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ISSUE 2
August 2021

Newsletter of The Histotechnology Society of NSW

HISTOGRAPH

Editorial

Hi and welcome to the second edition of the Histograph. Hope all our member's are staying safe and well during this challenging and unprecedented times due to COVID-19 pandemic.

Who would have thought ammoniacal silver could be explosive. Enjoy reading Tony's 2 informative articles on Ammoniacal Silver and Hollande's Fixative.

IBSA group in conjunction with the Histo Society are conducting webinars to seek feedback about the National training for basic surgical cut-up. See Flyer on how to register for one of the 3 zoom sessions.

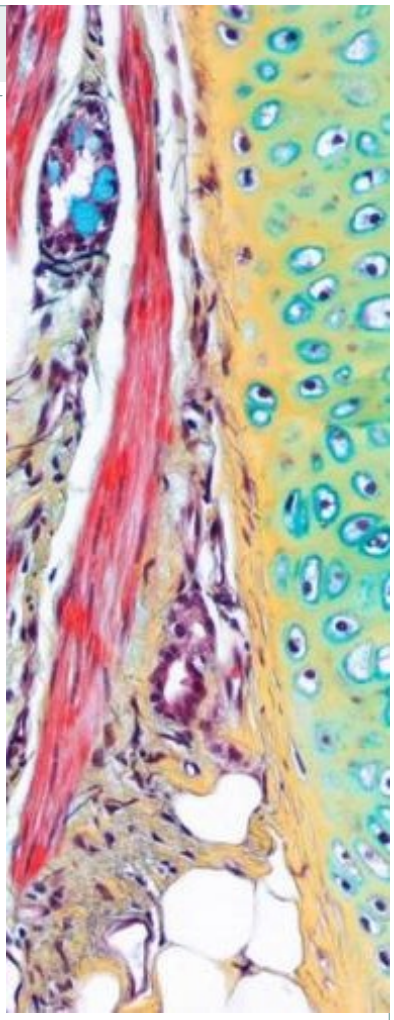
Leica conducted a case study on "Slide Labelling Automation Reduces a Laboratory's Hands-On Time".

Anyone having issues renewing your membership please read the article on "Membership Renewal and How to find next payment" .

I'm sure everyone solved the Test & Teach from the last issue. Answers are now published.

Hope you enjoy reading this issue. Feel free to email me on linda.prasad@health.nsw.gov.au with any feedback or any articles you would like to publish. Stay Safe

Linda Prasad
Editor



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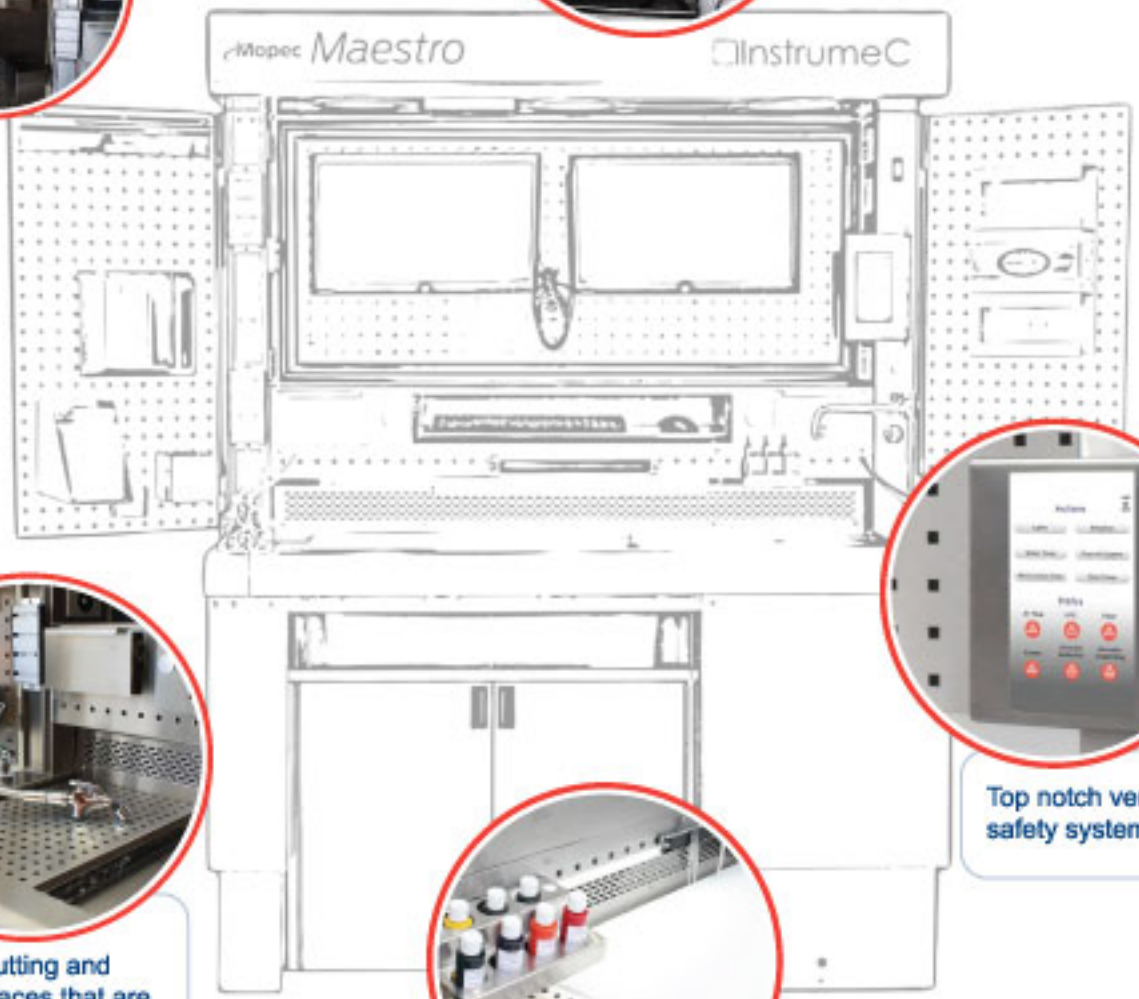

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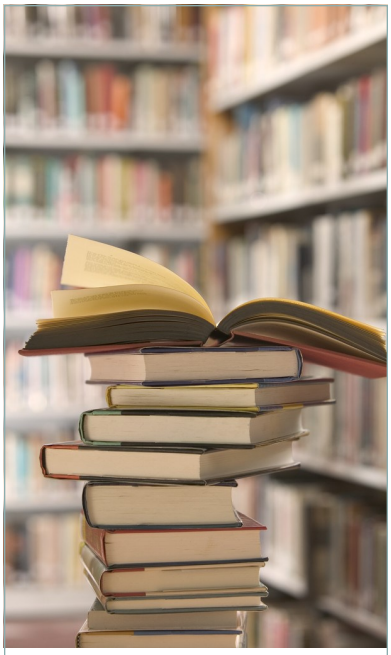

Histotechnology Society of NSW COMMITTEE MEMBERS are volunteers who work tirelessly to promote histotechnology and provide educational opportunities for continuing professional development. Thank you team for the GREAT JOB

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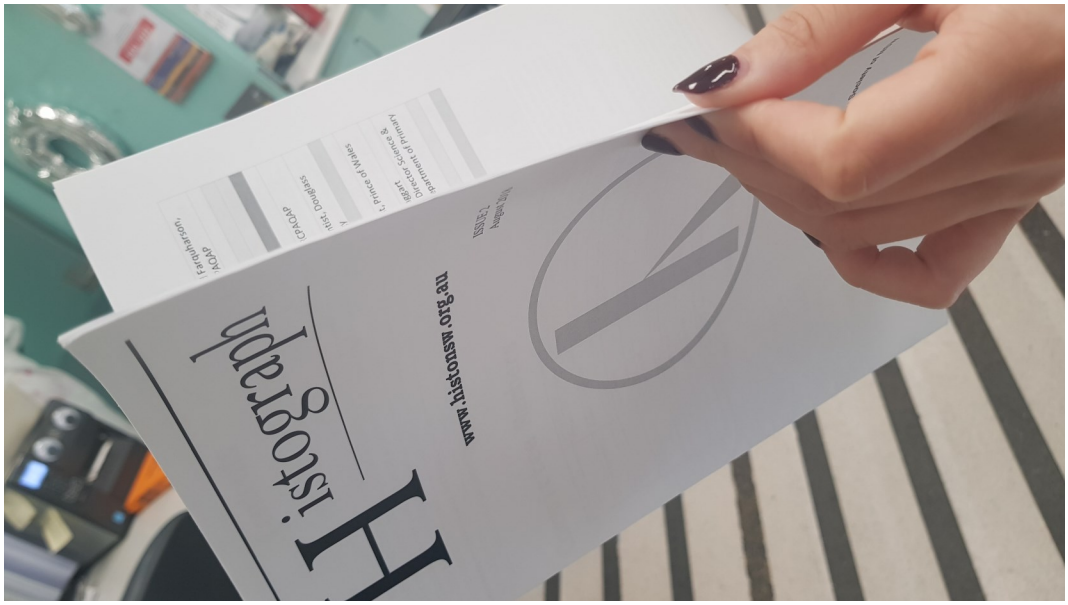

Chairman's Report

Our committee made the decision to cancel our second attempt at holding the next National Conference in Sydney. This was done with the support of Histology Group of Australia (HGA). The plan was to hold the Conference at the Sofitel Hotel in Darling Harbour and the Conference dinner at Doltone House in March 2022. Both arrangements have been cancelled. The Conference planning is now back with the HGA to make a decision on where and when the next Conference will take place. In these uncertain times with COVID-19 and no coverage in contracts relating to COVID-19, deposits can be lost.

On the positive side we have commenced working with Doltone House on holding a night time function/dinner there to utilise the deposit we have with them.

The HGA at a committee meeting decided that we should make a change to the name. With our involvement with "The Australian Council for Certification of Medical Laboratory Scientific Workforce"[ACCMLSW] and other professional bodies, it was felt we needed a more professional name. Overseas Histology organisations are also contacting us. Several names were proposed and reviewed by the State committees and HGA. The name agreed on is the "Australasian Association of Histology and Histotechnology" [AAHH]. This was recently approved by NSW Fair Trading.

HGA is currently looking into membership categories and changes to the constitution. This is being done in conjunction with the State committees.

Our NSW committee is in the process of reviewing our website. Part of this review is looking into financial membership [categories, financial membership dates, and renewals] and involvement of our membership officer.

Two webinars have been held since our last Histograph:

In May an Industry Webinar on Embedding Equipment and Embedding Media. We have to thank Mark Mullin for organising the industry involvement and UTS for incorporating the webinar into their program. Companies involved included Leica, Abacus dx and Bio-strategy.

We are hoping to hold more of these. In July we held a webinar on the Reticular Endothelial sys-tem. Dr Tamara Sztynda, presented a very interesting discussion on Primary and Secondary lymphoid organs.

These Webinars have been well received and are an important part of our current program under the challenging conditions with COVID-19.

Cheers,

Trevor Hinwood. Chairperson

Histotechnology Society of NSW

LEICA BIOSYSTEMS CASE STUDY

SLIDE LABELING AUTOMATION REDUCES A LABORATORY'S HANDS-ON TIME WHILE POTENTIALLY IMPROVING PATIENT SAFETY

Introduction

Researchers estimate that more than 160,000 adverse patient events occur each year in the United States because of patient specimen identification errors involving clinical laboratories.[1] Specimen labeling errors within the laboratory can occur at several points of specimen processing, and handwriting slides can lead to transcription errors.[2] Using lean processes in the clinical laboratory can help reduce errors while removing non-value-added wastes, thus driving toward an improved, efficient system.[3]

This assessment aimed to analyze the amount of hands-on time required to label slides for patient identification and compare that to two automated solutions. The first solution was a batch process using a high-capacity slide printer, and the second was an on-demand process using compact slide printers at each microtome station.

Methods

This study, conducted by the Leica Biosystems Content and Evidence Team, occurred at a dermatopathology laboratory. The laboratory was interested in finding automated solutions to their current labor-intensive slide labeling process. The team performed time and motion studies for the current process, which included handwriting slides at microtomy and relabeling slides at case assembly. The same time and motion study was completed for the proposed processes of batch slide printing and on-demand slide printing.

Results

The data from the time and motion studies are summarized in the following tables and chart:

Hands-On Time for Slide Labeling Processes

	Per Slide (secs)	Per Day (mins)	Per Year (hours)
Handwriting Slide	9.5	86.5	374.8
Relabel with LIS Label	3.3	30	130
Total Current Labeling Process	12.8	116.5	504.8
Batch Slide Printing	4.4	40	173.3
On-Demand Slide Printing	2.5	22.8	98.8

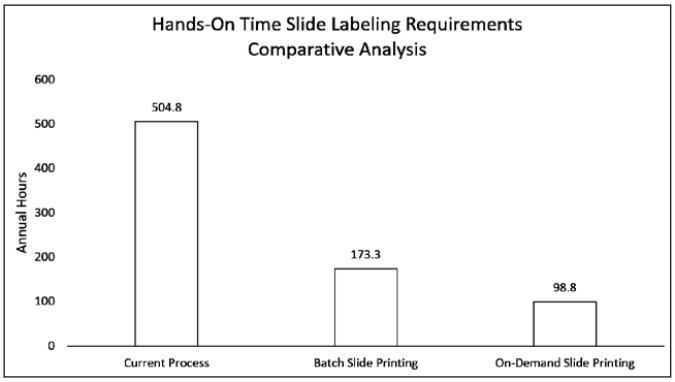

Fig. 1: Comparison of hands-on time requirements for each observed process

Projections and Realized Results are specific to the institution where they were obtained and may not reflect the results achievable at other institutions.

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LEICA BIOSYSTEMS CASE STUDY

Discussion

Upon reviewing the data, technologists following the current process were spending an average of 9.5 seconds handwriting each slide during the slide labeling process at microtomy. As the laboratory generates an average of 546 slides each day, it amounts to 86.5 minutes per day. Over the entire year, this adds to 374.8 hours of required technologist hands-on time. Additionally, the technologist working at case assembly must also correctly match and relabel the slide with an LIS-generated label. This activity requires an average of 3.3 seconds relabeling each slide, or 30 minutes a day. Annually, this single process requires an additional 130 hours of technologist's hands-on time.

The slide labeling process using a batch printing process takes 4.4 seconds of technologist time per slide or 40 minutes per day with an annual total of 173.3 hours. The slide labeling process using an on-demand slide printing process takes 2.5 seconds of technologist time per slide or 22.8 minutes per day for an annual total of 98.8 hours. Both automated solutions would eliminate the matching and relabeling of each slide at case assembly.

Transitioning to a batch printing process would allow the lab to save up to 8.4 seconds per slide or a 65.7% improvement over their current slide labeling process. However, implementing the on-demand slide printing process could save this laboratory up to 10.3 seconds per slide or an 80.4% improvement over their current slide labeling process.

Conclusion

Laboratories can reduce hands-on time and eliminate possible matching errors through automating manual slide labeling processes.

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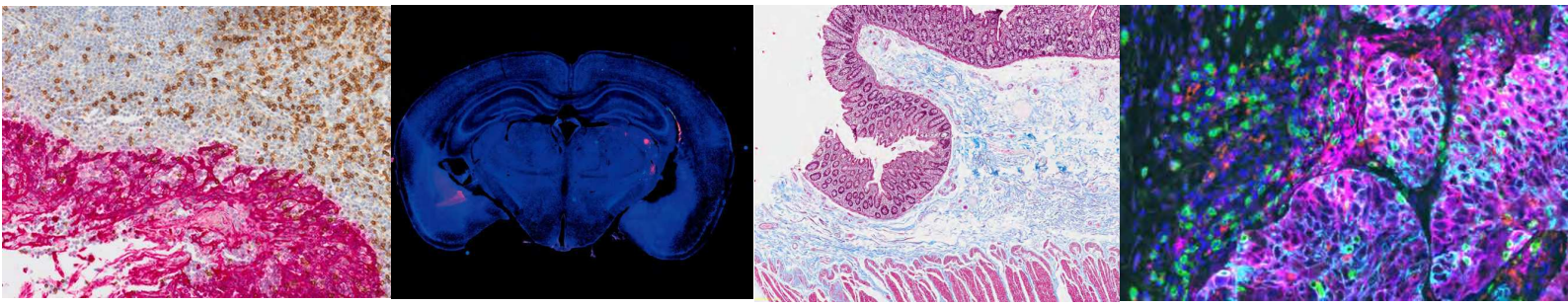

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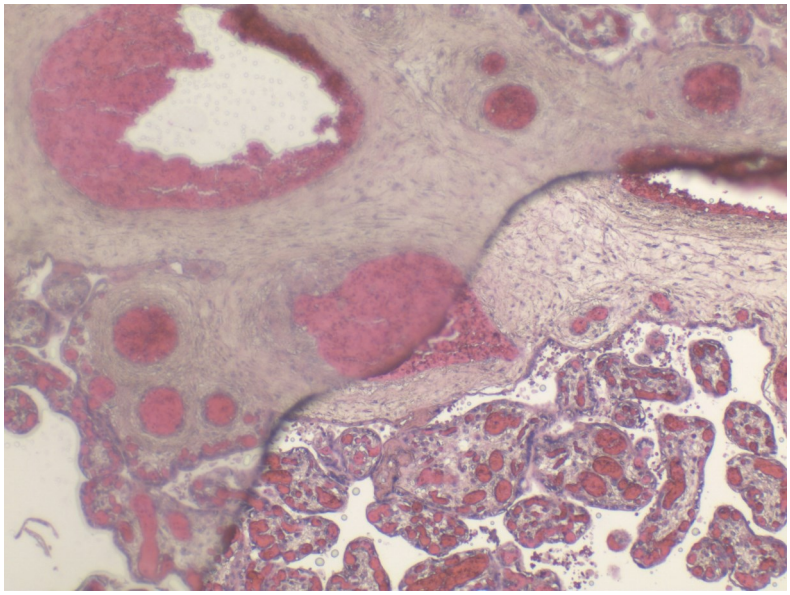
TEST AND TEACH - ANSWER FROM ISSUE 1

ANSWERS

1. **What is the tissue?**
   - Placenta
2. **What is the stain?**
   - H&E
3. **What is the artifact?**
   - Water present in the tissue section before cover slipping



HISTOTECHNOLOGY SOCIETY OF NSW

Webinars still to come for 2021

Sept-14th,Surgical Cut-up National Consultation

Nov-13th, Lymphomas

We look forward to your involvement.

Register Now

Surgical Cut-up National Consultation

Industry consultation webinar

Tuesday 14 September 2021

IBSA Group (Innovation & Business Skills Australia) co-facilitated by Histotechnology Society of NSW will be holding a national webinar. There will be 3 sessions (all sessions will be the same to ensure that the various shift workers can attend).

Webinar Date: Tuesday 14 September

Times: 9.00 am–10.00 am [AEST]
1:00 pm – 2.00 pm [AEST]
7.00 pm – 8.00 pm [AEST]

To register for one of our three Zoom webinar sessions:

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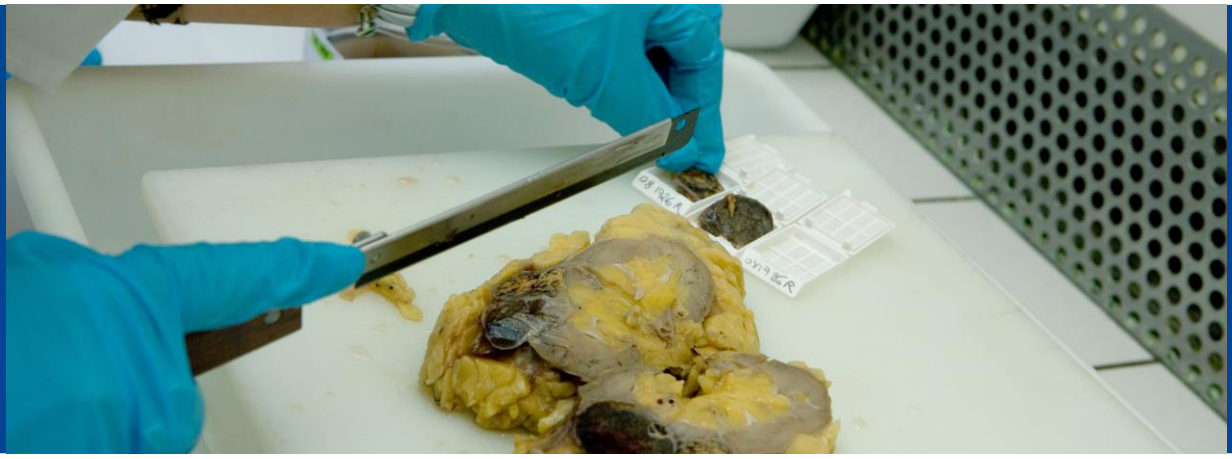

National training for basic surgical cut-up Industry consultation webinars

We are seeking feedback from industry on proposed:

- Creation of two units of competency:
  - ✓ Simple transfers in surgical cut-up
  - ✓ Simple (non-complex) surgical cut-up
- Creation of one skillset:
  - ✓ Basic surgical cut-up skills
- Update of two qualifications to incorporate the new units of competency:
  - ✓ MSL40118 Certificate IV in Laboratory Techniques
  - ✓ MSL50118 Diploma in Laboratory Technology

Providing feedback

Please register for one of our three Zoom webinars sessions here

Webinar Date: Tuesday 14 September

Times: 9.00 am–10.00 am [AEST]
1:00 pm – 2.00 pm [AEST]
7.00 pm – 8.00 pm [AEST]

Co-facilitated by the

HISTOTECHNOLOGY SOCIETY OF NSW

For more information:

Mark Shaddock | Training Package Development Manager
P: 0499 599 298 | E: manufacturing@ibsa.org.au

Leah Simmons | Laboratory Operations Industry Reference Committee
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Hollande's Fixative - a Blast from the Past

Tony Henwood, Principal Scientist, Histopathology, the Children's Hospital at Westmead, Sydney. NSW.

Where would we be without formalin fixation? It's been around for over 100 years and would have to be the preeminent preservative for routine histopathology. Yet, there are regular reports of alternative "molecular-friendly" fixatives that purport to produce results that rival formalin (1,2). Sometimes we are confronted with a fixative from the past. This is the case with a fixative that was first described in 1942 by Hollande (3) and bears his name. This fixative contains picric acid, formalin, acetic acid, and copper acetate.

According to Freda Carson (4), this modification of Bouin's solution is stable and will decalcify small specimens of bone. It has been used as a fixative for biopsy specimens of the gastrointestinal tract. Hollande's-fixed tissue can be stained successfully with most special stains. The cupric acetate present in the solution stabilizes red blood cell membranes and the granules of eosinophils and endocrine cells, so that less lysis occurs than with Bouin's solution (4).

The recipe for Hollande's fixative (4,5):

Copper acetate	25 g
Picric acid	40 g
37-40% Formaldehyde	100ml
Acetic Acid	15ml
Distilled water	1000ml

Gatta et al. (1) presented a formulation that appears at odds to others in that it has mercury chloride rather than acetic acid. I am not sure whether this is a typo or not.

McMahon et al (5), in their studies on demonstrating immune complex deposits using fluorescence microscopy of H&E sections of Hollande's fixed renal biopsies suggested that picric acid is a well-known dye and is a component of several histologic fixatives including Bouin's and Hollande's, reacts with basic proteins to form crystalline picrates that can be replaced by other acid dyes including eosin. Hollande's is known to induce clumping of nuclear chromatin, which gives H&E-stained tissue sections such good nuclear detail. Picric acid and glacial acetic acid components of Hollande's may also induce denaturation and clumping of immune complex proteins that does not normally

occur with fixation in neutral buffered formalin. Such clumping would permit better eosin staining and easier visualization of immune deposits using H&E. Copper acetate was originally added to Hollande's to improve the dissolution of picric acid but also was found to stabilize red blood cell membranes and certain cytoplasmic granules. Copper acetate may also act in a way like copper sulfate, which has been used as a mordant to promote color uptake in the textile dye industry.

Historically, Haggitt (6) in 1982, recommended Hollande's solution for the routine fixation of gastrointestinal biopsies. Murphy et al. (7) in 1993 suggested that Hollande's was a better fixative for diagnostic bladder and prostate biopsies. More recently, Rowe et al. in 2001 (8), used Hollande's to fix cell blocks prepared from PreservCyt preserved fluids. McMahon et al. (5) in 2002, using Hollande's fixative, described brightly fluorescing immune complex deposits within glomerular basement membranes and mesangial matrices that correlate well with the results of standard direct immunofluorescence on frozen tissue and electron microscopy. This phenomenon was not seen after routine formalin fixation. At the Cleveland Clinic, Hollande's fixative is preferentially used in the histologic processing of most tissues including kidneys because of superior nuclear detail (5).

In 2020, Rostoker et al. (9) studied the accuracy of hepatic iron load measured by signal intensity ratio and R2* relaxometry MRI in dialysis patients using tissues fixed in a range of fixatives including Hollande's solution. In 2021, Liu et al. (10) studied the tissue microenvironments of Barrett's esophagus premalignant tissue biopsies using digital pathology and found that there was an equal performance of Hollande's fixative with formalin.

Unfortunately, there are issues with using picric acid-containing fixatives such as Bouin's and Hollande's. These solutions are known as "Hard" fixatives, compared to "Soft" fixatives such as neutral buffered formalin. Soft fixation predisposes to nuclear "bubbling" artefact due to chromatin coalescence. Hard fixatives tend to produce brighter Haematoxylin and Eosin and Giemsa staining. Hard fixatives cause coarse clumping of the cytoplasmic matrix and nuclear chromatin. As noted by Epstein (11) the problem with using such fixatives on tissues such as prostate is that they enhance nuclear detail not only of the malignant glands but of the benign glands as well. Therefore, visible nucleoli are seen in benign glands quite readily whereby it becomes less clear what constitutes prominent nucleoli in the atypical glands. In using fixatives that enhance nuclear and nucleolar detail, the threshold for what constitutes prominent nucleoli diagnostic of carcinoma becomes elevated.

A second issue is that, even though Hollande's fixative works well for histochemistry, it is not suitable for molecular applications such as FISH (1).

Finally, Hollande's fixative generates several laboratory hazards because of the picric acid component that is toxic and potentially explosive (12).

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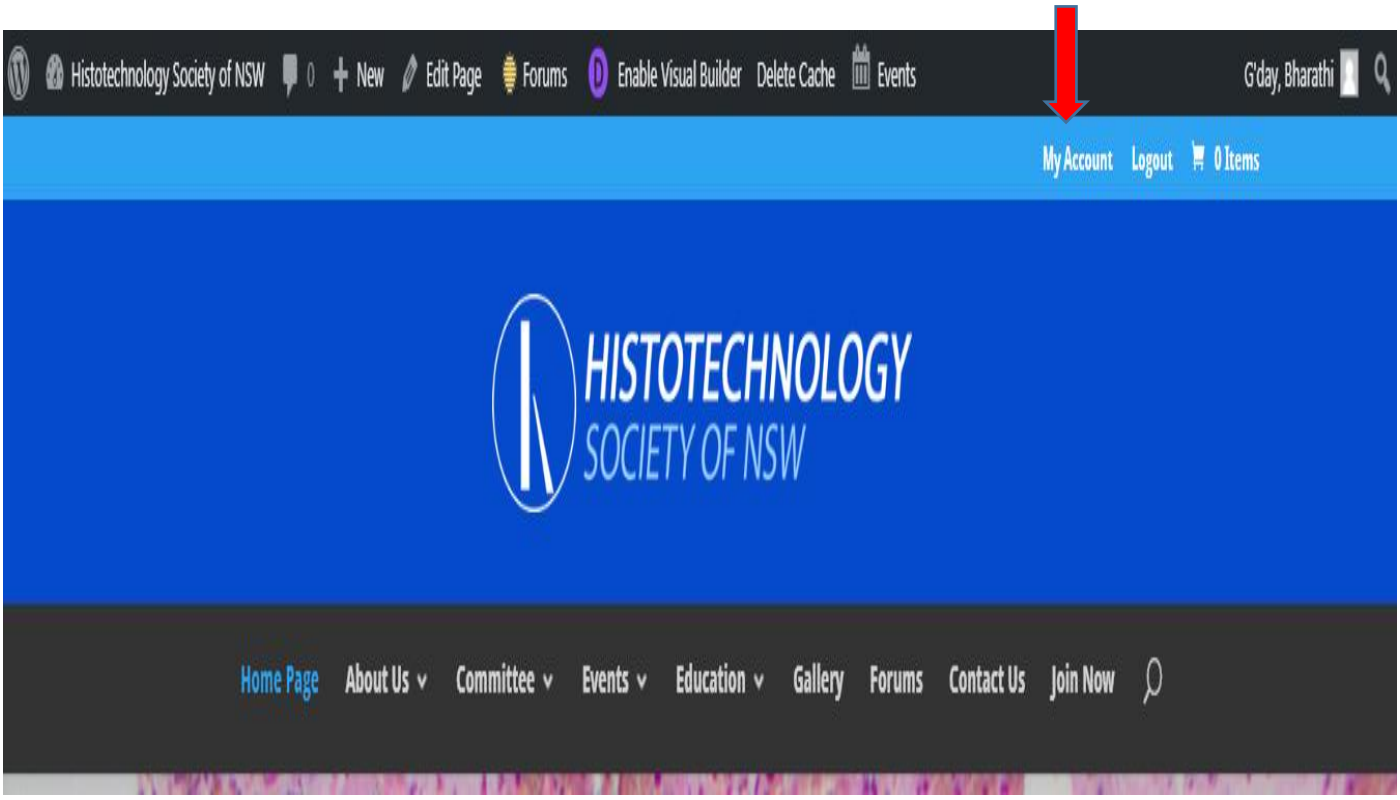

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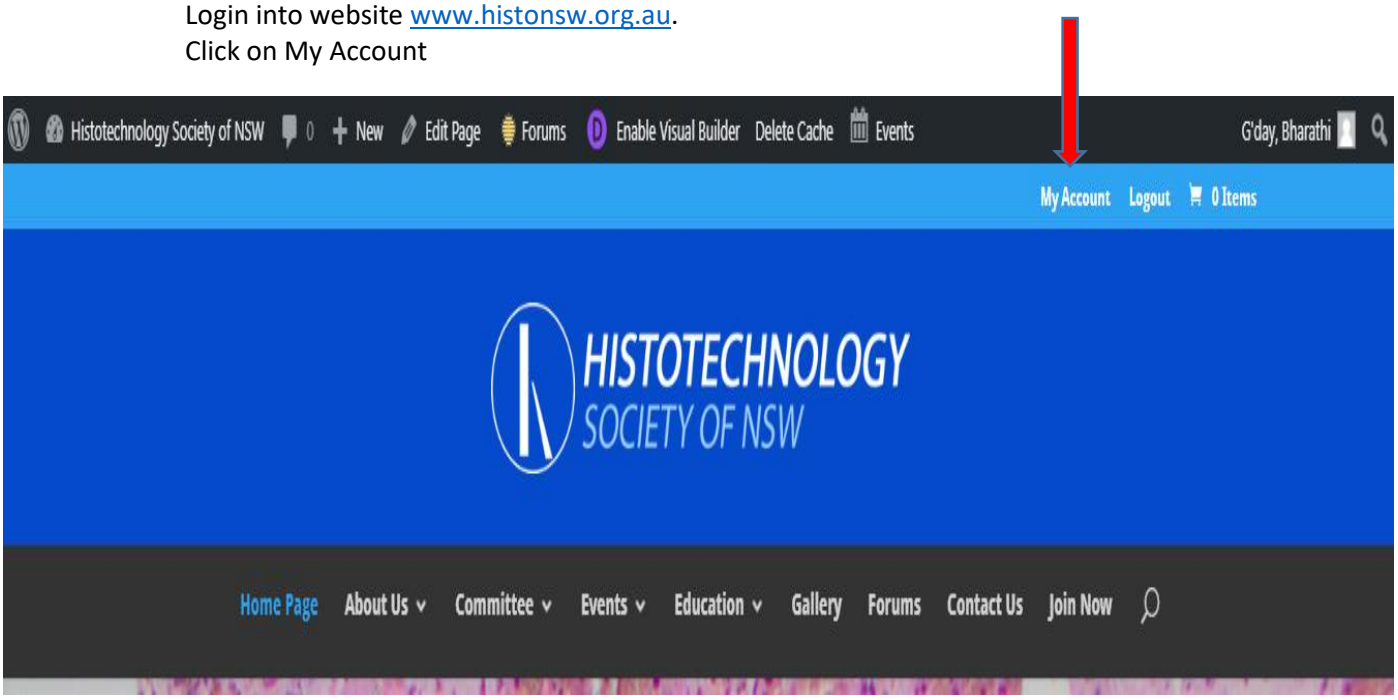

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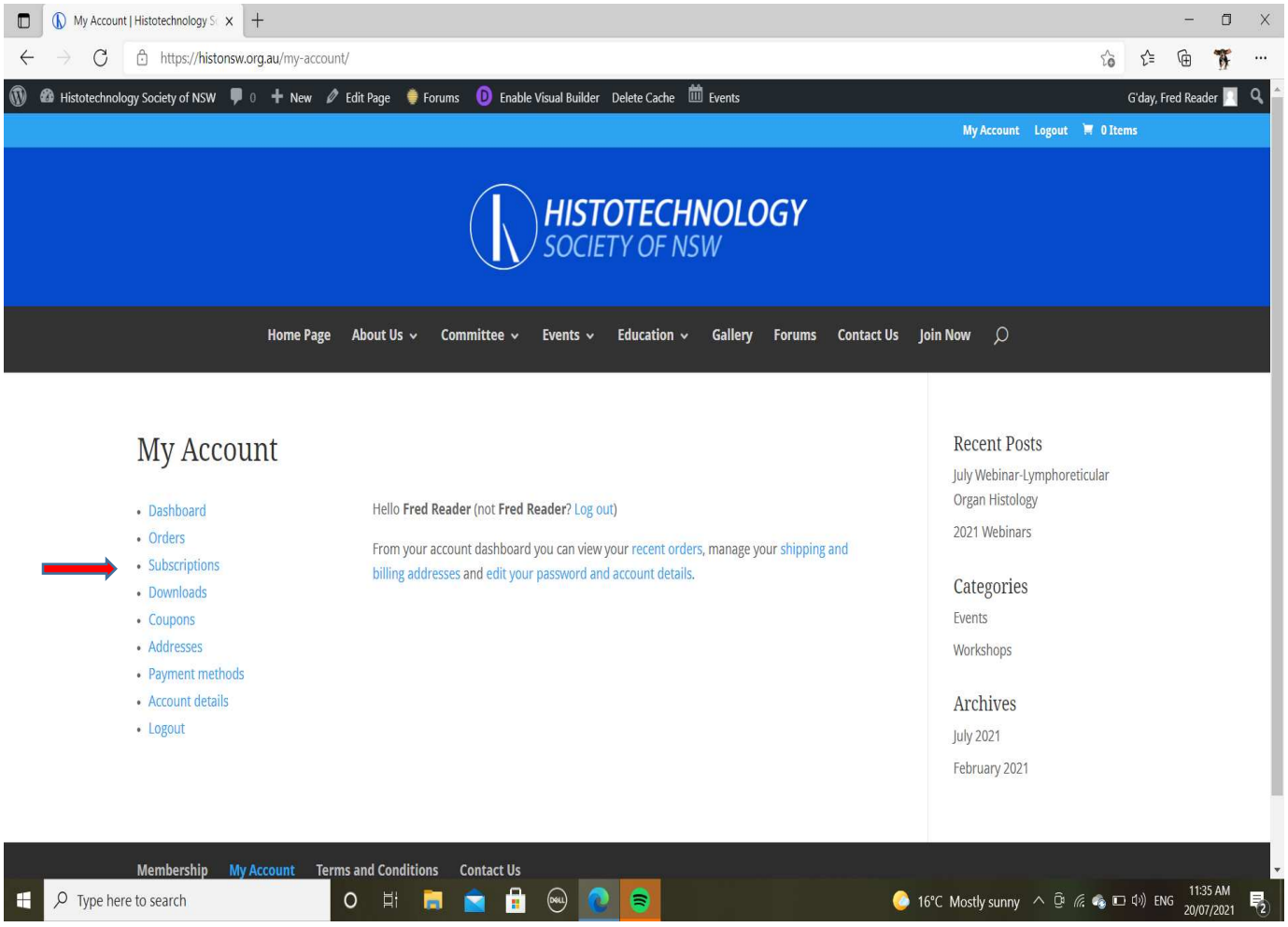

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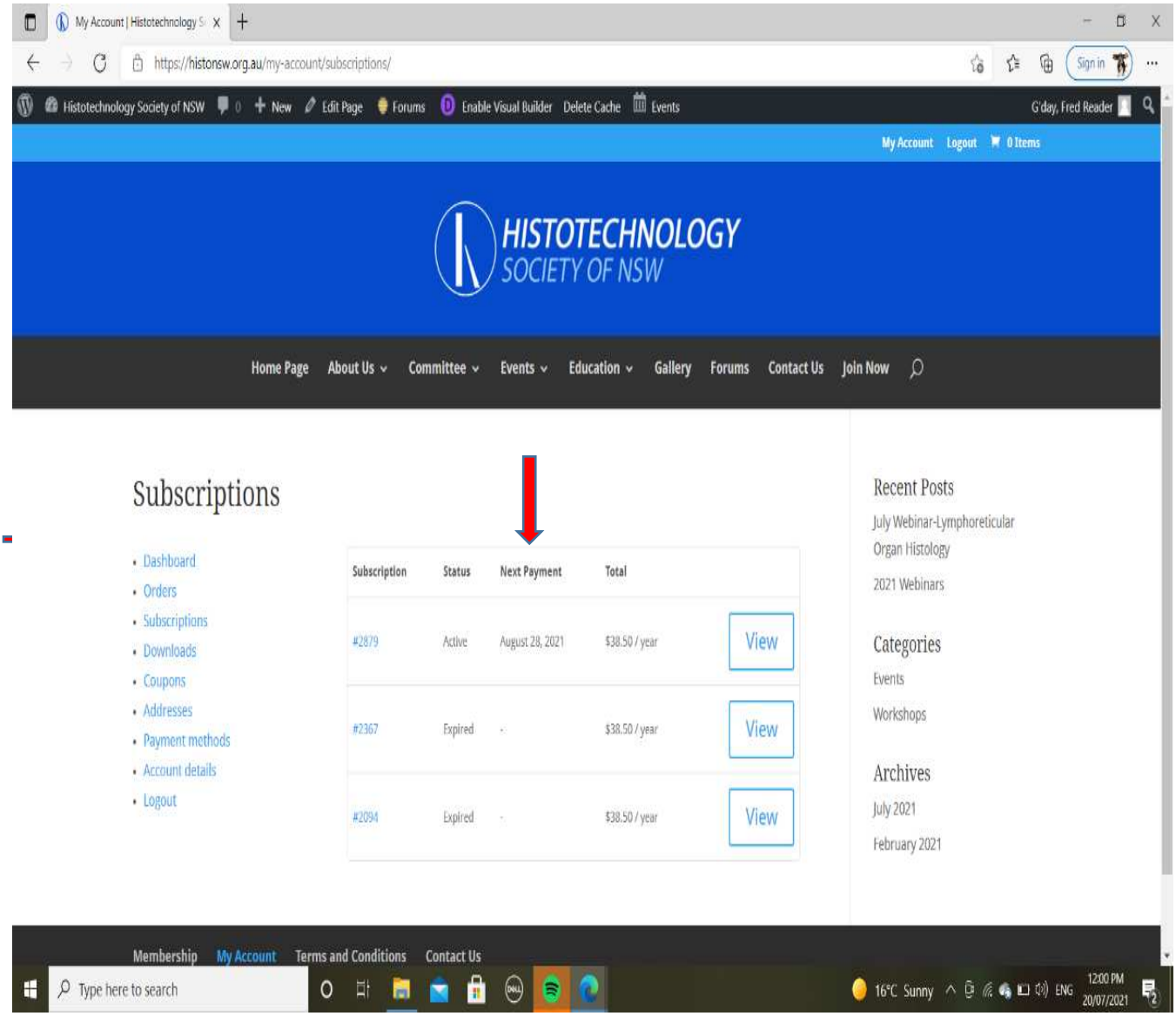

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Ammoniacal Silver - Safety Concerns

Silver can be a health hazard in more ways than you think.

In 1934, James Rice reported an explosion of a silver solution used in producing mirrors. The culprit was an ammoniacal silver solution. The instability of the compounds formed in silvering baths leaves the nature of their identity in doubt. However, as early as 1767, Kunckel described "fulminating silver" formed in ammoniacal silver solutions. This compound is not silver fulminate but more probably silver nitride, Ag_3N, or silver imide, Ag_2NH, or a mixture of both (1).

In 1930, Marshall (2) reported an explosion of a silver reticulin solution. The staining dishes containing the ammoniacal silver oxide solution were inadvertently left standing in the sun from Saturday noon until Tuesday morning. There were traces of alcohol in the silver solutions carried there by the sections of tissue. The sunlight hastened a chemical reaction between the silver, the ammonia and the alcohol and there resulted from it a highly explosive, very sensitive and unstable compound, silver fulminate (C=N-O-Ag). Silver fulminate can be distinguished from the so-called fulminating silver, which only explodes on drying. When the dish was taken up to be emptied and cleaned, it was warm from the sun. The mere movement of the liquid was responsible for the explosion.

Ammoniacal silver solutions with metallic deposits, whether on the glassware (silvering) or floating on the surface are very dangerous as the silver material is the explosive compound. It is believed that this silver material is silver azide.

The reactivity of silver nitrate is high. It combines readily with many other chemicals. When dissolved in water, it behaves like an acid. When combined with ammonia, as in many reticulum methods, it may form shock-sensitive compounds. Because of its ability to form explosive compounds, it is classified as incompatible with the following laboratory chemicals: ethyl alcohol, nitric acid, hydrogen peroxide, oxalic acid, and tartaric acid (3).

Ammoniacal silver nitrate can be explosive. Three factors increase the risk of an explosion (3):

1) If the solution is left to evaporate, crystals that form may explode. These are most likely silver nitride or silver azide (fulminating silver).
2) The formation of fulminating silver is accelerated using unclean mirrored glassware and exposure to light.

3) As the crystals take time to form, it is wise not to prepare ammoniacal silver for future use, nor to leave solutions of it sitting around.

Wallington (4) offered the following advice:

1. All ammoniacal silver solutions should be prepared immediately before use, in clean vessels and not in 'silvered glassware' which is especially dangerous. Flexible plastic containers offer greater safety. Wallington (1965) believes that the recommendation to coat staining jars with black paper, bound with strong tape (for storage of solutions) might lessen the effect of an explosion but would not prevent (at least) subsequent blackening of person and surroundings.
2. Solutions should never be exposed to sunlight.
3. Any unused reagent should be immediately inactivated by the addition of excess dilute hydrochloric acid or a solution of sodium chloride and discarded. Refrigeration does not prevent formation of the explosive compound.
4. All new staff and students should be instructed in the above.

Senba (5) recommends the addition of sodium thiosulphate to inactivate used ammoniacal silver solutions. Sodium thiosulphate accelerates the formation of stable $AgS_2O_3^-$, $Ag(S_2O_3)_2^{3-}$, and/or $Ag(S_2O_3)_3^{5-}$.

Staples and Clark (6) minimized the danger of producing potentially explosive chemicals by substituting 1% silver nitrate for 5% silver nitrate in the reticulum procedures.

Silver is a naturally occurring metallic element that has no known physiological or biological function in the human body. It is present widely in the human environment; therefore, low concentrations are also present in the human body. Normal healthy people have silver levels of <3 μg/L (7).

Argyria is the most widely publicized clinical condition associated with silver accumulation in blood and soft tissues. It commonly occurs in individuals exposed to high levels of silver occupationally, or consuming or inhaling silver hygiene products (including colloidal silver products) for long periods. Silver is absorbed into the body and deposited in the perivascular regions of the skin and other soft tissues as black granules of silver sulfide or silver selenide. The resulting slate grey discoloration of the skin occasionally associated with melanogenic changes, is semipermanent and cosmetically undesirable but is not known to be life-threatening (8).

The acute toxicity due to exposure to large doses of inorganic silver salts such as silver nitrate is similar to corrosive effects leading to irritation of the gastrointestinal tract (if

ingested) manifested in the form of epigastric burning, vomiting and diarrhea or respiratory tract irritation (if inhaled), severe toxicity will cause decreased blood pressure and decreased respiration ultimately leading to convulsions and shock (7).

Lech (9) has described a fatal case of silver poisoning after ingestion of 160ml of a suspect liquid. In the body, silver binds to high molecular weight proteins and metallothionein in cytosol fractions of tissue. It forms firm bonds with sulfhydryl groups of proteins and enzymes and precipitates tannins and alkaloids. It is known, based on experiments with rats, that silver incorporated into the rat body is strongly deposited in the basement membranes and combines primarily with sulfur-containing polyanionic glycoproteins (i.e., proteoglycans) (9).

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