porous material dark. The contrast variations across the metal layer sequences reveal that density increases from low-k, to SiCN, to capping material and Cu. In addition, for CMP sample, we observe that ULK is darker at the bottom than at the top, whereas the same contrast is observed across the capped ULK, indicating that CMP step introduces either density or chemical variations. In figure 2 zero-loss peak and plasmon region line-spectra recorded across the capped and CMP ULK are displayed. The shape and the intensity of the zero-loss peak as well as the plasmon region appear identical at any level in the capped ULK layer, indicating that neither significant density nor chemical composition changes occur. On the contrary, for CMP sample, the zero loss and plasmon loss region intensities increase by a factor of 2, from the bottom to the top, but no change is observed neither in the shape nor in the plasmon peak energy. The contrast variation across the CMP low-k layer can be thus attributed to density variation effects. SiOCH compounds are known to be porous materials. Thus a likely interpretation is that the chemical solution used for CMP step, penetrates into ULK and slightly dissolves ULK. Pore volume fraction is thus increases and density decreases [4].

Band Gap Measurement of ULK

Figure 3 displays the calculated SSD spectra representative for ULK layer in capped sample and for ULK at the top and bottom.
regions in CMP sample. For each one of these spectra two significant intensity jumps are observed within the 0–10 eV range. For the capped ULK, the first jump is located at 2.4 eV and the second at 8.5 eV while for CMP ULK, the first is located at 1.1 ± 0.1 eV and the second at 8 and 8.5 eV depending on the acquisition place. To validate the method, the same band gap determination is applied to spectra recorded from the well known phases present in the stack i.e. SiCN and amorphous-SiO₂. For both, band gap and low-loss structures energies are in agreement with values reported in the literature. The presence of any artefacts in the ULK SSD spectra due to Cerenkov effects or surface coupling effects can be excluded [5], since refractive index of SiCOH compound is reported lower than 1.4 and TEM foil thickness is measured to be 55 +/- 5 nm. This confirms the reliability of our results. Comparing ULK, SiO₂ and capping material SSD spectra, we find that they are not very different in the energy range above 10 eV, where plasmon contributions dominate the spectrum. The typical structures located at 10.5, 14.6 and 18.1 eV observed in am-SiO₂ spectrum are also particularly well reproduced, indicating that SiOCH electronic behaviour is close to the SiO₂ one. The only difference is that the SiOCH plasmon energy position is shifted about 1 eV to lower energy. This is in agreement with the expected chemical composition effects resulting to C and H atoms addition to SiO₂. The interpretation of the spectra in the energy range below 10 eV is less straightforward. The signatures located at 8 and 8.5 eV for CMP and capped ULK are naturally identified as the band gap since it is the case for the intensity jump in am-SiO₂ located at 8.9 eV. While as expected, the intensity falls down to zero in am-SiO₂ for energy lower than 8.9 eV, a surprisingly significant signal remains in this energy range from 1.1 to 8 eV and 2.4 to 8.5 eV for CMP and capped ULK respectively. Taking account of these signal intensity level, it would be unrealistic to attribute it to band tail effects. The most probable explanation to electron states in the band gap region, are dangling bonds created at the surfaces of the pores in the ULK. Therefore, to our knowledge no calculations supporting these assumptions have actually been carried out. After processing each individual spectrum of line-scans across capped and CMP ULK, a mapping of the band gap and defects signatures as a function of the depth can be established (fig. 4). While in the capped ULK both signatures are constant across the layer, a significant decrease of the band gap from the bottom to top occurs for the CMP ULK. Moreover, the defect signatures energy position is always about twice lower in CMP than in capped ULK, independently of the location in the layer, like as this signal was not impacted by the density variations. Additional experiments are carried out to shine light on this result.

**Conclusion**

The impact of CMP on ULK material in a state of the art device is investigated using HAADF imaging and EELS in HR-TEM. STEM-EELS line profiles allow an accurate mapping of three fundamental features of ULK material: density, band gap and electronic defect signatures. We have first proven that the capping layer plays its protection role since no densification variations are detected and a flat band gap profile is observed across the capped ULK. In the contrary, a significant modification of the contrasts and electronic properties are observed in the CMP ULK layer. As a consequence, a negative impact on the dielectric constant of ULK unprotected before CMP is thus expected.

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Objective Design

Meeting the Demands of a Live Cell Imaging Station.

Today’s advanced microscopes can be configured to create complete imaging workstations. With immediate access to a range of imaging techniques, researchers can gather multi-dimensional image information to increase understanding in their research. New imaging techniques and capabilities, however, often require specialist objectives for optimum results. Here we describe some of the new objectives that have become available for live cell imaging in recent years and show how complementary microscope and objective design enhance imaging capabilities to accelerate research.

Making the Most of an Imaging Workstation

The ability to insert a number of imaging modules into the light path of today’s advanced microscopes has had a profound impact on imaging capabilities, allowing single microscopes to be transformed into multi-purpose imaging stations. With the Eclipse Ti inverted microscope, for example, users can switch easily between brightfield, darkfield, phase contrast, Nomarski DIC, Hoffman modulation contrast, widefield fluorescence and confocal imaging to enable techniques such as TIRF, FRET, FRAP, FLIP and photoactivation studies. Selecting the appropriate lens for live cell imaging applications is important for ensuring optimal results.

Objectives for TIRF

When laser light is used to illuminate a sample at an incident angle greater than the critical angle, total internal reflection occurs. Under these conditions an evanescent wave is generated within about 100 nm of the coverslip-specimen interface, and can be used to create fluorescence images with an extremely high signal-to-noise ratio. Nikon’s TIRF objectives (CFI Apochromat TIRF 60x and 100x) for use with a standard coverslip and immersion oil have an extremely high N.A. of 1.49, enabling the capture of even single molecule fluorescence and allowing time-lapse imaging of detailed molecular trafficking and cell membrane events. Both 60x and 100x TIRF objectives use a correction ring to correct for deterioration in image quality caused by deviations in the thickness of the coverslip or temperature fluctuations. Optimal performance is ensured even at physiological temperatures.

TIRF imaging in combination with the Ti’s Perfect Focus System (PFS) ensures in-focus images even over prolonged observations. PFS overcomes focus drift, one of the biggest problems in time-lapse imaging, by instantaneously correcting focus in the desired Z-plane.

The high N.A. of TIRF lenses makes them highly versatile, able to capture high resolution, high signal-to-noise ratio images (fig. 2) not only in TIRF imaging but also in episcopic or confocal fluorescence, Nomarski DIC observations and, in the case of the 100x objective, for laser tweezers microscopy.

The PFS system uses 870 nm light to detect the coverslip interface allowing near infra red (I.R.) fluorescent dyes, such as Cy5.5, to be used. The improvement in optical characteristics enables the use of a wider range of objectives for PFS in applications from Ca2+ concentration measurements in the ultra violet (U.V.) to laser tweezers in the I.R. region.

Author’s background

Peter Drent is General Manager, Biosciences, at Nikon Instruments, Europe BV. He has a special interest in digital imaging technologies and is a frequent speaker at Nikon’s Digital Imaging Seminars.
New Options for Phase Contrast

One of the most innovative features of the Ti is the incorporation of an external phase contrast unit. This incorporates a phase ring in the microscope body instead of in the objective lens and allows the use of high N.A. objectives for phase contrast imaging. High N.A. TIRF objectives, for example, can be used to capture exceptionally high-resolution phase contrast images. Because phase contrast observation is possible with the same objective used for TIRF observation as well as DIC observation, phase contrast images are captured with less oblique background shading than that in DIC observations, allowing high-precision data processing and image analysis. Even water immersion lenses can be used for phase contrast, with refractive index-matched, minimal aberration imaging of deep specimen areas. Four types of phase ring are available to suit the objectives used.

For alternative phase contrast options, Nikon has recently introduced its apodised phase contrast series and CFI S Plan Fluor ELWD/ELWD phase contrast objectives. The CFI Plan Fluor ADH 100x (Oil) objective is ideal for critical phase contrast applications where users need to visualize minute structures in unstained low-contrast specimens. The objective includes an apodisation phase ring to reduce halos which can reduce the quality of the phase contrast image and improve contrast to twice that of comparable lenses. Time lapse imaging of cellular process, such as activities in cell division or mitochondrial transport, can now be observed without being obscured by halos. The Plan Fluor ELWD/ELWD series have a newly-developed broadband multilayer coating that provides high transmission from near U.V. to near I.R. wavelengths. A correction collar allows these objectives to be used with culture vessels and specimens of different thicknesses. These lenses can, in addition, be used with a wide range of illumination techniques.

New to the Plan Apochromat Violet-corrected (VC) Range

A new 20x Plan Apochromat violet-corrected (VC) objective has recently been added to the VC range. VC objectives are ideal for multi-stained fluorescence specimens, brightfield and DIC observations and, because they are corrected axially from 405nm to 660nm, they are also suitable for confocal applications and phototoactivation studies. The impact of Plan Apochromat (VC) series objectives on the interpretation of laser scanning confocal images can be appreciated in figure 4, which compares consecutive cross-sectional XY and XZ images acquired with a conventional lens and a VC lens. With the conventional objective, DAPI fluorescence (blue) may shift in the Z-axis direction due to axial chromatic aberration, making it appear outside the plane of the cell. With the VC objective lens, axial chromatic aberration has been corrected up to the violet range allowing DAPI fluorescence (blue) to be accurately visualized within the cell. Because aberration correction takes place right up to the edge of the captured field of view, these objectives are ideal for digital imaging.

Choosing the Right Lens for Fluorescence Applications

Correct selection of objective for each application will help ensure high quality results.

Apochromat VC series objectives and zoom optics become very important in balancing digital and optical resolution. When using fluorescence applications, however, a choice needs to be made between maximum signal collection or the ability to use probes that work at U.V. or I.R. wavelengths. When the signal is not limited, Plan Apochromat lenses are preferred.

Important in live cell imaging, fluorescent proteins enable an array of techniques such as FRAP, FLIP, iFRAP, FLIM, TIRF and laser ablation techniques. Certain probes require Plan Apochromat VC optics for activation and imaging including CFP, GFP, YFP, Cherry-FP, Tomato-FP, PA-GFP, Kaede, and PS-CFP. Confocal is the technique of choice for high resolution 3-D fluorescence imaging. The most common laser scanning confocal microscopes typically use blue (488 nm), green (543 nm) and red (633 nm) excitation. At these wavelengths correction and transmission are optimal in Plan Fluor and Plan Apochromat series lenses. When using I.R. excitation (as in 2-photon laser scanning microscopy) efficient I.R. transmission is crucial. Plan Apochromat VC lenses not only work well in the violet part of the spectrum, but also perform well in the I.R., enabling greater depth penetration in the specimen. These objectives, capable of accommodating blue to red parts of the spectrum, are also useful for quantum dot deep cell imaging techniques.

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Nikon’s Eclipse Ti Series inverted microscopes can capture images at two different wavelengths simultaneously, using dual cameras. Accelerating image acquisition while maintaining full frame resolution, the system is ideal for FRET and the capture of rapid dynamic cellular events using calcium or other ion-targeted probes, ratio probes, dual emission ratiometric dyes etc. The two cameras are positioned on the instrument’s back and side ports. Perfect registration between the two cameras is assured on installation to ensure that no information is lost during imaging. No further realignment or specialised alignment software is required. Even when the intensity difference between wavelengths is large, high-quality images can be captured by adjusting camera sensitivity for each wavelength.

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Complete Online Pathology Resource

Olympus is offering the Slidepath Digital Slidebox interactive digital slide application software solution. This comprehensive multimedia management system allows users to create their own web-based pathology resource in minutes. Furthermore, the software can be used to exploit the power of digital slides in education and training, offering maximum teaching efficiency in all histological applications. The software package allows academic tutors to build extensive libraries of slides and pathology cases for review by students using the web. Developed with key academic institutes, its design facilitates the creation and editing of educational content, quickly and easily, and is an interactive resource for teaching in any discipline that uses glass slides.

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Scientific Imaging Webinar Series

Media Cybernetics announced their 2009 Imaging Webinar series. The free webinars focus on the latest image analysis and image processing tips and techniques. “By attending our free imaging Webinar programs from their own lab or office, researchers can gain a better understanding of both basic and advanced digital image processing techniques,” said Kathy Hrach, Marketing Manager for Media Cybernetics. “Attendees will be able to ask questions and interact with our imaging specialists during these live presentations.” Upcoming Webinars Include: Deconvolution of Confocal Fluorescence Microscopy Images (March 25), Beginners Guide to Recording a Macro (April 7), and Deconvolution of Widefield Fluorescence Microscopy Images (May 6).

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Nanosensors has added a Carbon Nanotube SPM probe to its scope of products. The Single or Double Walled Carbon Nanotubes (CNT) at the end of the tips are grown by chemical vapor deposition and are ready to use, no shortening or post processing is necessary. The Single/Double Wall Carbon Nanotube SPM probes have a tip diameter between 2 and 3 nm. Compared to other Carbon Nanotube probes available on the market today that are mostly multiwalled carbon nanotubes the tip radius of the Company’s Carbon Nanotube AFM tips is considerably smaller. They are therefore very suitable for high resolution measurements of nanometer-sized features.

Asylum’s MFP-3D Nanoindenter has been selected as one of the top five products exhibited at the AVS International Symposium. It is a true “instrumented” indenter that does not use cantilevers as part of the indenting mechanism. These characteristics and the use of state-of-the-art AFM sensors provide substantial advantages in accuracy, precision and sensitivity over other nanoindenting systems. Unlike cantilever indenters, the system drives the tip perpendicular to the surface. This vertical drive avoids the lateral movement and errors that are inherent in cantilever-based systems. The positioning accuracy is subnanometer using the system’s closed loop nanopositioning sensors. The Identer can operate under fluid and at temperatures up to 300°C.

Mad City Lab’s Nano-SP30 positioning system is particularly suited for nano-indentation and nano-manipulation applications. It is a single axis integrated positioning system combining the advantages of stepper driven motion with piezo precision. For coarse approaches, the Nano-SP30 offers 25 mm of travel with a step size of 95 nm. High resolution (<0.1 nm) positioning over 30 microns is achieved via the piezo driven stage. The system can be mounted vertically or horizontally and has been designed to be compatible with vibration isolation tables and optical fixturing. It is equipped with high resolution position sensors with real time read back and closed loop control enabled for the piezo component. In addition feedback from external sensors (such as force sensors) can be accepted by the Company’s USB enabled controller, giving the user high versatility for many demanding nano-metrology applications.
**Small, Fast, and Rugged Smart-Camera**

The Matrox Iris-GT is powered by an Intel 1.6 GHz Atom processor and runs Windows CE 6.0. It features an integrated graphics-controller with VGA output, 256 MB DDR2 memory, and 1 GB of flash-disk. For connectivity to external devices, the camera includes a 10/100/1000 Ethernet port, a USB 2.0 port, an RS-232 serial port, an opto-coupled trigger input and strobe output. The camera also supports Ethernet/IP and Modbus over TCP/IP communications to directly interact with PLCs and other automation equipment. It is housed in a sturdy, dust-proof and washable casing and is initially available in two sensor configurations: Matrox Iris GT300 – 640x480 @ 110fps 1/3” monochrome CCD and Matrox Iris G1200 – 1280x960 @ 22fps 1/3” monochrome CCD.

**Microscope Digital Camera**

Olympus’ new DP72 digital camera is highly flexible and features advanced image processing capabilities. It is particularly suited for pathology applications, where faithful color reproduction is essential for clear and consistent diagnoses. The re-mastered pixelshift technology provides high-definition images with the single click of a button. Capturing a 12.8 megapixel (4140 x 3096 pixels) full-resolution image with excellent clarity and detail is achieved in less than 2.5 seconds. The camera provides a high range of image resolutions to suit all magnifications and experimental requirements. In live-mode, 1360 x 1024 pixel images can be displayed at 15 frames per second (FPS) without any color shift during sample movement, making it ideal for live imaging documentation.

**CCD Microscopy Camera**

Lumenera Corporation has released a new ultra sensitive high color fidelity CCD scientific camera. This newest USB 2.0 offering from the Infinity family of microscopy cameras is designed for low light applications such as fluorescence, and where precise color reproduction is needed. The Infinity 3-1U offers large 6.45u x 6.45u pixels in a 2/3” format with a maximum resolution of 1392x1040. It employs the Sony ICX285 1.4 megapixel CCD sensor – the market leader for fluorescence and other challenging low light applications. In addition, the camera’s very good color rendition makes it a useful addition to any clinical laboratory. It is suitable for both fluorescent and brightfield imaging.

**Portable 1064 nm Raman Spectroscopy**

Andor Technology announced the successful outcome of a collaboration on a new portable spectroscopic instrument that allows up to three times greater collection efficiency than standard Czerny-Turner spectrographs. The new instrument is dedicated to 1064 nm Raman Spectroscopy. Based around the Company’s Idus Ingaas detector array camera and Bayspec’s high throughput Volume Phase Holographic Gratings (VPHG) spectrograph, the instrument offers performance benefits whilst also boasting thermo-electric detector cooling down to –85°C, USB2.0 plug and play connectivity, F/1.8 aperture and spectral resolution of 8 cm–1 alongside with 3,000 cm–1 spectral coverage.

**LED Lighting Modules**

The Imaging Source has announced a series of cost effective LED lighting modules, which seamlessly integrate with its USB, Firewire and Gige camera. The series offers very bright LEDs, and direct connection to the camera. All parameters can be set via the shipped software, IC Capture and IC Imaging Control or by custom built software. It is compatible with the company’s USB CCD cameras, Firewire cameras, and Gige cameras. All cameras ship with IC Capture and IC Imaging Control. The former is an end-user application, with which it is possible to set all cameras parameters, save singular images and image sequences and display live video streams. IC Imaging Control is an SDK, consisting of a Net component, Active X control and C++ class library for Windows XP and Windows Vista.
Surface Analysis and Nanometrology

The LNE, the French National Metrology Institute, and Digital Surf have signed a technical cooperation agreement. The Institute will join Digital Surf’s Mount Shasta program with internationally reputed laboratories that use Digital Surf’s Mountains Map surface imaging and analysis software.

Enhanced OEM volume rendering technology

Siemens Healthcare’s 3D volume rendering engine QVIA is now available as an OEM product. This software module can be incorporated into end user applications to visualize 3D microscopic data and is based on off-the-shelf graphics cards. Multiple 3D channels can be fused using individual transfer functions and combined with surface based visualizations. QVIA provides third party vendors with fast and high quality visualization technology without remarkable investment. The shown murine macrophages data are provided by courtesy of Dr. Birgit Kraus at Lehrstuhl Pharmazeutische Biologie, Universität Regensburg.

Automated Virus Detection and Identification

Item Solution Virology provides a new technological approach in automated detection and identification of the gastroenteritis viruses in highly textured electron microscopy micrographs. Using digital TEM image acquisition and analysis, an automated virus detection workflow can be setup. Such a workflow automates the process of ocular inspection of TEM images, increasing speed and reliability in the diagnostic procedure. Viruses cause epidemic outbreaks such as the “winter vomiting disease” in many countries every year. Detecting and identifying of the known gastroenteritis viruses and other unknown viruses have all become of critical global interest. Identification of viruses with PCR (Polymerase Chain Reaction) and ELISA (Enzyme-Linked Immunosorbent Assay) can be troublesome due to frequent virus mutation rates, spurred by the hundreds of millions of new infections occurring every year.

Small Cantilever AFM

Asylum Research introduces the Cypher AFM, the first new small sample AFM in over a decade. It is the world’s highest resolution AFM, combining the accuracy and control of closed loop with the power of atomic resolution for the most accurate images and measurements possible today. Included are Spoton automated laser alignment, high-speed AC imaging with cantilevers smaller than 10 µm, integrated acoustic/vibration isolation, and thermal control for image and measurement stability.

Nano-View™/M Series

- XY Microstage
- Multi-axis Nanopositioning
- Larger Aperture
- 300 microns nanopositioning
- Digital Control
- Scan Synchronization
- Accessories included!
Fluorescence Microscopy in a New Light

The Leica Fluo LED 4000 and Fluo LED 100 LED illumination systems offer very high image quality for fluorescence applications while being maintenance-free, compact and easy to operate. In addition to its flexibility for fluorescence applications in cell biology and neurobiology and for use in imaging facilities, the LED 4000 offers five precentered LED modules, that can be freely combined for multi-color experiments with different wavelengths. The compact illumination unit is directly connected to the microscope. If an additional wavelength is required, individual LED modules can easily be exchanged. For applications requiring one fluorochrome only, the Fluo LED 100 is a solution. In pathology, cytology, microbiology and immunohistochemistry, where usually only fluorescein is used, this ultra compact and user-friendly LED illumination with its excitation wavelength of 470 nm is suited.

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Filter Switching Light Source

Agilent Technologies announced the availability of the Polychrome 3000, an ultrafast filter-switching light source for fluorescence microscopy applications. The light source uses high-precision galvanometer-driven mirrors to direct white light to different filter positions. The setup allows changing between filters in milliseconds. A filter slider on the front of the system allows users to exchange up to five filters in a single pass. All filter positions offer equivalent brightness and homogenous illumination of the specimen. The filters used are standard 25 mm diameter filters that can be up to 5 mm thick. This instrument uses an optimized light path combined with advanced condenser technology and a stable quartz fiber light guide. This leads to the highest brightness in the specimen plane.

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Convection-cooled Illumination System

Dolan-Jenner Industries introduces the Fiber-Lite Mi-30SG Unit. Designed for laboratory use, the 30 watt illumination system is one of several Fiber-Lite Illumination Systems that the company produces. It offers the price-conscious consumer many of the features found in the Mi-150, with the added benefit of a rugged, convection-cooled enclosure which results in zero noise and vibration. The quartz halogen unit comes as a single goose-neck model and delivers over 10,000 foot candles of intense, cold illumination. The lamp has an average life of 200 hours, and the unit weights just 7.25 pounds, making it portable and versatile. The unit can be fitted with an optional filter holder, and color filters can be ordered as a complete set or individually.

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Yellow and Orange Diode Lasers

Toptica offers lighting solutions based on frequency-doubled grating stabilized diode lasers. According to “take infrared and make orange out of it”, lasers of the type DL-SHG (Diode Laser – Second Harmonic Generator) provide output powers from several mW to a few ten mW – depending on the design wavelength in the range of 555 nm to 625 nm. Such lasers can be coarse-tuned by a few nm and allow for some 10 GHz modehop-free fine-tuning. The proprietary design guarantees frequency drifts below 200 MHz/K and coherence lengths of several hundred meters. Main applications of these high-end lasers are spectroscopy and interferometry but also laser cooling or optical pumping of atoms and ions.

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New Hardcoated Tripleband Filter Set

The new blue-green-orange multiband filter set designed from Semrock Inc. will be offered very soon from AHF analysentechnik AG. Due to the special hardcoating technique this multiband filter set is optimized to provide simultaneously stunning brightness e.g. for DAPI/FITC/TRITC or corresponding dyes like DAPI/Cy2/Cy3. Striking contrast, and superb color balance of multiple fluorophores can be analyzed by eye or with a high-quality color CCD camera. Full multiband sets are very helpful tools for routine analysis and quick screening of samples. This blue/green/orange version can be delivered as well with singleband excitors for quick electronic control. Please contact www.ahf.de for offering a DEMO unit.

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Nikon and Jeol Join Forces

Nikon Instruments Europe and Jeol have announced an agreement that appoints Nikon as official European distributor of the Neoscope SEM. Whether used by trained electron microscopists as a compact screening instrument, or by lab technicians seeking a higher resolution alternative to the light microscope, the microscope is designed to help accelerate the pace of research in all fields. Offering simplicity, affordability, and convenience, it is suited for sampling inspection, failure analysis of manufacturing materials, materials research, metallurgical laboratories, medical devices, forensics, bioscience research, pathology and environmental laboratories.

Scanning Electron Microscope

Fei’s Magellan extreme high-resolution scanning electron microscope (XHR SEM) allows scientists and engineers to quickly see 3D surface images at many different angles and at resolutions below one nanometer (about the size of ten hydrogen atoms, side-by-side). Most importantly, the microscope images samples at very low beam energies, avoiding distortions otherwise caused by the beam penetrating into the material below. The Company’s TEM, SEM, and Dualbeam solutions were created specifically for materials science, life science, and mining.

3D/4D at upcoming congresses

At this year’s annual congress of the German Neuroscience Society, to be held in Göttingen, Germany, March 25 – 29, and at the 2009 conference of the Focus on Microscopy Society, in Krakow, Poland, April 4 – 8, Bitplane AG will be participating in the scientific exhibition. They will be happy to demonstrate their flagship Imaris 6.2, and cordially invite participants to an introduction to the fascinating world of 3D & 4D imaging at their booth.

Looking back, acting forward:

- Compositional Analysis
- Electron Microscopy
- Image Processing
- Light Microscopy
- Scanning Probe Microscopy

We are very grateful to our partners from industry and academia, and the community, for their commitment and loyal collaboration.

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**Stereo Zoom Microscope**

The stereo zoom microscope SX45 from Vision Engineering is designed for budget conscious decision makers. Continuing costs for running the microscope are kept to a minimum through long-life LED illumination. A trinocular viewing attachment to be utilised for the microscope, with the option for a digital or USB camera to be used simultaneously. Multimedia solutions are available for image archiving, acquisition, processing, analysis and documentation. Available in a variety of stand options including: the bench stand for optimum stability and substage illumination; the boom mount for greater working distances and added versatility; the articulated arm, ideal for large and bespoke work stations/benches requiring extended reach. The zoom ratio is 6.3:1 with the greater magnification of x50, twinned with long working distances and a large depth of field, offers operators optimised functionality for inspection and rework.

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**Microscope Accessories**

Jasco released two new FT-IR Microscope accessories, the IRT-5000 and IRT-7000, which can be easily interfaced with either the company’s FT/IR-4000 or FT/IR-6000 spectrometers. These microscopes feature innovative functions such as “Smart Mapping”, newly developed for infrared micro analysis and IR imaging. The IRT-5000 is an FT-IR microscope system featuring dual detector capability and multiple objectives with excellent signal to noise ratio. The IRT-7000 is a multi-channel IR microscope for IR imaging, and with the combination of the FT/IR-6000 and step scan option offers an advanced capability for dynamic imaging as well as time-resolved measurements of a specific area. In addition to high-performance and ease-of-use operations, both microscopes feature a “Smart Mapping” function as standard, which enables mapping analyses of a limited area without the use of an automated sample stage.

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**High-resolution Multiphoton Microscopy**

The LSM 7 MP Laser Scanning Microscope from Carl Zeiss is tailored to the needs of multiphoton microscopy, offering flexibility, a compact design and ease of use. It generates high resolution microscopic images for a broad range of experiments in biomedical and basic research. Thanks to the use of two scanners in one compact system, two lasers with different wavelengths can be utilized sequentially or simultaneously for specimen imaging and manipulation. The range of detectors and their filter equipment, as well as an extensive line of microscope accessories, allow customization of the microscope system. Applications include e.g. high resolution 3D imaging in long-term process observations, and functional imaging in conjunction with simultaneous photo-manipulation.

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**Raman Microscope**

The DXR Raman microscope from Thermo Fisher Scientific is equipped with fully integrated, pre-aligned components for fast and easy field installation and configuration. The instrument is designed specifically to help non-specialist users achieve rapid sampling and analysis of particles, down to one-micron spatial resolution. It utilises several patented features to automate and simplify the steps an expert would go through to optimise a measurement. Interchangeable Smart components require no operator adjustment and ensure automated system configuration. Patented auto alignment and auto calibration ensure reliable results. A fiber probe option is available for remote sampling. Furthermore, the microscope utilises the company’s Valpro complete system validation package, allowing for compliance with cGMP and FDA regulatory requirements. The company also offers a large collection of Raman spectral libraries to aid in sample identification.

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DISCOVER THE FULL SPECTRUM: THE NEW AND IMPROVED cell* IMAGING SOLUTIONS.

Imaging solutions for life sciences must be reliable user oriented and above all flexible, in order to react to growing demands. That’s why cell* from Olympus now has more functions and is even easier to use, providing you with a whole new spectrum of exciting possibilities. Put simply, cell* is the optimum system solution for modular software and hardware in microscopy. It offers both functionality and technology, from the simple capturing of images, through analysis, measurement, documentation and archiving – even as far as the free programming of analysis procedures and the real-time observation of live fluorescent cells. Let yourself be inspired by a colourful palette of new possibilities – cell* imaging solutions from Olympus open up new perspectives for your work.

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