PATHOLOGY & DIAGNOSTICS NEWSLETTER
EUROPEAN EDITION

reSOLUTION

- Digital Microimaging Device
- Fixation & Dissection
- Process Automation
- Cryomicrotomy
Dear Reader,

We are proud to present the first reSOL uTion – eurOpean Pathology & Diagnostics Newsletter. The aim of this magazine is to provide a balanced mix of information from the world of technology combined with users’ experiences made in their daily working environment. In this edition you will find a feature on easy-to-use disinfection techniques in cryomicrotomy, besides an article on a new fixation method and the technique of Laser Microdissection. We also report on laboratory workflow solutions, which are playing a more and more important role in helping pathologists and laboratory personnel perform their responsible job and cope with the increasing workload in a safer and more relaxed way.

Giancarlo Migliore –
european Marketing Director, Leica Microsystems

More Information From Tissue Samples

HOPE fixation and laser microdissection open up new horizons for conventional and molecular pathology

Immunohistochemical and molecular-pathological examinations are becoming more and more popular among today’s pathologists for diagnosing diseases. By analysing antigenic structures or nucleic acids, tumour tissue, for instance, can be unambiguously characterised and differentiated. However, tissue samples preserved with the usual morphologically oriented formalin fixation are not suitable, as formalin masks epitopes and also degrades nucleic acids. This makes antibody staining or reproducible RNA/DNA analysis with the PCR and the RT-PCR (Reverse Transcriptase-PCR) technique problematic and sometimes even impossible.

Formalin-free fixation preserves proteins and nucleic acids

In cooperation with Dr. Jürgen Olert from Mainz University Hospital and Prof. Ekkehard Vollmer from Research Center Borstel, Dr. Klaus Hermann Wiedorn, head of the department of Molecular Pathology at the Institute of Pathology at the Klinikum Stuttgart, Germany has developed an alternative to formalin fixation. Now patented, this new method called HOPE fixation (Hepes glutamic acid buffer mediated Organic solvent Protection Effect) yields excellent results for special examinations, as proteins are preserved practically in their native state and DNA/RNA are much better...
preserved than with the formalin fixation, therefore allowing the extraction of high molecular weight nucleic acids applicable for downstream applications like PCR and RT-PCR. The HOPE method is equally suitable for routine pathology, as the sample morphology is just as good as that of conventional fixation methods. With HOPE fixation, the sample is incubated in a formaldehyde-free solution and then preserved by “gentle drying.”

**Homogeneous analysis material**

As well as suitably fixed tissue sections, reproducible PCR gene expression analysis results also require homogeneous analysis material so that RNA data can be clearly referred to cell type. For this reason, Dr. Wiedorn uses a Leica LMD600 Laser Microdissection system, which allows selective isolation of cells.

The diode laser integrated in the microscope enables fast and highly precise selection of extremely small areas of a sample. The required cells can be labelled and cut out on screen in a matter of seconds. The material falls into a collection device for subsequent extraction and PCR analysis.
Obtaining cell-specific analysis material with the laser scalpel

Easy-to-use laser microdissection system Leica LMD6000

The origin and course of many diseases depends on specific genetic activities and interactions of proteins in single or few cells. For unambiguous and reliable diagnoses, it is therefore necessary to isolate cell populations selectively to be able to obtain material for cell-specific analysis of RNA, DNA or proteins in the required quantity and quality.

With Leica’s easy-to-use laser microdissection system it is possible to select and cut extremely small areas (even single cells) from tissue sections. A pulsed laser beam is focused through the objective of an upright microscope onto the sample, destroying the tissue in the focus.

A series of many pulses creates the desired cutting line. The laser hits the sample only for extremely short intervals of less than 1 ns, so that there is absolutely no build-up of heat within the surrounding area.

Besides the laser and the control software for selecting the dissectates, the upright microscope has been equipped with a special stage accommodating three specimen slides and the corresponding collection devices. Laser microdissection samples are normally prepared on a membrane, so that they can be easily and effectively removed – the cut areas fall into a collection device underneath the stage simply due to the force of gravity.
The recent development of an automated tissue embedding system specifically adapted for HOPE fixation is a further important step on the way to using HOPE for high-throughput applications in everyday routine.

More information on HOPE fixation: k.wiedorn@katharinenhospital.de

HOPE fixation yields excellent results for special examinations, as proteins are preserved practically in their native state and DNA/RNA structures remain highly molecular.

The Leica LMD6000 also dissects probes directly from the glass slides, e.g. from older archive specimens.

The powerful diode laser is ideal for fast and highly precise dissection. In the Leica LMD6000, the cutting movement is not controlled via the stage, but via special prisms. Precision therefore increases in direct proportion to the objective magnification. For an objective with 63x magnification, a cut width of about 1 µm is attained, for a dry objective with 150x magnification the width of the cut is even less than 1 µm.

More information on Leica LMD6000: LMD@leica-microsystems.com
Excellent Diagnostics with a New Degree of Freedom

New network imaging solution Leica DMD108

For more efficient microscopic diagnostics Leica Microsystems is continuously developing new imaging techniques and timesaving solutions. The new network imaging solution Leica DMD108 addresses this growing workload in today’s busy laboratory. It reduces physical discomfort and speeds daily workflow with its modern solution for image and data sharing.

Brilliant images directly on display

The user can see high-resolution and colour true images directly on a monitor without having to look through eyepieces. The superb image quality rivals that of conventional microscopy thanks to an integrated digital camera and a custom designed hardware and software embedded imaging system. Colour temperature remains constant even when the Leica DMD108 automatically adjusts light intensity according to the objective in use. The integrated colour management system adapts to individual staining methods.

New level of comfort and easy quantification for diagnosis

At the push of a button or of the footswitch, the Leica DMD108 changes magnification and adjusts to the optimal aperture diaphragm and light intensity.

Users’ opinions of the Leica DMD108

Innovative presentation and communication tools facilitate tumour diagnosis

PD Dr. med. Jens Uwe Alles, Manager of the Institute for Pathology and Cytology, Wetzlar, Germany

“My colleagues and I are particularly pleased with the convenient discussion and presentation facilities of the Leica DMD108 as well as the network solution for direct communication.

We discuss cases which are difficult to interpret – usually tumour specimens – every day. The excellent screen presentation is extremely helpful for our assessment. Thanks to the beamer port, we can also use the microscope for discussions in larger groups, which is something we will be doing more often in future. The medical sector is being increasingly organised in specialised centres, and this demands intensive exchange of information. Our institute, for example, is a nationwide joint practice with locations at three hospitals. I therefore often send images by e-mail via the network link of the Leica DMD108 when I want to discuss a case within the practice or with an external pathologist.

My daily work as a pathologist is made much easier by the flexible documentation and image processing provided by the Leica DMD108. I am able to take photographs at great speed and annotate the images with all the relevant information. Every week we have a presentation connected with the breast cancer screening; using the Leica DMD108 I can produce images suitable for presentation in no time at all. For assessing tissue sections I like to use the...
The intelligent design allows a very comfortable body posture. Images can instantly be saved – even during examination – and compared with other saved images in split-screen mode. Quantification of structure sizes, measurement of distances and areas are also very easy. The macro navigation function shows the location of the image portion being viewed.

**Direct communication with colleagues**

Diagnoses can be audio-recorded with an integrated microphone and all data connected can then be transferred to the local network. Whether sharing images, comments or diagnostic information, the Leica DMD108 connects the user with colleagues via LAN, USB or integrated e-mail. The system offers an additional digital output for a second monitor or digital projector to discuss cases.

More information on Leica DMD108: Peter.Schmitt@leica-microsystems.com

precise measurement feature. For example, I measure the thickness of a melanoma and add markers of different colours to document key information together with the image.

The integration of computer and camera makes the Leica DMD108 compact and easy to handle. Last but not least, an important criterion influencing our decision to buy the product was its outstanding price-performance ratio. We plan to buy another one soon.”

Dr. Mauro Truini, Director of the Advanced Technology Diagnostic Department, IST National Cancer Institute, Genoa, Italy

“The Leica DMD108 is an ideal instrument for discussion and presentation of cases. We use it a lot for training of students in oncology and pathology. The instrument is also utilised to assess relevant morphological characteristics in malignant tumour tissue which are difficult to interpret and are therefore discussed by several experts together. The excellent screen presentation is extremely helpful for this application.

The Leica DMD108 is fast and easy to use. The system’s ergonomical design makes viewing and processing the images a real pleasure. The results we get on the screen are outstanding whatever the magnification. Another good feature is the macro overview with its crosshair that shows where we are in the specimen. The user-friendly software offers further helpful functions, such as the quantitative measurement and the direct comparison of live images with stored photos.

With the expected integration in our LAN and LIMS system, I am also looking forward to using the Leica DMD108 more in our diagnostic routine and to exchanging opinions, as I believe it can significantly improve the overall workflow and our daily personal work.”
Improving Laboratory Workflow

Good results with automated printing systems integrated in LIMS

Situated in the Adige valley on the edge of the beautiful Dolomite region, the histopathology lab of the Santa Chiara Hospital, Trento, Italy, directed by Professor Paolo Dalla Palma, performs all the laboratory diagnosis for the North part of the province of Trento, including all PAP TEST screening.

With over 60,000 cases and a responsibility towards other hospitals in the province as well, it was an important requirement to integrate an information system in the pathology lab. That is why, almost 10 years ago, Prof. Dalla Palma was among the first to adopt a specific LIMS (Laboratory Information Management System) for histopathology and cyto-diagnostic labs, which today is widely used in many Italian hospitals.

“Installing a LIMS meant re-organising the work process and defining procedures and standards, which enhanced the organisation of the work tre-

Enhanced safety and efficiency in laboratory processes

The automated printing systems Leica IPC and Leica IPS

Leica Microsystems has recently further enhanced its two special printers for clinical laboratories with new features: the cassette printer Leica IPC and the slide printer Leica IPS. The two printers perfectly respond to the increasing need to cope with increasing throughput requirements in histology and cytology while improving specimen safety and traceability. The systems use a patented ink which is resistant to solvents and other chemicals. The Leica IPS and IPC print alphanumeric characters, logos, mono- and bi-dimensional bar codes and even pictures directly on standard histology cassettes or slides with a printing field.

The Leica IPC is equipped with six magazines with a total capacity of 480 cassettes. Cassettes can be used with and without lid attached, thanks to the tiltable print angle. The Leica IPS works with three magazines with a total capacity of 450 slides. All magazines allow automatic management of different colours connected to different specimens and visual control of the specimen workflow in the lab. Both printers can be equipped with an integrated unload station that arranges the printed objects on ten individual trays, enabling more than 100 printed objects to be stored. Both systems work either with PC stand-alone software, or integrated in the local LIMS, where they are particularly easy to use due to the availability of Windows drivers and many functions. The printers enhance standardisation processes and slim down the laboratory workflow without losing the necessary flexibility to cope with the increasingly complex preparation techniques available for diagnosis.

Left: The Leica IPC for versatile printing of tissue cassettes, right: The Leica IPS imprints standard frosted microscope slides.
mendously,” Dr. Roberto Togni, one of the pathologists, explains. “The recent integration of the Leica IP C cassette printer in the LIMS has improved the process even further and brings the benefit of more software features, such as automatic direct printing of pre-defined quantities of bio-cassettes through simple input of tissue types, or preparation methods without reducing flexibility. With the increase of new diagnostic methods, flexibility is particularly important.”

Another colleague, Dr. Enzo Polla, remarks on the automatic selection from the printer’s loaders of appropriate and different colour cassettes. “This feature is linked to the existing colour coding adopted in the lab and also visible in the software, and it has speeded up the process considerably. This allows on-screen control of everything that’s happening in the lab.”

Perhaps the most interesting comment came from laboratory technician Claudio Beducci who performs the specimen preparation. “Initially I was very sceptical about the printer and thought there was nothing faster and more practical than a felt-tip pen in my hand. But now with the printer of Leica Microsystems it is much easier for everybody to follow the process, and we have been able to avoid many mistakes.” One result that is even more important for him: he feels more relaxed and safe in doing his job.

Claudio Peducci, Dr. Enzo Polla and Professor Paolo Dalla Palma (left to right) from Santa Chiara Hospital, Trento, Italy, are pleased with the Leica IP C cassette printer integrated in the Laboratory Information Management System.

Contest

Your Opinion is Valuable!

Win an iPod nano Red Special Edition* or other nice prizes.

Dear Reader,

Please give us your comments on this European Pathology & Diagnostics Edition of reSOLUTION magazine. Send us your complete name and address of the institute where you work, along with your comments by going to the following link by 31 August, 2007:

www.leica-microsystems.com/EU-Pathology

Winners will be drawn from all completed entries.

* Apple contributes $10 from the sale of each iPod nano RED to the Global Fund to help fight HIV/AIDS in Africa.
Reduced Infection Risk

UVC disinfection effective for destroying clinically relevant pathogens

Chemical disinfectants are not only toxic for the user but are also awkward to use and dispose of. Leica Microsystems has therefore developed cryostats with UVC disinfection to enhance safety and efficiency when cutting cryosections. The method is simple to use and requires no warming of the cryochamber. To prove the efficiency of UVC radiation, Dr. Ingo Maier, ecoscope, Laboratory for Microbiology and Ecotoxicology (Amtzell, Germany) carried out test series with Leica cryostats. The results confirm the germicidal effect of UVC disinfection.

Disinfection after 30 to 40 minutes

As there is no worldwide standardised testing method, germs commonly used to prove the efficiency of chemical disinfectants were used as “biodosimeters”. In the first test series, ecoscope tested the effect of UVC on the standard test germ Staphylococcus aureus ATCC 6538. Thirty drops of in total 0.5 ml test suspension were symmetrically applied to stainless steel plates and surface-dried. These plates were placed in four different positions (Fig. 1) inside the cryochamber and irradiated at a temperature of –20°C. The bacteria suspension used was adjusted to a density of about 10⁹ colony forming units per millilitre. A retrieval rate of 70% was established without UVC disinfection after preparation, freezing and retrieval. According to the European DIN eN 1040 standard for chemical disinfectant suspension testing, a germicidal reduction by at least 99.999% is required. This value was achieved in the Leica CM1850 UV after 30 minutes of radiation, and in the Leica CM1900 UV after 40 minutes.

Cryostats for routine histology and clinical pathology

Leica CM1850 UV and Leica CM1900 UV

Reproducible results in just a few minutes – it’s time that counts in today’s histology and pathology labs. To be able to prepare samples efficiently it’s essential to have a high-performance cryostat. Leica CM1850 UV and CM1900 UV cryomicrotomes are ideal for ultra fast freezing and cutting of fresh tissue, are easy to use and incorporate state-of-the-art safety features. The integrated UVC irradiation for efficient surface disinfection in the cryochamber together with the user’s personal protection provides added safety when dealing with infectious samples. To be completely on the safe side, smooth surfaces in the cryochamber can additionally be sprayed with commercial disinfectant. The slot cover of the splash-protected microtome keeps infectious material out of the casing.

AgProtect™ coating

The Leica CM1850 UV offers the user even greater protection due to the patented antibacterial AgProtect™ coating of relevant outer surfaces. Silver (AG) ions inhibit the growth of bacteria, effectively reducing the spread of germs on the microtome.

Key features of the Leica CM1850 UV:

• UVC disinfection with safety sensors
• AgProtect™ coating
• Splash-protected microtome
CRYOMICROTOMY

The original virus titre had an average value of around $10^{-6.8}$/ml. The retrieval rate determined in order to calculate the virus titre reduction afterwards was practically 100%. After an irradiation time of 95 minutes in Position B (Fig. 1), the titre decreased by 99.99%.

Taking other test positions as well as a safety factor into consideration, ecoscope recommends an irradiation time of 180 minutes for the Leica CM1850 uV and 240 minutes for the Leica CM1900 uV in order to achieve high-level disinfection at which all microorganisms are destroyed. A comparison with UVC doses for germ reduction from scientific literature (Tab. 1) indicates the disinfection efficiency of the UVC lamp in Leica cryostats. In the case of the Hepatitis B Virus (HBV), for which no measured data is currently available, size or structure of genome allows conclusions to be drawn on the UVC sensitivity. Like SV 40, HBV is an extremely small dsDNA virus that is likely to be destroyed by similar UVC doses.

Tab. 1: UVC dose values for the disinfection of microorganisms and viruses by 5 log₁₀-steps (99.999%) for bacteria and fungi and 4 log₁₀-steps for viruses (99.99%).

<table>
<thead>
<tr>
<th>Species</th>
<th>Examples</th>
<th>UVC dose (mWs cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Staphylococcus aureus</td>
<td>10–28</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>3–48</td>
</tr>
<tr>
<td></td>
<td>Mycobacterium tuberculosis</td>
<td></td>
</tr>
<tr>
<td>Bacterial Spores</td>
<td>Bacillus ssp</td>
<td>36–371</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Candida albicans</td>
<td>22–177</td>
</tr>
<tr>
<td></td>
<td>Cryptococcus neoformans</td>
<td></td>
</tr>
<tr>
<td>Mold Spores</td>
<td>Aspergillus fumigatus</td>
<td>14–550</td>
</tr>
<tr>
<td>UVC-sensitive</td>
<td>Herpesviridae</td>
<td>13–58</td>
</tr>
<tr>
<td>Viruses</td>
<td>Influenza A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poliovirus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vaccinia</td>
<td></td>
</tr>
<tr>
<td>UVS-resistant</td>
<td>Simian Virus 40</td>
<td>440</td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoviridae,</td>
<td>Coronavirus</td>
<td>31–240</td>
</tr>
<tr>
<td>Retroviridae,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reoviridae,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

More information on the Laboratory for Microbiology and Ecotoxicology: Ingo.Maier@ecoscope.de

Separate specimen temperature control

Users who work with changing specimen temperatures will appreciate the Leica CM1900 UV. Two separate refrigerating systems for cryochamber and specimen head allow independent control of the specimen freezing. The specimen temperature can be set quickly and easily in increments of 1 K between −10°C and −50°C without time-consuming alteration of the chamber temperature.

Key features of the Leica CM1900 UV:

- UVC disinfection with safety sensors
- Splash-protected microtome
- Optional specimen orientation
- Disposable blade holder with optimised anti-roll guide
- Chamber temperature down to −35°C
- Quick-freeze shelf down to −45°C
- Separate specimen temperature control down to −50°C
- Separate manual defrost cycle for chamber and specimen head
- Automatic hot gas defrost cycle
- Removable, heatable sliding window
- Ergonomic design and self-explanatory control panel

More information on Leica CM1850 UV and CM1900 UV: Claudia.Dorenkamp@leica-microsystems.com

The Leica CM1900 UV rapid sectioning cryostat: Extremely rapid freezing through a quick freezing shelf for up to ten specimens and independent specimen cooling for brief and frequent temperature changes.
Pathology & Diagnostics Events

Please also visit our website www.leica-microsystems.com/EU-Pathology for information on Leica workshops in Europe

Sociedade Portuguesa de Anatomia Patologica (SPAP) 2007
May 3–5, Coimbra, Portugal

XXX Congreso de la Sociedad Española de Anatomia Patológica y Academia Internacional de Patología 2007 (SEAP)
May 16–19, Tarragona, Spain

Pathology Meeting 2007
May 18–20, Gothenburg, Sweden

Assises de Pathologie 2007
May 24–25, Bordeaux, France

Jahrestagung der Deutschen Gesellschaft für Pathologie 2007
May 30 – June 1, Magdeburg, Germany

IX Congresso Técnico de Anatomia Patológica
June 1–3, Porto, Portugal

ESHRE 2007
July 1–4, Lyon, France

Themacongres VAP 2007
July 2, Utrecht, Netherlands

British Pathology Society
July 3–4, Glasgow, Scotland, United Kingdom

21st European Congress of Pathology (ECP) 2007
September 8–13, Istanbul, Turkey

British Society of Clinical Cytology (BSCC) 2007
September 17–19, Cardiff, United Kingdom

II Congreso Internacional IVI 2007
September 19–21, Barcelona, Spain

Scanlab 2007
September 24–26, Stockholm, Sweden

IBMS 2007
September 24–26, Birmingham, United Kingdom

ILMAC 2007
September 25–28, Basle, Switzerland

Symposium of the Society for Histochemistry 2007
September 26–29, Freiburg, Germany

IV Congresso Nazionale SIAPEC
October 5–9, Milano, Italy

Morphologie-Histologie Tage 2007
October 12–13, Kassel, Germany

33rd European Congress of Cytology (ECC) 2007
October 14–17, Madrid, Spain

Midland CELLibration 2007
October 31, Midland, United Kingdom

Jahresversammlung der Schweizer Gesellschaft für Pathologie 2007
November 8–11, Winterthur, Switzerland

Medica 2007
November 14–17, Düsseldorf, Germany

Carrefour de Pathologie 2007
November 19–23, Paris, France

IV Congreso ASEBIR 2007
November 21–23, Bilbao, Spain