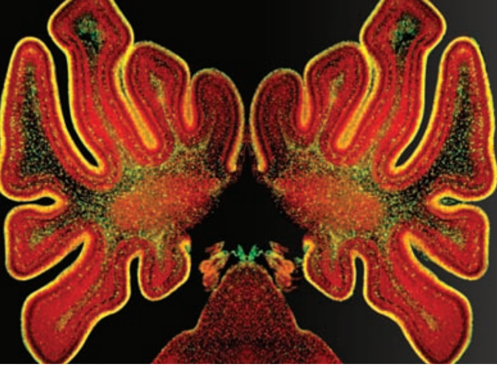

microScience Imaging

June 2006 No 1

Leica MICROSYSTEMS

Welcome Life Scientists!

microScience Imaging is designed to be a resource for those conducting research in the life sciences. High-quality images have become central to documenting cellular events and invaluable in conveying information in grants and publications.

This bi-monthly eNewsletter will cover general topics as they relate to imaging along with applications specific to Developmental Biology, Neuroscience, Protein Crystallography, Cell Biology, Entomology, and more. Leica will strive to keep you up-to-date on exciting new technologies in the field of imaging. Educational opportunities will be covered along with answers to your imaging questions. Our goal is to relay the most comprehensive imaging news to you in the most concise format. If you find this resource valuable to your science, please feel free share this information with a friend.

We value your feedback! If you have any questions or comments, please contact: microscience.imaging@leica-microsystems.com.

With my best regards,

Robert Wick, Ph.D.
VP, Microscopy

Epigenome Research Assisted by Laser Microdissection

By Christopher Vega, Ph.D., *Leica Product Manager*

Although the Human Genome Project has identified the genes (and their nucleotide sequences) within human DNA, the epigenetic regulation of human genes is still not fully understood. During an AACR workshop held in June 2005, leading cancer researchers developed a strategy for investigation into the epigenome. “A Blueprint for the Human Epigenome Project,” published in the December 2005 issue of *Cancer Research*, describes their goals (*Jones & Martienssen, 2005*). In summary, the researchers sought to understand the relationships between chemical epigenetic changes and pathological states, including cancer.

Epigenetics refers to the study of reversible modifications that result in the activation or silencing of a gene. Typically, these modifications take the form of nucleotide methylation, specifically cytosine of cytosine/guanine dinucleotide pairs (CpG) (Reviews: *Warnecke & Bestor, 2000*). Modifications of the histone core proteins that package DNA in the form of chromatin, also effect epigenetic regulation of the cells (*Fraga & Esteller, 2005*).

As such, each cell type has unique epigenetic modifications across its genome, which maintain the delicate balance of homeostasis. Genes with unmethylated CpG are more accessible to transcription. Methylation of CpG can result in DNA silencing; however, excessive methylation of a cell’s repair and tumor suppressor genes has been correlated with cancer. Hypomethylation can result in chromosomal instability and allow the activation of oncogenes (*Feinberg, 2004*).

Epigenetic factors are heritable, and DNA methylation can fluctuate due to changes in diet and environment. For example, deficiencies in folate and methionine metabolism have been linked to several cancers, e.g., colorectal cancer (*Kim, 2005*). Additionally, a variety of environmental pollutants have been found to lead to epigenetic changes (*Sutherland & Costa, 2003*).

The epigenome provides a layer of transcription regulation over the genome. Understanding the relationship between epige-

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Epigenome Research

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nomic variations and pathological states, i.e., cancer, presents a challenge to both researchers and clinicians. However, epigenomic methods may provide novel diagnostic and treatment possibilities for a variety of disorders in the future (*Rodinhiser & Mann, 2006*).

As epigenetic research relies heavily on molecular techniques to quantify the changes in CpG methylation and histone acetylation, tools which improve isolation specificity of cells or tissues allow researchers to perform unambiguous downstream analysis of epigenetic variations. One of these tools is laser microdissection (*Becette et al., 2004*).

Laser microdissection employs standard microscopic techniques to visualize changes in nuclear morphology or to localize epigenetic markers with immunohistochemistry or FISH (Chaumeil et al., 2004). Specific cells or tissues can be dissected via a precisely steered laser beam and collected in a reaction vessel for molecular analysis. Automated cell recognition software (optional) detects and dissects samples with minimal human interaction for higher laboratory throughput. The Leica LMD6000 was designed as a tool to address research in this area and proves to be a valuable addition to many labs conducting highly specific molecular research.

Upcoming Events

Visit Leica Microsystems at the following exhibitions:

- Zebrafish Development and Genetics, Madison, WI (**June 14**)
- Developmental Biology, Ann Arbor, MI Booth #808 (**June 17**)
- Inter/Micro Chicago, IL (**July 11**)

For more events:
http://www.leica-microsystems.us
(click on Company, then Events)

Open Forum

How do I clean my compound microscope objectives?

The first step is always to look closely at the objective to assess how badly grease, oil or resins are built up. The easiest way to do this is to remove the objective from the microscope and use the microscope eyepiece upside down as a magnifying glass to examine the objective.

To clean, first remove all dust from the lens with canned air. This will prevent the dust from scratching the objective and causing damage.If residue remains on the lens, first try cleaning with distilled water and a Q-tip®, moving the Q-tip in a circular motion from the inside of the lens out. If this does not remove the residue, we have had good results using lighter fluid, again rubbing in a circular motion with a Q-tip around the lens. After using lighter fluid, clean off any excess residue with distilled water. Now enjoy crisp, clean imaging!

We welcome your questions! Submit a question to the Open Forum section and receive a FREE Leica Lens Cleaning Kit! If your question is chosen for use in a future issue, you also will receive a **$500 Leica Product Voucher** on your next purchase!

For You

Special product offers currently available from Leica Microsystems:

New Investigator Program
Starting up a new lab? Beginning a new research project? Contact your local Leica Microsystems Sales Executive to see if you qualify for thousands in discount!

FREE Fluorescence Illuminator Promotion
Purchase a completely configured upright research, inverted research, or stereomicroscope with fluorescence (DM5000 B, DMI4000 B, or MZ16 F models) and receive a FREE Leica EL6000 fluorescence illuminator.

FREE Modulation Contrast Upgrade
Purchase a Leica DM IL inverted tissue culture microscope and receive a FREE upgrade to Integrated Modulation Contrast (IMC), which provides relief contrast with plastic specimen holders such as Petri dishes.

Demonstration Equipment 40% Off
Contact your local Leica Sales Executive to find out about certain microscope accessories that are available from our demonstration stock at a 40% discount.

Technology Fast Track

4Pi Confocal Elucidates HbC's Fight Against Malaria

by Lon Nelson, *Leica Marketing Manager*

Researchers from NIAID and NIST investigating the effect of Plasmodium falciparum malaria on red blood cells (erythrocytes) may have found a mutated hemoglobin form which provides in vivo protection against this disease (*Tokumasu et al., 2005*). The mutated Hemoglobin C (HbC) differs from normal Hemoglobin (HbA) in that it is more susceptible to accelerated oxidation and removal from the body. Tokumasu, et al. hypothesize that malaria infection causes mutated HbC erythrocytes to undergo a change in the distribution of surface membrane molecules, or 'band 3' molecules. The clustering of band 3 molecules may enhance the autoantibody response against these erythrocytes, thereby starting a cascade where these red blood cells are destroyed and removed from the system.

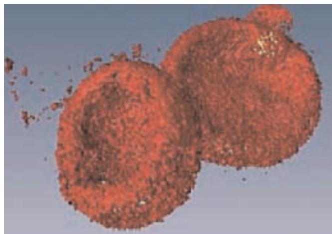


Figure 1: Parasitology: Human red blood cell – healthy and malaria infected – early stage.

During this study, for the first time researchers were able to visualize band 3 molecular aggregation using the new Leica 4Pi confocal microscope. Since these aggregates are well beyond the resolution of a traditional confocal microscope, the 110 nanometer maximum resolution of the 4Pi system was ideal for this experiment. The 3-D rendering (Figure 1) of the erythrocytes (8 micron diameter) provides more information than ever before possible.

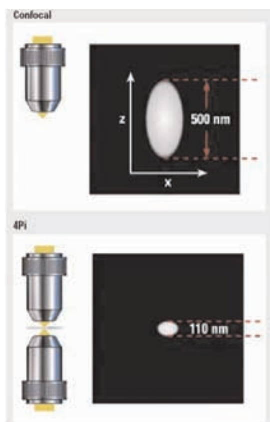


Figure 2: Z axis resolution of confocal vs 4Pi microscopes.

The Leica 4Pi innovation won the 2005 German Business Innovation Award; the third time that Leica Microsystems has been thus honored. The 4Pi concept, devised by Prof. Stefan Hell, Director of the Max Planck Institute of Biophysical Chemistry, consists of two high numerical aperture opposing objectives (Figure 2). This design provides the maximum lateral resolution of any system currently available.

For more technical information on Leica's 4Pi system, visit: **http://www.leica-microsystems.com**

For the entire article, visit:

http://jcs.biologists.org/cgi/reprint/118/5/1091?gca=118%2F5%2F1091&sendit=Get+All+Checked+Abstract%28s%29&

The Private Eye

CCD vs. CMOS Technology for Digital Imaging

by Rob Kimura, *Leica Product Manager*

There are currently two leading image sensor technologies used for digital imaging in microscopy; CCD (Charged Coupled Device) and CMOS (Complimentary Metal-Oxide Semiconductor). Each technology offers unique performance features that tailor them to specific applications. The basic concept is the same for either technology, detecting and collecting light (photons), then turning that detection into electrons. Once converted to electrons, data is transferred to the computer in the form of electrons per pixel, and finally, the computer software interprets this data and outputs an image.

CCD sensors offer greater performance for low-light applications such as fluorescence and live cell imaging. A CCD reads out its pixels starting at one corner of the sensor and scans the entire length of the sensor. An analog-to-digital (A/D) converter then translates the charge values into a digital value. This is not a comparatively fast process, but does yield very good sensitivity. CCD sensors are designed with tiny microlenses that funnel photons into each pixel, similar to using a funnel to collect water. This microlens technology, along with the chip readout process, helps collect every possible photon. This shortens exposure times, and thus, reduces (electronic) image noise and improves captured pictures.

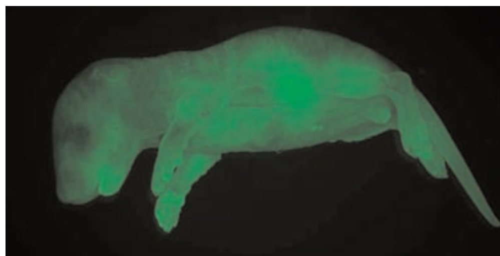
CMOS sensors offer great benefits in frame-rate speed and data transfer for quick acquisitions. Therefore, CMOS technology is perfectly matched for high-speed time-lapse, digital video recording, and general brightfield applications. Instead of scanning across the entire sensor, each pixel is read out at the same time in CMOS chips. Every pixel on this type of sensor has several transistors, which amplify the charge collected by the pixel before the A/D conversion takes place and data is sent to the computer. Although this design greatly increases the speed readout of the sensor, the use of these sensors does invite some electrical noise which would not be welcome in low-light applications. In fact, the speed output is so fast that there are cameras in existence that can acquire up to 500fps (frames per second).

Generally speaking, CCD sensors are best for low-light applications where every photon counts, and CMOS sensors are best for high frame-rates where the amount of light is not a limiting factor. Leica offers a complete line of microscope-grade digital cameras using both of these technologies. Contact your local Leica Sales Executive to discuss your application and find out which technology would be best for your application.

Botox Opens New Door to Cancer Treatment

by Craig Wollschlager, *Leica Product Manager*

A study published in the February 15, 2006 issue of *Clinical Cancer Research* introduces the use of Botox to assist the effective delivery of chemotherapies and radiation to resistant tumors.



Transgenic mouse expressing green fluorescent protein (GFP). GFP is typically used to determine tumor size in mouse models. GFP filter, size of mouse: 2.5cm. Group of Dr. Daniel Metzger, Professor Pierre Chambon, and Imaging Centre of IGBMC.

Until recently, cancer research focused mainly on depriving tumors of oxygen and nutrients as a means of starving them to death, requiring increasingly heavier dosages of chemotherapy and radiation. This increase in toxic dosages has mirrored the increase in tumor resistance to these therapies, calling for more research into new anti-cancer drugs and more efficient methods of delivery.

Transgenic mouse models expressing GFP using long-pass filter. Samuel Lunenfeld Research Institute, University of Toronto.

Gallez, et al. found that by injecting Botulinum neurotoxin type A into tumors implanted in mouse thighs, the tumors' cellular vasculature opened, allowing chemotherapeutic drugs to enter and destroy the previously resistant cancer cells more effectively. Rather than constrict the blood vessels to starve the tumor, Botox briefly opens the blood vessels that feed the tumor to more effectively allow therapeutic agents and the oxygen necessary for effective radiation into the tumor. The combination of Botox and the chemotherapeutic agent cyclophosphamide showed substantial increases in tumor oxygenation and perfusion and, more importantly, stunted tumor growth after just three days.

Researchers used MRI and Patent Blue staining to obtain pixel-by-pixel perfusion maps, providing data on tumor growth or shrinkage in this study. Another effective and accepted means of visualizing tumor growth is via fluorescence protein labeling and subsequent fluorescent imaging. Fluorescence imaging offers numerous advantages over other detection methods. Fluorescent stains and dyes, such as green fluorescent protein (GFP), are far more sensitive than colorimetric methods. Recently, fluorescent imaging of mice, rats, and other larger model organisms became even easier.

The Leica MacroFluo. A Macroscopic Fluorescence system combines the features of long free working distance and a large field of view with a high-resolution vertical imaging path, which benefits the imaging of larger specimens like never before possible. Not only can researchers image tumor fluorescence expression in multiple adult mice simultaneously, but they can then zoom in (16:1 optical zoom) to capture ultra-high resolution (up to 1.4 micron). The MacroFluo is one of the most versatile tools for fluorescent imaging in cancer research, serving not only as an optical microscope, but as a large field imaging workstation.

References: Gallez B, et al. *Botulinum Toxin* Potentiates Cancer Radiotherapy and Chemotherapy. *Clinical Cancer Research* 2006; 12(4): 1276-83.

Upcoming Science Courses in 2006:

Marine Biological Lab:

- Neural Systems and Behavior (**June 10-August 6**)
- Neural Development & Genetics of Zebrafish (**Aug. 13-26**)

http://www.mbl.edu

Cold Spring Harbor:

- Molecular Embryology of the Mouse (**June 7-27**)
- Neurobiology of Drosophila (**June 30-July 20**)

http://meetings.cshl.edu/courses.html

Mount Desert Island Biological Laboratory:

- Quantitative Fluorescent Microscopy (**June 3-10**)
- Stem Cell Symposium (**Aug. 11-13**)

http://www.mdibl.org/courses

The Jackson Laboratory

- Frontiers in Microscopy (**July 5-8**)
- The Second International Human ES Cell Workshop (**Aug. 3-6**)

http://www.jax.org/courses/events

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Note: *We are interested in your comments and thoughts about the newsletter. Please feel free to email your comments to:* ***microscience.imaging@leica-microsystems.com***