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First International Histology Congress in Central America

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I am pleased to report that the first International Histology Congress held in San Jose, Costa Rica, Central America, June 14-16, 2012, was a huge success. With more than 100 participants from 8 countries in the Americas in attendance, the presenters and participants (from as far south as Chile and as far north as Canada) helped to make a truly international educational event.

Fig. 1. Histology students from the University of Costa Rica Medical School, San Jose, Costa Rica.

I would like to personally thank everyone who helped make this Congress a reality. Along with many companies from the United States and Central America, we had strong representation from our National Society for Histotechnology (NSH). Brenda Royce, Jean Mitchell, Beth Sheppard, and Jerry Santiago all did an outstanding job at promoting membership in the NSH and in encouraging participants to attend our upcoming symposium/convention in Vancouver, British Columbia, Canada, this fall.

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I have been asked why and how this meeting came to be held in Costa Rica. I lived in Costa Rica, Panama, and Colombia for almost 10 years back in the 1970s and 1980s. I met and married my wife, Ana, in San Jose, Costa Rica, and we periodically return to visit family and friends. Approximately 4 years ago, I began working with and conducting classes for students and histologists in San Jose during my visits. In June 2011, on short notice prior to one of my visits, I called Adonay Jaen, Supervisor of Histology at the San Juan de Dios Hospital in San Jose, to ask him if he would like me to present a couple of classes while I was in town visiting family in San Jose. We set a date and he told me that he was going to invite a few students and histologists from around the city. To my surprise, when I arrived to give the class, I walked into a classroom of more than 60 individuals including histology students, pathologists, and medical personnel from the University of Costa Rica Medical School, as well as those from Nicaragua and Panama. Accomplished with only 2 weeks notice, this session demonstrated the real hunger for education in pathology and histology that exists in this part of the world.

Fig. 2. Opening Ceremony of the Congress. (Left to right) David J. Davis, Pathology Manager, University of Costa Rica Hospital; Msc. Xinia Alvarado, Director, Medical Technology School, University of Costa Rica; Adonay Jaen, IHC Lab Manager, San Juan de Dios Hospital, San Jose, Costa Rica; and Dra. Lidia Ugalde, Pathology Director, Hospital Mexico, San Jose, Costa Rica.

Fig. 3. David J. Davis (left) with Juan Carlos Araya, from Santiago, Chile, President of Pan-American Medical Technologist Society of South America.

After the class over a coffee, Adonay and I talked about the success of this 1-day meeting and what we might be able to accomplish if we had a year to plan for a future meeting, and so planning for the 2012 International Congress had officially begun. (Note: In this part of the world, a symposium or convention is referred to as a Congress [Congreso].) Funding for such an educational event was high on the list of things to do and we ultimately received outstanding support from the hospitals in San Jose, the University of Costa Rica, many of the local and international companies from Central America and the United States, and from the NSH. We were fortunate to have received 3 different awards at the 2011 NSH that went toward our meeting in San Jose: the Lee Luna Foreign Travel Scholarship Award sponsored by Leica Microsystems awarded to May Chin, the Ventana Medical Systems Immunohistochemistry Scholarship awarded to Adonay Jaen, and the Leica Leadership in Management Award awarded to David Davis. Fortunately, things came together at the right time. No one person made this meeting happen. It took a real group effort from some truly dedicated companies and people.

Our objectives for the Congress were to provide an opportunity to:

- Expand and augment knowledge in the field of histology, including the evolution of in situ hybridization and other molecular techniques
- Create and strengthen important networks within the South, Central, and North American histology communities

Fig. 4. Students at the Congress promoting the National Society for Histotechnology (NSH).

Fig. 5. Vendor area interactions.

I would like to share part of my welcome letter, which was handed out at the Congress.

"It is clearly an exciting time in the histology laboratory with both the continued use and utility of well established techniques and what feels like an almost daily expansion of markers (e.g. new antibodies for immunohistochemical evaluation, new probes for in situ hybridization) as we in histology continue to meet the challenges of the modern Pathology Laboratory.

Histology is our profession. It took a great deal of dedication and individual commitment for each of you to attend this meeting. Each of you needs to be commended on taking that extra step to be that professional. Let's work together to remove borders and language barriers to promote our World and our Profession."

Based on participant feedback, we feel that the meeting accomplished our goals and, in addition, ignited an interest in the community to continue with future conferences. Adonay and I have already started to work on the next two meetings scheduled for June 2014 in San Jose, Costa Rica, and 2016 in Panama City, Panama. We are considering Venezuela or Chile in 2018 or 2020. By holding this meeting every 2 years, we hope that that time frame will allow us to muster the support we will need from within the region and internationally. These next two meetings will be our barometer to see where, when, and if we will continue with this Congress. I truly hope this is just the beginning of many more to come.

Financially, the 2012 Congress essentially broke even, with minor deficits covered by the hosts. It was well worth every dime and every hour we spent planning for and holding the Congress. Anyone interested in contributing talent, time, and funding is invited and encouraged to join us for the 2014 and 2016 Congresses. I encourage others to get involved and help with the planning of the meeting and presentation of talks. It is my expectation that participants will find the efforts rewarding. We each have something to offer and each of us can make a difference by sharing what we know. I feel that participation in such outreach efforts provides a great way to learn about other cultures, other people, different countries, and ourselves. In that spirit, here is a little saying I give to each of our new resident pathologists when they join our team at the University of Colorado Hospital:

"Don't be afraid your life will end; be afraid that it will not begin."

Fig. 6. The vendor area was popular and extremely busy between classes.

Fig. 7. Each day breakfast and lunch were provided to all who attended. Fresh fruits of Costa Rica and Central American dishes were enjoyed by all.

Direct Measurement of Tissue Thickness on Stained Slides (Measurement in 3 Dimensions) for Determination of Tissue Hypoplasia and Hyperplasia and Calibration of Microtomes

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Introduction

In this report we describe a method for measuring the thickness of a stained tissue section with an optical microscope and image analysis software for subsequent tissue and cell characterization and diagnosis. This technique also allows for monitoring thickness variation in microtome settings.

For a number of quantitative optical microscopic techniques it is relevant to obtain an estimate of the thickness *t*' of the tissue section; distribution of tissue components is dependent on tissue thickness and will differ if only the surface area is measured. Thickness measurement in soft connective tissues is a continuing problem due to the apparent compression of the tissue by micrometer-type gauges. Cell counting is an important procedure that has many applications in histology and histopathology. Most of these measurements are done in 2 dimensions by evaluating the length and breadth of cells and tissue structures.

Tumor growth is determined by the rate of cell proliferation and cell death. The apoptotic index (AI) may be used as an additional prognostic indicator in invasive breast cancer. Apoptosis plays an important role in tumorigenesis. De Jong et al counted the number of apoptotic cells in hematoxylin and eosin (H&E)-stained tumor sections in a series of 172 grade I and II invasive breast cancers with long-term follow-up.[1] The number of apoptotic cells in 10 high-power fields was converted to the number of apoptotic cells per square millimeter (mm^2) to obtain the AI. The AI showed a positive correlation to the mitotic activity index (MAI) (P=0.0001), histological grade (P<0.0001), and worse tumor differentiation.[1]

van de Schepop et al counted apoptotic cells in 4 µm thick H&E-stained tissue sections from 12 breast cancers.[2] Apoptotic cells, recognized by morphological criteria, were counted in consecutive fields of view at 1000X magnification in a marked area in the most poorly differentiated region of tumor. These counts were regarded as the gold standard.[2]

Evaluation of proliferation activity of the neoplastic cells gives important prognostic information, especially in breast cancer.[3] Many studies have demonstrated that the MAI is an independent prognostic factor for recurrence-free survival.[3] There are many ways to measure cell proliferation, including the determination of the mitotic rate by counting the mitotic figures.[3] Mitotic counts are performed by counting the number of mitoses from 10 high-power fields (MAI), or by expressing the count per mm^2, which produces the standardized mitotic index (SMI), or volume fraction corrected mitotic index (M/V_v index).[3]

Elzagheid et al collected data from different studies and tried to evaluate the relative significance of different prognosticators in lymph node positive/lymph node negative (LN+/LN-) patient groups.[3] In LN+ patients, HER2/neu and E-cadherin immunohistochemistry were statistically the most significant prognosticators followed by proliferation-associated features (mitotic counts by SMI or MAI).[3]

Various histological grading systems for breast carcinoma have been described. The majority of tumor grading systems combine nuclear grade, tubular formation, and mitotic rate. Lee and Langdon have measured the thickness of soft connective tissue,[4] which is a continuing problem due to the apparent compression of the tissue by micrometer-type gauges. The authors have compared 5 methods for the measurement of thickness[4]: (1) a Mitutoyo nonrotating thickness gauge; (2) a custom-built instrumented thickness gauge that was strain-gauged to measure contact force; (3) a commercial Hall effect probe (Panametrics® Magna-Mike®); (4) a custom-built electrical resistance probe; and (5) measurement of fresh frozen histological sections under polarized light. Using bovine pericardium as a test material, all of the methods examined were adequate to assess both sample-to-sample and location-to-location differences in thickness. They found that the resistance gauge gave significantly greater thicknesses than did the other methods, with little or no compression; indeed, extrapolation to zero load of thickness readings from the instrumented gauge yielded identical thickness.[4] Thickness measured in frozen sections was indistinguishable from thickness measured with the nonrotating gauge, the instrumented gauge under 0.5 to 1.2 g compressive load, or the Hall effect probe.[4] With the correct technique, the simple and inexpensive nonrotating gauge remains a pragmatic choice for thickness measurement in planar soft tissue.

Dedicated breast computed tomography (bCT) is an emerging technology with the potential to improve the detection of breast cancer in screening and diagnostic capacities. Typically, the 3-dimensional (3-D) volume reconstructed from the scanner is displayed as sectional images. Packard et al evaluated the effect of section thickness on the detectability of simulated masses using a prewhitened matched filter (PWMF) as a model observer. This study showed that PWMF detection performance of a known lesion size is quantitatively influenced by section thickness in dedicated bCT.[5]

Accurate analysis of the 3-D architecture of developing organs is critical to understanding how developmental defects can be linked with structural abnormalities. Sims-Lucas described a 3-D reconstruction technique for the developing kidney including the outer kidney capsule, ureteric epithelium, and developing nephrons.[6] This 3-D reconstructive process involves generating serial sections of the developing kidney, followed by histological

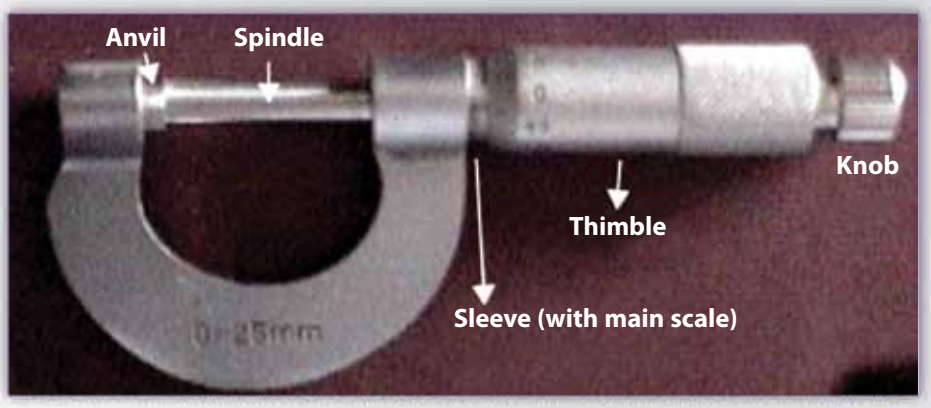

Fig. 1A. A micrometer, depicted above, is a device that uses a calibrated screw for precise measurement of small distances.

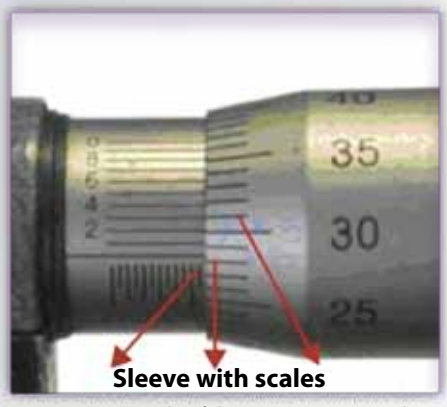

Fig. 1B. An example of the measurement scale on a micrometer.

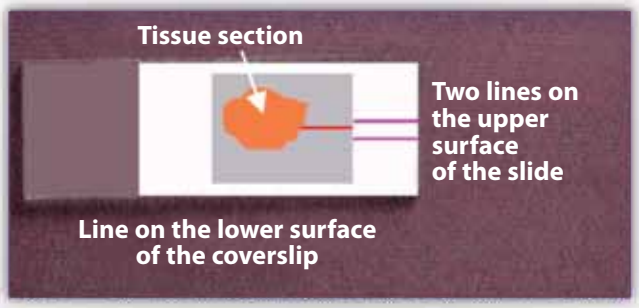

Fig. 2. The image illustrates the markings that are placed on the top of the slide and the bottom of the coverslip to allow thickness measurement of tissue with the micrometer.

staining. Each serial image is projected on the monitor and each tissue lineage or structure is traced. The kidney tracings are aligned and a 3-D image is rendered. Each reconstructed tissue/lineage can then be subjected to quantitative analysis (eg, surface area or volume). The reconstructed ureteric epithelium can be skeletonized to determine the branching architecture.[6]

Mitotic index and apoptotic cell counting are used for drug evaluation and studies in cell culture.[7]

Materials and Methods

Calibration of Microscopic Vertical Axis (Z-Axis)

1. A micrometer is required for this process (Fig. 1A, 1B).
2. Measure the thickness of the coverslip using the micrometer (Tc = 0.145 mm).
3. Use a glass slide without a tissue section but with a coverslip. Draw three lines: two on the upper surface of the slide and the other on the lower surface of the coverslip (Fig. 2). Measure its thickness using the micrometer (Tg = 1.07 mm). Make sure that the Z-axis is adjusted to zero (fine adjustment knob is held on zero).
4. Select an objective lens that has a high numerical aperture (40X).
5. Focus carefully on the lines on the upper surface of the glass slide.
6. Note the current Z focus position (position of the fine adjustment knob) (Fig. 3).
7. Focus carefully on the line on the bottom of the coverslip.
8. Using this technique will ensure that your thickness measurements are not biased by the thickness of the depth of field of the objective lens (value of measurement A).
9. Measure the slide and the coverslip using a micrometer.
10. Calibrate the graduations scale on the fine adjustment knob (Fig. 3) with the measured value of the micrometer (Tg + Tc = 1.215) to determine the value of each division on the microscope. This value (thickness final, Tf = 1.215) is always subtracted from the final thickness (glass slide + section + coverslip) in order to arrive at the section thickness.

Fig. 3. The microscope's fine focus adjustment can be used to measure the distance between the lines marked on the slide and the line marked on the bottom of the coverslip.

Measuring Tissue Section Thickness

Histological sections are cut from embedded tissues on a microtome and placed on glass slides. Tissue section thickness can be measured by using the same calibration procedure described on page 25. Mount a tissue section on a glass slide and cover with a coverslip. Then follow steps 1-5 on page 25. The difference between the Z focus position when focused on the upper surface of the slide and the lower surface of the coverslip is equal to the thickness of the tissue section (value of measurement B). Tissue section thickness is calculated by subtracting measurement A (see page 25) from measurement B as follows:

Tissue section thickness = B - A

Determination of Surface Area

For this purpose, we have used ordinary flatbed scanners (Mira 6) and image analysis software (ImageJ 1.36b). To start working with the scanner (MiraScan 5.0), follow the steps below:

1. Click "Start" on the Windows task bar > "Programs" > "MiraScan V5.0." Select freehand selection to drag the image to fit in the preview window.
2. Place the original slide on the scanner glass plate (coverslip downward) and cover with a piece of blank white paper. An example specimen (fowl intestine) is shown in Fig. 4.

   Image type—mode is "color"

   Output size—resolution is 600 dpi

   Select the exact size for your scanned images

   Brightness—select 1

   Contrast—we used 85

   Saturation—choose 53
3. Digitized image is then processed using image analysis program (ImageJ).
4. Calculate the total surface area (analyze > measure > set scale, cm = 306 pixels).

Fig. 4. Image analysis software and a flatbed scanner can be used to measure the area of stained tissue of any size or shape. The image above is fowl intestine, stained with H&E.

Results

Formalin-fixed paraffin-embedded (FFPE) whole tissue sections are ideal for monitoring the 3-dimensional structure of tissues. Lymph node and ovary tissues were fixed in 10% neutral buffered formalin for at least 48 hours, and then chemically processed and embedded into paraffin blocks. Duplicate slides sectioned at 5 to 7 µm were prepared on a rotary microtome using disposable microtome blades; the tissue sections were mounted onto glass microscope slides and stained with H&E.

Normal cells, apoptotic cells, and neoplastic cells were recognized by morphological criteria and counted in fields of view at a microscope magnification 1000X. Counts are given per field of surface area. All fields of view were counted at image resolution of 630X and 400X. Mitotic counts (MAI) were expressed as the number of mitotic figures per mm^2. Surface areas of digitized images were similarly measured at the same conditions. The reproducibility was tested by repeated measurements at these magnifications in 10 systematically selected fields of view. The thickness *t*' of the section differed from the actual distance "*t*" between the two cuts that generate the section. The selection of blades, knife angle, cutting speed, and other variables depends on the study material and user experience.

Discussion

The quantitative assessment of morphological features in biomedical samples is an important topic in microscopic imaging. There is a possible correlation between morphological aspects and 3-dimensional reconstruction. Morphological changes of the brain,[8,9] lymph nodes,[10] prostate,[11] and many other organs have been reported.

Quantitative characterization of the 3-D structural attributes of acquired macroscopic tissue specimens at a micron-level resolution using light microscopy[12] is performed with the help of image analysis programs.

The method we described allows the researcher to gain insight into important morphological parameters such as cell density and size and their relationship with surrounding tissue through the visualization of the tissue in 3 dimensions.

Information about the distribution and quantity of normal and apoptotic cells[1,2] and tissue components are valuable in growth and development studies of normal structure and in tumorigenesis.[3,7]

The determination of tissue thickness from paraffin blocks in the histology laboratory has been based primarily on visual estimates. Kong et al[12] and Bacus and Bacus[13] have developed a radiographic method to determine tissue thickness in tissue microarray (TMA) donor paraffin sections. In this report we described a method and instrumentation necessary for measuring the thickness of a tissue section with an image analysis system. This technique can be used for analyzing cell objects within a cell of interest or to assess structure variation within tissue samples. This method can also be used for comparing tissue section thickness to microtome thickness setting as an indicator of machine performance, which allows for adjustments in microtome operation in order to yield optimal sections for study. This technique is suitable for the study of a wide range of tissues from various species.

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A Discussion of Quality Control Outcomes From Recycled Reagents

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Abstract

As part of an initiative into Green Practices, our Core Lab investigated the benefits of using recycled reagents for tissue processing and staining. Solvent recycling is certainly not a new concept. It has been shown to be both cost-effective and user-friendly for a field of science known for its chemically harsh methods—histotechnology. Therefore, recycling seemed a natural progression to responsible stewardship for our facility and for the environment. With preparatory investigation, we chose a well-known recycling system in widespread use in many histology laboratories. The system was cost-effective and uncomplicated to use. However, during the Core Lab's evaluation, we began to notice some differences in our stains at a time when there had been no other changes in our normal staining protocol. This prompted us to undertake an organized internal review of outcomes from the use of recycled reagents.

Introduction

Nearly 20 years after recycling was introduced into the field, differing opinions and questions still exist as to the quality of the tissue samples derived from the use of recycled reagents. Recycling methods range from complex fractional distillation processes for solvents to simple gravity recycling systems for alcohols that do not use electricity or heat.[1,2] Although the recycling of formalin fixative has become more commonplace, the commentary on recycled reagents is still going on.

There is no doubt that the recycling of formalin has made a difference to the environment and to responsible budgeting. Yet the cloud around quality outcomes from some recycled reagents still exists,[3] and has become a point of some contention between supporters and nonsupporters of reagent recycling. Common complaints are of fuzzy nuclear detail and changes in the color or hue of cytoplasmic staining with hematoxylin and eosin (H&E). These complaints, however, are met with a typical response that "if your system is working adequately and is well maintained," a recycled product of good quality is indistinguishable from a fresh reagent.[4]

Our Core Lab has been somewhat behind the times in these discussions as we have not previously considered recycling for a research core lab, where samples are mostly snap-frozen cryostat sections. However, a journey that has taken us from using Lean processes to Green processes has brought us to the crossroads of recycled reagents and quality outcomes.[4]

We approached recycling with the following questions:

- Would recycling be cost-effective?
- Would recycling be time-effective?

…and, most importantly,

- Would the quality of the end product be better than or equal to the current Core Lab quality standard?

We began our investigation with an open mind, ready to collect the data needed to make an informed decision about recycling for our Core Lab.

Materials and Methods

After a thorough inquiry into which system we wanted to trial, we chose a well-known recycler that had been used for many years in numerous labs. The system selected uses fractional distillation for recycling.[5]

- The system was set up by the vendor
- The staff was given a comprehensive in-service, complete with hydrometer to record results on alcohol purity (a necessity if you are recycling)
- Staff faithfully tested the purity of the alcohol, which averaged an acceptable 97%, and adjusted for our standard protocol

With controls in place we began our study. We immediately noticed that our slides looked different when processed in recycled reagents. Our gross impression was that the slides had taken on a pinkish-orange hue, different from what we'd expect from our normal H&E staining. Would there be a difference under the microscope? Or was this gross impression just that—an *impression*?

We brought our lab's recycling dilemma to the Histonet but found more questions than answers. Our slides, which were prepared with recycled reagents, seemed bright in comparison to slides prepared with our normal stain; however, we noted many other complaints with recycled reagents that centered on the fading of slides over time. We decided to review our test slides again, at time points of 3 months, 6 months, and 9 months, to assess stain fading before drawing any conclusions.

Study Design

Mouse tissue was collected from a single animal and fixed in 10% neutral buffered formalin (NBF). Samples of lung, heart, brain, spleen, liver, and kidney were harvested. Samples were processed with our standard protocol on a closed system tissue processor. Sections were infiltrated and embedded in Paraplast® X-tra and sequential sections were cut on a rotary microtome at 5 µm. All slides were stained with H&E on the Sakura Tissue-Tek® DRS-601 Slide Stainer (Sakura Finetek USA, Inc., Torrance, CA).

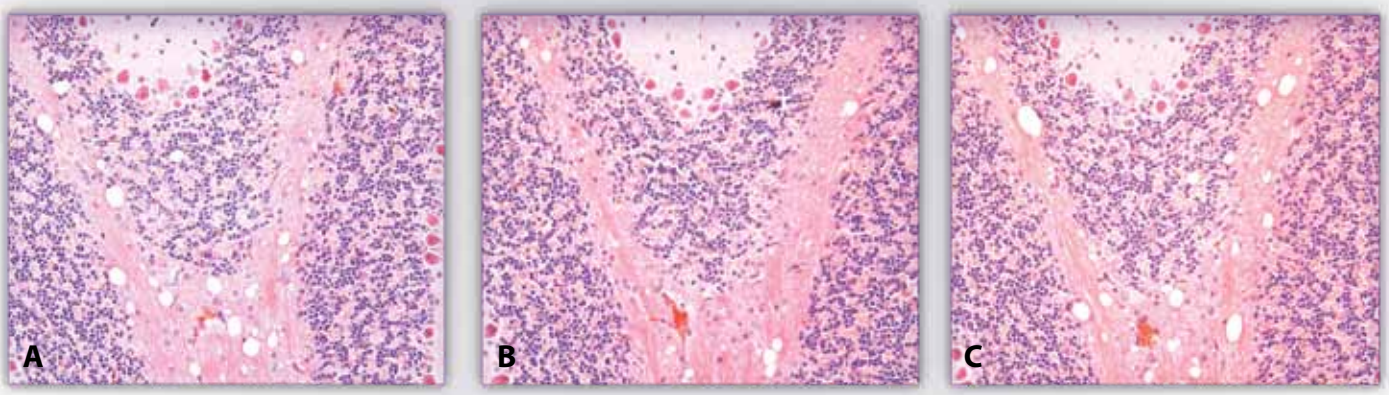

Fig. 1. Mouse brain tissue fixed and stained with H&E, 20X; Day 0—A) Baseline sample prepared with all fresh reagents (alcohols and clearing reagent); B) same as baseline sample except recycled Histo-Clear clearing reagent was used; C) same as baseline sample except recycled 80% and 95% ethanol were used.

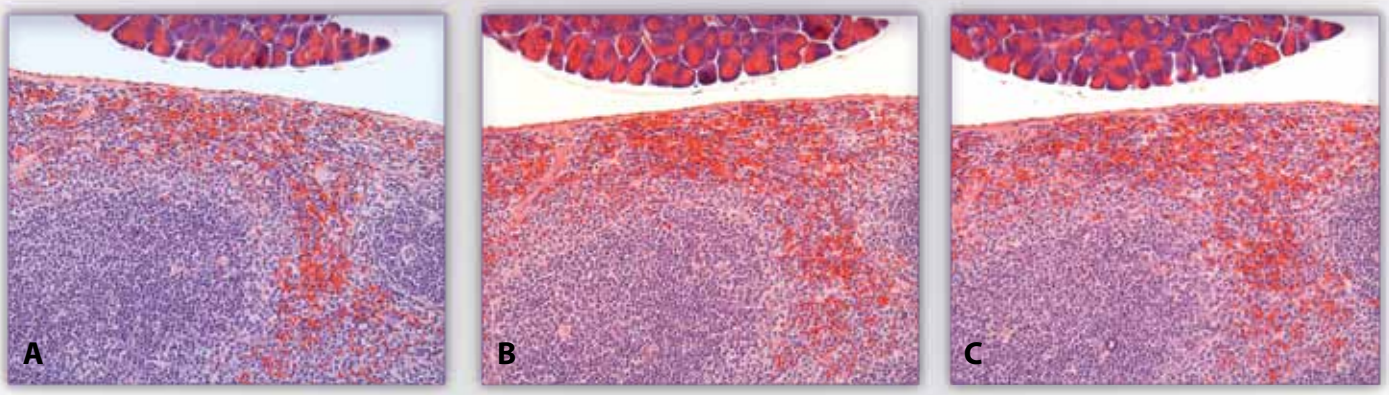

Fig. 2. Mouse spleen tissue fixed and stained with H&E, 20X; 3 months—A) Baseline sample prepared with all fresh reagents (alcohols and clearing reagent); B) same as baseline sample except recycled Histo-Clear clearing reagent was used; C) same as baseline sample except recycled 80% and 95% ethanol and recycled Histo-Clear were used.

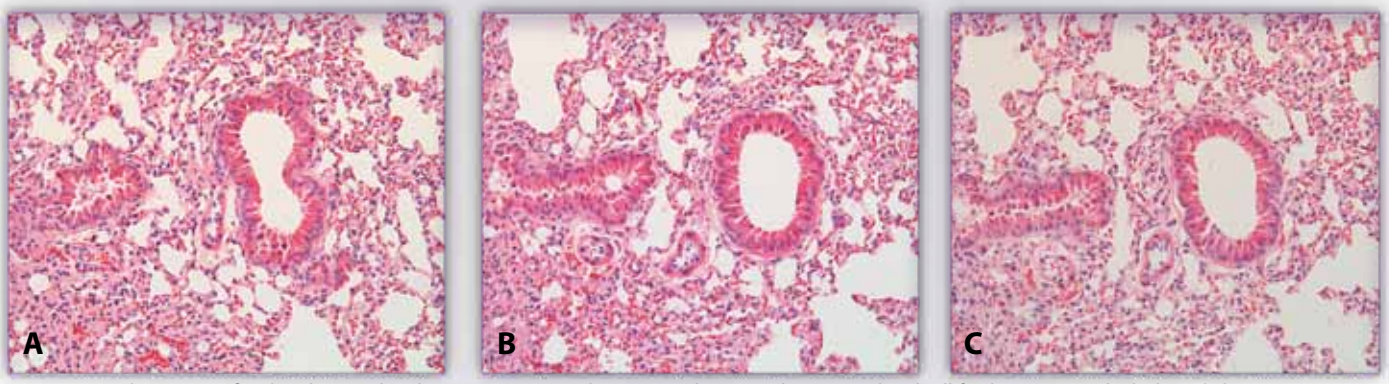

Fig. 3. Mouse lung tissue fixed and stained with H&E, 20X; 9 months—A) Baseline sample prepared with all fresh reagents (alcohols and clearing reagent); B) same as baseline sample except recycled Histo-Clear clearing reagent was used; C) same as baseline sample except recycled 80% and 95% ethanol and recycled Histo-Clear were used.

- Set #1: Stained with all fresh alcohols and clearing reagent (as our baseline control)
- Set #2: Stained using recycled alcohols and fresh clearing reagent
- Set #3: Stained using fresh alcohols and recycled clearing reagent
- Set #4: Stained using both recycled alcohols and clearing reagent

We photographed each set at 3 months, 6 months, and 9 months for comparison. All slides were viewed with an Olympus BX/51 microscope at 20X, and photographed with a QImaging Retiga Camera 2000R (QImaging, Surrey, BC, Canada) and Image-Pro Plus® software, version 6.2.

Scoring

At the conclusion of our study, 3 different reviewers evaluated and compared each set at each time point to our baseline fresh reagent set using the following criteria:

- Change in color or hue
- Change in the sharpness and clarity of nuclear staining
- Fading of the stain in relation to time

We asked reviewers to score slide sets as either better than the baseline (+), equal to the baseline (=), or failing in any of the designated criteria (-), when compared to the original baseline slide for each tissue type.

Scores and Corresponding Samples

Brain—Color and hue are affected. Only the fresh reagent set maintained standard H&E integrity for all criteria (Fig. 1A). Scores for both recycled reagent sets scored lower for clarity (Fig. 1B, 1C).

Spleen—An obvious color change is noted by reviewers in the samples stained in recycled reagents (Fig. 2B, 2C). Scores for the samples in both the recycled alcohols and the clearing reagent showed a shift from baseline in the color and hue compared to the baseline fresh reagent set (Fig. 2A).

Lung—Fading is evident in the samples that used recycled clearing reagent (Fig. 3B) when compared to the fresh reagent set (Fig. 3A). Scores for fading with the recycled samples were lower among all reviewers. Cytoplasmic color appears lighter in the sample using recycled clearing reagent (Fig. 3B) than in the sample with both recycled 80% and 95% ethanol (ETOH) and clearing reagent.

Results

These results reflect the data collected from all tissues sampled. In every case, reviewers found the slides stained with fresh reagents (baseline) to have the highest scores for all criteria: color/hue, clarity of nuclear staining, and fading of stain over time. Each one of the slide sets—#2 (recycled alcohols and fresh clearing reagent), #3 (fresh alcohols and recycled clearing reagent), and #4 (recycled alcohols and clearing reagent)—regardless of tissue type, demonstrated some deficiency over baseline slides (fresh reagent set). Sets #2, #3, and #4 all scored below the baseline on color/hue, clarity of the nuclei, and/or generalized fading. Of all the tissues tested, the spleen sample seemed to compare best with the fresh reagent set; therefore, some of the noticeable fading may actually be dependent on tissue type. However, throughout the duration of the study, regardless of the use of fresh or recycled reagents, reviewers reported no fading in any sample set from the first time point. After some discussion, this was attributed to the stability of H&E staining over time.

Conclusion

The authors know that there are many reagent recycling systems to be tested; however, they believe the initial sample number was too small to draw any hard conclusions. But the Core Lab agreed that there was enough definitive information from this brief study to say that there is evidence of a change in quality when using recycled reagents.

This study also expected to address the question of cost-effectiveness and time efficiency. In this 9-month study, our Core Lab saved about $525.00 in reagent costs; but, when we calculated lost technician time (for the hands-on recycling process) and disposal of the remaining sludge, our actual savings amounted to only $157.50 over the course of the study. As a result, we will continue to look into other recycling methods and hope to eventually find a system that will allow us to be an environmentally friendly and more cost-conservative lab.

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Acknowledgments

The authors wish to thank Nemours/Alfred I. duPont Hospital for Children, Department of Biomedical Research for their interest in continuous quality improvement. This project was funded through the Histotechnology Core Lab R&D account.

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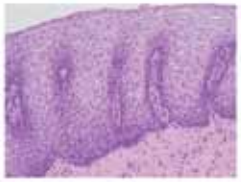
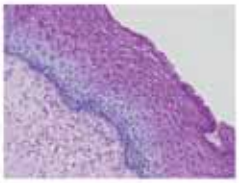
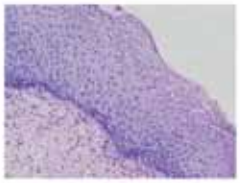
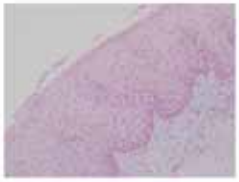
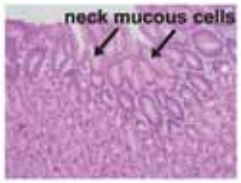
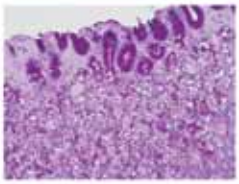
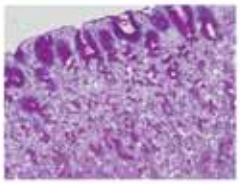
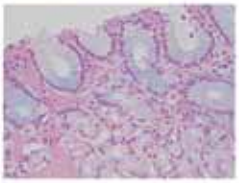
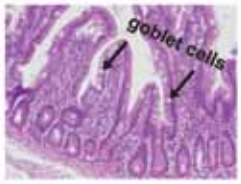
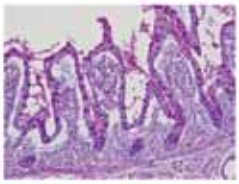
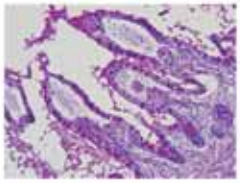
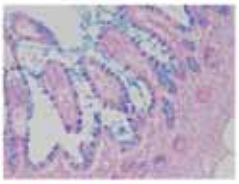
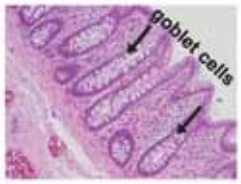
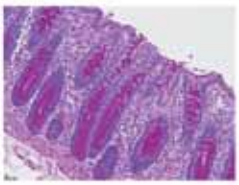
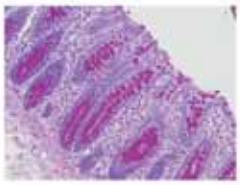
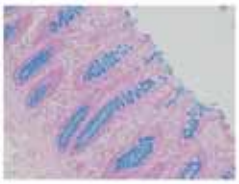
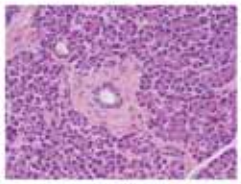
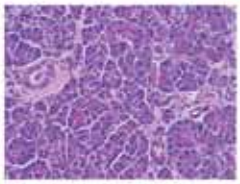
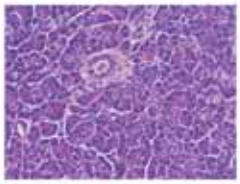
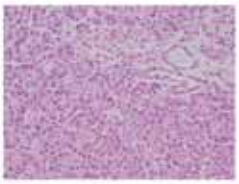
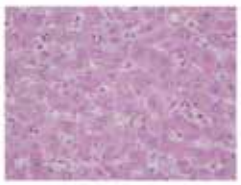
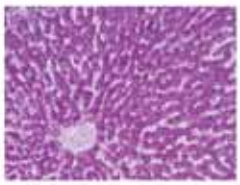
Carbohydrate Profile of the Normal Human Gastrointestinal Tract: A Histochemical Study

Rashmil Saxena, BFA, HT(ASCP)CM

Division of Transplantation, Department of Surgery
Indiana University School of Medicine, Indianapolis, IN

rasaxena@iupui.edu

Tissue Type	H & E	PAS	PAS With Diastase	Alcian Blue pH 2.5
		Demonstration of neutral mucosubstances including glycogen **Neutral mucosubstances:** pink (color depends on amount) **Nuclei:** blue	Demonstration of glycogen **Glycogen:** unstained **Neutral mucosubstances:** pink (color depends on amount) **Nuclei:** blue	Demonstration of acidic mucosubstances, both carboxylated and sulfated **Acidic mucosubstances:** deep blue **Background:** pink-red
ESOPHAGUS				
STOMACH				
SMALL INTESTINE				
COLON				
PANCREAS				
LIVER				

All images are 100X.

Introduction

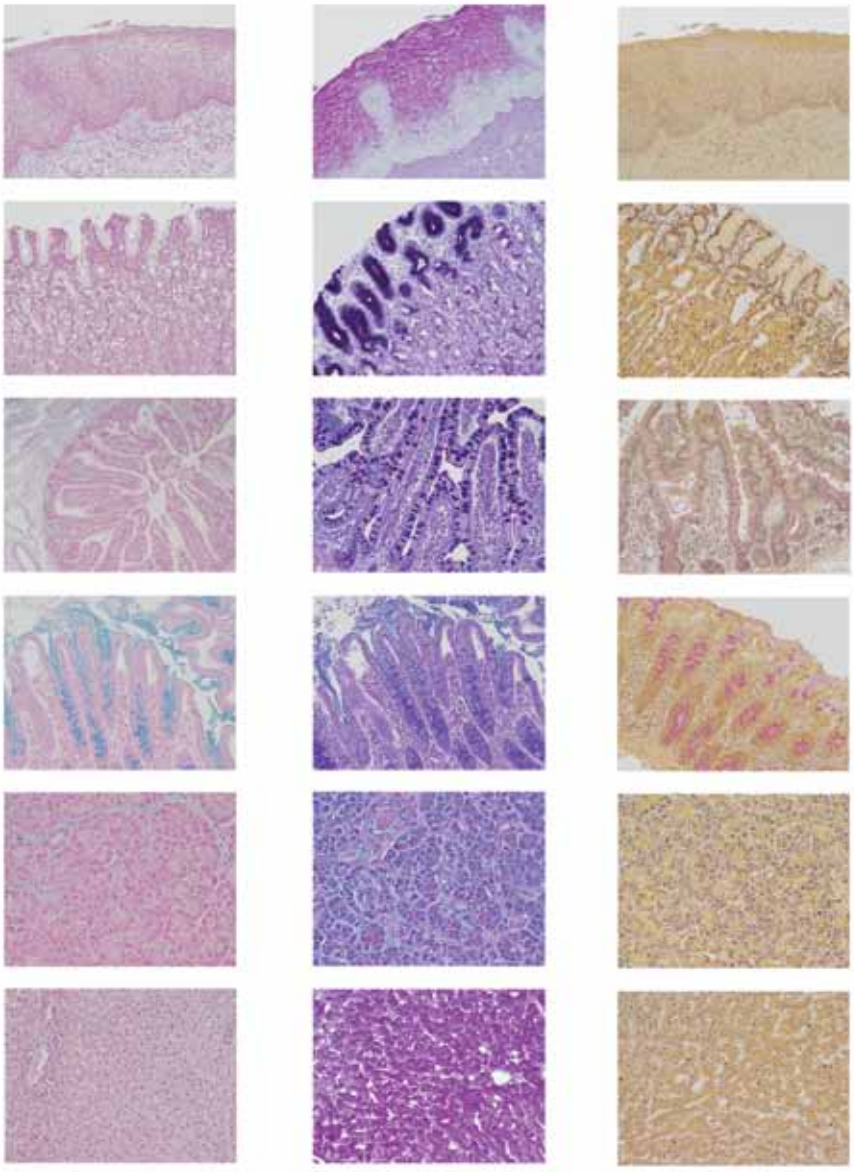
The gastrointestinal tract contains a large amount of carbohydrates. Most of these are large molecules complexed to other chemical substances such as lipids, proteins, or both lipids and proteins. These complex molecules may be neutral or acidic.[1]

In this study, the various organs of the gastrointestinal tract were stained with the most commonly used histochemical stains for carbohydrates to identify the carbohydrate content of the normal esophagus, stomach, small intestine, colon, pancreas, and liver.

Materials and Methods

Four-micron thick sections were cut, deparaffinized, and hydrated. Each organ type was stained for PAS, PAS with diastase, alcian blue at pH 2.5, alcian blue at pH 1.0, alcian blue/PAS, and mucicarmine. Standard methods of staining were used.

Alcian Blue pH 1.0	Alcian Blue/PAS	Mucicarmine
Demonstration of sulfated mucosubstances, which are highly acidic **Sulfated mucosubstances:** pale blue **Background:** pink-red	Simultaneous demonstration of neutral and acidic mucosubstances **Neutral mucosubstances:** pink **Mixture of neutral and acidic mucosubstances:** magenta	Demonstration of acidic epithelial mucin, both carboxylated and sulfated **Acid mucosubstances:** pink-red **Nuclei:** black **Background:** yellow



Conclusions

The squamous mucosa of the esophagus contains abundant glycogen. There are no acidic mucosubstances.

The stomach mucosa contains abundant neutral mucosubstances. There are no acidic mucosubstances.

The small intestine contains abundant neutral and acidic mucosubstances. Sulfated mucosubstances are present in the stroma.

The colon contains abundant neutral and acidic mucosubstances. The goblet cells contain abundant sulfated mucosubstances.

The pancreas contains some neutral mucosubstances. Sulphated mucosubstances are present in the stroma.

The liver contains abundant glycogen. There are no acidic mucosubstances.

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Remembering Charles Joseph Churukian

Histotechnologist of the Decade, Innovator, Scientist, Author, Educator, Spiritual Advisor, and Friend

Diana Scott, HT(ASCP)

University of Rochester Medical Center
Rochester, NY

diana_scott@urmc.rochester.edu

The discipline of histotechnology lost Charles Churukian on February 23, 2011. His thirst for knowledge, his kind, unassuming manner, his passion for histology, and his sharing nature left us all richer for having known this remarkable man.

For me, Charles is "Chuck," the special stains guru; he has also been a mentor to pathology residents and attendings, and histotechnologists and technicians around the world. I first met Chuck in 1977 at the University of Rochester Special Stains Laboratory. I was introduced to him by Elizabeth Mayle, my instructor at the Monroe Community College Histotechnology Program. I had just graduated and was preparing my slides for the ASCP HT certification exam. Chuck was a reviewer for the practical portion of the exam, when it was still required to submit slides to the Board of Registry in order to become certified. I was sure that Chuck had seen and reviewed thousand of slides, so I asked him to take a look at my work. He offered an inspirational comment to me. "You just need a little practice, these don't look too bad." Encouraging words!

I remember my job interview with Chuck: "So, how long do you think you'll stay with us if given the position?" he asked. Of course the answer for me was "forever," and as it turns out, I have been here ever since. That was just what he needed to hear and he sent me to interview with "the one and only" Dr. Eric Schenk—the codeveloper of the Churukian-Schenk stain for demonstrating neuroendocrine tumors and coauthor of several important articles with Chuck.[1-5] Chuck always looked for long-term commitments from individuals. He sought out those who were eager to learn and had the ability to produce only the best quality slides. "Be economical, don't waste, always follow the instructions...the results will be foolproof every time!"

Chuck devoted his professional life to making things better and with little or no added cost. He pioneered the development of microwave special stains methods, especially those that took hours to perform. Chuck was a giver—a giver of knowledge and of himself. He never kept his findings secret. He shared his results and made all of our lives easier with his rapid staining methods and foolproof procedures. His lab ran virtually at no cost, except for his time. He was frugal and knew where to find the products and people necessary to make the lab and the techniques work reliably.

An academician throughout his career, Chuck authored or coauthored more than 55 manuscripts and presented over 100 lectures at scientific conferences and meetings. As a consultant to the Biological Stain Commission, Chuck's contributions have left a long-lasting legacy of excellence. The Biological Stain Commission has distributed his *Manual of Special Stains* to almost every teaching hospital in the United States and around the world. His name is familiar to many histotechs and pathologists in the United States.

Chuck was one of the founding members of both NSH and the New York State Histotechnological Society, and in 1989 was appointed as Fellow of the New York State Histotechnological Society. Over the years, he received numerous awards and accolades from his peers and colleagues for his tremendous body of work: the Golden Forceps Award, J.B McCormick Award, *Journal of Histotechnology* Editor's Award, *Journal of Histotechnology* Diamond Cover Award,

Histotechnologist of the Year Award, and most recently he was honored at the 2010 NSH Symposium/Convention where he was presented with the prestigious Histotechnologist of the Decade Award.

Chuck gave of himself every day of his life. Across the globe, his wealth of knowledge and spirituality have touched us all. Chuck would tell me, "I don't know where the information comes from; it is the Lord and his spiritual guidance—I wake up and the ideas are there." Chuck provided weekly counseling and guidance to those at the county jail who were afflicted with substance abuse; he would follow up with them after their release. Chuck had a calling to serve both his community and those in medicine. A truly remarkable attribute!

Not a day goes by when our lab doesn't mention or think of him. We still refer to the desk in the Special Stains Lab as "Chuck's desk" or someone will ask, "Where did Chuck put that control?" His spirit through the many handwritten notes he left behind embraces us every day. It is difficult to believe that it has been more than 1 year since his passing, but his vision and character live on in all of us who had the opportunity to share in his humor, intellect, and life. He is sadly missed.

In 2012, Region I Inc. graciously renamed one of its annual scholarships in honor of Chuck. The Charles Churukian Memorial Scholarship Award is given to an individual who demonstrates motivation, has financial need, is active at the state, regional, or national level in promoting histotechnology, and wants to attend a meeting in order to increase his or her knowledge. Simply, this award is for a person who Chuck would be proud and honored to call a histotechnology professional and a colleague.

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Letter From the Editor

Submitting articles to *HistoLogic*®

On behalf of *HistoLogic*, I would like to express my appreciation to all of you who have supported this publication through the submission of manuscripts over the past 40+ years. *HistoLogic* was created in 1971 in an attempt to keep histology practitioners around the globe connected to one another through the sharing of relevant, practical scientific and technical information. Since that time, it has been read worldwide by more than 1,000,000 individuals. It is my sincere hope that the information that has appeared within these pages during my editorship has been of benefit to you in your work.

We understand that many of our colleagues may not have the opportunity to further their professional development through attendance at symposia and workshops. For this reason, the appearance of sound scientific information in your mailbox becomes all the more important to aid you in keeping your theoretical knowledge complete and up to date. As a result, we at *HistoLogic* remain committed to providing you with the highest caliber publication possible.

Few things contribute more to the advancement of science and technology than the sharing of knowledge and ideas with our colleagues. Indeed, this communication is the very foundation upon which new ideas are stimulated and new developments and discoveries are built. I believe that as scientists we have a responsibility to share what we know. It matters not whether you publish your work in *HistoLogic*, the *Journal of Histotechnology*, or any other scientific publication, only that you do, in fact, share your knowledge. Knowledge shared in print will always remain one of the most effective tools for reaching large numbers (often tens of thousands) of individuals.

This is where you come in. If you have never considered submitting your work for publication, I urge you to consider the opportunity. I know that some may be daunted by the perception that it is very difficult to get one's work published. *HistoLogic* is interested in any manuscript that is scientifically valid. I've conversed with some colleagues who believe that they don't write well enough to successfully publish an article. However, as the scientific editor, I am available to assist you in any way possible. I am happy to help you construct your article, so please do not allow this concern to discourage you from coming forward with your ideas.

For those who are interested in submitting a manuscript to *HistoLogic*, I urge you to contact me directly at dellav@musc.edu, or you may correspond with me via the mailing address included on the back of this issue. I prefer that manuscripts be prepared using word processing software such as Microsoft® Word or Corel® WordPerfect.®

Articles that appear in *HistoLogic* are required to have relevant scientific or technical references that should be listed in the order in which you cite them in your article. References must be sufficiently detailed as to allow the reader to locate the information you used when constructing your manuscript. We prefer that references are formatted according to the *AMA Manual of Style*, and I can assist you with getting your references into that format, if necessary. We encourage you to include color images that serve to illustrate key points in your article for the reader. While we prefer these in electronic format, including jpeg and tiff, we can also work with high-quality color prints and slides. All slides should have a caption that includes such relevant information as tissue type, stain used, magnification, and any remarkable features to be brought to the attention of the reader.

Most important of all, please be sure to include the most expedient way for me to communicate with you. Email is preferred over postal mailing, but if email is not available, please include a telephone number where you can be reached.

I hope that you will seriously consider making a contribution to the histology community by submitting a manuscript to *HistoLogic*. I look forward to hearing from you.

Vinnie Della Speranza, Scientific Editor

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Educational Opportunities in 2013

JANUARY

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Quality Management Using Barcoding in the Histology Laboratory**
Speaker: Clifford M. Chapman
Strata Services
Lexington, MA

23 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Lung Cancer: Immunohistochemistry and Molecular Testing**
Speaker: David Tacha, PhD, HT(ASCP)HTL
Biocare Medical
Concord, CA
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

FEBRUARY

15 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **State of Histology**
Speaker: Glenda Hood
Tarleton State University
Fort Worth, TX

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Stretching Your Knowledge of Elastin Stains**
Speakers: Jamie Pert and Amy VanDeWiele
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org
Email: histo@nsh.org

MARCH

8-9 **Indiana Society for Histotechnology**
Site: Hilton Indianapolis North
Indianapolis, IN
www.indianapolisnorth.hilton.com
Contact: Debra Wood
Phone: (317) 491-6311
Email: demwood@iupui.edu

15 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Keep the Bathwater, It Has Metabolites In It**
Speaker: Dean Troyer, MD
Sentara Norfolk General Hospital
Norfolk, VA

27 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Training for the Revised Hazard Communication Standard (HCS)**
Speaker: Ada Feldman
Anatech Ltd
Battle Creek, MI
Phone: (443) 535-4060 or register online at www.nsh.org

APRIL

12-14 **Georgia Society for Histotechnology**
GSH 40th Anniversary Celebration
Site: Oceanside Inns & Suites
Jekyll Island, GA
Contact: Wanda K. Simons
Email: wandrous@att.net
Website: www.histosearch.com/gsh/

12-14 **Texas Society for Histotechnology**
Site: Houston Marriott
Houston, TX
Contact: Kathy Dwyer or Veronica Davis
Phone: (972) 768-6279 or (972) 579-8291
Email: kdwyer3322@aol.com
veronida@baylorhealth.edu

APRIL (cont.)

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Quality Assurance in Surgical Pathology**
Speaker: Bonnie Whitakier
Ohio State University College of Medicine
Columbus, OH

19-20 **Colorado Society of Histotechnology**
Spring Symposium
Site: La Quinta
Fort Collins, CO
Contact: Stacey Langenberg
Phone: (303) 577-2303
Email: stacey.langenberg@ucdenver.edu

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Mohs: What's It All About?**
Speaker: David Kemler
In-Office Pathology (IOP)
Lake Forest, IL
Phone: (443) 535-4060 or register online at www.nsh.org

24-26 **Tri-State Meeting (Iowa, Minnesota, Wisconsin)**
Site: Hotel Julien Dubuque
Dubuque, IA
Contact: Judith Stasko (IA), Sheri Blair (MN), Jean Mitchell (WI), Dawn Schneider (exhibitors)
judith.stasko@ars.usda.gov
sheriblair1@netzero.net
JMitchell@uwhealth.org
dawn.schneider@ministryhealth.org

26-27 **Histology Society of Ohio**
Site: Westlake Holiday Inn
Westlake, OH
Contact: Amy Aulthouse
Email: a-aulthouse@onu.edu

MAY

16-17 **Illinois State Meeting**
Site: Harrah's Hotel & Casino
Joliet, IL
Contact: Lori Bellafiore
Phone: (217) 202-3820
Email: labellafiore@yahoo.com

16-19 **Florida Society for Histotechnology**
Site: Bonaventure Resort & Spa
Weston, FL (Fort Lauderdale)
Contact: Charlotte Kopczynski, President
Phone: (727) 580-1738
Email: violetfem@yahoo.com

17 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Role of Immunohistochemistry in Breast Pathology**
Speaker: Prashant A. Jani, MD, FCAP, FRCPC
Thunder Bay Regional Health Sciences Centre
Thunder Bay, Ontario, Canada

22 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Emotional Intelligence**
Speaker: Lisbeth O'Malley
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org

30-Jun 1 **Missouri Society for Histotechnology**
36th Annual Spring Symposium
Site: Hilton Garden Inn
Columbia, MO
Contact: Janci Wellborn or Sharon Walsh
Email: jwell1958@msn.com
userwalsh@sbcglobal.net

✔ Mark Your Calendar!
Educational Opportunities in 2013

JUNE

21 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Digital Evolution: Taking the Pathologist From the Basement to the Bedside**
Speaker: Damien Matusiak
Leica Biosystems
Buffalo Grove, IL

26 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Identifying Histology Look-Alikes**
Speaker: Amy Aulthouse, PhD
Ohio Northern University
Ada, OH
Phone: (443) 535-4060 or register online at www.nsh.org

JULY

19 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Green Histology**
Speaker: Lawrence Patton
Leica Biosystems
Buffalo Grove, IL

24 **NSH Teleconference 1:00 pm Eastern Time**
Title: **How Did This Slide Contamination Happen?**
Speaker: Violet Swazer
Detroit Medical Center
Detroit, MI
Phone: (443) 535-4060 or register online at www.nsh.org

AUGUST

16 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Effects of Pre-Analytical Factors on IHC**
Speaker: Damien Matusiak
Leica Biosystems
Buffalo Grove, IL

28 **NSH Teleconference 1:00 pm Eastern Time**
Title: **What Should a Good H&E Look Like?**
Speaker: Robert L. Lott
Skin Pathology Associates
Birmingham, AL
Phone: (443) 535-4060 or register online at www.nsh.org

SEPTEMBER

18 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Immunohistochemistry, Antibodies, and Pathology**
Speaker: George Yang, MD
Cell Marque
Rocklin, CA
Phone: (443) 535-4060 or register online at www.nsh.org

20 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Histology Process Improvement: Tissue Processing From Conventional Overnight to Continuous**
Speaker: William DeSalvo
Collaborative Advantage Consulting
Tempe, AZ

20-25 National Society for Histotechnology Symposium/Convention
Site: Rhode Island Convention Center
Providence, RI
Contact: Aubrey Wanner
Phone: (443) 535-4060 or register online at www.nsh.org
Fax: (443) 535-4055
Email: aubrey@nsh.org

OCTOBER

18 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Safety in the Histology Laboratory**
Speaker: Terri L. Braud
Holy Redeemer Hospital
Rockledge, PA

23 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Building Effective Teams**
Speaker: Louis Anderson
Johns Hopkins Medical Institutions
Baltimore, MD
Phone: (443) 535-4060 or register online at www.nsh.org

NOVEMBER

15 **University of Texas Health Sciences Ctr/San Antonio**
Teleconference 12:00 pm Central Time (800) 982-8868
Title: **Role of Pathologist in Colorectal Cancer Diagnosis and Management**
Speaker: Prashant A. Jani, MD, FCAP, FRCPC
Thunder Bay Regional Health Sciences Centre
Thunder Bay, Ontario, Canada

20 **NSH Teleconference 1:00 pm Eastern Time**
Title: **An Introduction to Commonly Used Immunohistochemical (IHC) Stains in Dermatopathology**
Speaker: Alison Uzeblo, MD
William Beaumont Hospital
Royal Oak, MI
Phone: (443) 535-4060 or register online at www.nsh.org

DECEMBER

18 **NSH Teleconference 1:00 pm Eastern Time**
Title: **Double IHC Staining**
Speaker: Charlie Dorner
Dako North America Inc.
Carpinteria, CA
Phone: (443) 535-4060 or register online at www.nsh.org

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The editor wishes to solicit information, questions, and articles relating to histotechnology. Submit these to: Vinnie Della Speranza, ***HistoLogic***® Scientific Editor, 165 Ashley Avenue, Suite 309, Charleston, SC 29425. Articles, photographs, etc, will not be returned unless requested in writing when they are submitted.