HISTOLOGIC®
Technical Bulletin for Histotechnology
SAKURA
Vol. XXXIV, No. 2, Nov. 2001

30 Years and Still Going Strong

Vinnie Della Speranza
Scientific Editor

This year ***HistoLogic***® celebrates its 30th anniversary! Over the years it has been read by close to 1,000,000 people worldwide. We believe that it has made a difference in spreading valuable information to histotechnologists and pathologists around the world.

In 1981 for this publication's 10th anniversary, Lee G. Luna, editor, reviewed predictions that had been made for the 1970-1980 decade. The original article can be retrieved from the Sakura Finetek U.S.A. web site at www.sakuraus.com. It is interesting to realize that some of the same conclusions made then also apply today. Faster, more automated processing systems are not yet here; automated stainers are now widely used, but their principles have not changed much; microtomy has not evolved significantly except for the more widespread use of motorized units.

There have been advances, however, with automated coverslippers. For example, by using the Tissue-Tek® SCA™ Film Coverslipper, slides can be viewed sooner under the microscope, thanks to the fast drying time. The fact that it coverslips one slide every 3 seconds also helps! Slide labeling now can be done without using paper labels and practical bar-coding is on the horizon. Other areas of rapid growth are immunohistochemistry and *in situ* hybridization.

What progress will be made over the next 10 years? From the development of the very first microscope, histopathology became a discipline largely of visual images. (Be sure to read "The Golden Age" in this issue.) Diagnosis continues to rest upon interpretation of those very images, in their spectacular color, which is the hallmark of what we do in the laboratory. With the ever increasing sophistication of computers, and as imaging devices become easier to operate with better quality and cheaper price tags (see "Histology and Digital Imaging - Present and Future Technologies" in this issue), the days of pulling (and refiling) glass slides for conferences, tumor board, or teaching may well be behind us. How long will it be before the glass slide becomes relegated to the museum display case?

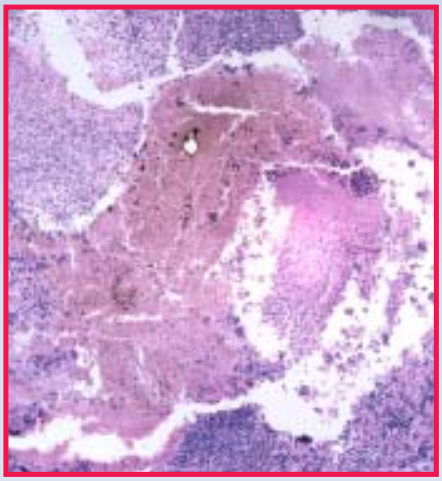
There are strong indications that automation of the embedding process will become reality in the near future. Faster, more efficient processing technologies are also at the testing stage. This could lead to drastic changes to the workflow of routine anatomic pathology laboratories. I can only wonder if today's sophisticated motorized microtomes are paving the way for a day when instruments will be able to section tissue at high speed without human intervention.

Since ***HistoLogic*** is here to stay for many years to come, it will be fun to revisit these predictions in 2010.

The Golden Age

Barry R. J. Rittman, PhD

My perspective is largely colored by my training as a medical laboratory technologist and histologist in England and the United States, including my training in electron microscopy and image analysis, and my teaching in histology and histologic technique. This is a somewhat different perspective than for most histotechnologists.



Most technologies have a golden age- an era when advances are continually being made which significantly move that technology forward. What do we consider to be the golden age of histologic technique? Depending on outlook, some might believe it to be the 19th century when independent work by "gentlemen scientists" developed early methods for examining tissues which still form the basis of much of our current histology work. Others might look at the developments within the past two decades and suggest that the explosive technological development that has taken place now puts us in the middle of an exciting golden age. Still others, in trying to look ahead, may think that the golden age is yet to come- that we are merely the pathfinders for ensuring the advance of histologic technology. All are correct in their own way and only vary in perception. It is certain that specific key events and discoveries at optimal times are critical in advancing technology and histologic technique.

Much of the work being carried out today is based on the discoveries made in the 18th and 19th centuries. It was not until the 1820s that microscope lenses which corrected for chromatic aberration were being manufactured, usually on a custom basis.[1] Slides used in the 18th and early 19th centuries were made of ivory with a central hole; the specimen was covered on both surfaces with small pieces of mica. By the 1830s, glass plates were in use. These were often known as sliders. The standard 3" × 1" slides still in use today were recommended by the Microscopical Society of London around 1839. Cover glasses were starting to be made thinner by the mid-1800s. In 1855, Chance manufactured cover glasses, but it was left to Beale in 1880 to produce cover glasses that were thin enough to take advantage of the high dry objectives then being manufactured.[1]

By 1840, the major cell structures such as mitochondria and chromosomes had been discovered and both mitosis and meiosis described. The activity of cells in embryology, fertilization, pathology, and physiology was the subject of investigation at about this time. The concept of fixation was not yet developed but tissues were "hardened" in alcohol, chromic acid, and formalin. Bracegirdle cites the following dates for the introduction of substances for either hardening or fixing tissues: alcohol 1743; mercuric chloride 1846; potassium dichromate 1860; picric acid 1879; Bouin's fixative 1897; and formaldehyde 1893.[2]

Despite the scarcity of stains prior to the 1850s, there are several publications with very detailed histology which came from meticulous observations of unstained tissues. van Leeuwenhoek is credited with using the first stain, where he showed contrasting muscle fibers with saffron around 1714-1719.[2] In the early years of microscopy, carmine and indigo were the most commonly

used stains, starting around 1850. Aniline dyes were not readily available until 1856 and many of these were adapted from the dyeing of fabrics. However, stains provided a technological leap forward allowing distinctions between tissue components whose existence up to that time had been controversial. [3-5] Some interesting observations can be found in documents from the late 19th century. The book by Miller (1887) opines, " nine-tenths of the microtomes are purchased because of failure in free hand work with a dull knife." [6] Miller used free-hand sectioning and hardening agents such as alcohol and chromic acid. Teeth were decalcified in chromic acid. Sections were produced by infiltrating from alcohol into Bayberry tallow or in celloidin. Wax sections were collected in benzol and treated as free-floated sections. Staining was done with hematoxylin and eosin, borax carmine, or carmine plus picric acid, and sections were dehydrated and permanently mounted. The permanence of some of these techniques is seen in a section (that I possess) from 1861 of cerebellum stained with borax carmine in which the details are still crystal clear. Weigert's method for myelin and Gram's stain are also from this period (1884). Metallic impregnations using silver and gold salts were used by some investigators during this era.

By 1890, optical microscopes had reached the level of theoretical resolution using transmitted light. Pearse cited the iodine reaction for starch by Colin and de Claubry in 1815 as one of the first histochemical methods.[7] In the 1930s and 1940s, there was a revival of interest in understanding the basis of histochemical reactions and developing new techniques. Staining techniques didn't progress rapidly until after the Second World War- before and during the war, aniline dyes were unavailable from Germany. The first transmission electron microscope was introduced by Knoll and Ruska (1932).

I began working in an oral histopathology laboratory in London, England, in 1957, transferred to the University of Iowa in 1968, and then to the University of Texas in 1989. While in London, pathologic specimens had to be produced in a timely manner but there was ample time for study, reflection, experimentation with new and old methods, and personal training. Technicians carried out all the histology, histochemistry, electron microscopy, museum technique, and tea making.

This was an exciting time, when new discoveries about the structure and physiology of the cell were being made on a regular basis. The electron microscope explored anew the structure of cells and tissue, and in many ways its application paralleled the use of the optical microscope in the 19th century. Along with the use of the electron microscope came the need for improved techniques to visualize tissue, as well as the development of ultramicrotomes, glass knives, new processing techniques, electron dense stains, and histochemical techniques that were applicable specifically for electron microscopy. A point of interest here is that Gustav Mann recommended the use of glass knives to prevent contamination of sections that would be tested for the presence of iron.[8] A useful reference in the early development of electron microscopy instrumentation and techniques is Hall.[9] The general belief was that the improved resolution of the electron microscope would revolutionize routine diagnosis. In most cases this has not been realized.

In light microscopy, the emphasis was on the quality of sections. Results needed immediately in the operating room used tissue fixed in hot formal-saline for 1 minute, followed by frozen sections and rapid staining to obtain a section within 4 minutes of receipt of the tissue. The introduction of the cryostat and its popularity due to the work of Everson Pearse revolutionized the production of uniform sections for frozen section diagnosis. While freezing microtomes using liquid carbon dioxide was in common use, the cryostat allowed for both the preparation of sequential sections that remained frozen and the comparison of histochemistry on unfixed sections with those fixed by various other methods.

Image analysis and three-dimensional reconstruction were carried out routinely using a camera lucida and a pencil, projecting images on flat sheets of wax. In this way individual features could be traced, and by cutting these out and sticking sequential sections together, a three-dimensional reconstruction was prepared. Some reconstructions of individual enamel spindles produced in this manner are still regarded as classic examples. The amount of work in tracing hundreds or thousands of features in serial sections can only be imagined. Stereology by manual analysis of individual images provided valuable information but again was exceedingly time-consuming.

Tissue had always been processed manually. An automatic processing machine, known as the Autotechnicon, was described in 1909[2];

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it offered automatic transfer of specimens to a new solution and then to baths of molten paraffin. However, the Autotechnicon only gained popularity in the United States, and was later replaced in the late 1970s with closed processors offering the capability of several different programs. Microtomy utilized rotary machines such as the MSE and the AO 820- the latter regarded by many as the ultimate microtome of its day. Cambridge Rocking microtomes were still used by many and a modified sturdier model formed the basis of the initial commercial cryostats. (The Rocking microtome was introduced in 1881 and the rotary microtome in 1883.)

During the 1950s and 1960s, paraffin wax sections were routinely cut at 6-7 microns. Double embedding was used routinely for hard specimens and celloidin for large specimens. Histochemical reactions that were used included von Kossa technique, Perls' Prussian blue, Periodic Acid Schiff, and the Feulgen reaction. Immunochemistry and immunocytochemistry came into existence during this time. In 1967, Gibb et al produced antibodies to dopamine ß-hydroxylase, and in 1969, Geffen et al used indirect immunofluorescence on adrenal glands.[7] During this period, enzyme-labeled antibodies were also developed. The historical development of histochemistry is summarized in Pearse.[7]

Monoclonal antibodies were introduced in immunohistochemistry by Cuello et al.[10] The use of lectins to demonstrate carbohydrate moieties started to become popular in histochemistry in the 1970s and 1980s when it was recognized that their specificity was similar to antigen-antibody reactions.[11] PCR and *in situ* hybridization are commonplace today and allow us to visualize not only where substances are located but also whether they are being synthesized. Automated machines for staining and also for immunohistochemical reactions are now in common use. Antigen retrieval techniques have, in many instances, circumvented many of the limitations imposed by fixation.

Today, confocal microscopy allows us to look at structures and localize some materials in intact blocks of tissue. The confocal microscope (originally known as the tandem scanning reflected light microscope) was introduced by Egger and Petran in 1967 using a Nipkow disk system.[12] Digital photography has largely eliminated the need for photographic film of gross specimens and sections. New antibodies are being produced almost on a daily basis. Automatic tissue processors and automatic stainers are commonplace.

In 1902, Gustav Mann suggested that " we had learnt almost all we could of the organelles in the cell." [8] During the 1970s when image analysis was rapidly developing, the suggestion was that automatic image analysis would allow rapid and reproducible diagnosis of conditions such as with the Pap smear. This has only partially materialized. Automated systems such as image analysis and receptor data can simplify the tasks and provide more information for us to use in diagnosis, however, they cannot replace the final arbiter- the pathologist. The histotechnologist not only understands the technology but is able to make it work and then provide that information in a reproducible and reliable manner.

There are usually rapid steps forward in instrumentation followed by a much longer period during which application of that technology occurs. I believe that single cell analysis such as laser scan microdissection is one technique that will be in common use. Antibodies will be used to localize most substances and their concentrations within blocks of vital tissue. Images will be scanned, analyzed, and compared with normal locations and values, all in automated fashion. The histotechnologist will still remain a vital link in this process. While many things can be automated, the ability of a good histotechnologist to solve problems is not yet matched by a machine.

In looking back at previous work in the 19th century, we can only be awed by the wealth of information gleaned by simple instrumentation and meticulous observation, but at the same time amused by some of the erroneous deductions by investigators. I wonder how future histologists will view our attempts with what we now regard as sophisticated instrumentation, techniques, and training.

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A Field of Poppies

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Recently, a histotech reported having difficulty with her trichrome, a procedure that had " always worked before." Only one change had occurred that coincided with the disaster: her original supply of red dye, which had been around for many years, had been consumed. A newly purchased dye of the same name had been used, with very unsatisfactory results. The red dye in question is ponceau S, one of the most confused dyes in histology. Ponceau is French for poppy, an allusion to the poppy-red color of the aqueous solution.

Poppies are red,
collagen is blue.
Choose the wrong dye
and you will be, too.

Conn's Biological Stains (9th ed., 1977) lists 17 dyes whose name starts with the word ponceau, but doesn't include three others that are available currently. Among all of those, there are two called ponceau S, a ponceau SS, and two given the designation ponceau SX. To compound the problem further, there is a crystal ponceau 6R, a brilliant ponceau G, and an acid ponceau. How is a person ever going to replace an old dye with an equivalent one? There are ways, better today than a few years ago, but still they are not foolproof. Here's why.

The names of dyes are assigned arbitrarily by manufacturers, most of whom are in business to serve the textile trade. Their customers do not care which dye is used, only that it produces a given shade on a particular fiber or fabric.

For example, Eddie Bauer wants to assemble a collection of mint green separates: a wool blazer, a cotton turtleneck, and a silk scarf. All have to be the same color, and each must be treated with a different dye to achieve that color because each fiber reacts with dyes uniquely. Eddie Bauer couldn't care less about the names of the dyes used to achieve that end, and the manufacturers pull names seemingly out of a hat. The same dye may be sold under any of dozens of names, and different dyes are given the same or nearly identical names.

Many dyes have letters appended to their names, e.g., ponceau S. Sometimes these have real meaning, designating a shade (eosin Y for yellowish, as opposed to eosin B for bluish). Occasionally, the letter denotes a chemical characteristic. Alizarin contains no ionic groups and is insoluble in water. Add a sulfonic acid group to the molecule and you have alizarin red S (S for sulfonic), the familiar, water-soluble dye used to stain calcium deposits. Sometimes the letter is doubled (ponceau SS) or a combination of letters and numbers is used (crystal ponceau 6R, used in Lendrum's MSB stain for fibrin); in many of these instances, a deeper, richer color is implied. Here is where a good bit of the problem lies.

To match Eddie Bauer's woolen color swatch, the dye manufacturer may make an extra pure batch to heighten the color, or might add inert ingredients to tone down the color. Chemically, it could be the same dye molecule. Meanwhile, another manufacturer could take a different dye, fiddle with its concentration to match the cotton swatch, and give it a name similar or identical to that used to dye wool.

Biological supply houses then buy small quantities of these dyes, sometimes keeping the name intact or changing it to one of their own. Years ago, histologists had little choice but to buy what was offered and hope for the best. In 1905, Curtis recommended ponceau S for trichromes in a paper written in French, but the chemical identity of that dye is unknown. Since then, dozens of red dyes have been suggested as Curtis' ponceau S. A strikingly parallel story began a few years later when Masson (also French) reported on the use of ponceau de xylidine in trichromes, another mystery dye that has come to be equated (somewhat arbitrarily) with ponceau 2R.

With that as background, is it any wonder that we have trouble obtaining the same product year after year? We can achieve some measure of consistency by relying on Colour Index numbers (C.I.#), numerical designations assigned to nearly all dye molecules by the Society of Dyers and Colourists. These numbers provide a standardized identification for each dye that transcends language, culture, and whim. If your old bottle has a C.I.# on it, order a new bottle with the same number. Really old bottles will show a three-digit number from the 1st edition of the Colour Index; current numbers from the third edition have five digits. *Conn's Biological Stains* has a table matching the two systems.

So what did our friend have that was labeled ponceau S? Here is a list of likely candidates:

C.I.#	Name
14700	ponceau SX
15635	ponceau S
16140	ponceau scarlet
27190	ponceau SS
27195	ponceau S

These differ from one another in several ways, as the molecular models show (Figs. 1-5). I have designed these models so that important features, such as ionic groups, are prominent. Sulfur is yellow, oxygen is green, nitrogen is blue, carbon is gray, and hydrogen is black. Each molecule has one hydroxyl group (green oxygen with one black hydrogen atom) but

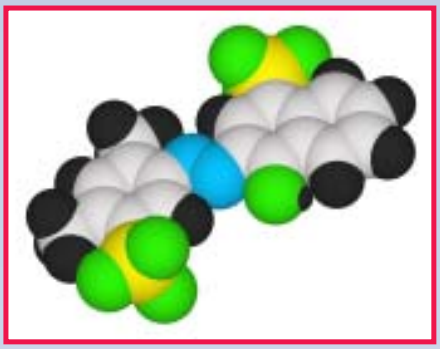

Fig. 1. Ponceau SX, C.I. 14700. Two rings, each with a sulfonic acid group.

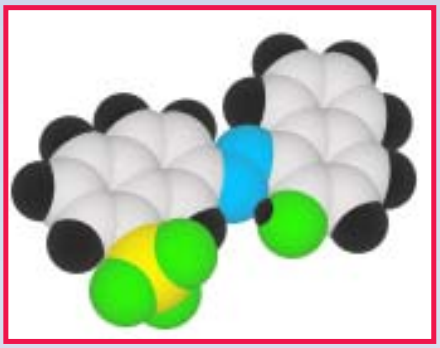

Fig. 2. Ponceau S, C.I. 15635. Two rings, but only one sulfonic acid group.

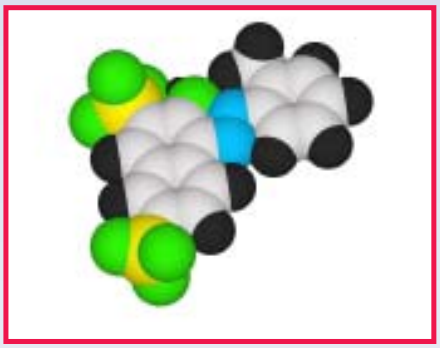

Fig. 3. Ponceau scarlet, C.I. 16140. Two rings and two sulfonic acid groups, but both are on the same ring.

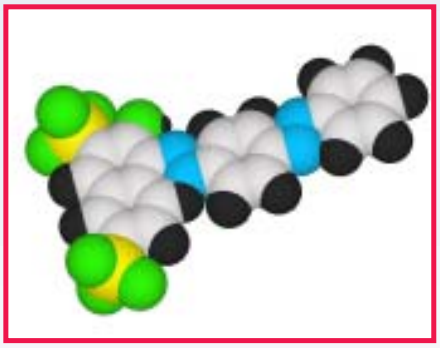

Fig. 4. Ponceau SS, C.I. 27190. Three rings and two sulfonic acid groups, but both are on the same ring, leaving a long floppy tail that will not bond to cytoplasm.

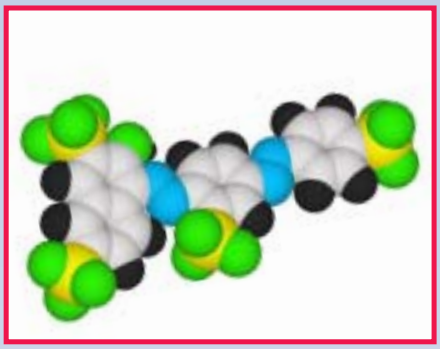

Fig. 5. Ponceau S, C.I. 27195. Three rings and four sulfonic acid groups, spread all over the molecule.

these probably play no role in our story. Sulfonic acids (yellow sulfur with three green oxygen atoms) are important because they bear a negative charge that could bind to cytoplasmic proteins.

See how the dyes vary? The first three molecules each have two sets of rings, while the rest have three sets. The number of sulfonic acid groups ranges from one to four. The number and placement of these groups directly affect how they interact with the tissue in a trichrome stain.

Trichromes work by competitive displacement of red dye by blue or green dye from select tissue elements (e.g., collagen). The red dye must be bound tightly to cytoplasm to resist displacement. Collagen is weakly ionic and attracts the blue or green dye by nonionic forces (probably some type of van der Waal's force). Cytoplasm is more strongly charged and attaches to dyes via ionic bonds. Red acid dyes that are well charged should bond strongly to cytoplasm, especially if their negative charges are spaced evenly around the molecule. Those with fewer charges, or with several charges concentrated at one end of the molecule, will be more easily dislodged by the blue or green dye. This will produce muddy cytoplasmic colors.

Compare C.I. 27190 with C.I. 27195 (Fig. 6). The former has two sulfonic acid groups stuck on one end, leaving a large, uncharged portion dangling free of the tissue. This is a less stable configuration because of the loose end. The latter has four sulfonic groups spread all over the molecule, providing a much more reliable fastening to cytoplasm.

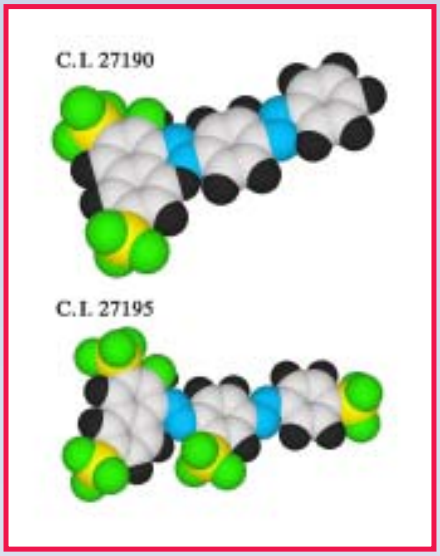

Fig. 6. Comparison of C.I. 27190 with C.I. 27195.

Now look at the remaining dyes. With only one opportunity for ionic attachment, C.I. 15635 (Fig. 2) probably will not provide as clean a trichrome as C.I. 14700 (Fig. 1) or C.I. 16140 (Fig. 3).

My guess is that our friend had C.I. 14700, 16140, or 27195 in the original bottle (if in fact the dye was one of those listed above). She probably purchased C.I. 15635 as the replacement, as this is commonly sold as ponceau S. If I were to use one of these in a trichrome, I would choose 27195 because of its uniform distribution of many ionic groups.

Acknowledgment

Peggy Wenk of William Beaumont Hospital, Royal Oak, Mich, first told me about the histotech who reported the problem to her following one of Peggy's workshops.

Histology and Digital Imaging - Present and Future Technologies

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Introduction

The practice of histotechnology is devoted to the creation of visual information. A change in image recording equipment as well as image storage, retrieval, and dissemination technologies fundamentally affect the usability and value of the histology lab's output. Few technologies have developed as rapidly as digital imaging. That in turn has created profound change in all imaging applications, from home pictures to magazine publication. Histology is certainly no exception. The advent of relatively inexpensive and easy-to-use digital microscope cameras has opened new possibilities for the pathologist and other consumers of histologic material.

Cameras

A discussion of digital imaging must first focus on the often confusing and rapidly expanding range of available cameras.[1] They all share the use of a solid-state sensor, most often a CCD (Charge Coupled Device).[2] In fact, a CCD produces an analog signal, so the creation of a digital computer image file requires the use of an A/D (analog to digital) converter.

Standard Video and Image Capture

When this conversion takes place outside of the camera, it is usually called image capture. Inexpensive "frame-grabbers" now cost less than one hundred dollars. They achieve simplicity of use through "plug-and-play" USB (Universal Serial Bus) interfaces and very user-friendly software. The output of any standard video source or camera can be captured quickly into a standard JPEG image file or a low-resolution streaming video output. Useful for simple low-resolution telepathology[3] and conferencing, standard video cameras do not produce an image of high enough quality to satisfy most diagnostic pathologists.

Digital Cameras

When the A/D converter is incorporated in the camera itself, it is considered a digital camera because the output is in a computer readable image file format. At the least expensive level, the imaging sensor differs very little from a standard video camera. To produce image resolutions greater than 640 × 480, larger nonvideo-standard sensors are used with resolutions that range into the millions of pixels (picture elements). Besides pixel count (image resolution), the cameras are further distinguished by the manner in which they form color images. These design differences are important to the cost, ease of use, and types of application, so they will be discussed individually. For each camera type, a specific commercial example will be offered from models that represent the current state of the art. These examples are offered for illustrative clarity and comparison. However, it is very important to be aware that there are other comparable competitive models available and new ones will be forthcoming.

Single Chip (One-pass) Color Camera

The most common method used to generate color is to incorporate color filters directly on the sensor so that different pixels are sensitive to red, green, or blue light (RGB). The actual array of filters is usually by rows of pixels, most often in a pattern of Green-Red-Green-Blue. This means that one-half of the pixels are sensitive to green light and only one-fourth each to red and blue. The rest of the image data is filled in by digital interpolation. A 1 million pixel count one-pass color camera produces a digital image file with 3 million data points (3 MB), but only one-third are derived from actual resolved image information. This has contributed to giving first generation digital cameras a reputation for inadequate resolution, especially for low-power microscopy.

Fig. 1. The Nikon Coolpix 995 is a high-end prosumer-grade fixed-lens digital camera that can be mounted on a microscope with an optical adapter. The half of the camera containing the LCD display can be freely rotated for the optimal viewing angle.

One solution is to increase the total number of pixels until the resolution becomes acceptable, despite the interpolated nature of some of the image data. Current models of prosumer-grade digital cameras have resolutions above 1500 × 2000 pixels (3 megapixel). With the addition of an inexpensive microscope adapter, they are converted to a surprisingly capable microscopic camera, even at low magnifications. The Olympus C-3030,[4] the Kodak MDS 290,[5] and the Nikon Coolpix 995[6] (illustrated) are all examples priced below $3000. The Nikon Coolpix 995 has the advantage in that the center pivot allows the microscope user to orient the view screen for the most convenient angle. The addition of a USB interface and simple control software allows the capture of images directly to the user's computer. These cameras do a reasonably good job of automatic white balancing for a neutral background, but they lack some of the image processing features of more specialized microscope cameras. One frequent complaint is the difficulty of focusing on the small LCD screen, which is not optimally designed for microscope imagery, especially at low power. These cameras are a good choice for a lab on a tight budget or one that needs a camera for occasional microscope use. One advantage is the camera can be easily removed from the adapter and used for other imaging chores around the lab.

Dedicated Single Chip Color Microscope Camera

Another group of cameras uses sensors of similiar size and performance to the prosumer cameras, but they differ in that they are designed for dedicated microscope use. These cameras use custom software to perform specialized operations like shading correction (subtracting an image of the microscope light field to ensure a flat uniform background). Often, shading correction, exposure time, and white balance can be stored for each microscope objective ensuring optimal quality at all magnifications. Full monitor screen focusing overcomes the handicap of the small portable LCD screen. The SPOT INSIGHT[7] color camera uses a 1600 × 1200 pixel mosaic color CCD that can operate at a rate up to 15 frames a second for near real-time focusing. At a price tag of some $4000, it provides a practicing pathologist with an easy-to-use, high-quality microscopic instrument which can produce images suitable for reports and records, teaching, research, and publication.

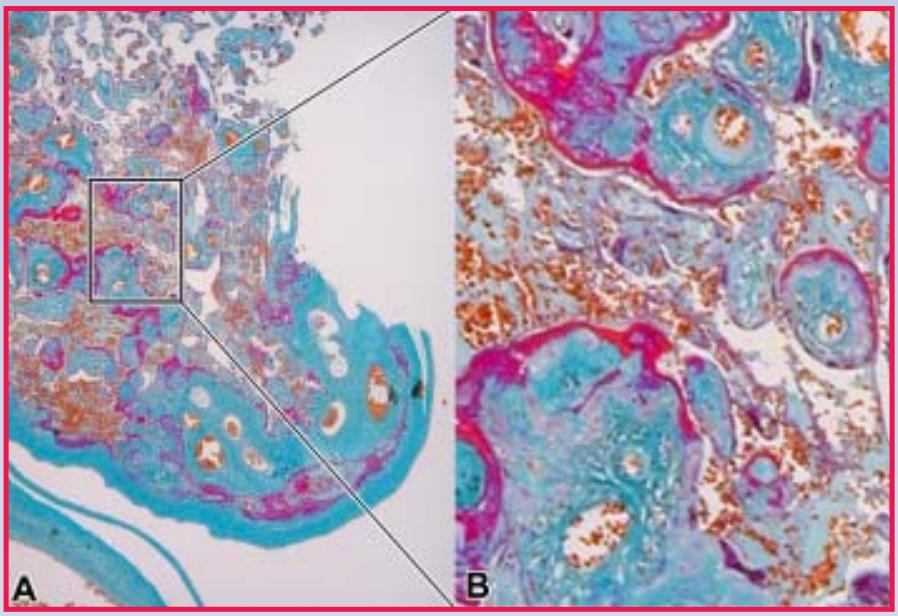

Fig. 2. (A) A low power (4×) image of placenta (Frazier-Landrum fibrin stain) taken with a relatively inexpensive Nikon Coolpix 995. (B) An enlargement of a small area of the image showing the surprisingly credible low power performance of the high pixel count (3.34 million) sensor of the type being used for top-quality mass-market digital cameras today.

Network Microscope Camera

The increasing popularity of telepathology[8] and image exchange with remote collaborators has created a need to integrate digital cameras directly into networks. The Nikon DN100 Digital Network

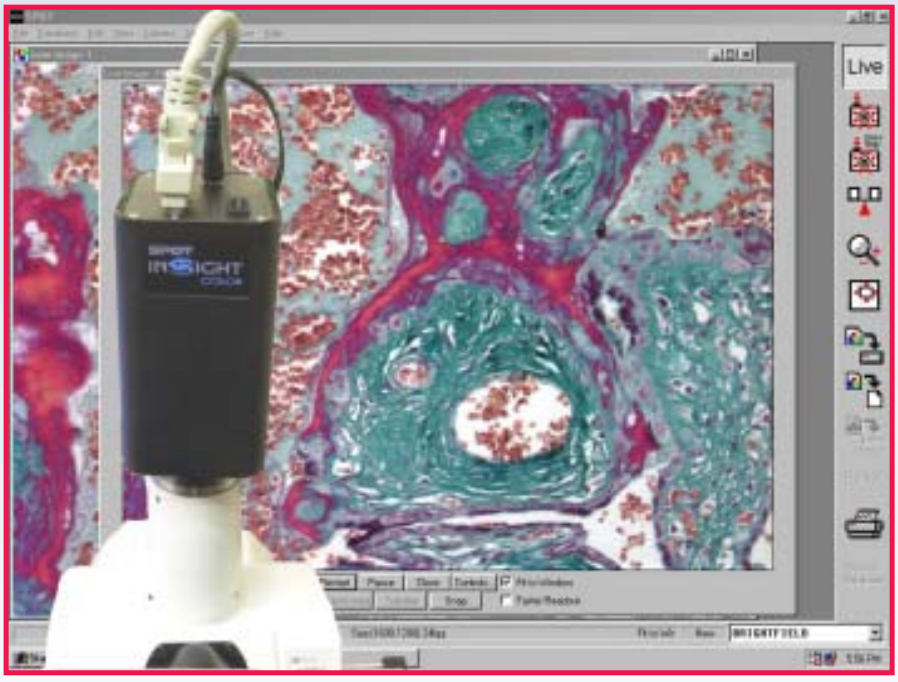

Fig. 3. The SPOT INSIGHT is a dedicated microscope camera. The computer interface and software provide full computer monitor screen focusing and advanced image processing features especially designed for microscope imagery.

Fig. 4. The Nikon DN100 is a dedicated network camera. A remote user can connect with it through a standard web browser to view and download images.

Camera[9] is an example of a new class of instruments that will undoubtedly grow rapidly. A 1280 × 960 pixel camera, not dissimilar to instruments already described, features a specially designed control box that allows the acquisition and transfer of images with or without the use of a host computer. It has a built-in network connection that allows the operation of the camera from a remote location via a standard Internet link. Essentially, the remote user calls up the camera as if it were a web site. Images can be viewed at a frame rate of one every few seconds (at a reduced resolution), and downloaded at full resolution. Operation is nearly as simple as a home web-camera, but with an image quality that will support serious histological diagnosis.

Fig. 5. The SPOT RT uses the same software interface as the SPOT INSIGHT but offers higher resolution images through three-pass color capture. In addition, it is electronically cooled for long exposures to support extreme sensitivity for weak fluorescent signals. This model has the optional filter slider that allows the camera to operate in a true monochrome mode, bypassing the color filters.

High Resolution Multi-pass Cooled Color Camera

There are two methods that can overcome the inherent resolution limitations of the single-sensor color camera. One method is to have three separate sensors with an assembly of prisms and filters to deliver the correct wavelength to each CCD. This allows for the simultaneous acquisition of all three colors. This is a very popular and successful strategy in the design of high-end professional video cameras. The expense and bulk of three high-resolution sensors make this option less practical in digital cameras.

A less expensive option is to have a single high-resolution monochrome sensor and a tricolor filter selector that acquire each color separately. This does not allow the camera to be used for moving objects because of the inherent time lag between color channels, but it is quite workable for static histology specimens. A popular example of this class of camera is the SPOT RT[10] with a 1600 × 1200 pixel count CCD that produces a 5.76 MB image, similar in size to many of the single-chip cameras.

The difference is that all of the pixels represent actual image data rather than interpolated data, producing a substantially higher quality image even though the file size is the same. In this class of research-grade cameras, the CCD is often electronically cooled, providing digital noise suppression for the long exposures required in fluorescent techniques such as FISH.[11]

Another adaptation to achieve higher resolution is to combine some of the characteristics of a digital camera and a scanner to offset or step the sensor to achieve higher sample sizes. Examples of this class of " pixel-shifting" cameras include the Zeiss Axiocam[12] and the Nikon DXM 1200.[13] These

cameras can generate extremely large image files that give excellent results even at very low magnifications. Coupled with powerful computer image processing and deconvolution software, these instruments are stretching the theoretical limits of optical light resolution.[14] Prices for this class of cameras vary from $7000 to $15000, making them more suitable for research and publication purposes than routine histological records.

This description of camera types is hardly exhaustive but does reflect the great range of pricing and capability that the modern histologist can choose from for digital imaging. A recent informal survey of practicing histologists and pathologists at the Medical University of South Carolina and private labs in the area indicated that approximately 79% of images in their work were currently digital as opposed to 21% film. Eighty percent of the respondents expected to abandon film images completely within five years, indicating continued growth for these instruments.

The Virtual Microscope

It is a safe prediction that digital cameras will continue to evolve with higher resolving power, greater ease of use, and lower prices. In addition, a new class of instruments is emerging- the virtual microscope.[15] The concept is to digitally capture and store all of the visual information contained in a histological sample and allow the field to be scrolled and the magnification varied in a manner similar to a microscope. Earlier versions have used a combination of low-power scanning with a transparency scanner[16] and indexed higher magnification images. Other systems use software to "tile" or "stitch" individual high magnification images in a manner similar to the software that stitches together individual snapshots to form a panoramic view. An interesting new instrument currently in prototype is the Aperio ScanScope[17] microscope slide scanner. The ScanScope uses an interchangeable microscope objective to scan an entire microscope slide at resolutions up to 54,000 pixels per inch. For comparison, a typical high-resolution 35mm slide scanner operates at a resolution of 2000 pixels per inch. The resulting slide contains all of the image information from the original tissue slide as if each field were photographed with a 20× objective. The resulting file is 3 GB in size and even with image compression, only about three images can be stored on a CD-ROM. Despite its exciting potential in research and teaching, the huge volume of data will require the development of cheaper network storage, writeable DVD-ROM disks, and higher bandwidth networks to support widespread adoption.

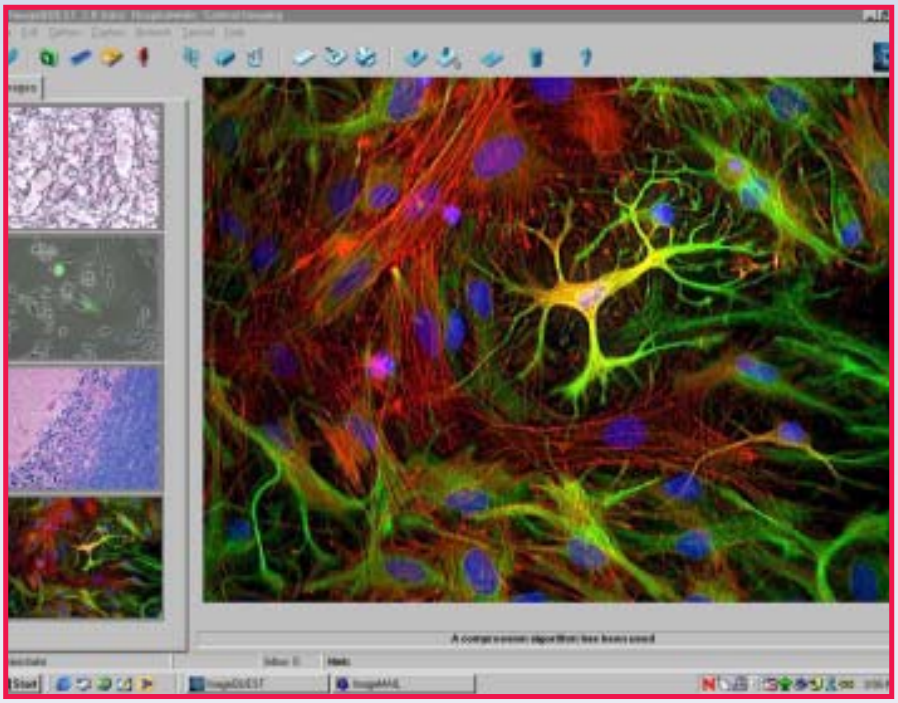

Fig. 6. A well-designed image database such as ImageQUEST is an essential component of any image archiving system. It maintains good organization and security for large and diverse image collections while making them instantly accessible through accession coding, demographic criteria, or Boolean search.

Picture Archiving and Communications System (PACS)

The efficiency and ease of use of digital cameras encourage their use for routine documentation. This quickly results in the rapid accumulation of large numbers of images. Fortunately, digital files have the potential for more compact and well-organized storage compared to the negative, slide, and print file cabinets of an earlier era. In addition, a well-designed image database can support recordkeeping, reports, research, and teaching[18] with different software packages drawing on the same image files. This can end the need for separate sets of transparencies for teaching, prints to file with reports, and negatives to archive. To achieve this level of functionality requires designing an integrated camera/database/network system[19] for the archiving and communication of medical images.[20]

For example, consider a surgical pathologist working in a teaching hospital environment. In this case, the system needs to be capable of acquiring and storing microscopic and gross images that will be used for reports, records, consultation, clinical/pathological conferences, quality assurance, teaching, and possibly research. The acquisition process must be streamlined to reflect the time and case flow pressures in the modern diagnostic lab. The image database must be integrated into, or at least

compatible with, the Laboratory Information System (LIS) and conform to the security and network protocols of the institution.[21] The system must also be compliant with applicable HIPAA[22] patient privacy regulations and DICOM[23] medical image interoperability standards. In addition, there will be oversight and regulation by academic accreditation bodies and various state and federal agencies. In brief, the system must provide a high level of security from unauthorized access[24] and other safeguards such as fail-safe image tracking and image-change logging to ensure the integrity and accuracy[25] of the images and the attached data.

ImageQUEST,[26] which allows the pathologist to operate his camera directly in the database using a TWAIN interface similar to that used to control a desktop scanner within a word processing program. The program supports the mapping of fields of information from other databases, as well as the dictated or typed entry of diagnostic dictation at the microscope. These data fields are then searchable by accession number, patient name, diagnosis, or any other attached data. By simple point-and-click, the pathologist can produce an illustrated report using premade templates with selected image and data information. This report can be forwarded to the referring

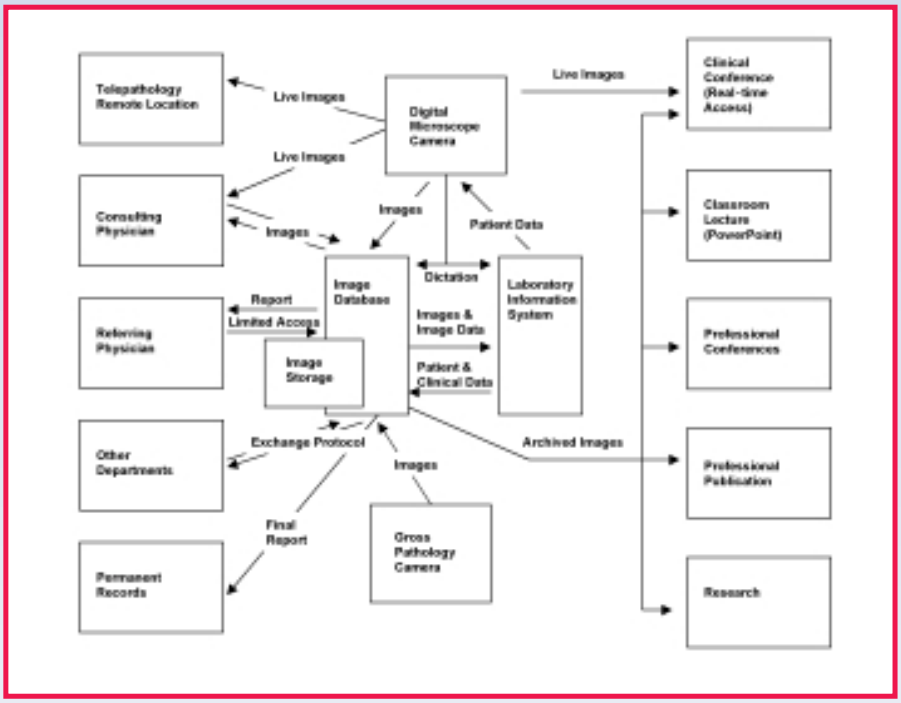

Fig. 7. A schematic diagram showing the theoretical flow of images and image data based on a possible pathology departmental system at a teaching hospital.

Fortunately, manufacturers of Laboratory Information Systems are finally recognizing the importance of integrating images with other clinical and demographic patient information. Other companies are producing stand-alone image database programs that can supplement and interface with the Laboratory Information System. A good example is

physician by either encrypted image email or accessed through a password-protected web site. With a networked conference room and a computer-driven classroom projector, the stored images can be presented directly from the database for a weekly clinical conference or downloaded to presentation software such as PowerPoint for teaching.[27]

Histological Digital Imaging in the Future

How might these technologies affect the laboratory of the future? With the rapid pace of camera and software development, it is difficult to accurately predict change over time. Still, long-term planning requires that the wise lab manager anticipates at least the general directions of growth. It is a reasonable assumption that a continued shortage of qualified histotechnologists,[28] managed care cost containment, and competitive pressures will make the histology lab of the future more automated. This, along with a growing concern for error reduction and patient security, will make embedded positive sample identification such as bar-coding necessary. Presuming that the histologic slide carries such identification, a suitable reader on the microscope will automatically access the Laboratory Information System and call up all relevant data that the pathologist needs to evaluate the specimen. The fully automatic digital camera will make all necessary adjustments for exposure, color balance, and focus in real time on a full screen display. Images will be snapped at the click of a foot pedal or voice command, allowing the pathologist's hands to always remain on the microscope. Each image will be inserted automatically into the database along with the other patient data and notes the pathologist dictates through voice recognition software. If required, the camera can also be used for real-time telepathology[29] or remote consultation. A fully illustrated surgical pathology report is automatically generated and filed. When performing final sign-out, the pathologist cross-checks the images with the report to reduce error and improve quality assurance. A notification is then automatically generated and sent to the referring physician by encrypted email. That physician can then use a preassigned password to access a secure web site to view and download the report. When the pathologist attends tumor board or a weekly clinical conference, he can call up the database from

the conference room computer and display the images and information as required in real time without spending additional preparation time generating a formal slide presentation. Later, when preparing for a lecture, professional conference, or publication, he can search the database by diagnosis or other criteria to find suitable images.

How far off in the future is such a system? Almost all of the equipment and software mentioned exists today, at least in a prototype form. Commercial vendors and individual labs are even now putting together systems with many of these features. As these systems demonstrate cost-effectiveness, improved quality control, and competitive advantage, other labs will rapidly adopt them. As usual in medicine, scientific curiosity will open the door, technology will create new opportunities, but in the end, it is always cost-savings that topples empires and creates new worlds.

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A Simple Method to Demonstrate *H pylori* and Mast Cells

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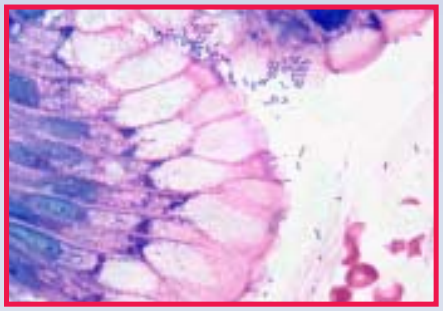
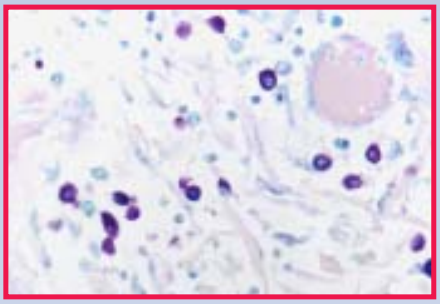
Discovered in 1983 and classified as *Campylobacter, Helicobacter pylori* (*H pylori*) is a gram-negative spiral-shaped bacteria 0.5 × 3.0 micrometers with 4 to 6 sheathed flagella attached to one pole. The spiral shape aids the bacteria in making its way into the mucous lining of the stomach and duodenum. It is the only bacteria able to colonize in the gastric mucosa.[1,2] In Perth, Australia, after months of trying, the "new" organism was finally cultured by Dr. Robin Warren and Dr. Barry Marshall because of a staff shortage over a holiday weekend. The two were frustrated in their efforts to prove *H pylori* responsible for many ulcers. To prove their theory, Marshall and a volunteer swallowed a solution containing the cultured *H pylori*. Within a week, Marshall was ill with gastritis. They published their experiment in 1985 under the title "Attempt to fulfill Koch's postulates for pyloric *Campylobacter*."[3,4] Not all of the postulates were fulfilled because neither one of them had developed ulcers.[1] The infection is found in 60% to 80% of patients with stomach ulcers and 90% of patients with duodenal ulcers. It is prevalent throughout most of the world and it is estimated that approximately 10% of the US population will become infected with this intestinal pathogen.



A number of methods to detect *H pylori* have been reported in the literature, yet individuals continue to inquire about what is the best method to demonstrate this organism. Few offer the simplicity, speed, and low cost of the method that follows. While many regard the silver stains as offering the best contrast, they are costly and capricious, making consistent and reproducible results problematic. With those methods the presence of any precipitate can obscure the bacteria, requiring repeat staining which can delay turn-around time and increase the cost to the lab.

What follows is an inexpensive and rapid method for the detection of *H pylori* that offers good contrast for organism identification and is simple to perform. Using this method, the contrast is sharp enough for the bacteria to be seen at 40×. It is also quite useful for the demonstration of mast cells. The use of the microwave to heat the stain reduces the staining time in the working Giemsa from 20 minutes in a 60° C oven to 2 minutes.



Method: Microwave Giemsa Stain

Fixation: 10% neutral buffered formalin

Quality Control: Tissue known to be positive for *H pylori*

Solutions:

1. Methanol, absolute
2. 1% Acetic Acid
   Glacial Acetic Acid ... 1.0 ml
   Distilled water ... 99.0 ml
3. Stock Giemsa Solution 2[5]
   Giemsa powder ... 1.0 g
   Glycerin ... 66.0 ml
   Methanol ... 66.0 ml

Mix glycerin and giemsa powder, microwave* on high for 1 minute, stir, and cool. When the solution has cooled to room temperature, add the methanol. If a microwave oven is not available, the solution may be prepared using a conventional laboratory oven by placing the glycerin-giemsa mixture at 60° C for 2 hours before stirring. Store the stock solution in the refrigerator.

* Sharp carousel model R-410DK, 1200 watts.

4. Working Giemsa Solution
   Stock Giemsa ... 7.0 ml
   Methanol ... 5.0 ml
   Distilled water ... 38.0 ml

Staining procedure:

1. Deparaffinize and hydrate to distilled water.
2. Rinse slides in methanol for 2 minutes.
3. Preheat the working Giemsa solution in the microwave on high for 45 seconds. Filter the hot solution into a coplin jar containing the slides. Stain for 2 minutes.
4. Rinse quickly in distilled water.
5. Dip slides once in 1% acetic acid.
6. Rinse quickly in distilled water.
7. Rinse in three changes of methanol.
8. Clear in xylene, and mount.

Results:

Red blood cells	pink
Nuclei	blue
H pylori, fungi, and other bacteria	blue
Mast cells	purple
Rickettsia	purple

In our hands, the use of methanol to dehydrate stained sections prior to mounting seems to provide lighter staining of surrounding tissue, offering better contrast with stained organisms.

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Concepts in Epitope Retrieval for Immunohistochemistry

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A newcomer to the world of immunohistochemical (IHC) staining will quite likely become quickly discouraged and overwhelmed. Attaining clean, specific immunostaining of all appropriate antigens with no distracting or misleading nonspecific staining is no small feat, even for the most experienced among us. The discussion that follows summarizes the relevant information needed to allow the reader to develop a coherent yet reliable method for antigen unmasking.

First appearing on the scene in diagnostic histopathology in the early 1970s, the application of IHC to routinely processed tissues represented a new era for the demonstration of antigens, allowing pathologists to correlate immunological findings with cytological and histological criteria.[1] In the decades that followed, there were various attempts to improve the sensitivity and specificity of the IHC method. These included the development of primary antibodies optimized for antigen binding in formalin-fixed tissues, enhanced detection strategies employing various enzyme-chromogen systems, and techniques for signal and chromogen amplification. Today, despite tremendous efforts by instrument manufacturers to bring this technology within reach of any laboratory, irrespective of staff expertise, IHC staining has become more complex, fraught with pitfalls for both the uninitiated and experienced alike. Thus, the ability to produce accurate, reliable, and reproducible staining with the vast array of antibodies to tissue and cellular antigens is tenuous at best.

Experts have long lamented the need for standardization of the IHC method to allow for comparable testing from one laboratory to another, but the many variables that can affect staining have stymied the Biological Stain Commission's efforts at standardization over the past decade.[2] The failure of all laboratories to use a standard fixation protocol for various tissues is but one illustration of the daunting challenge facing proponents of IHC standardization.

The widespread appeal of formaldehyde as a routine fixative during the past century has been largely due to its superior ability to preserve morphological detail. Although the exact mechanism of formalin fixation is still unclear, the chemical process leading to protein fixation by formalin appears to involve multiple chemical reactions among formaldehyde, various amino acid residues, and even peptide bonds. An additive fixative, this small molecule may offer an efficient means, through cross-linking, of retaining localized antigen in tissue. This is exemplified

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by the reported observation that cytologic samples fixed in alcohol will demonstrate improved IHC staining when post-fixation with formalin is used, followed by antigen retrieval.[3]

The chemical actions of the formaldehyde molecule in tissues are largely time dependent. Cross-linking is believed to require at least two distinct chemical reactions involving amino acid NH_2 groups, as well as their side chains which vary among the amino acids. Thus, the combined results of these binding actions upon varied chemical constituents make it difficult to completely predict formaldehyde's effects on tissue antigens. It is generally accepted that, as a consequence of formaldehyde-induced cross-linking of proteins, a highly dense protein scaffold results, which can sterically hinder the accessibility of antigens.[4] The time-sensitive nature of these combined reactions, especially when considering the variable fixation times for surgical tissues in the clinical laboratory, adds to the challenges of antigen demonstration with IHC. This contributes to the difficulties often encountered when working with archival tissues.

In order to fully appreciate the impact of cross-linking fixation on antigen staining, it is essential to consider the three-dimensional impact of the attachment of the formaldehyde molecule to protein. The protein scaffolding continues to build upon itself over time which may, in effect, throw a "net" over the antigens of interest. This can result in steric hindrance and lead to a conformational (shape) change (denaturation) of the epitope you are trying to visualize, which might prevent binding of the antibody molecule being used for staining. Despite these shortcomings, formalin-induced cross-linking of protein appears to serve as an efficient means of *in situ* preservation of antigen in most circumstances, despite the simultaneous masking of antigenicity.[5]

The past decade has witnessed a proliferation of reports in the scientific literature describing strategies and methods for restoring tissue antigenicity which will undo the denaturing effects of various fixatives. Attempts by industry to identify a fixative that does not denature antigens while providing acceptable morphology has thus far been unsuccessful. This voluminous body of literature, combined with a number of competing proprietary methods to achieve epitope unmasking, can all serve to confuse the user who wants to develop a simple but reliable method for routine application. Disparate reports in the literature recommend the use of either proteolytic enzymes, chelating agents (EDTA), or high heat (Heat Induced Epitope Retrieval - HIER)- with the proponents of each method touting theirs as best.

The early work of Fraenkel-Conrat[6] suggested that the denaturing effects of formaldehyde fixation, ie, the cross-linkages, could be disrupted at high temperatures or with strong alkalis. This observation laid the foundation for the current approaches to antigen unmasking. While the development of one standard retrieval method that is effective for all antigens has not been forthcoming thus far, sufficient data have emerged to enable the reader to develop a sensible strategy for attaining unmasking at the bench.

Epitope retrieval methods may be broadly characterized as those that require heat and those that do not.

Epitope retrieval without heat:

Alkaline solutions

The exposure of celloidin-embedded tissues to a strong alkaline solution (NaOH/methanol ratio 1:5 for 30 minutes) resulted in improved immunostaining.[7] This demonstrated that there is an inverse relationship between concentration of alkali (***C***) and time (***t***), ie, the greater the concentration of sodium hydroxide, the less time required to achieve the desired result. However, strongly alkaline solutions may have a decidedly negative effect upon section adherence to glass, and more importantly may be deleterious to tissue morphology.

Proteolytic Enzymes

Treatment of formalin-fixed, paraffin-embedded tissue sections with proteases has been demonstrated to effectively unmask some antigens. Studies have shown that the required time of exposure to enzyme is dependent upon the duration of formalin exposure which, unfortunately, is not known in many instances, especially for archival tissues. Overexposure to proteases can destroy tissue antigenicity, creating a false negative result. Optimal exposure times must be determined and tailored to each laboratory's fixation and processing protocol.

Incubate hydrated tissue sections to trypsin or pepsin for 20-30 minutes (as a starting point) at 37°C. Prepare enzyme as follows:
40 mg pepsin in 100 ml 0.01N HCl
10 mg trypsin in 100 ml 0.1 M Tris-buffered saline (pH 7.8).
Following enzyme incubation, rinse sections in three changes of Tris-buffered saline (pH 7.4).

Heat Induced Epitope Retrieval (HIER):

Heat induced antigen retrieval has been purported to offer the best means for a standardized approach to antigen unmasking. A number of reported observations regarding the use of heat for epitope retrieval warrant emphasizing.

1. **The relationship between temperature (*T*) and time (*t*) is inverse.** That is, the higher the temperature, the shorter the time needed to achieve beneficial results. Temperatures below 80°C are not significantly beneficial. A temperature of 100°C is optimal. One recent report indicated that intense IHC staining can be achieved with the following conditions: 100°C × 20 minutes; 90°C × 30 minutes; 80°C × 50 minutes; 70°C × 10 hours.[3]

2. **Retrieval solution pH is important.** A number of markers appear to stain well regardless of retrieval solution pH. These include L26, PCNA, AE1, EMA, and NSE.[3] Other antigens, including MIB-1 and ER, exhibit diminished staining at neutral pH but strong staining at very acidic or very alkaline pH. A last group of antigens, such as HMB-45, shows weak focal positivity at low pH but yields intense staining at high pH. These data suggest that, with few exceptions, a retrieval buffer (Tris-HCl or sodium acetate buffer) of pH 8.0-9.0 may be suitable for most antigens. Low pH buffers appear better for some nuclear antigens, such as retinoblastoma protein, estrogen receptor, and androgen receptor. In this instance, acetate buffer at pH 1.0-2.0 or glycine buffer (pH 3.6) appear superior to citrate buffer.

3. **The significance of retrieval solution composition is unclear.** High heat using distilled water alone will achieve improved IHC staining, suggesting that the chemical composition of the retrieval solution is not important in many cases. When heat induced epitope retrieval was first reported, buffer solutions containing zinc (sulfate) or lead (citrate) salts were recommended. However, it is now generally agreed that the use of solutions containing such toxic salts offers no advantage over simple buffer solutions and should therefore be avoided. While many laboratories have adopted the use of citrate buffer, in some instances this solution has appeared less effective than acetate buffer, Tris-HCl, or glycine-HCl.

4. **The heating source is not significant to outcome.** Despite what others may tell you, the manner in which required temperature is achieved and maintained (vegetable steamer, microwave, or pressure cooker) is more a matter of convenience. Microwave users may maintain that they can complete retrieval faster, but keep in mind that 2 to 3 cycles of heating and cooling (to avoid the boiling over of retrieval solutions) are necessary for optimal IHC staining. One report indicates that microwave antigen retrieval may yield unanticipated artifact staining patterns.[8]

If you are using a domestic pressure cooker with 103 kPa/15 psi, keep in mind that you are at temperature when pressure is achieved. Excessive incubation of slides at high heat and pressure can result in compromised histology, particularly in skin where collagen and elastin can appear fragmented and disrupted. Pressurized boiling for 1-2 minutes is sufficient for epitope unmasking.

With a household microwave oven, it is important that you determine (calibrate) the time needed to achieve temperature with your particular oven. Repeated boiling cycles have been reported to be more effective than extending the boiling time of a single cycle. Heat a plastic coplin jar or other microwave-safe vessel until the solution begins to boil, taking care not to boil the solution out of the vessel. If your device takes 5 minutes to boil, allow 1-2 minutes in between heating cycles to maintain temperature without boiling over. Repeat again for a third cycle of heating.

The tendency among some vendors to give their retrieval solutions proprietary names may prevent the user from knowing what buffer has been purchased, which will put you at a disadvantage. You haven't purchased some special magic in their proprietary concoction, but simply the convenience of having the buffer solution premade for you. Insist on knowing what you are using.

EDTA:

A recent report by Morgan et al[9] suggests that calcium ions bound to proteins inhibit IHC staining of those proteins. This observation has been confirmed by others.[5] Parallel frozen sections were stained for MIB-1 and thrombospondin after prior incubation in 50 mM $CaCl_2$ at 4°C overnight. Sections that did not receive exposure to calcium stained intensely positive for these markers, yet those exposed to calcium chloride did not. Furthermore, when sections receiving calcium chloride pretreatment were subsequently treated with EDTA, a calcium chelator, intense immunostaining was restored, suggesting that calcium may induce conformation changes in antigen that are reversible.

Conclusion

Immunohistochemical staining techniques continue to be an evolving science. While attempts are under way to identify a means for standardization of this technique, the complexity of tissue fixative interactions, lack of standard fixation protocols from laboratory to laboratory, and the wide array of clinically relevant antigenic markers and detection strategies make standardization unlikely in the short term. It is left to each of us to determine a rational and reproducible protocol for antigen unmasking that is tailored to the fixation and processing schemes employed in our respective laboratories.

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Marine Histopathology at Cooperative Oxford Laboratory

Carol B. McCollough
HT/HTL(ASCP)

It's 4 PM on a crisp October afternoon when the white pickup truck rolls into the driveway at Cooperative Oxford Laboratory (COL). Stacked in the bed are 10 onion sacks filled with dirty brown lumps- oysters. Roy Scott comes to my office and announces, " I've got some samples for you," so I head out to the lot to help haul the sacks into the Wet Lab to store them in tanks with flowing seawater from the Tred Avon River. This is the true beginning of a day in the life of the Diagnostics & Histology Laboratory at COL. Next morning, my staff and I begin the annual process of determining the health of Maryland's oyster population. By the time we are finished in mid-December, we will have examined 1330 individual animals for the routine samples, plus another 360 ± 60, all using routine paraffin histology and microbiological assays for oyster diseases.

The US Bureau of Commercial Fisheries established the Oxford Laboratory in 1960 for the primary purpose of investigating oyster diseases that struck Chesapeake Bay and Delaware Bay in the late 1950s. It became the Cooperative Oxford Laboratory in 1987, through an agreement between Maryland Department of Natural Resources (MD-DNR) and the NOAA National Marine Fisheries Service to share the facility and cooperate in research. Currently, administration of federal programs at COL is carried out by NOAA's National Ocean Service Center for Biotechnology. Scientists at COL investigate health problems of finfish, shellfish (including oysters, various species of clams, scallops, and blue crabs), and other aquatic life in the Chesapeake Bay and along the Atlantic Coast. They also collaborate with scientists nationally and internationally to improve understanding of aquatic animal health and to develop management strategies to prevent and mitigate diseases. The laboratory participates in the National Marine Mammal and Sea Turtle Stranding Network, investigating strandings of these rare and endangered animals in Maryland, and sharing information, samples, and expertise with other institutions and coastal states. New techniques for classifying and mapping critical reef habitats in Chesapeake Bay have been developed recently at the laboratory. These methods are now being applied in Maryland's oyster restoration efforts.

The Diagnostics & Histology Lab is a subproject of the Oyster Disease Research Project (ODRP) of the Fisheries Division within MD-DNR. ODRP actually encompasses investigations into the health of any and all species of aquatic resources, as required. There are four state of Maryland staff members: Charlie Gieseker, MS, and I are pathologists; Sue Tyler, HT, and Jud Blazek are histotechs. NOAA also has a histotech on staff, Dorothy Howard, HT. The state staff provides technical support to both state and federal projects. The Immunoassay Laboratory is also part of ODRP. This lab is primarily concerned with the development and adaptation of clinical immunological techniques, molecular biology, and shellfish pathology. Lee Hamilton is the immunology technician. Chris Dungan, MS, is the research scientist who develops studies for and administers the ODRP. Federal scientists include Gretchen Messick, MS, HT, crustacean pathologist; Shawn McLaughlin, PhD, shellfish and coral pathologist; Jay Lewis, MS, shellfish pathologist; and Fred Kern, Fishery research biologist.

Fig. 3. Jud Blazek embeds razor clams in paraffin.

Fig. 1. Charlie Gieseker trims paraffin blocks after embedding crab tissue.

Fig. 2. Carol McCollough sections paraffin blocks of oyster spat.

Work on oysters is focused primarily on two protozoan pathogens, *Perkinsus marinus* (dermo disease) and *Haplosporidium nelsoni* (MSX disease). A pair of us necropsy each 30-animal sample, measuring and shucking each oyster carefully, noting macroscopic anomalies, and collecting two tissue samples. The oyster's rectum is excised and cultured for *P marinus*, since it is believed that the route of infection

is through the gut epithelium. A cross-section through the mantle, gills, and digestive diverticulum is collected and fixed for histology. After embedding and sectioning, these tissues are all stained manually with H&E. Charlie and I then examine them for the presence of *H nelsoni* and any other microscopic pathology. After incubation, the rectal biopsy is fished out of the culture tube, macerated on a glass slide, and stained with Lugol's iodine. The starchy cell wall of *P marinus* stains black and is easily seen at 40×. Some animals are infected so heavily that we can see the black color develop with the naked eye. In these cases, the host oyster is in the terminal stage of the disease and not likely to survive longer than two weeks. *P marinus* is difficult to visualize with an H&E, but *H nelsoni* is readily identifiable. Its plasmodium looks like a small purple potato and has multiple nuclei. The name "MSX" stands for multinucleate sphere, unknown. Typically it first invades the gills, and once past the basement membrane of the gill epithelium it becomes systemic. Sporulation occurs infrequently, typically in young hosts, and the eosinophilic and slightly refractile spores are found in the epithelium of the digestive diverticula. These spores are also acid-fast; this is a characteristic of *Haplosporidia*. In animals with heavy infections, the H&E cross-sections appear homogeneous to the naked eye, while normal and less heavily infected animals show a great deal of tissue detail and contrast.

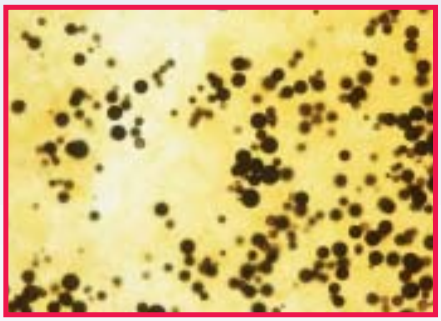

Fig. 4. *Perkinsus marinus* in oyster (Crassotrea virginica). Wet mount with Lugol's iodine. 40×

Work in the immunology laboratory has focused on the detection of *P marinus*, both in oyster tissue and in the water column. A polyclonal antibody was developed to *P marinus* and has been used for flow cytometry studies of parasite load in natural water, an ELISA assay, and DAB streptavidin-biotin and FITC immunostains useful on paraffin-embedded tissues. This antibody has also been useful in shedding light on the taxonomy of the *Apicomplexa* and their possible relationship to the dinoflagellates. Efforts are now under way to develop a monoclonal antibody, and to isolate and propagate *Perkinsus* sp. that infect other shellfish hosts to determine if these hosts harbor the same parasite species as oysters, or others.

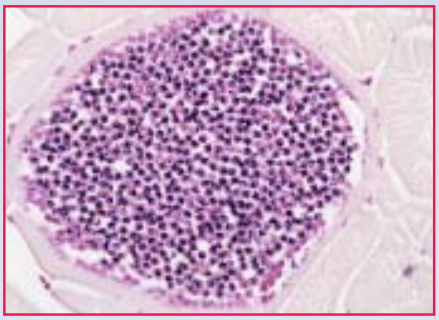

Fig. 5. Cyst containing spores of *Kudoa* species infecting muscle of Atlantic menhaden (Bevoortia tyrannus). Gram's stain; note gram-positive polar bodies. 40×

Lest you think that oysters occupy all of our interest, be assured that other aquatic species also receive our scrutiny. During the recent *Pfeisteria piscicida* scare in Maryland, the histology staff necropsied and fixed, embedded, sectioned, stained, and examined hundreds of Atlantic menhaden exhibiting dermal lesions. No histological evidence linking *P piscicida* with these lesions was found, however, spores of *Kudoa* sp., a myxosporean parasite, were found in the musculature of all specimens with lesions. In addition, a novel plasmodium was detected in many of the lesioned fish, and work to identify this organism is presently under way in collaboration with senior scientists at US-FDA and The Johns Hopkins University. A broad suite of special stains is being used to characterize the organism, including PAS, Protargol (Bodian), Feulgen, and alcian blue. The histology staff also works in the field collecting and fixing lesioned fish for EM. We recently collaborated with scientists from the Maryland Department of Agriculture in identifying a new species of *Mycobacterium* in wild striped bass, performing hundreds of AFB stains in the process. These fish also exhibited severe dermal ulcerations. Occasional fish kills occur in wild or aquaculture populations of finfish, and our staff prepares and examines the histological slides to determine causes of mortality. Planned studies of fish health and population status are also part of our repertoire. One recent study to characterize American eel populations in Maryland's Chesapeake Bay had us preparing hundreds of H&E slides of eel gonads. Great cocktail party conversation!

Work with crustaceans at COL emphasizes the health of blue crabs, those feisty, tasty morsels that are the mainstay of summer seafood in the mid-Atlantic. Crabs have been of interest at COL for many years, and a definitive treatise entitled "Histology of the Blue Crab: A Model for the Decapoda" was authored by now-retired Phyllis T. Johnson. Recent research has focused on *Hematodinium* sp., a parasitic dinoflagellate found in both the hemolymph and tissues of sick crabs. Watermen from Maryland coastal bays have reported crabs dying in crab pots. *Hematodinium* sp. infections were found in adult and juvenile crabs from the coastal bays of Maryland, Delaware, and Virginia. Other crustaceans affected by *Hematodinium* spp. include amphipods and green crabs, plus Tanner crabs and other commercially important species. These parasites are easily visualized in both cytology smears and tissue sections using an H&E stain. They exhibit a characteristic clumped chromatin that stains dark blue with hematoxylin.

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Work in the Diagnostics & Histology Lab at COL is varied and interesting. We work on a diverse group of species exhibiting a variety of diseases and pathologies. While most of the work consists of tinctorial stains on FFPE tissues, we are expected to execute necropsies, immunostains, and microbiological assays. No day is ever the same, even when working on the same project, because each sample is different, and one never knows what fascinating discovery lies in the next animal or on the next slide. Or what unique situation or question might have to be addressed. The question this morning was from a field biologist who had just finished surveying a pond and had discovered the skeletal remains of several young sturgeon- "How should I preserve the skulls to use as show-and-tell material?" What fun!!

Ups and Downs of a Histology Student

Richard Sparling, AS, HT(ASCP)

In 1994 I decided to make a major career change in my life. As a father of two, working full-time on the night shift to help defray expenses, I entered the Histology Program at State University of New York's College at Cobleskill. It was at this time that I began my experience in the exciting world of histology. I started out feeling interested, curious, excited, and most of all, a bit scared. As the first semester began, I realized that the path I had chosen to take in life was going to be a greater change than anything else I had ever done.

In my two years at SUNY Cobleskill, I experienced many ups and downs. I would like to share some of these with you and I'm sure that you will be able to relate to them. Starting this training at the age of 44 was different, to say the least, but it was also a slight advantage. I was here to learn a career that would allow me to help others even if it was behind the scenes. My emotions ran high as I began my biology courses, which explained our physical makeup and why our bodies function the way they do. The knowledge that I was gaining kept me thinking about my future career in histology, even when I wasn't at school. I would meet old friends and begin explaining what I had learned and how it was a great change in my life. And with this positive attitude I made it through the first year with confidence that my change in careers was a good choice.

In the fall of 1995, I began the semester with a renewed exuberance. Then the test began! I started my second year and encountered the histology course. I had no idea that the professor expected me to know ALL of the cells and tissues in the human body. Now the thoughts of whether or not I had made the best choice by entering the field of histology began to surface. But I wanted this new career and I buckled down to work. Ultimately, the semester went quite well and I mastered the material. And then, during the final semester came the part of the training that I had long awaited- Microtechniques, the hands-on portion of my training. Learning the proper way to preserve, process, cut, and stain tissues was so exciting. The professor began showing us how to set up the processor and cut the proper size tissue for cassettes. None of this seemed to look all that difficult. As the weeks went on and we were taught the way to section, stain, and coverslip, through both lecture and demonstration in lab, I felt that these also looked quite easy. Well, reality set in quickly! How could something that the professor made look so easy, be so very difficult for me to do? I thought that there must be something physically wrong with me. Try as I might, the sections kept sticking, folding, and tearing, until I finally got the section I had long awaited. I carefully moved it toward the waterbath, only to have the air currents whip the ribbon against my arm! After many frustrating attempts, I finally managed to get a ribbon of wrinkle- and scratch-free sections onto the waterbath and then onto the slide.

Now that I had the "perfect sections," I was ready to stain them before turning them in to the professor for grading. All was going well, and then I noticed that my tissue was no longer on the slide but floating its way to the bottom of the coplin jar. I persevered and stained another slide. This time the tissue remained on the slide, but the differentiation was terrible. Who ever said a Masson's was easy?

After many attempts, I finally began to get a grasp on the staining. The next glitch was coverslipping. It looked easy and I thought I did a good job. Then I noticed the professor looking at one of my slides, so I decided to ask her how it looked. She said that my staining was all right and that she wanted me to look and tell her what I thought of the slide. As I started to look in the scope I heard her chuckle and soon saw why. There was this small, perfectly round circle surrounded by a dark line right in the center of the tissue. Slowly I was learning to be humbled. It really was an art and I needed to learn to become the artist.

It was only after leaving college and starting work in a histology lab that I truly realized why the professor had required that students learn so many details in her courses. I worked at that job for a year and then had an opportunity to return to SUNY Cobleskill as a lab technician for the biology courses, including all of the histology courses. My favorite course is Microtechniques- the one I struggled through years ago. Now my job allows me to be in a position to help students with the same problems I used to have. It is very gratifying and I feel that my presence is an asset to the training program. I have the expertise of a certified histotechnician, but I am still on a similar peer level as the students. I watch students going through the same ups and downs that I had. When they are experiencing the downs, I am now able to ease their anxieties and help them to persevere by providing technical advice and sharing my experiences with them. I explain that they aren't having any problems that thousands

of histotechs before them have not experienced. It is beneficial to all students to hear similar explanations not only from the professor, but also directly from a former student. Sometimes it is an advantage to me as the technician to not be in a position of authority. I feel that being a student, working in the field, and now returning to the college to work with the students is one of the most rewarding experiences I have ever had.

Latex Allergy

Wanda Shotsberger, HT(ASCP)
Medical University of South Carolina

In 1987, the Centers for Disease Control issued its recommendations for latex glove use as part of universal precautions against HIV infection.[1] This action, coupled with OSHA's Bloodborne Pathogen Standards, increased latex glove use by 17-fold,[1] and thus, dramatically increased healthcare workers' exposure to latex allergens. The result has been an alarming increase in the reports of latex allergy among healthcare workers. Although allergy to latex is considered uncommon among the general population with less than 1% prevalence,[2] among healthcare workers and others with high rates of exposure to latex the rate is as high as 17%.[1]

Latex is a plant derivative. It is produced from the sap of the tree *Hevea brasiliensis*.[1] Thus, latex contains plant proteins, many of which are now believed to be plant defense proteins. The proteins protect plants by causing unpleasant reactions, which keep herbivores from returning to a particular plant.[2] These proteins are also thought to be the cause of latex allergies, as they are present in latex gloves and other products made from latex.

As with any medical condition, prevention is preferable to treating a condition or attempting to cure one, but preventing latex allergy is difficult. In a hospital setting, most devices have a latex component somewhere in them. Gloves are obvious culprits, but syringes have rubber stoppers, stethoscopes have rubber tubing, tourniquets are often latex strips, and even the floor mats in entryways have rubber backs. Thus, prevention of latex allergies can be a daunting task. Fortunately for healthcare workers, many hospitals have adopted a latex-safe policy and have switched to latex-free devices wherever possible. These hospitals have outlawed latex balloons in their facilities, and have switched to vinyl and nitrile gloves in place of latex for many purposes, such as food handling and patient exam room use.

Prevention efforts are noble in intent, but what should you do if you suspect they have not helped you? If you suspect you have a latex allergy, there are several steps you should take. The first step is to have your allergy diagnosed. From there the steps you take will depend on the severity of your reaction.

Diagnosing a latex allergy is not easy. There are only two accepted ways to diagnose a latex allergy: blood work, which is the allergen-specific IgE test, and skin prick testing. IgE testing has a high false negative rate, so skin prick testing has been shown to be the more reliable test,[2] however, it carries a risk of anaphylactic shock and should only be administered by an experienced professional.

Reactions to latex vary from sub-acute reactions to anaphylaxis. Sub-acute reactions are rashes that are usually limited to areas of skin directly exposed to latex. An example would be dry, itchy skin on the hands limited to the area covered by gloves. These reactions are referred to as " irritant contact dermatitis." [1]

Next in severity is " allergic contact dermatitis," characterized by redness, swelling, and weeping sores. This reaction is not limited to the area directly exposed to latex and can cover the arms and chest of an allergic person who has simply donned a pair of latex gloves. This reaction generally increases in severity with each exposure. It is also called delayed hypersensitivity, or Type IV allergy. Symptoms may not appear for 1 to 3 days after exposure with this type of reaction.[1]

The most severe reaction is Type I, or immediate hypersensitivity. This reaction can manifest as hives, asthma, or a respiratory allergic reaction similar to hay fever. The patient can experience itchy, watery eyes, runny nose, and excessive sneezing. Anaphylaxis is the most severe of the Type I reactions, where a rapid drop in blood pressure is the hallmark symptom. Immediate medical intervention is required with this reaction, as severe shock or death can occur.[1]

Once diagnosed, the second step for the latex allergic patient obviously is to limit exposure to latex, but this is not as easy as it may seem. Latex gloves are the most obvious threat to the allergic healthcare worker, but the list of products, both medical and household, containing latex is extensive. Blood pressure cuffs, Band-Aids, catheters, condoms, ACE bandages, some balloons, T-shirt iron-ons, the handles on " comfort grip" tools- all of these and many, many more products contain latex, which may cause a life-threatening reaction. Consider the following suggestions:

1. Switch from powdered latex gloves to nitrile gloves. Vinyl gloves are permeable to many of the chemicals we use in the histology lab, so they are not an option for laboratory use.
2. When eating out, ask your wait-person if latex gloves are used in the kitchen. Food handling leaves latex proteins on food, which can cause an allergic reaction.
3. Avoid toys (balloons, Koosh® balls, tennis balls) that contain latex.
4. Avoid other household products that contain latex such as stretchy Band-Aids, rubber tub mats, rubber dish draining

products, Playtex® gloves, and even pantyhose. Obviously, the list of products containing latex is extensive, and eliminating exposure is impossible. Exposure often comes from unlikely sources, and patients do not always know what has caused a reaction.

Many patients also experience cross-reactions with different foods. These reactions are thought to result from the plant proteins discussed earlier. These foods include, but are not limited to: banana, kiwi, avocado, peanuts, and fruits known as the stone fruits, which include peaches and cherries.[3] These fruits and other foods that cross-react are best avoided.

Latex exposure is not limited to direct exposure. People with latex allergies are at risk from airborne exposure as well. Latex particles attach to the powders in gloves and become airborne with donning and removal of gloves, especially if gloves are "snapped" off.[1] Exposures of this type are not limited to the work environment, however. Walking into an auto parts store where hoses, floor mats, tires, and windshield wiper blades are unpacked, transferred, and stored is also a hazard. If you have a latex allergy, consider carefully what is inside a place of business before you even open the door.

The final step in treating a latex allergy is necessary only for those with a Type I hypersensitivity. These patients should wear a Medic Alert® -style bracelet engraved with "Latex Allergy," and should carry an epinephrine syringe in case of a reaction. These patients should also call the fire rescue unit nearest them and report their allergy. This will allow that station to stock non-latex gloves for any calls to the home of the allergic individual. These may seem like extreme measures, but for those with a Type I hypersensitivity, treatment by a latex glove-wearing paramedic gives a whole new meaning to a life and death situation.

Latex allergy is a difficult condition. The difficulties in diagnosing the condition are problematic, and prevention and treatment are quite a daunting task. The statistics will continue to show a rise in latex allergy symptoms among those people continually exposed unless latex is greatly reduced in hospital and industrial settings. Considering the difficulties this presents, it is not likely to happen in the foreseeable future.

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Reprocessing of Tissue Blocks

Gilles Lefebvre, Managing Editor

At times, a tissue block may need reprocessing. Many factors can lead to blocks unsuitable for diagnosis, including inadequate fixation time, a specimen that is too thick, and improper reagent rotation schedule. Some of the well-known results of improper processing are:

- Depression in the block once it has cooled. Sometimes the depression may take 24 hours or more to develop.
- The specimen is "mushy."
- The color of the block is not uniform, appearing more opaque where the tissue is located.
- Sections are difficult to obtain because there are holes where the tissue should be.
- Sections "explode" when floated in the water bath.

To reprocess the specimen, the paraffin block surrounding it must first be removed. To do so, place the block in a base mold that is sized to accept the solid block. Place the base mold onto the warming plate of a tissue embedding console. The block will take approximately 20 minutes to melt. Once the paraffin is melted, transfer the specimen in a properly identified cassette. Place the cassette in the tissue processor basket.

With some commercial tissue processors, the operator can set up a program reversing the processing steps. However, this reversed process does cause reagent contamination. On the Tissue-Tek® VIP® Series tissue processors, the use of the built-in cleaning cycle makes the procedure fast and economical since the processing reagents are not used. The Tissue-Tek® VIP® will automatically prompt the operator at the end of the processing cycle to start a cleaning cycle. A cleaning cycle can also be done on demand. Simply place the basket containing the specimens that need to be reprocessed into the processor and start a cleaning cycle. Automatically, the Tissue-Tek® VIP® system will first heat the retort for 10 minutes to remove any residual paraffin. Then, nine changes of xylene will take place, followed by five changes of alcohol. On the latest model, the Tissue-Tek® VIP® 5 system, the user can program the number of changes for each of the cleaning stations.

Once the cleaning cycle is completed, the specimens can then be processed again starting with a lower grade alcohol, such as 70%, to avoid buffer salt precipitation within the tissue. The reprocessing should not be performed with the standard overnight process typically used with fresh tissue. The extent of chemical exposure renders the specimens dangerously over-dehydrated, to the point that they may become unsuitable for diagnosis. A shorter program should be used, with immersion times at least half the original ones.

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Educational Opportunities in 2002

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Title: " **Temporal Artery Biopsy**"
Speaker: Peggy Wenk, HTL(ASCP)
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January 18 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Mohs Histographic Surgery**"
Speaker: Clifford M. Chapman, MS, HTL(ASCP)QIHC

February 15 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Handling Chemical Hazards in Histology**"
Speaker: Maureen Doran, BA, HTL(ASCP)

February 20 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
Title: " **Laboratory Microwave Ovens: Theory and Non-staining Applications in Histology**"
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March 15 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
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Speaker: David Tacha, HT(ASCP)HTL

March 15-16 — **KENTUCKY SOCIETY FOR HISTOTECHNOLOGY**
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March 20 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
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March 21-24 — **NSH Region III hosted by TENNESSEE SOCIETY FOR HISTOTECHNOLOGY**
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Email: Mike_Lafriniere@memorial.com

March 22-23 — **IOWA SOCIETY OF HISTOTECHNOLOGY**
Annual Spring Symposium
Site: Best Inn & Suites, Des Moines, IA
Contact: Debra Foster (563) 244-5665
Email: pdfoster@cis.net

April 11-13 — **NSH REGION II & NEW JERSEY SOCIETY FOR HISTOTECHNOLOGY**
Site: Holiday Inn, Atlantic City, NJ
Contact: barbara.bujak@bms.com
mary.uberti@bms.com

April 17 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
Title: " **The Highs and Lows of pH**"
Speaker: Betty Tallardy, HT(ASCP)QIHC
Contact: NSH office (301) 262-6221

April 19 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Histopathology of the Prostate**"
Speaker: Dean Troyer, MD

April 26-27 — **WASHINGTON STATE SOCIETY FOR HISTOTECHNOLOGY**
Site: Seattle area
Contact: Linda Cherepow (206) 587-0430 ext. 5044
Email: cherepowl@immunex.com

May 4-6 — **FLORIDA SOCIETY OF HISTOTECHNOLOGY**
Site: Deerfield Beach Resort, Deerfield Beach, FL
Contact: Tonia Breckenridge (850) 937-9995
Email: tabrecken@aol.com

May 15 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
Title: " **Cytochemical Detection of Apoptotic Cell Death**"
Speaker: Mark C. Willingham, MD
Contact: NSH office (301) 262-6221

May 17 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Special Stains and the Liver Biopsy**"
Speaker: Michael Lovell, MD

May 17-18 — **GEORGIA SOCIETY FOR HISTOTECHNOLOGY**
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Contact: Connie Waurin (404) 686-2385
Email: Connie_Waurin@emoryhealthcare.org

June 6-8 — **NSH REGION III/ARIZONA SOCIETY FOR HISTOTECHNOLOGY**
Site: Sheraton Phoenix Airport Hotel
Contact: Andi Grantham (520) 626-4415
Email: algranth@u.arizona.edu

June 15 — **ALABAMA SOCIETY FOR HISTOTECHNOLOGY**
Site: Birmingham, AL
Contact: Rita Humphrey (205) 939-9639

June 19 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
Title: " **Basic Troubleshooting of the H&E Stain**"
Speaker: Joan Vesey, HT(ASCP)
Contact: NSH office (301) 262-6221

June 21 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Paraffin or Plastic: Concepts for Optimal Management of Bone Specimens**"
Speaker: Robert A. Skinner, HTL(ASCP) and Frances Swain, HT(ASCP)

July 17 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
Title: " **Competency Assessment for the Histotech**"
Speaker: Daniel Huan
Contact: NSH office (301) 262-6221

July 19 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Quality Asurance in Anatomic Pathology- It's Everybody's Job**"
Speaker: Joseph W. Galbraith, MBA, HTL(ASCP)MT

August 16 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Space- the Final Frontier**"
Speaker: Elizabeth Sheppard, BA, HT(ASCP)

August 21 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
Title: " **Immunohistochemistry**"
Speaker: David Tacha, HT(ASCP)HTL
Contact: NSH office (301) 262-6221

September 20 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Tissue Culture**"
Speaker: Gillian Rittman, ONC

September 28-October 3 — **NSH SYMPOSIUM/CONVENTION**
Site: Long Beach, CA
Contact: NSH Office
4201 Northview Drive, Suite 502
Bowie, MD 20716-2604
(301) 262-6221
Fax: (301) 262-9188
Email: histo@nsh.org
Web page: www.nsh.org

October 18 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **The Conversion of Special Stain Procedures to Immunohistochemistry**"
Speaker: Nancy L. Shellhorn, BA, MT(ASCP)

November 3 — **CONNECTICUT SOCIETY FOR HISTOTECHNOLOGY**
Site: Mid State Hospital, Meriden, CT
Contact: Cindy DeRiso (203) 694-8587 ext. 3017

November 15 — **UNIVERSITY OF TEXAS HEALTH SCIENCES CTR/ SAN ANTONIO**
Teleconference: 12:00 pm Central Time 1-800-982-8868
Title: " **Breast Cancer- The Role of the Histology Lab**"
Speaker: I-Tien Yeh, MD

December 18 — **NATIONAL SOCIETY FOR HISTOTECHNOLOGY TELECONFERENCE**
Title: " **Cytoprep for the Histotech**"
Speaker: Beth Cox, SCT(ASCP)
Contact: NSH office (301) 262-6221

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Technical Bulletin for Histotechnology