TISSUE PAPER

"Bridging Histology Laboratories since 1982"

There's more to me than Histology?

Our editor, Anthony van Zwieten puts another unsuspecting person under the microscope. See Aubrey Daybell's testimony in this edition.

Ask the "GAN MAN"

What do you do when you're feeling blue due to BLUE HUE? It is a question asked by many in Histology. David Gan investigates and presents his findings.

"Roaming Reporter"

Anthony van Zwieten was our On-The-Field correspondent for this edition to give us a full report on the recent AIMS/ HGQ joint scientific meeting.

Latest Products

See the latest products & updates from the Trade.

Newsletter Design: Jerres Alcober

2016 HSNSW/HGQ conference - Port Macquarie

President's Report - Jerres Alcober

Welcome to this edition of the Tissue Paper. 2016 has been an event-packed year for the HGQ. 4 scientific meetings & 1 state conference have been held during the course of the year. The HGQ committee would like to thank all that contributed to the well attended, educational and enjoyable scientific meetings with Pathology Queensland (RBWH), Mater Pathology, Australian Institute Medical Scientists and Australian Society of Cytology. I would also like to thank the Histotechnology Society of NSW for a successful state conference at Port Macquarie, NSW. It has been a privilege and a pleasure to have been part of this joint venture. The 2017 National Histology Conference (NHC) in November 2017 will be another joint venture with all respective histology groups across Australia. The 2017 HGQ committee will be looking forward to working with all groups to make the NHC in Hobart, TAS an event that is talked about in years to come. I would like to take this opportunity to thank the 2016 HGQ committee for all their hard work and effort this year. I am excited to be working along side the elected 2017 HGQ committee. The new energy and experience will help drive the HGQ forward to hit new heights. 2017 HGQ committee - President: Jerres Alcober (TPCH); Secretary: Amanda Marsden (PAH); Treasurer: Becca Hobbs (BQ); Committee members: David Gan (QML), Melissa Hillas (Mater), Kellie Vukovic (SNP), Barry Madigan (RBWH), Jason Tu (PAH), Greg Viggers (GCUH), Arin Chandra (TPCH), Lloyd Blundell (Trade), Brett Harrison (Trade). Keep up to date with the HGQ with free membership. Become a member at www.hgq.org.au . Until the next edition, take care, stay safe and enjoy!! Happy reading ;)

Dako

Agilent Pathology Solutions

Dako's integration with Agilent

Agilent will complete its operational integration of Dako by merging Dako Australia into Agilent Technologies Australia on November 1, 2016.

You will continue to work with the same account managers, applications specialists and field service engineers but you will need to update some of the information in your systems to place orders and process payments. Details of the changes are listed on our website at **www.dako.com/dako-is-agilent**.

We remain committed to providing quality products and services as your trusted partner and your contacts will continue to provide the support you have come to expect from Dako.

If you have any questions, please speak with your Account Manager or contact the Customer Care team at CustomerCareAU@agilent.com

Read more at **www.dako.com/dako-is-agilent**

Agilent Technologies

Histotechnology Group of Queensland

Executive Committee 2016

President:

Jerres Alcober - TPCH

Secretary:

Michael Staunton - RBWH

Treasurer:

Becca Hobbs – Biosecurity QLD

Editor:

Anthony van Zwieten – TPCH

Web Admin:

Jerres Alcober - TPCH

Committee:

Jason Tu – PAH

Melissa Hilas – Mater Pathology

Jessica Hurst – RBWH

David Gan – QML

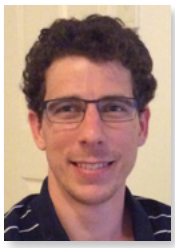
Greg Viggers – GCUH

Kellie Vukovic - SNP

CK Tan - Trade

Lloyd Blundell – Trade

Secretarial Report - Michael Staunton



Hello and welcome to all of our readers and members, both old and new. 2016 has been busy for the HGQ with several successful and well attended events throughout the year.

A big congratulations goes out to everyone involved in the joint conference with the NSW group that saw a lovely conference hosted in Port Macquarie and enjoyed by over 80 attendees. Further thanks and congratulations go out the Mater Pathology group, AIMS and the ASC for their contributions to the numerous scientific meetings throughout the year that gave us gems of knowledge such as the numerous effects tattoo ink can have in your body and the ability to transfer gut bioflora via fecal donation. I hope everyone enjoyed the presentations as much as I did.

Looking to the future, keep an eye out for the next scientific meeting early in 2017 and don`t hesitate to get in touch if you have an idea that you would like to present. Make sure you keep up to date with the HGQ by registering your details on our website (it's easy and best of all, free) and keep an eye out for updates and announcements as we move into the New Year.

I wish each and every reader a very merry Christmas and hope that everyone has a safe and happy holiday period. Hope to see you all happy and well in 2017.

Publication Guidelines

Newsletter Correspondence

Histotechnology Group of Queensland
Anatomical Pathology Department
Level 2 – Clinical Sciences Building
The Prince Charles Hospital
Rode Road, Chermside, QLD 4032
admin@hgq.org.au
(07) 3139-4379

Guidelines to Contributors

Please forward submissions in Microsoft Word or compatible program either via email and/or CD & DVD. For any attached photos, please also include these in a separate file. Include your name and address if required. Submissions can be in the form of a brief note, letter or as a complete article.

Advertisement Rates

Single A4 page - $50
Double-sided A4 page - $100

"Roaming Reporter"

AIMS/HGQ Scientific Meeting - The Pineapple Hotel

Anthony van Zwieten - Pathology QLD - TPCH

The HGQ held a combined scientific meeting with QLD AIMS for the second consecutive year at the Pineapple Hotel in Woolloongabba on a Tuesday evening in late August. There were approximately 50 people in attendance who are involved in the Pathology industry from students to laboratory managers. Thanks to Brett Harrison and Jessica Unwin for their support on behalf of Leica to sponsor the evening. The theme of the talks were "Malabsorption" from different pathological perspectives.

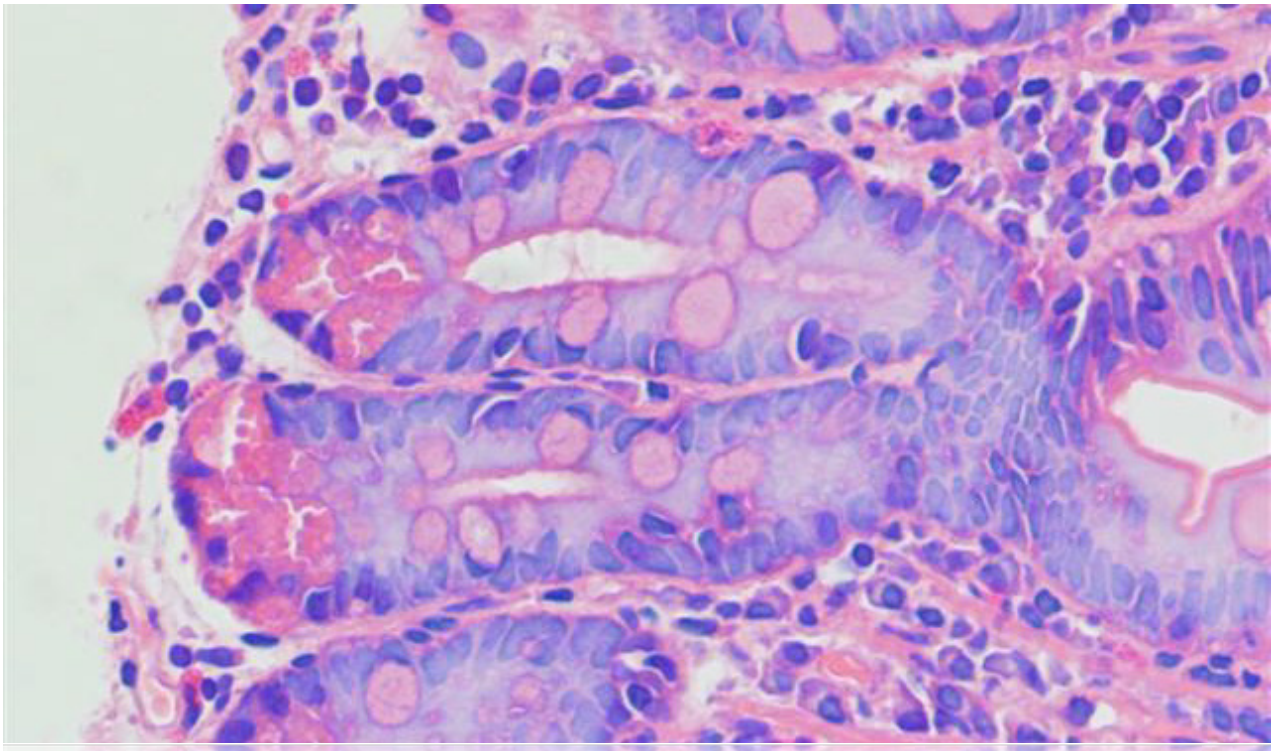
The first speaker was Dr Jason Stone, a histopathologist from QML who has presented for the HGQ on multiple occasions and as always he didn't disappoint. His talk was titled "Pale, Bulky and Offensive" and centred on a case study of coeliac disease being diagnosed on duodenal biopsy. Jason reminded the attendees that intraepithelial lymphocytes (IELs) are the major histopathological diagnostic feature of this condition but serological and genetic testing are equally important for the detection of true gluten intolerance. I walked away from Jason's talk that I would be safe to consume the carbohydrate-rich canapés and beverages that were on the horizon.

The second speaker was to be Dr Michelle Bryan, Director of Haematology at the Gold Coast university Hospital. Unfortunately she was unable to attend, but Anne-Marie Christensen was able to assist and did a fantastic job with little preparation. As lecturer in Haematology at QUT, Anne-Marie excelled in presenting the Haematological investigations behind malabsorption which can be subtle and range from iron deficiency anaemia to vitamin B12 and folate deficiency. I respect haematology as a department but blood films are quite boring to look at as a histologist.

> "Surprisingly she didn't seem that interested in the idea…"

The final speaker was Dr Elise Pelzer from QUT, a microbiologist who specifically researches the microbial environment of the human body. She spoke clearly and confidently about the effect of poor dietary choices and antibiotic therapy on the normal gastrointestinal microbial flora. She stated that if a person undergoes 10 bouts of antibiotic therapy before the age of eighteen, the normal microbial flora of the GIT is completely replaced by other introduced bacteria. As a radical solution to treating malabsorption, faecal microbial transplantation in the form of a tablet is a proven remedy. Usually the faecal bacteria pill is "donated" by a family member or friend who is exposed to a similar living environment. I mentioned this to my wife and surprisingly she didn't seem that interested in the idea.....

As us in the pathology industry usually do, we concluded the evening with a bite to eat and a few drinks after talking about bodily fluids and the like.



Ask the "GAN MAN"

Blue Hue

David Gan - QML Pathology

Recently, a few people have asked me why nuclei of some biopsies have a sky blue hue to them instead of the traditional dark blue to purple colour of a routine hematoxylin and eosin stain. This blue hue is usually associated with poor morphology and upset Pathologists.

Blue hue, also known as "smudgy nuclei", "nuclear melt down" and even "Mr Blobby", is not a new problem in the Histology laboratory. For many years, people have talked about it, written about it and on occasion, our laboratory has unfortunately suffered from it. There are multiple ways that nuclei may be adversely affected. It is usually caused during processing but may also be from drying out before fixation and even overheating the section before staining. All tissue types may be affected, but I find that in our laboratory, it most often occurs in the mucosa of small gastric or intestinal biopsies. There are varying degrees of blue hue, from a slight change of colour in some nuclei (Fig.1.) to large areas of tissue with no nuclear detail making the tissue impossible to diagnose (Fig.2.).

This problem seems to occur more often during the cooler months so growing up in the 80's, I like to call it "A Hazy Shade of Winter". Some of you oldies are saying that it was Simon and Garfunkel in the 60's but I do have a soft spot for the Bangles! Younger readers may have to Google it. Either way, the words of the song ring true for us in this instance. "Time time time, see what's become of me. While I looked around for my possibilities. I was so hard to please..." Finding a suitable fix for affected nuclei

has been difficult. Reprocessing has been the best option but this is time consuming, wasteful of tissue and may lead to over-processing and the tissue becoming brittle. We have been looking to stop the problem from occurring before fixing is required.

As soon as you look into it, you find that this is a very confusing problem with many variables. If this is more than likely a processing issue, why does it not happen to all of the tissue on the processor? Why does it happen to tissue that is small and more than likely fixed in formalin immediately? Why is it seen more often in certain tissues? Why does it happen more often during the colder months? So many questions so few brains.

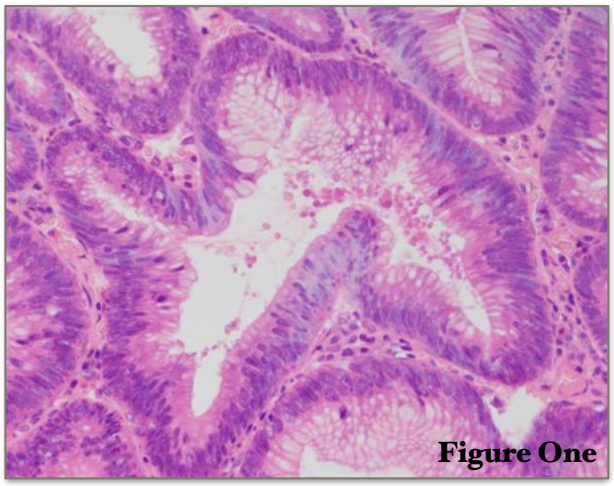


Figure One

We have tried to find the cause of the problem by looking at every step of the process. We looked into factors before the specimens arrived in the laboratory and found no connections. The affected specimens, although mainly gastrointestinal biopsies, came from different doctors using different batches of formalin on different days. The tissue was definitely fixed adequately before processing.

Our grossing system was carefully checked and even with various changes from marking dyes to wrapping methods, the incidence of blue hue was not noticeably reduced.

Tissue processing was the most obvious area of concern. Increasing the processing time for small biopsies from 2 to 3 then 4 hours helped minimally but did not solve the problem. The use of recycled xylene in the processors was stopped but the issues continued. Problems were not limited to one processor and all of the maintenance schedules for the processors were up to date. Partial changes of the solutions were not helpful but total changes including all waxes were successful. At last, some good news! (At this stage I should warn you that if you are looking for an answer to all of your problems you are going to be disappointed, sorry.)

Drying and staining of the tissue after sectioning was addressed and after multiple experiments with temperatures and solutions we believe that our issues were not caused any time after embedding.

So in our instance, we believed that it was a processing issue. Even though the recommended solution changes were performed on time and the instruments were serviced regularly, it was occurring.

We did make a few observations about the processing when issues arose.

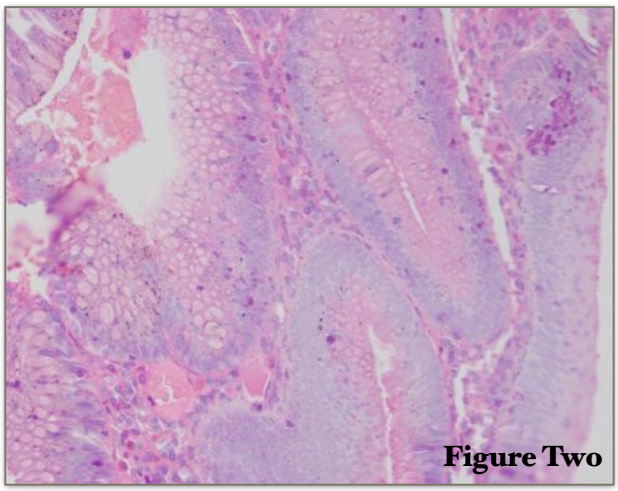


Figure Two

* On occasion, small amounts of an aqueous solution were observed in the xylenes.

* On rare occasions, after a processing run, the volume of the formalin reservoir on the processor was reduced by up to 20%!

* Once, a batch of solid wax pellets appeared to contain moisture.

* The solutions on the processors were changed as recommended, not early.

This all pointed towards contamination being the culprit. The contaminated wax pellets were a one off occurrence and a quick check of each batch is an easy solution. The occasions where there was a great deal of formalin carry over, were probably due to mechanical failure of the pump system. So where was it coming from if the solutions were being changed regularly? One unproven theory is that increased condensation may occur at some stage due to the greater difference in temperature between the atmosphere and solutions. This could occur in the processors or even in the wax of embedding machines and external molten wax reservoirs. Quite unlikely, but still worth thinking about if there was no other reason.

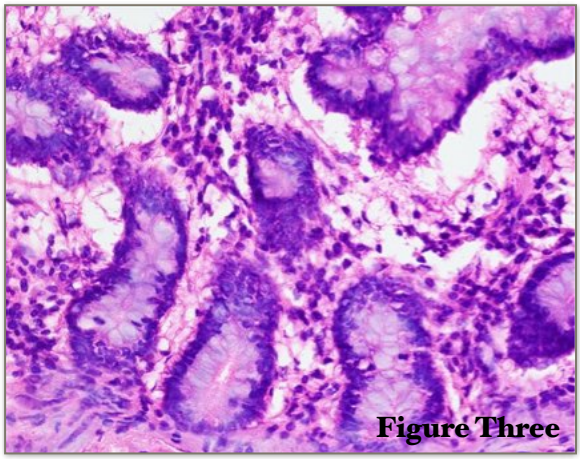


Figure Three

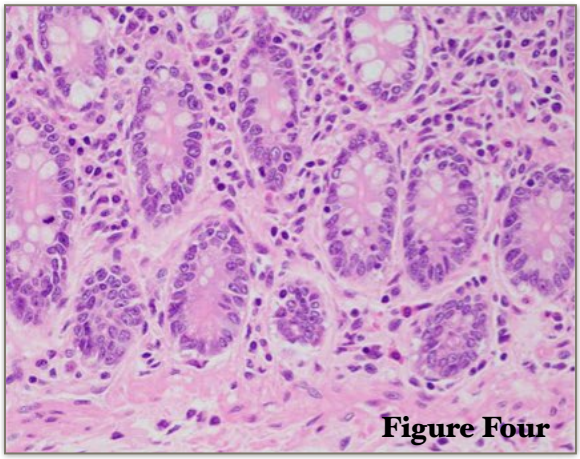


Figure Four

An even more unlikely theory was that the drop in temperature in some way was causing the solutions to penetrate the tissue at a slower rate. This theory was thrown out the door after the increases in processing times did not solve the problem.

The aqueous fluid that sometimes contaminated the xylene proved to be formalin, proving that it was not contamination caused by moisture in the atmosphere condensing in the processor. This explained why partial solution changes did not fix the problem. The formalin was in the xylenes so could easily contaminate further xylenes and waxes. Have you ever noticed that an aqueous solution will settle to the bottom of xylene and tend to stay there when the xylene is drained?

To try to prove the contamination theory and try to solve the problem, I set up a few basic experiments. I hand processed pieces of small bowel (approximately 12x5x2mm) and introduced different contaminants throughout the process to try and reproduce the artefact. The most difficult part was hand processing so our processors would still be able to be used for routine work. This is not great science since it is not reproducing a standard processing run, but give me a little credit for doing it in staining pots without vacuum (Fig.4.)! When was the last time that you hand processed tissue?

The basic findings were:


Figure Five

No artefact was seen when the last ethanol was contaminated (1 part in 100) with water.

No artefact was seen when the last xylene was contaminated (1 part in 100) with ethanol.

No artefact was seen when the last wax was contaminated (1 part in 100) with ethanol.

No artefact was seen when the last wax was contaminated (1 part in 100) with xylene.

The artefact was present when the last xylene was contaminated (1 part in 100) with water (Fig.3.). This was reduced at a contamination of 1 part in 1000.

The artefact was present when any amount of water was in contact with the tissue in any of the wax steps. There was no artefact present when the tissue did not come in contact with the water during a wax impregnation. This resulted in quite a variation in processing in the same run (Fig.5. note the lower right piece of tissue in the block is poorly processed and is white, when stained with H&E it appears darker (Fig.5.1) while the other piece is processed and stained optimally (Fig.5.2))

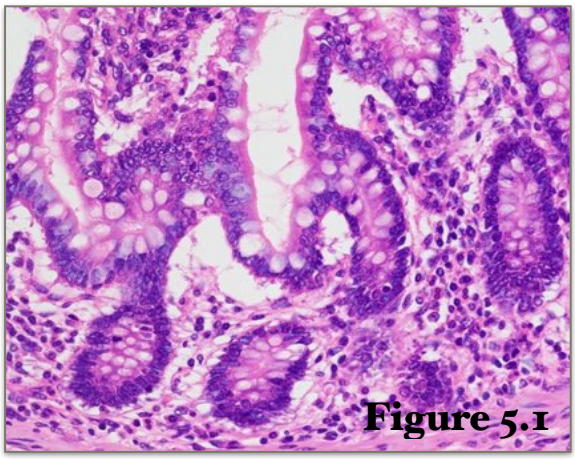


Figure 5.1

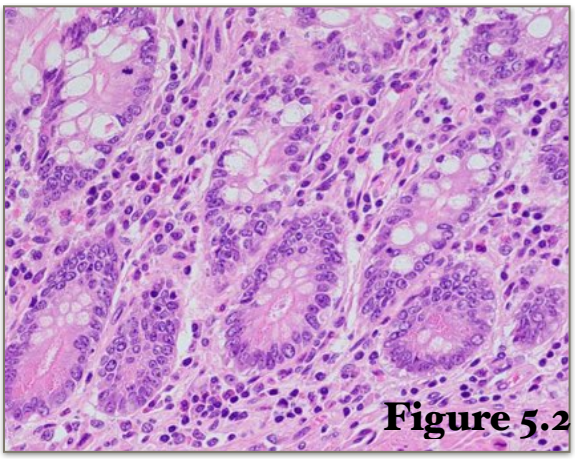


Figure 5.2

Results were catastrophic when water was in contact with the tissue in the last wax even for less than 5 minutes (Fig.6.– Fig.6.2). It was note that the water appeared to adhere strongly to the tissue and was very difficult to dislodge after the initial contact. This is worthwhile thinking about in the context of the artefact occurring more often in certain tissue. Is there some chemical connection between the formalin (aldehydes) and mucosa etc?

So what have we learnt from all of this?

*In our instance the blue hue is caused by poor processing.

*Most likely it is caused by formalin contaminating the xylene on the processor.

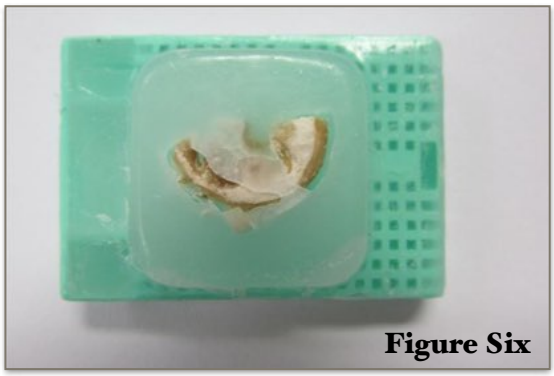


Figure Six

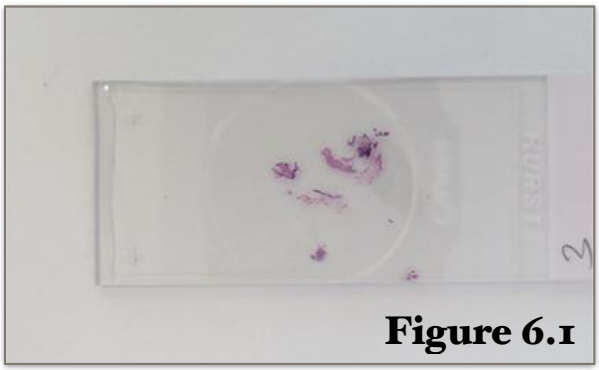


Figure 6.1

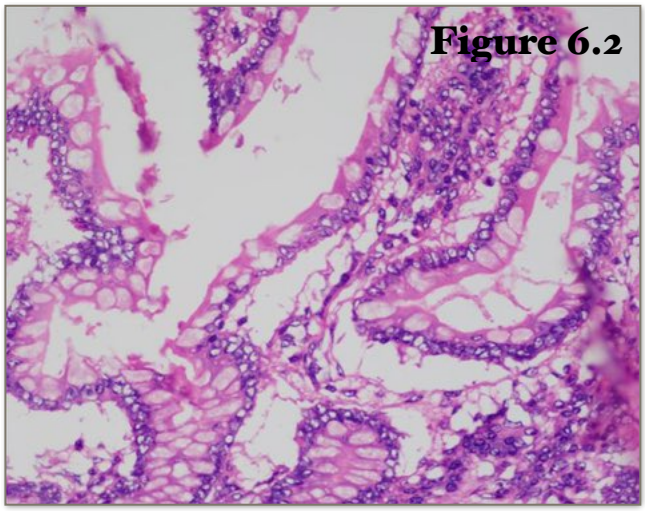


Figure 6.2

*A quick check of the volumes in the reservoirs will help in discovering mechanical errors on a processor.

*Even a small amount of formalin in the wax could create the artefact.

*Changing the solutions on the processors before their recommended thresholds was the best way to prevent the artefact. Since we have been doing this, only very minimal blue hue has been seen, touch wood! If the artefact appears, empty all solutions on the processor and ensure reservoirs are cleaned including the wax chambers before refilling.

The good news is that we are developing a method to reverse the artefact from the original H&E slides. This speeds up diagnosis and does not waste any tissue from small biopsies. The results vary depending on the severity of the artefact but the method has certainly assisted greatly in the diagnosis of severely affected tissue (Fig.7.1 Fig.7. & Fig.8. Fig.8.1). I will keep you up to date with further developments.

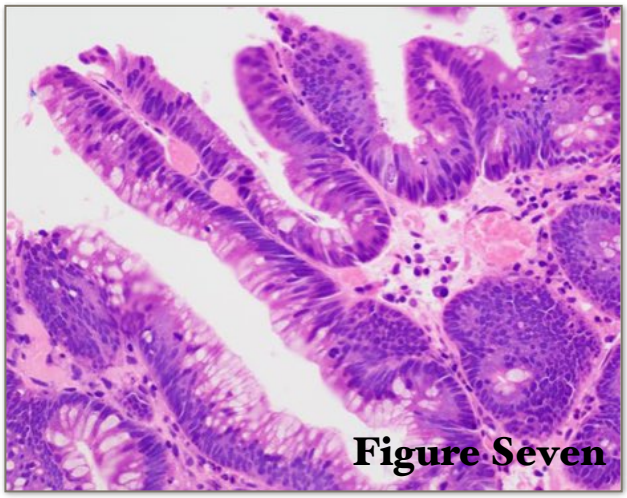


Figure Seven

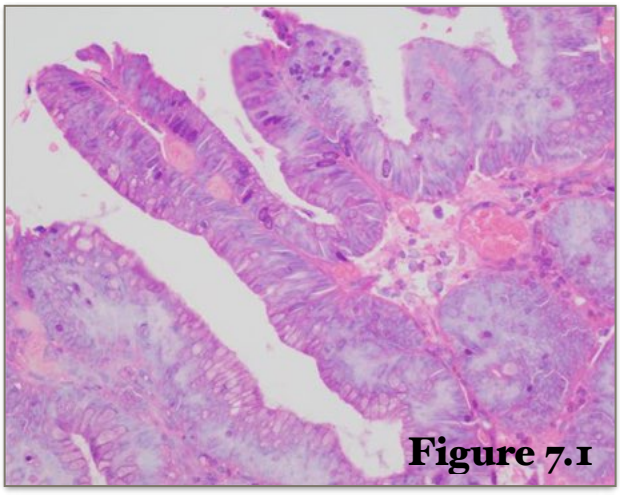


Figure 7.1

I would be very interested to hear about other people's experiences and if they have found a solution for this problem.

Geoffrey Rolls has spoken and written about this and many other artefacts. "Artefacts, Faults and Failures" and "101 Steps to Better Histology" are great reads and excellent resources if you have access to them.

Special thanks to Ian Pullen for his expert assistance and brain power.

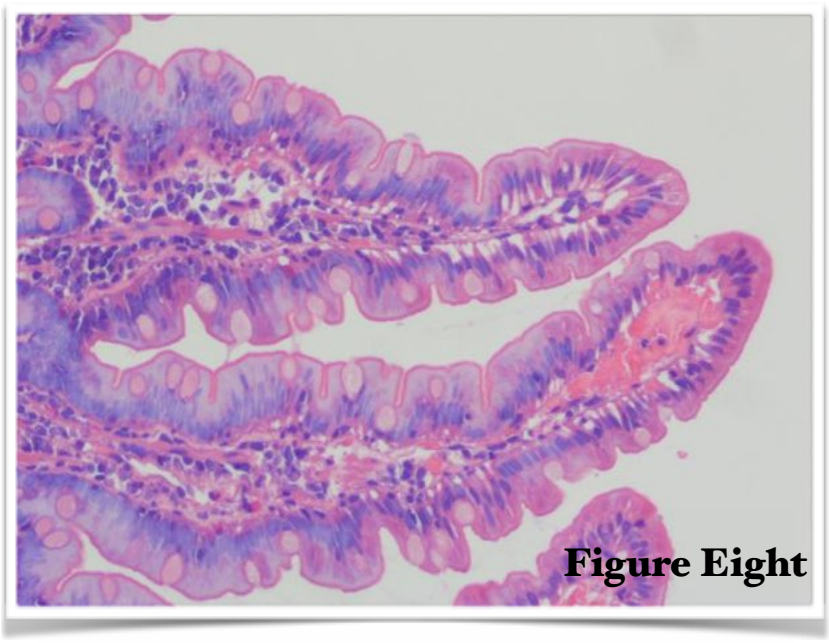


Figure Eight

"Also known as Smudgy Nuclei, Nuclear Melt Down and even Mr Blobby"

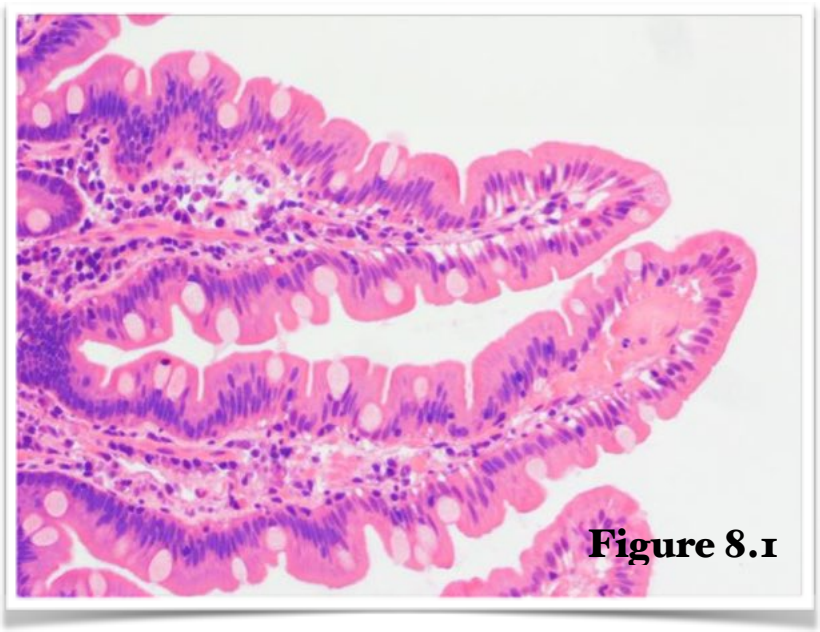


Figure 8.1

There's more to me than Histology

Aubrey Daybell - Scientist

Pathology Queensland - Sunshine Coast

How long have you worked in Histology?

I've been in Histo for the best part of 10 years. The only reason I know that is because the LSL column has started to appear on my pay sheet. I started at Coastal Pathology doing my initial training with Glen Hopper. I was then lucky enough to nab a job with Pathology Queensland and started working at the hospital which is where I've been ever since.

When people ask, "So, what do you do?" How do you explain Histology?

I used to try an make it sound really complicated and dropped words such as 'Immunohistochemistry' (the only 8 syllable word I know) and 'Medical Scientist' but then I didn't really know what either of those words really meant. So now I simply say that I make slides derived from body tissue or bodily fluid for doctors called Pathologists and they diagnose those slides of any cellular anomalies.

What is a skill you'd like to learn and why?

In terms of Histo I would love to learn surgical cut up of complicated specimens. Along with literally getting my hands dirty it would also be a good opportunity to prove to the pathologists that there may possibly be a way to cut up a bowel without inking everything from the head piece microphone to the poor lab assistant.

In terms of life in general, I would really like to be able to say that I am skilled at listening to what ever it is my wife yabbers on about and play x-box at the same time.

If you could witness any event of the past, present or future, what would it be?

I think it would have been fascinating to have been around when Christ was doing his teachings. Just to see how he delivered then and to what extent his charisma carried. I could only imagine it to have been quite significant due to the fact his teachings were documented, collated and rehashed time and time again to the point they were made into arguably one of the most important and dynamic pieces of literature in history.

What is the best conference you have ever attended?

I really enjoyed the HGQ conference back in 2008 held at Caloundra. I was a relatively new scientist back then and the theme happened to be "Back to Basics" so it was very relevant to where I was in terms of my career.

What is your dream holiday destination and why?

Bora Bora, simply because it looks like a paradise. I'm inherently lazy and the Maldives looks to me like a place that would really suit my personality in terms of lazing around drinking long island ice teas in one of those over the water bungalows.

"I am skilled at listening to what ever it is my wife yabbers on about and play x-box at the same time"

Have you worked at any other labs during you career?

I have worked for both SNP and QML as a student working part time as a phlebotomist. I also worked under a haematologist in London for a company called "The Doctors Laboratory". My role there was the liaison officer for the Dr Sari's patients. I would ring them and give them there dosage requirements for their Warfarin. the company serviced the "High End" of London's Elite and some of the people I had to ring were well known celebs. I then came home and worked for Coastal Pathology where as I have already mentioned started my Histo training.

What was your first part time job?

My very first part time job was at the Spirit House at Yandina where I continued to work for nearly 15 years on and off. I would go in as a 14 year old and hose off the foot paths because back then they used to have coal brassier that would leave soot all over the ground and so I had to pump water up from the creek and rid the whole restaurant of the black dust. I eventually moved to the kitchen which was brilliant in terms of learning from really experienced chefs. Then eventually out onto the floor as a waiter,

What is your favourite restaurant?

The Spirit House, Yandina

Manual or semi-automatic microtome?

Manual, but the semi-automatic definitely has its advantages.

You have a very busy life outside of work. Can you explain a typical week for you and how you are able to achieve a work/life balance that is the envy of many?

I have a very busy schedule outside of work as my wife Catherine and I have 4 children that are constantly being run around the Sunshine Coast. I think their ages range from 3 through to 10 and are involved in a lot of extra curricular sports. My eldest son Jesse trains with me in Martial arts, and swimming. My only daughter Ari is a competition gymnast who is required to train nearly 7 hours a week., My 3rd plays soccer and the youngest is the most unpredictable and has a professional career in visiting the ER with broken arms and the like. They all also do swimming lessons.

Catherine is a mortgage broker and I train and teach Silat Perisai Diri which is an Indonesian martial arts 3 times a week. I'm exhausted just from typing out all of the stuff out.

However we make it an important point to have Sundays off where we go to church and spend quality time doing things like bike rides. In terms of a work life balance I think it's important to treat each other with respect and also don't take yourself too seriously.

I think people can get caught up in their work to the detriment of their loved ones and interests. However as a professional I also think we need to hold what we do with a good deal of importance. Medical Scientists do have a great responsibility to the role and especially to the patient. In conclusion to this question someone wise person (the editor) once told me that one should work hard and play hard.

Advanced Notice

Wide Bay Scientific Group 2017

Scientific Conference

Location:
Sunshine Coast
24-26 Feb 2017

Celebrating and supporting medical laboratory personnel, and **Students** in the Wide Bay Region.

Early bird specials and discounts for Students available.

Workshops, and Social Events

Please contact us now if:

- You are interested in presenting
- There are topics you would like to see presented
- You wish to be added to the electronic mailing list
- Your company is interested in sponsorship opportunities

Email us: widebaysg@gmail.com

Image provided by 'Visit Sunshine Coast'