

microScience inSight

Issue #10 April 2009

The Objective



Dr. George Paxinos

Interview with Dr. George Paxinos

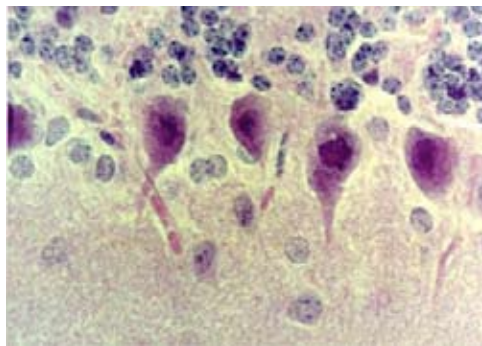
Dr. George Paxinos paved the way for future neuroscience research by being the first to produce an accurate 3-D stereotaxic atlas to guide placement of electrodes and injections in the brain of experimental animals. His atlases are now international standards. Click here to read a recent interview: [paxinosinterview.pdf](#)

To find out more about the products featured in this article, click here: www.myNeuroLab.com



Produce frozen sections of paraffin-quality

by Charles W. Scouten, *Innovation Manager – Biosystems Division, Leica Microsystems, Inc.*



The CryoJane Tape-Transfer Method

Cryotomy has changed very little since its inception in the early 1900's. In 1905, Dr. Wilson at the Mayo Clinic developed a protocol for freezing tissue, rather than hardening it with paraffin. Laboratories still use variations of this method today. The CryoJane Tape-Transfer System (developed by Instrumedics, Inc., now a Leica Microsystems company) has been called the most significant improvement in cryotomy since the development of the cryostat over 35 years ago. Adaptable to most cryostats, CryoJane enables the user to produce frozen sections of paraffin-quality by keeping a snap-frozen tissue section frozen until fixation. Conventional frozen sections are melted onto slides prior to fixation. Coupled with slow freezing, this uncontrolled melting is a key factor in the formation of ice crystal artifact. Other common artifacts such as compression, folds, and wrinkles also make serial frozen sectioning a frustrating task.

The CryoJane Tape-Transfer Method helps avoid these artifacts by utilizing cold adhesive tapes and slides. The cold adhesive tape is placed on the frozen block face, and it supports and adheres to the section as it is cut. The adhesive tape routinely captures sections without folds or wrinkles and allows the technician to transport sections to cold adhesive slides. This makes serial sectioning easy. Once the section is adhered to the slide, the slide is exposed to an 8ms pulse of long wave UV light (360nm). The UV exposure activates a photo catalyst in the slide's adhesive, which instantly converts to a hard, solvent resistant plastic. The still frozen section is now permanently bonded to the glass slide, preventing section loss even during challenging staining protocols. The tape is then peeled away; leaving the section still frozen and fully intact on the slide. The slide is immersed in a room temperature fixative inside the cryostat chamber. This allows the section to fix as it rehydrates; not melt and then fix as in conventional frozen sections. The technician can follow any protocol for processing the section. The cured adhesive on the slide was designed to be inert and is highly recommended for H&E, ISH, IHC, and other histological stains. This method is ideal for protocols such as Laser Capture Microdissection (LCM) because the technician can readily visualize and capture desired cells from the entire surface of the flat section.



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Using cryo-preparation methods to help solve cell biology questions

by Kim Rensing Ph.D., *Application Specialist, Leica Microsystems, Inc.*

The production of wood cells in trees involves a number of steps that result in a connected series of hollow tubes, which move water from root to leaf for cellular metabolism. The first step is the creation of new cells in the cambium, a tissue that surrounds the stem just beneath the bark. The internal cells of the cambium retain their initial characteristics and continue to produce new cells through cell division, while the cells to the inside of the cambium expand and produce elaborate cell walls.

On maturity these highly-elongated cells go through programmed cell death, leaving functional xylem (wood), which is predominantly composed of cellulose and lignin. Together these two polymers create the strength and durability of timber, the primary source of building material in North America. When lignin is removed from wood, cellulose remains, which is the base material for many paper-related products. Considering the importance of this natural resource, scientists are only just beginning to understand the underlying cellular processes. Studies have shown that gene expression, its products, and other regulatory biochemicals vary across the different tissues in plant stems. However, to make valid observations about the effects of such products at the cellular and sub-cellular level, advanced cryo-preparation techniques are necessary.

Ultrastructure

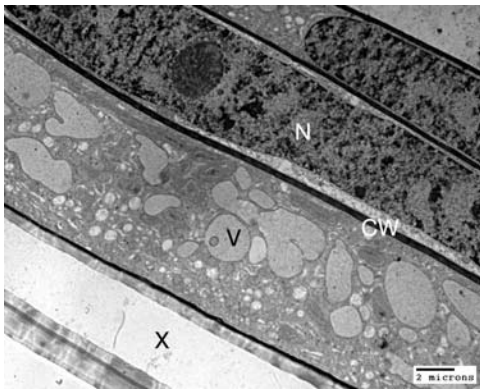
Cambial and immature xylem cells are extremely delicate and highly susceptible to damage during standard chemical processing for EM. Live cell studies from the early 1900's showed that cell structure changes dramatically between active summer and dormant winter periods. During the growing season, a huge aqueous vacuole confines the cytoplasm to a very thin peripheral layer, but during dormancy, the cells contain generally-dispersed cytoplasm, which surrounds many smaller vacuoles.

TEM of chemically-fixed tissues confirmed the annual alternations in cellular structure, but "new" structures were also observed, particularly in the dormant stage. Membranes were highly in-folded with a gap between the outer membrane and the cell wall. Scientists speculated as to the function of these structures, but high pressure freezing and freeze substitution of the cambial cells demonstrated that these structures were actually artifacts. Chemical fixation causes delicate small vacuoles to fuse with the plasma membrane. In reality, the cell membrane always stays tightly associated with the cell wall (1).

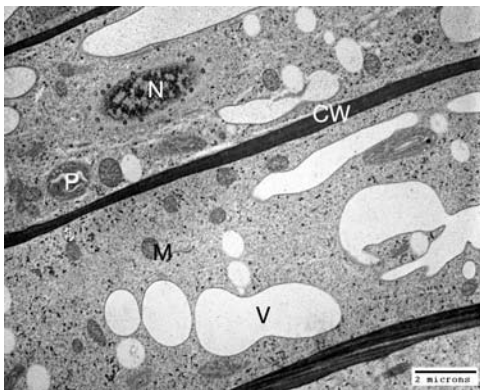
This was the same situation in studies of dividing cells. The tiny vesicles which fuse into a membranous sheet that becomes the dividing cell plate, were randomly joined by chemical fixation. The real stages of vesicle fusion during cell division are now well established from high pressure frozen and freeze substituted samples (2).

Immunogold and enzyme-gold localization

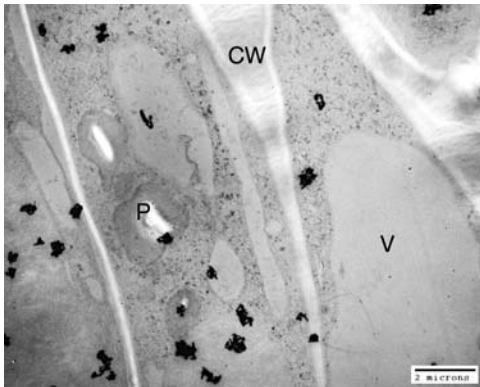
Room temperature processing for immunolocalization relies on the incomplete fixation of formaldehyde. This fixative has less effect on protein conformation than the more commonly used glutaraldehyde, but results in poor ultrastructural preservation. Post-fixation with osmium tetroxide usually destroys all protein reactivity in the tissues, so this step is generally omitted, which makes preservation even worse. Immunolocalization of proteins at the TEM level (immunogold labeling) has thereby been a compromise between adequate labeling and adequate structural preservation. In comparison, high pressure frozen samples can be substituted while



Dormant cambium. Large nuclei and a great number of small vacuoles and vesicles dominate these cells. There are no gaps between the cytoplasm and the cell walls. CW – cell wall, N – nucleus, V – vacuole, X – xylem



Cambial cells just prior to resumption of seasonal activity. Smaller vacuoles coalesce and vesicles disappear. Cytoplasm is still against the cell walls. CW – cell wall, M – mitochondria, N – nucleus, P – plastid, V – vacuole



Active cambial cells labeled by autoradiography for ³H-phenylalanine. The black irregular shapes are silver grains at the sites of radioactive decay events. Large vacuoles are predominant and organelles such as plastids are readily observed. CW – cell wall, V – vacuole

continued on page 3

frozen, to remove cellular water without the use of chemical fixatives such as glutaraldehyde or osmium tetroxide. If high molecular weight compounds like uranyl acetate are added to the substitution medium, the ultrastructure preservation is similar or better than chemical fixation with glutaraldehyde and osmium tetroxide, yet there is often a higher level of antigenicity in the target molecules. This method was successfully used to locate molecules involved in activating lignin precursors in the developing cell walls. Using these preparation methods, it was even possible to detect active enzymes by incubating prepared tissue sections with their colloidal gold-linked substrates. Specific cellulose enzymes have been situated using such immuno-enzyme assays (3).

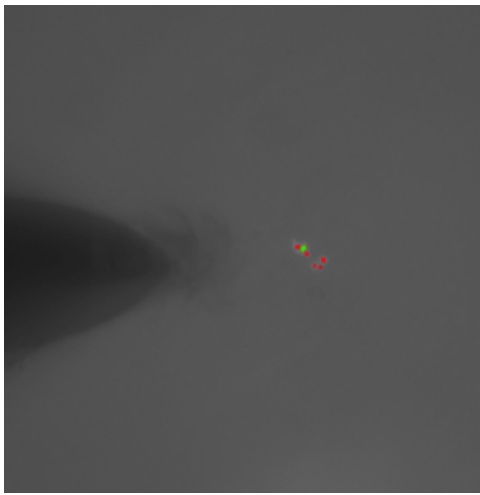
Autoradiography

Radioactive compounds can be fed to live tissues and to any downstream products localized by autoradiography. Following standard preparation techniques, sections are coated with a thin photographic emulsion. Radioactive decay causes deposition of silver grains from the emulsion at the site of the emission, and these can be observed by both light and electron microscopy. Early autoradiography studies using radio-labeled lignin precursors in chemically-fixed wood were inconclusive. During chemical fixation, membrane vesiculation and solute migration retard precursors. High pressure freezing immobilizes cellular constituents in milliseconds, and subsequent freeze substitution keeps these constituents in place while cellular water is substituted with a solvent such as acetone. Embedding in plastic resins requires changing the solvent for a liquid resin that is subsequently polymerized. However, room temperature resin infiltration still results in solute migration and extraction. Alternatively, samples can be infiltrated with special acrylic resins at temperatures as low as -70°C. This was shown to eliminate the extraction of radio-labeled compounds that occur with room temperature resin infiltration. The results of these studies support membrane-based transporters rather than an organelle (Golgi) based mechanism, as was previously surmised for moving the precursors out of the cytoplasm to the cell wall where they are polymerized (4).

References

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- (4) Kaneda M, Rensing KH, Wong JCT, Banno B, Mansfield SD, and Samuels AL (2008) Tracking monilignols during wood development in *Pinus contorta* var. *latifolia*. *Plant Physiology* 147: 1750-1760.





A mixed-species bacterial community on tooth enamel. A micro-manipulator tip approaches from the left in preparation for capture of the cell cluster, which contains 3-4 cells of two different types as identified by red- and green-fluorescent quantum-dot-labelled antibody conjugates.

A cleaner mouth: understanding bacterial communities in biofilms

by Dr. Robert J. Palmer, Jr. *National Institute Dental Craniofacial Research – NIH*

Bacteria love community living. In fact, the majority of bacteria colonize into groups referred to as biofilms. In your mouth, these biofilms are known as dental plaque. In an effort to thrive in the oral cavity, bacterial communities come together through an intricate form of communication using specific lectin-like interactions. These interactions occur when a streptococcus with a lectin-like adhesion recognizes partner species' receptor polysaccharide (RPS). Through these interfaces, bacteria are able to recognize each other, colonize, and grow. In your mouth, a 3-species bacterial community is one of the building blocks for plaque. The secret, and the goal of the research discussed here, is to understand these building blocks to reduce or eliminate disease-causing dental plaque while encouraging the growth of "healthy" plaque.

In a recent publication in the *Journal of Bacteriology* (1), several difficulties in isolating these bacterial communities for further study were conquered. One approach to capturing the community is micromanipulation. The difficulties are:

- many of the different species are cocci and thus impossible to distinguish based on morphology alone
- that one might have to look at the sample for extended periods during manipulation
- that the substratum on which the bacteria sit (tooth enamel) is opaque and solid
- that the target is very small (a few bacteria together in a clump; imagine a circle roughly 10 micrometers in diameter)

The first difficulty was solved by using primary immunofluorescence and long pass filters to identify the different cell types. This allowed the research team to target communities of a particular combination of species. The second difficulty was solved by using Quantum Dots as the antibody-conjugated fluorors. These inorganic fluorors are very bright compared with even the brightest organic fluorors, and they are virtually impossible to bleach under routine conditions. Equally important is that, regardless of emission wavelength, the fluorors can all be excited by a single low-wavelength, i.e., 488nm, source.

Leica Microsystems provided the solution to difficulties three and four; micromanipulation with an upright microscope (certainly not the convention when considering micromanipulation) and a high NA water immersion "dipping" lens. The team used Leica Microsystems' 63x/0.9 NA water immersion objective with 2mm working distance. It has resolution sufficient to distinguish single bacterial cells. For the micromanipulation, steel manipulator tips with 25µm diameter, e.g., those from Minitool Inc., were employed. These proved essential because they are more resistant to breakage than glass tips during inevitable 'crashes' of the tip into the chip surface. Also, these tips are available formed into a variety of shapes such as shovels. During these experiments, the tips were held in twin manipulators, one on each side of the specimen, and inserted into the meniscus drawn up by the lens. Even with an anti-vibration platform (an inexpensive, yet very effective platform from Vistek Inc.), the meniscus can and does break, but it is typically stable for about 4 minutes. The bacterial colonies are very sticky and remain affixed to the tip even as it is withdrawn through the air-water interface of the meniscus.

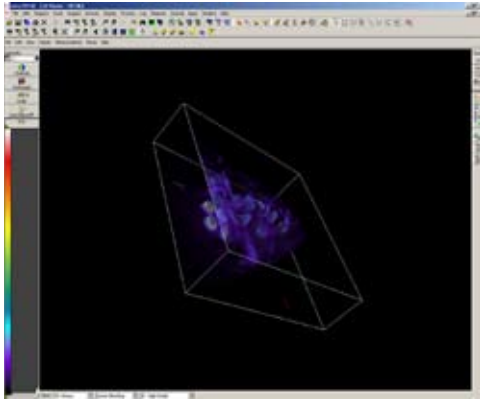
This hybrid micromanipulation system could be used to capture microbial communities from any substratum, and is applicable to a broad spectrum of biofilm research. In addition to the instrumentation described above, these investigators use a Leica Microsystems' confocal microscope for high-resolution imaging of the fluorescent bacteria colonies. In the end, we learned that RPS-mediated recognition between bacteria in the mouth sets up the formation of communities within dental plaque biofilms. This finding could lead to new approaches in fighting the battle against cavities, gingivitis, and periodontal diseases in your mouth.

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- (1) Chalmers N I, Palmer, Jr., R J, Cisar, J O, and Kolenbrander, P E (2008.) Characterization of a *Streptococcus* sp.-*Veillonella* sp. Community Micromanipulated from Dental Plaque. *J. Bacteriol.* 190: 8145-8154.



Technology Fast Track



Leica MM AF Drosophila 3D image

Extend your image processing, analysis, and reporting capabilities

Capturing pictures of cells, tissue sections, or whole model organisms requires versatility from the software that drives the imaging devices and analyzes the photos. Leica Microsystems has recently partnered with Molecular Devices (a division of MDS Analytical Technologies), the industry leader in imaging applications and developer of the Metamorph® line of imaging products. The result of this collaboration is Leica MM AF (Metamorph Advanced Fluorescence), a highly robust image acquisition and analysis package. It is a stand-alone system and an additional module that augments the currently available Leica LAS AF series of software products. The software is scriptable, so each experiment can be customized. Leica MM AF also vastly extends the image processing, analysis, and reporting capabilities that Leica Microsystems offers to research customers. To learn more, click here: [Leica MM AF](#)



Leica TCS SP5 X

Scientists call Leica TCS SP5 X supercontinuum confocal one of the best innovations of 2008

According to a panel of scientists, the Leica TCS SP5 X Supercontinuum Confocal Microscope is one of the most innovative products on the life science market. *The Scientist* magazine presented the ten winners – including the Leica TCS SP5 X, which was launched on the North American market February 2008. The confocal microscope integrates a white light laser that provides users with the full spectrum of wavelengths between 470 and 670 nm for simultaneously viewing of up to 8 fluorescence proteins, for example. To learn more, click here: [Best Innovation](#)



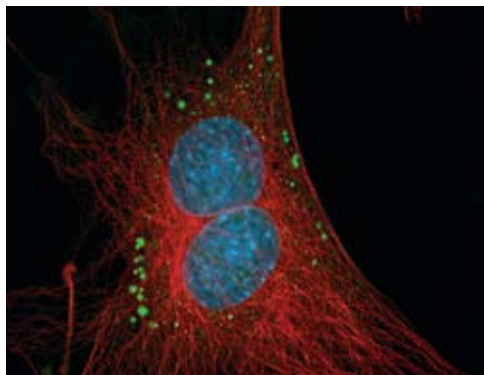
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Prize-winning technology: high resolution and high depth of field, simultaneously

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Leica Microsystems 2009 Confocal Microscopy Course Schedule

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The cost includes all materials, daily lunch, and one group dinner.

Essential Confocal Microscopy (\$945.00)	May 19 to 22 July 21 to 24 September 22 to 25
Advanced Confocal Microscopy (\$1,245.00)	April 7 to 10 November 10 to 13

For information or to register e-mail: lsr-applications@leica-microsystems.com

See Leica Microsystems, Nanotechnology at the following workshops:

Biomedical Research Equipment and Supplies Exhibit in Ft. Detrick, MD	April 29 to 30
Neurobiology Course at MBL	June 6 to August 9
UC Berkeley Cryo EM Course (hosted by Hitachi)	June 18 to 27
Microscopy and Microanalysis Sunday Workshop (details to follow)	August

See Leica Microsystems at the following Trade Shows:

Experimental Biology	April 18-22
American Association of Cancer Research, Denver, CO	April 19-22

Visit the following websites for life science courses supported by Leica Microsystems:

http://www.mbl.edu/education/courses/special_topics/aqlm.html
http://www.mbl.edu/education/courses/special_topics/om.html
<http://www.cbi.pitt.edu/qfm/index.html>
<http://www.3dcourse.ubc.ca/2009/public.php?page=pawley>



Ultimate Precision Trade-up Offer:

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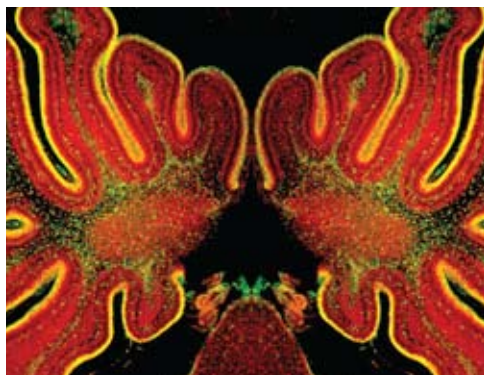
Leica Microsystems offers a 10% discount off the list price of the following products: Leica CM3050 S Cryostat, SM2000 R Microtome, and the VT Series Vibrating Blade Microtomes. This offer is valid on orders received until **April 30, 2009**.



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