



# MICROTIME

**The Georgia Society for Histotechnology**

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*Callarway Gardens is  
Beautiful in the Spring!*

## *President Letter....*



Dear Members;

March 25-27 is fast approaching and we will soon be at the GSH 2011 meeting at Callaway Gardens....I hope you plan to attend. The Hotel has graciously offered to honor the \$99 room rates as long as they have rooms. Make sure you tell them you are with GSH to ensure you pay only the \$99 rate. The rooms have been renovated and are very attractive. A room entitles you to admission to the garden attractions and a continental breakfast. This is going to be a great meeting with good speakers and lots of vendors. Hopefully we can enjoy the beauty of springtime in the gardens. Callaway tells me the Azaleas should be blooming and I know the butterfly house is a great place to go. The Birds of Prey is an opportunity to see some magnificent birds in flight.

I encourage each and every one of you to attend and convince your coworkers and friends to attend also. This is a good opportunity to accrue CEU's, renew old friendships and make new friends. See the latest in technology for your laboratory. If you cannot come both days, come for one day, either Saturday or Sunday. The HT/HTL Review on Friday always proves helpful to students as well.

Make plans to attend the membership meeting Saturday afternoon and share your thoughts, concerns and needs with your Officers and Board of Directors. We not only welcome your thoughts, but encourage your suggestions and ideas. Our profession and our Society are only as strong as each and every one of you.

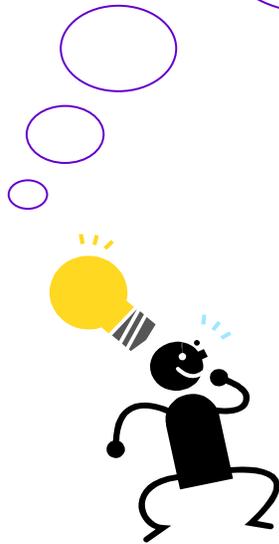
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Mike Ayers

President GSH



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them to the newsletter.....



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GSH EDUCATION COORDINATOR and  
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Carl Sagasser  
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 FX: 229-317-6682  
 Email: carl.sagasser@darton.edu

**HISTORIAN**

Janet Hobbs  
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 PH: 706-721-8515  
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**GSH BYLAWS & LEGISLATIVE CHAIR**

Larry Fields  
 Marietta, GA  
 fields932@gmail.com

**MICROTIME EDITOR**

Carole Fields  
 Marietta, GA  
 PH: 404-895-9604  
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Fran Adams  
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 PH: 770-994-1360  
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## **Tissue Processing Without Xylene - Part 1: Processing With Alkanes and Terpenes**

by René J. Buesa B.Sc., HTL (ASCP) (Ret.)

Starting in 1869, the standard procedure for tissue processing consisted of infiltrating the tissues with paraffin wax to assure the required consistency needed in microtomy but this was easier said than done because animal tissues contained from 65 to 90 percent water by weight and paraffin did not mix with water. Consequently the first step is to remove the water using alcohols but since they are polar substances (the same as water did not mix with paraffin determining that intermediary substances, miscible with both alcohol and paraffin, had to be used <sup>(1)</sup>.

Starting in the 1950s many of such substances like aniline oil, benzene, chloroform, dioxane, and toluene were used on dehydrated tissues until the late 1970s when there were great concerns about their safety, so much so that xylene became the “safer alternative”. Now it is known that xylene is as, or even more dangerous than the substances it initially substituted, which has determined using other substances to substitute it <sup>(2)</sup>.

Presently around 41% of US histolabs use xylene substitutes which are divided in two large categories: alkanes and terpene derived.

**Alkane substitutes:** are saturated hydrocarbons with a variable number of carbons arranged in straight line (aliphatic), branched (isoparaffinic), or with one or more cycloalkane carbon rings (naphthenic) with physical and chemical properties dependent on their structure and number of carbons in the molecule, none containing benzene, making them non aromatic hydrocarbons.

There are about 33 brand names used by 68% of histolabs using xylene substitutes the most frequent being naphtha (white gasoline) derivatives. They are between 0.12 to 2.56 (average of 0.92) times cheaper than xylene and the most popular brand names are Clear-Rite, Formula 83, and ProPar used by 67% of histolabs using alkanes. Some have been used satisfactorily for more than 10 years (Clear-Rite 3) or even 20 years (Formula 83) but there have been some problems with them.

In general, although they have low odor level, are recyclable, not very oily, and with a lower hazard level than xylene, are less effective at dewaxing during staining and if used to clear stained sections are usually incompatible with xylene or toluene-based mounting media, and cannot be used in automated coverslipers. There is some anecdotal evidence that they cause skin, eye, and respiratory tract irritation.

From the histotechnique stand point some alkanes are known to dry, shrink, and make tissues brittle requiring processing modifications. Other cause difficult to open wrinkles in sections, while others fade stains. There are some alkanes that absorb too much moisture, are flammable, and feel oily and greasy.

All in all, although less toxic and cheaper than xylene, alkanes do not represent an ideal substitute.

**Terpene based substitutes:** also called terpenoids, are isoprene polymers found in essential oils of plants. They were the first clearing agents used in histology and include turpentine, initially known as “terpentine” (after which the whole group was named), and oils of bergamot, cedar wood, clove, oregano, terpineol, and thyme, among others all sharing a high cost that make them impractical for automated tissue processors.

The ones used presently as xylene substitutes are "Limonene" derivatives obtained by steam distillation of the liquid slurry resulting from citrus fruit peel pressings. Limonene consists of two isoprene units and exists as dextrorotatory (D or "+") and levorotatory (L or "-") racemic mixtures with the "D" or (+) form being the main component in the citrus peel oil.

Limonene is produced in technical, refined, and food grades and when in the late 1970s xylene dangers became an issue, many manufacturers started to produce D-Limonene based alternatives because it was readily available, cheaper than other terpenes, and had already being incorporated in many household dewaxing and cleaning products which determined the existence of an industrial capability that could accommodate a product diversification.

There are about 18 brand names used by 32% of histolabs using xylene substitutes. They are between 0.93 and 7.44 (average of 2.2) times more expensive than xylene. The brands AmeriClear, Histo-clear, and Histosolve X (BioClear) are used by 67% of histolabs using D-Limonene derivatives.

Generally speaking there are many health complaints with D-Limonene derivatives all stemming from its strong orange smell found nauseating by many and that in some cases have produced asthma and headaches, as well as sensation of "metallic taste". D-Limonene can oxidize with other substances used in histology and produce hand dermatitis.

From the histotechnique perspective it requires processing modifications and needs special mounting medium and some complain that it hardens brain, liver, and spleen samples. It also has poor dewaxing properties and the stained sections fade requiring special mounting medium. Some brands have the tendency to turn yellow and leach oily deposits.

Objectively, both alkanes and D-Limonene derivatives are not good xylene substitutes, so "What to do?" you may ask. The answer to this question and the presentation of the best xylene substitute available will be the subject of "Tissue Processing Without xylene - Part 2".

Part II to follow next issue.

#### References:

- 1- Buesa, RJ: Histology safety: now and then. *Ann.Diag.Pathol.*, 2007; 11(5):334-339
- 2- Buesa, RJ, Peshkov, MV: Histology without xylene. *Ann.Diag.Pathol.*, 2009; 13(4):246-256



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## Charles J. Churukian



### **Brighton: May 11, 1928 - February 23, 2011, Charles J. Churukian 82.**

Predeceased by his parents, Joseph and Christin Aintablian Churukian, and sister, Sally Churukian Taroni. He is survived by his wife, Irene Billings Churukian of 41 years and his sister, Rose Churukian Milone as well as sisters-in-law, nieces and nephews. Charles will be sadly missed by family and friends.

As a PFC in the infantry, he served as a heavy machine gunner in World War II receiving the Army of Occupation Medal and Victory Medal.

Teacher, mentor, poet, editor, innovator, "guru of special stains", Charles worked in histology laboratories for 54 years and supervised the Histotechnology Lab at the UR Medical Center for the last 40 years. He was recently presented with the "Histotechnologist of the Decade" Award by the National Society of Histotechnology for his contributions to the laboratory science field having numerous publications, presentations and awards to his credit. He devoted his career looking for ways to modify the art of special stains for the benefit of patient care. In addition to his professional life, he offered spiritual guidance to many inmates at the jail.

On Saturday, March 5, at 11:00, friends are invited to attend a Memorial Service at Asbury United Methodist Church at 1050 East Ave. followed by a

reception in 1010 East Avenue, adjacent to the church. Interment, White

Haven at the convenience of the family. Charles' family would like to thank the staff at UR Wilmot Cancer Center, and LifetimeCare Agency for their loving compassionate care

In lieu of flowers, donations may be directed to Good News and Jail Ministry of the Rochester Area, AMAA Scholarship Fund (for university students in Armenia), Asbury Outreach Programs (Dining Caring Center,

Storehouse) or James P. Wilmot Cancer Center.



**GSH REGISTRATION FORM FOR 2011 MEETING**

March 25-27, 2011

Mountain Creek Inn, Callaway Gardens, Pine Mountain, Georgia

Call for hotel reservations at 1-800-225-5292

Rooms \$99 which includes Continental Breakfast and Admission to the Park

Please fill out a separate form for each attendee ***completely*** and send with payment or an indication of payment through PayPal

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**NONREFUNDABLE REGISTRATION FEE: \$35 (includes the Awards Luncheon on Saturday and applies to HT/HTL review and attending vendor reception only)**

**FEES: Friday HT/HTL Review - \$25 plus \$35 registration fee = \$60**

**Saturday only - \$60 plus \$35 registration fee = \$95**

**Sunday only - \$50 plus \$35 registration fee = \$85**

**Both Saturday and Sunday - \$100 plus \$35 registration fee = \$135**

**Students Both days - \$60 plus \$35 registration fee = \$95**

**STUDENTS: Your Instructor must sign here to be eligible for student rates:**

Name: \_\_\_\_\_ School Name: \_\_\_\_\_

**PLEASE CHECK WORKSHOPS DESIRED: You can only attend a total of 3 for both days.**

Saturday a.m.: Workshop #1 \_\_\_\_\_

Saturday p.m.: Workshop #2 \_\_\_\_\_

Sunday a.m.: Workshop #3 \_\_\_\_\_

Sunday a.m.: Workshop #4 \_\_\_\_\_

PLEASE TOTAL: Registration fee for all attendees \$ 35.00

# of Extra Luncheon tickets: \_\_\_\_\_ \$ \_\_\_\_\_

HTL Review Session - \$25.00 \$ \_\_\_\_\_

Saturday only - \$60.00 \$ \_\_\_\_\_

Sunday only - \$50.00 \$ \_\_\_\_\_

Both days - \$100.00 \$ \_\_\_\_\_

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**March 25, 2011 - Friday**

**1 to 5 p.m.: HT/HTL Review Session for Students – Taiquanda Winbush, AS, HT(ASCP)**

Are you preparing to take the HT/HTL exam and you are nervous about it? In this review class you will learn how to study and what to study. The class will consist of 3-4 hours of Q&A along with presented slides. A handout will be included as part of the program.

Topics included in this workshop will include discussion on fixation, microtomy, staining, laboratory operations, safety and processing/embedding. Also included will be general discussion of histology at the microscopic level including images of representative tissues. Students taking this course should have a general knowledge of histological technique and will be taking the National Registry exam in the next few months.

**5 to 7 p.m.: Meeting Registration**

**7 to 9 p.m.: Vendor Reception in Vendor Area**

**March 26, 2011 – Saturday**

**Seminars on Saturday will be held in Room A**

**7:00–8:00 a.m.: Meeting Registration**

**8:00-9 a.m.: Lamar Jones – Histo Tats & Toos**

This lecture will teach the participate the history, origin, application, identification of tattoos. The use of the dyes utilized, histological application and the removal of tattoos will also be discussed

**9:00-10 a.m.: Dr. Katja Lehmann - "What is the Buzz about Lean?" Part I**

This talk will educate the audience about the definitions of Lean, Six Sigma and Lean Six Sigma as well as teach standard LEAN concepts that can be applied in a laboratory environment in order to improve the goals important to that specific laboratory (e.g. decrease Turn Around Time, decrease defect rates, etc.) The last part will have an outlook into the laboratory of the "future". The audience will then have the opportunity to use the skills learned and practice leaning a "mock laboratory" in an hands-on exercise, improve the current processes in the laboratory to make it LEAN. Part II after break.

**10:00-10:30 a.m.: Break in Vendor Area**

**10:30-11:30: Dr. Katja Lehmann - "What is the Buzz about Lean?" Part II**

The last part will have an outlook into the laboratory of the "future". The audience will then have the opportunity to use the skills learned and practice leaning a "mock laboratory" in an hands-on exercise, improve the current processes in the laboratory to make it LEAN. Part II after break.

**11:30-1:00 - GSH AWARDS LUNCHEON – Tickets required**

**1:00-3:00 p.m.: Wanda Jones – Dermatopathology – Skins from Beginning to End**

**3:00-3:30 p.m.: Break in Vendor area**

This mini workshop will take the attendee through the dermatology process from the beginning to the end.

3:30-4:30 p.m.: John Stock – Basic Troubleshooting for Histology Laboratory Equipment

*This module will go over general maintenance covering most equipment found in a histology Laboratory. The participant will; Identify common maintenance issues found in a Laboratory environment and encourage the User to think about his or her actions of maintenance. Suggest methods and procedures of maintenance that will prevent downtime of the units and meet regulatory certification. Review what tools are helpful and necessary to perform daily maintenance, and their possible uses.*

**4:30-5:00 p.m.: GSH General Membership Meeting with GSH Board Meeting to Immediately Follow**

**Workshops on Saturday will be held in Room B**

**Workshop # 1: Room B**

**SATURDAY, 8-12 noon, Room B**

**#1: Microanatomy and Tissue Identification – Diana Fry, BS, HT/HTL(ASCP), Duke University**

The workshop will begin with overview of five tissue types: epithelial, connective, muscular, nerve, and blood. Special focus will be given to classification of epithelium and its use in identifying tissues. Organ systems covered will be gastrointestinal, respiratory, cardiovascular, integumentary, endocrine, skeletomuscular, excretory, reproductive, nervous, and lymphatic. The organs involved will be located and briefly described grossly, then microscopically. Function or physiology will be covered only where it may help the technician to understand the microanatomy.

**Workshop #2: Room B**

**SATURDAY, 1-4, Room B**

**#2: - CSI – Case Study Immunos – Mike Reichenbach, HT(ASCP) QIHC, Ventana Medical Systems & Debra Flynn, HT(ASCP) QIHC, Biocare Medical**

Through IHC panels, attendees will have a better understanding of how complex or “unsolvable” cases can be diagnosed, leading to a better course of treatment for patients. It is our intention to motivate attendees to understand how important they are to the overall outcome of patient care. Join our team of investigators as we assist our pathologists in determining correct diagnoses through the use of Undifferentiated Tumor Panels and Secondary Tumor Panels in IHC. Actual patient cases will be presented, which may have been relegated to the “unsolved mysteries” file, were it not for the technology of IHC. Basic IHC theory, covering topics of antibody validation, control tissues, explanation of tumor panels and general IHC antibodies, and actual patient cases will be covered during this lecture. Review of latest FDA-cleared therapies will also be covered.

**March 27, 2011 – Sunday Workshops**

**7:00-8:00 a.m.: Meeting Registration**

**Workshop #3: Room A**

**SUNDAY, 8-12**

**#3: When Paraffin Won't Cut It – A Hands On Approach to Working with Resin in Hard Tissue Applications - Jack Ratliff, BA**

If paraffin won't cut it, what do you do? Believe it or not, this is a common question that has haunted many histotechnologists from both a clinical and research perspective. It seems all too many times a specimen is received into the lab only to find out later that it is either difficult or impossible to obtain usable sections when embedded in paraffin. In fact, if the specimen doesn't catch on the blade and pop out of the wax, one will surely experience an immediate dulling of the blade that creates an unacceptable knife artifact in the tissue if a section is obtainable or some type of shredding with a complete loss of usable tissue. This is a common experience when working with bone and even when decalcification techniques are employed because the bone is just too dense.

**10:00 – 10:30 a.m. Break**

This workshop is intended to provide an application usage of methyl methacrylate (MMA) resin as a suitable embedding media for working with “hard tissue”, specifically bone, while focusing on specific methods for cutting 5 micron sections using a rotary microtome. The first half of this workshop will identify specimen preparation, dehydration, infiltration and embedding requirements for working with resin (MMA) and bone. The second half will focus on a live demonstration of thin section microtomy utilizing a specially configured rotary microtome used to cut bone specimens embedded in resin (MMA) and willing participants will have a chance to participate and produce thin sections of their own. Participants are encouraged to bring their own polymerized specimens, although not necessary, and will also have the option to retain their cut slides upon completion of the workshop so that they may be able to return back to their labs and complete any desired staining techniques. Individuals with a current or future intent to process and cut undemineralized tissue or tissue containing foreign implant materials are strongly encouraged to attend.

## **Workshop #4: Room B**

**SUNDAY, 8-12**

### **#4: Standardization of Antigen Retrieval Techniques - Lamar Jones, BS, HT(ASCP)**

This workshop will discuss the history and application of antigen retrieval techniques for immunohistochemistry. This workshop can have a “wet” portion utilizing some of the instruments available for different types of antigen retrieval. Handout, “wet” Kodachrome slides.

**10:00 – 10:30 a.m. Break**



GEORGIA SOCIETY FOR HISTOTECHNOLOGY

MEMBERSHIP OR RENEWAL FORM

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NSH MEMBER \_\_\_\_\_ YES \_\_\_\_\_ NO \_\_\_\_\_

GSH recommends that you also consider joining NSH in order to be eligible to serve as a representative of GSH in the House of Delegates at the annual Symposium and actively participate in your society. Only NSH members who are GSH members can serve in the HOD.

\*\*\*\*\*Date: \_\_\_\_\_ for year 2011 \*\*\*\*\* REQUIRED

Membership is free to anyone working in histology in Georgia but you must fill out a form each year to be a member. If you do not live in but work in Georgia, you qualify. If you do not work in Georgia you may still be a non-voting member with other member privileges such as discount rates at meetings.

MEMBERSHIP YEAR IS FROM JANUARY TO DECEMBER

Are you interested in serving on a committee? \_\_\_\_\_ Do you prefer mail sent to: Home? \_\_\_\_\_ Work? \_\_\_\_\_

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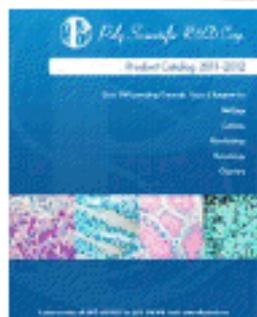
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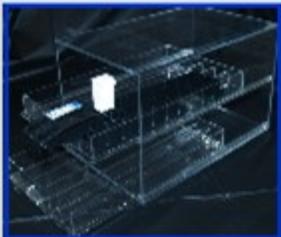
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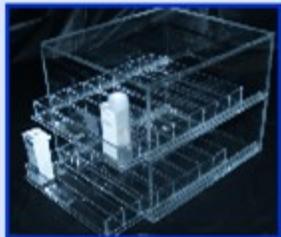






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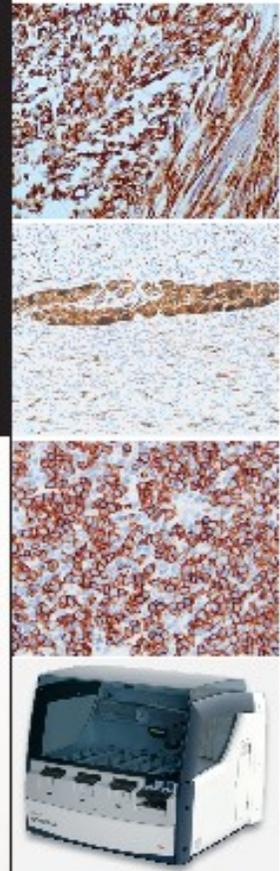


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