The Leading Investigator

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A Powerful Vision

The Future of Forensic Training Programs

An Interview with Skip Palenik, President and Senior Research Microscopist of Microtrace LLC

by Wayne Buttermore, *Leica Microsystems Product Manager, Forensic Microscopy*

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Skip Palenik

Recently, Wayne Buttermore, Marketing Manager for Forensic Microscopy, Leica Microsystems, interviewed Skip Palenik to hear his perspective on the benefits of continuing education in forensic science. Palenik, President and Senior Research Microscopist of Microtrace LLC, sits on the Board of Directors at the McCrone Research Institute (McRI), Chicago. Palenik conducts courses in photomicrography, applied polarized light microscopy, identification of small particles, advanced crystallography, and scanning electron microscopy, among others.

For more than thirty years, Palenik has worked as a research microscopist. His work has helped provide crucial evidence in many criminal investigations, and Palenik has testified in and worked on many high-profile cases. As a lecturer and writer, he has also contributed to current education and literature regarding microscopy and chemistry.

Buttermore: The excitement generated by today's forensic TV programs and current events have stimulated the public's interest in forensic science. What impact does this have on recruiting people into the McRI training programs?

Palenik: Forensic TV shows have had a major impact, but it's a double-edged sword. The good thing is that a lot more people apply for and attend educational programs and schools. In general the forensic community sees more people from academic institutions with basic science training that want to explore a career in forensic science. There is a much larger number of students applying for short

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courses such as those we teach at McRI, although we target and attract people already working in the forensic field. Microscopic trace evidence is my specialty, and I assume that anyone with at least a four-year degree attending one of my classes has the fundamental science skills mastered.

The flip side of the coin is that some people are surprised that they have to learn chemistry, biology, and physics to work with trace evidence. I have friends that are university professors of forensic science, and they have had new students that are actually appalled at the prospect of studying science.

Buttermore: How do programs like McRI ultimately benefit the forensic community?

Palenik: Most university programs don't offer in depth forensic science training. For example, when I conduct specialized training in microtracing, I put my students in situations where they see how different science disciplines are used. Physics courses do not talk much about chemistry – chemistry courses don't talk much about physics or biology – but applied, analytical, and forensic microscopy shows students how the concepts of light, electricity, chemical behavior, biological compounds, etc. work together.

Buttermore: How do McRI courses supplement the training that goes on everyday in the lab?

Palenik: Like the lab, McRI courses takes science and applies it to real life. Naturally, everyone obtains a lot of training on the job in the crime lab. But, you are also limited by the particular experience of the people that work in your laboratory. That is why many people opt for specialized courses, at least in microscopy, where they get the benefit of being taught by world-leading scientists.

Buttermore: Clearly there is a need and expectation from the forensic community for continuing education, right?

Palenik: Absolutely. Some organizations have certification programs to help its members keep current and proficient through course work, meetings, and training. One of the requirements under the FBI's SWGMAT guidelines is that crime labs achieve certain minimum levels to ensure that, for example, new scientists starting to work in trace analysis receive training in polarized microscopy and more.

At the moment there are very few options other than McRI for someone wanting to learn and polish their skills in analytical microscopy. As we touched on earlier, academic institution are simply not providing high-level microscopy training for forensic scientists. High-level standards of analytical microscopy were set forth by Walter McCrone in all of his writing and lectures over the years. He continually sought to expand the field of application but also the understanding and application of the microscope – in all areas of science where problem solving is involved.

Buttermore: Do you tend to train more on applied practice or theoretical knowledge at McRI and Microtrace?

Palenik: My training will definitely remind students of the things they learned in college physics; for example, light, atomic, and molar refractivity. But I show students how it applies – measuring the particular index of refraction of a fiber and why it is useful when comparing known fiber with unknown fiber. Students connect college physics with the work in a crime lab and learn why they do what they are asked to do. I give them the reason behind it and hopefully it helps transform them from forensic technicians into true forensic scientists.

Buttermore: The forensic community backs continuing education with regional, national, and international meetings. Do agencies accurately budget for their people to participate in meetings and McRI courses?

Palenik: I am aware of many forensic scientists who struggle throughout their careers to get training. So the fact that we now have accredited laboratories that must train personnel helps. There are forensic labs with very dedicated people that were never shown any techniques and do not have the experience and expertise in house. They were being asked to do things they simply did not know how to do. They find out they were completely wrong in the way they handle certain pieces of evidence when an expert comes from somewhere else and does it correctly.

There are still labs that are not getting technical training. I know one lab on the west coast that has a budget of $600.00 per person for its people to attend one advanced training course a year. But a trip anywhere, apart from the tuition, could cost over $1000.00. People want to attend courses, but the funds are not available, so they use vacation time and their personal funds to obtain more training. This is not at all unusual for forensic scientists.

Forensic Detection of Sperm from Sexual Assault Evidence: A New Approach

by Karl Reich, Ph.D., CSO, *Independent Forensics, Hillside, IL*

The impact of modern scientific methods on the analysis of crime scene evidence has dramatically changed many forensic sub-specialties. Arguably one of the most dramatic examples is the impact of molecular biology on the analysis of biological evidence. The techniques required to process biological evidence and generate a DNA profile are beyond the scope of this article, but require several impressive looking pieces of equipment with flashing lights, computer interfaces, and robotized arms. Somewhat obscured by all this technology is the fact that the methods and procedures for screening biological evidence, a necessary precursor to finding the best item of evidence to process for DNA analysis, are essentially unchanged for the past forty years.

Forensic laboratory personnel are well aware that the entire structure of DNA profiling begins with the identification of a questioned stain from an article of evidence or from the analysis of an evidence swab. There are both criminalistic and laboratory procedural reasons to identify the source of the biological material that will be processed for DNA, as coming from blood, saliva, semen or sperm. As fully half of all forensic biology laboratory analysis involves sexual assault evidence, the identification of semen and sperm are particularly important. Recall that sperm is a specialized cell with distinctive morphology that is also the source of the overwhelming majority of DNA-containing cells in human ejaculate.

Once seminal fluid has been identified on sexual assault evidence, the DNA analyst must attempt to determine not only whether sperm are present, but which item of evidence or swab has the most sperm, in order to identify the sample most likely to provide a DNA profile. Here again, the forensic methods involved are unchanged for forty years, and current forensic identification of sperm uses a generalized cell staining method coupled with brightfield microscopy. In theory, this should be sufficient to identify sperm, in practice sperm isolated from sexual assault evidence has lost many, if not all, of its distinctive sub-cellular organelles upon which morphological identification depends. Hence, DNA analysts spend many hours searching for sperm using a less than optimal microscopic technique.

In order to provide a more scientifically and procedurally robust sperm searching technique, Independent Forensics has developed a fluorescent monoclonal antibody-based kit, SPERM HY-LITER™, for the microscopic identification of sperm from sexual assault evidence. SPERM HY-LITER™ is designed to provide positive identification of sperm using a unique monoclonal antibody that has been chemically tagged with an Alexa 488 fluorophore. The kit incorporates a second fluorescent dye, 4',6-diamidino-2-phenylindole (DAPI) that will stain all cell nuclei; this is a fluorescent analogue of the KPIC stain currently used in most DNA forensic laboratories. By combining both fluorescent dyes, SPERM HY-LITER™ provides several visually confirmatory steps for the identification of sperm. Sperm can be visualized in the fluorescein channel (the fluorescent spectra of Alexa 488 falls conveniently within the emission maximum for fluorescein); all cell nuclei can be seen in the DAPI channel; and using specialized dual filter 'cubes,' epithelial nuclei and sperm can be visualized simultaneously.

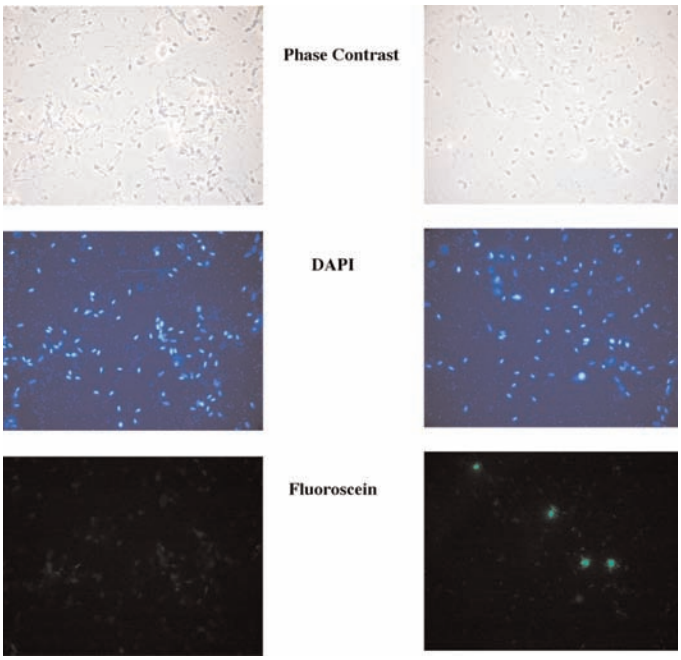

Fig.1: A mixture of canine, feline, bovine, equine, caprine, ovine, porcine, murine (left panels) and a mixture of canine, feline, bovine, equine, caprine, ovine, porcine, murine, and human sperm (right panels) were stained with SPERM HY-LITER™ and visualized using phase contrast, DAPI, fluorescein filters (top, middle, and bottom, respectively). Note that only the mixture containing human sperm is labeled in the fluorescein channel thus demonstrating species specificity of SPERM HY-LITER™ staining. Photomicrographs taken on a Leica DM2500 microscope fitted with A4 and L5 filters. Final magnification: 400X

The monoclonal antibody used in SPERM HY-LITER™ provides an unprecedented degree of specificity that allows the identification of human sperm from previously unsearchable samples. Furthermore, while the signal-to-noise advantage of fluorescent microscopy (and the very low background seen with the fully optimized SPERM HY-

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Forensic Detection of Sperm

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LITER™ kit) increases the sensitivity of sperm detection by orders of magnitude compared to current brightfield microscopic techniques. The incorporation of both DAPI and Alexa dyes was designed for image processing software such that sperm recognition could be essentially automated. By using computer-aided image analysis software, SPERM HY-LITER™ stained preparations can first be scanned for 'features,' i.e., fluorescent signals above background – and second, these features can then be analyzed further for the color (or hue) of the observed fluorescence. Only those features that have both DAPI (from the DNA) and the Alexa 488 (from the monoclonal antibody) fluorescence would be scored by the software as sperm.

SPERM HY-LITER™ provides all required solutions for slide staining in pre-calibrated dropper bottles – two slightly different version allow staining of smear slides (often included in sexual assault evidence kits, 'rape kits') or of extracts made from evidence swabs or identified stains. The addition of phase contrast to the method, although not required, gives less experienced crime laboratory personnel the ability to visualize cells, nuclei, and sperm in one image.

As an illustration of the specificity and sensitivity of the SPERM HY-LITER™ method, we show a mixture of sperm from a variety of animal species, with and without human sperm, stained with SPERM HY-LITER™ (**Fig. 1**).

The sensitivity and cell type specificity of SPERM HY-LITER™ is demonstrated from images provided by a crime laboratory case work validation study of SPERM HY-LITER™ (**Fig. 2**). Here a smear slide made by a sexual assault nurse examiner from a vaginal swab collected from a sexual assault victim was stained using SPERM HY-LITER™. These types of slides are notoriously difficult for crime laboratory personnel to analyze for the presence of sperm, as the cell density, collection method, and storage conditions all conspire to destroy sperm cell morphology and inhibit KPIC staining, making standard sperm identification methods all but impossible. The series of images demonstrate the complexity of the original slides (see phase contrast image), the ability to detect sperm in the preparation (see combined phase and FITC image), as well as confirmatory steps in the process where both epithelial and sperm cells can be simultaneously identified (see combined dual cube and phase contrast image). SPERM HY-LITER™ stains sperm in all layers of the preparation.

The job of the forensic analyst often involves screening many items of evidence in a case. Although current forensic laboratory protocols vary, screening for sperm is usually performed with 40X objectives (400X final magnification). Here again, SPERM HY-LITER™ provides an advantage over current methods as stained preparation can be easily visualized using 10X and 20X objectives (100X and 200X final magnification) greatly increasing the field of view and therefore decreasing the time needed to scan stained slides. In fact, the signal from SPERM HY-LITER™ stained slides is such that sperm can scanned using appropriately configured fluorescent-capable stereomicroscopes (**Fig. 3**)! The stereomicroscope can be fitted with either a traditional mercury light source or newer fiber-optic metal arc lamps, and accept the same filter cubes as traditional fluorescent compound microscopes. Given the field of view and working distance of these instruments (and therefore the speed and ease of slide manipulation on the stereo microscope), this approach promises to dramatically change the way in which crime laboratories search for sperm from sexual assault evidence.

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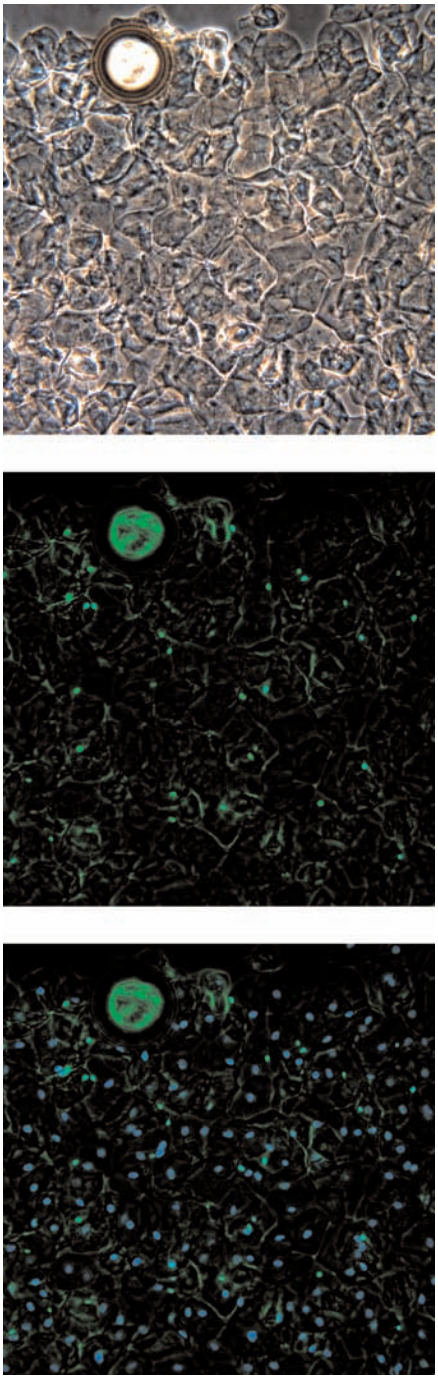

Fig.2: SPERM HY-LITER™ staining of a sexual assault smear slide performed by forensic DNA crime laboratory. Smear slide was stained according to SPERM HY-LITER™ supplied protocol. Photomicrographs taken with PAX-IT 2 camera using DAPI, FITC and dual DAPI/FITC cubes. Note air bubble at top of image.

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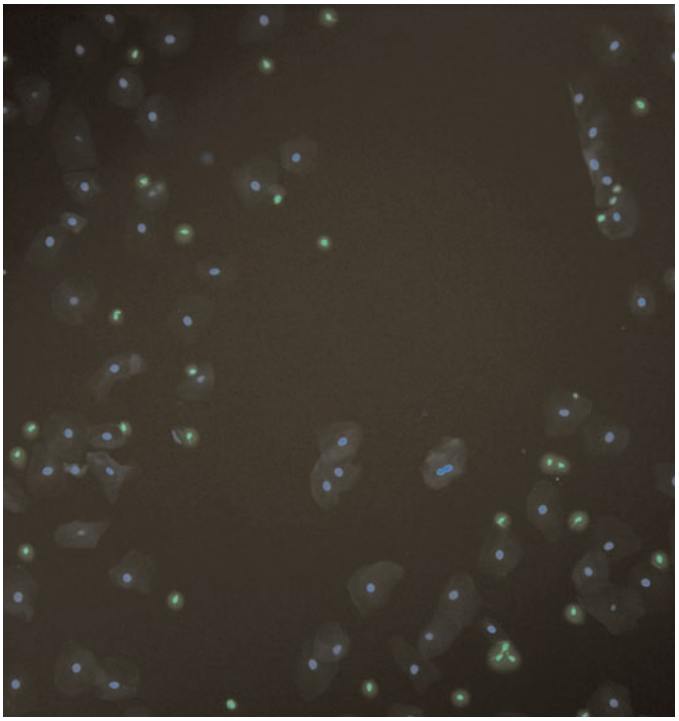

Fig. 3: Stereo fluorescent microscope view of SPERM HY-LITER™ stained slide illustrating both the increased field of view and signal-to-noise of SPERM HY-LITER™ stained preparation. Photomicrograph taken on an Olympus MVX at 120X final magnification with a dual DAPI/FITC cube. Light source: 100 W mercury lamp. Image collected with a PAX-IT 2 CCD camera.

Additional details are available at **www.spermhy-liter.com**

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Industry News

The **2008 ASQDE Meeting** will be held August 16-21, 2008 at the Renaissance Asheville Hotel in Asheville, NC. The Program Chair for this meeting is Grant Sperry, and the Site Chair is Charlotte Ware. The meeting will include presentations and workshops related to the field of forensic document examination. The meeting will also include an optional evening trip to the world-famous Biltmore Estate. Attendance is limited to members and invited guests.
More information: **www.asqde.org**

The **2008 Annual Meeting of the Southern Association of Forensic Scientists** will be held September 21-26, 2008 at Sam's Town in Shreveport, Louisiana. Sam's Town is located on the historic Red River in downtown Shreveport, just blocks from museums, Botanical Gardens, the IMAX Theater, and Festival Plaza.
More information: **www.southernforensic.org**

The **2008 Midwestern Association of Forensic Scientists 37th Annual Meeting** will be held September 28-October 3, 2008 at the Hotel Fort Des Moines in downtown Des Moines, IA, only a short distance to many restaurants and entertainment options.
More information: **www.mafs.net**

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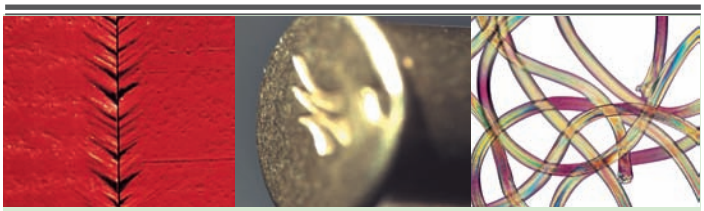
Nothing Remains Invisible!



Click below to enter the Leica Microsystems Photo Contest!

Send us your most beautiful application photo, taken with a Leica Microsystems microscope, and have the chance to win our quarterly prize!

www.leica-microsystems.com

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