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PARAFFINALIA

Volume 21 Number 1

February, 2016

Contents:

Editor: Elizabeth Baranyai

"The HGV aims to provide a dynamic continuing education program in which all persons with an interest in Histology and Histotechnology are freely invited to participate.

Committee Page

The members of the Histology Group of Victoria 2016 are:

Name	Institution
Adrian Warmington	St. John of God Pathology (Victoria)
Mark Bromley	Melbourne Pathology
Elizabeth Baranyai	Cabrini Health
Samantha Arandelovic	St. John of God Pathology (Victoria)
James Cvetkovski	RMIT
Kellie Vukovic	Peter MacCallum Cancer Centre
Sue Sturrock	Peter MacCallum Cancer Centre
Kristy DeGeorge	Austin Pathology
Jesenka Jefic	Austin Health
Maria Flynn	Melbourne Pathology
Meghan Leo	Histolab
Kellie Madigan	Leica
Aysha Yang Du	Box Hill Hospital
Hoang Yuyin	Walter & Eliza Hall Institute of Medical Research
Kerrie Scott-Dowell	Dorevitch Pathology/Leica
Alison Boyd	St. Vincent's Pathology

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BLURB FROM THE BUSH

So what will 2016 bring? The committee has commenced arranging the National meeting for 2017 in Hobart and will continue to focus upon this throughout the year. Our usual array of scientific meetings is being organised. Our Trivia night will see us through during the cold winter months. We continue to work upon improving our information technology and other aspects of membership that hopefully ends with advancing our communications with our members and making things easier for our volunteer committee.

Adrian Warmington

HGV President

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Under the Microscope with Sue Sturrock

Anatomical Pathology
Scientist in Charge
Peter MacCallum Cancer Centre

1. What was your first part-time job?

I worked with Clyde Riley at the Royal Women's over a Summer break to get some experience in the lab while I was still studying. It was a mini-Prof Practice experience. Clyde revealed the wonders of histology to me as he did for many others and he plied me with liqueur chocolates – I have a had a real problem with them ever since. Thanks Clyde.

2. How long have you worked in histology?

It's funny, because some days it feels like I just started yesterday. Then I realise I'm working next to someone who wasn't born when I finished at RMIT. I graduated in 1986. You do the maths.

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

If I'm feeling naughty I say "I cut up people's bits", but generally I say "I'm a Scientist working in Pathology – you know when they cut off a skin lesion….". Both responses have the same effect – a quick move to another conversational topic. I'd rather talk about what they do anyway.

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

Coding. So I know what my teenage daughter is talking about.

5. If you won the lottery, what would you do?

Go on a World Tour of the most famous sporting events held, hire a personal trainer and let Nugget know that I have left a control testing for ZN in Carbol Fuchsin and ask her to finish it off for me for when I get back.

6. Who do you most admire in life?

Dr Renate Kalnins – I want to be just like her.

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

The siren sounding as Carlton wins its next premiership over Collingwood by a kick, after the ball has gone over the boundary line. I will be delighted when that happens again.

8. What is the best conference you have ever attended?

AIMS-HGV-HDG 2012 at Cape Schank. I won an ipod, had a ride in a Porsche and can't remember much else due to over-partying. It was fabulous! Of course the NSH at Austin, Texas was over-the-top impressive, but the Americans keep themselves too nice!

9. If you could only keep five possessions, what would they be?

This is relevant because the beach house burnt down on Christmas Day and we did a practice evac the weekend before. Then, I took my 2 kids, husband, phone & charger, Jean Paul Gaultier fragrance and the Mikimoto pearls. I would also want my pussy so I guess the husband can fend for himself.

10. What is your dream holiday destination and why?

Whistler Canada for a skiing adventure before the knees seize up or anywhere with pool boys. What's not to understand about pool boys?

Reported by: Kellie Vukovic

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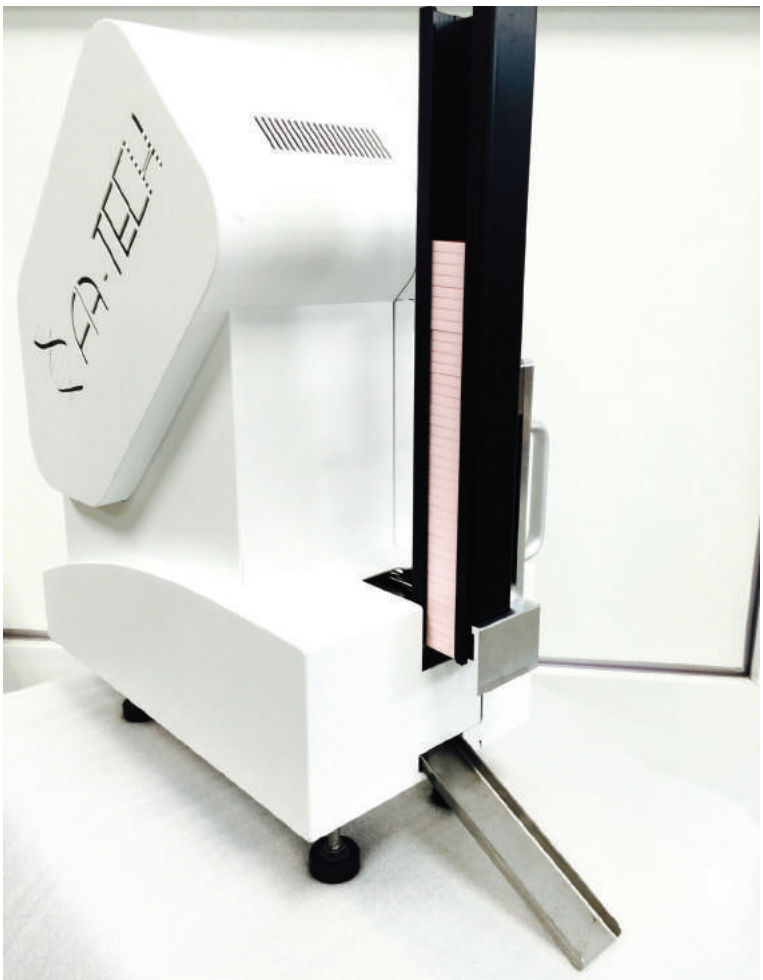

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NSH Review by Judy Brincat, Peter Mac

The 41st National Society for Histotechnology Annual Symposium/Convention was held at the Gaylord International Resort and Convention Centre, National Harbour, around 12 miles (the US still uses imperial measurements) from the national capital, Washington D.C. The location tempted me to submit a couple of abstracts, and much to my surprise, one of them was accepted. I was going to NSH, as a presenter once more. The prospect of delivering a 3½ hour workshop is daunting to say the least, and requires countless (weekend) hours of preparation, and a lot of help! I am very grateful to the HGV for their continuing support for life members.

The venue was magnificent, and as always the technical support for speakers was excellent. There are about 10 workshops running concurrently for each day's sessions, as well as a substantial vendor exhibition. I have learnt to firstly not over-commit myself to attending workshops, particularly the ones that start at 8am, and secondly allow myself plenty of time to thoroughly explore the vendor exhibition and read the posters. It's well worth the time.

Roche-Ventana hosted a soiree featuring a cancer survivor who shared his journey with us, highlighting the role histotechnologists play in pathology testing and the delivery of personalised medicine.

The hotel accommodation was luxurious, and my room had a view over National Harbour waterfront and the Potomac River.

Attendance at most workshops attracts a charge, however the C.F.A Culling Memorial Keynote Lecture is free, and always worth attending. 2015's was no exception, delivered by Mario Livio, author of "Brilliant Blunders" a book describing "colossal mistakes by great scientists that changed our understanding of life and the universe". The book was also a gift to all speakers at the symposium, and I am grateful to own a copy.

Other workshops I attended included an IHC troubleshooting workshop which I found extremely valuable, as well as Richard Cartun's workshop " *Use of Immunohistochemistry for the detection of gene alterations*-Is there a role for IHC in the molecular era?"

The social highlight of the convention is the Awards Banquet, usually held in a large ballroom within the complex. I was extremely privileged to be sharing a table with two award winners Neil Hand and Guy Orchard, both of whom have been guest presenters at National Histology meetings conducted by the HGV in previous years.

Photo courtesy of "NSH in Action " Fall 2015.

Neil accepting his award

After the hard work was done each day, I enjoyed spending time with other delegates, investigating the many local restaurants and watering holes at the resort and nearby.

After it was all over...time for sightseeing and a cooling ale at the Willard Hotel!

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Scientific Meeting Review by Kerrie Scott-Dowell

Pathobin

It was our privilege to have Dr Shane Battye speak at the November HGV meeting on Digital Pathology.

Digital Pathology is the development and implementation of an innovative image capture in both the areas of macroscopy and microscopy.

Macroscopy

3D photogrammetry is obtained using structure for motion (SFM) technology in Adobe Reader. The 3D image is made from the 2D photos and scene reconstruction. Constructing the 3D model using cameras with known geometry and structure for motion (SFM) gives new ways of visualising specimens.

A 2D photo gives no depth. SFM means multiple photos from a fixed camera with a rotating specimen. This requires a motorised turntable, a camera (digital SLR on f stop 7), a computer and software. The SFM workflow is1) acquire a photo set, 2) run 3D scene reconstruction and 3) use dense point cloud. For example with a prostatectomy specimen, 28 images are taken on the turntable rotating 360^{o}, and then use the software 1) visual SFM and 2) MeshLab. When you execute SMF you get an image using recognition points and you get a sparse point cloud. A dense point cloud is then run before the mesh generation. The MeshLab parameterization and mapping result in a 3 d image. If a specimen is firm enough it can be flipped and rerun such that 2 photosets are obtained and a whole image of the tissue can be made. You can also 3D print the tissue and accurately make a 3D model that can show the spread of disease.

SFM is low cost, speed to capture has lower physical surface detail, it has readily available software and can be done in natural or good lighting. This has shown to be a useful tool at grossing with complicated specimens and allows the Physician and Pathologist to visualise the specimens after cut up.

Microscopy

There is a need for digital microscopy because of storage issues, the diagnostic advantage of rapid consultation, for research and education. Most methods for digitizing takes several minutes per slide. However, there are quicker cheaper methods, such as flatbed scanners, which do low magnification, or digital camera/smart phones.

On a regular microscope camera you get an image of 2-3 megapixels and there is a significant cropping of the visible field. Alternatively you can point a camera ,or smart phone down an eyepiece of the microscope and get a better wider image. Pathobin sells a smartphone adaptor (it can adjust for an iphone or an ipod touch) and it costs $25. It can do a panorama image of a whole tissue core on the X10 objective with excellent diagnostic clarity.

The ipathobin can be downloaded free from itunes. Images can be manipulated using GIMP (General Image Manipulation Program) to get the white balance. Images can be stitched together and uneven illumination corrected using Pathobin. The stitching together also helps to alleviate lens distortion and apply the correction for iphone and ipad. There is also a hub for sharing of Macro and Microscopic images so as to build a pathology atlas and reference site. In the future they will be looking at digital pathology for image analysis, particularly complex analysis such as clinical evaluating the staining of Ki67 in patients.

No review of this talk could do justice to the images that were shown and the level of excitement that the prospect of implementation of this innovation into Pathology Departments, had on those attending. I urge everyone to check out the Pathobin website and take any opportunity to play with a digital imaging system.

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See page U9, cat. no. UM-WBG

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See page U5, cat. no. UM-TP

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See page U5, cat. no. UM-EC2800

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As of June 30, 2015

	Jun 30, 15
ASSETS	
Current Assets	
Chequing/Savings	
Histology Group of Victoria	8,757.20
ING	49,328.21
Total Chequing/Savings	58,085.41
Accounts Receivable	
Accounts Receivable	950.00
Total Accounts Receivable	950.00
Total Current Assets	59,035.41
TOTAL ASSETS	**59,035.41**
LIABILITIES	
Current Liabilities	
Accounts Payable	
Accounts Payable	-223.91
Total Accounts Payable	-223.91
Total Current Liabilities	-223.91
TOTAL LIABILITIES	**-223.91**
NET ASSETS	**59,259.32**
EQUITY	
Opening Bal Equity	61,136.71
Retained Earnings	-10,324.05
Net Income	8,446.66
TOTAL EQUITY	**59,259.32**

HGV inc.
Profit & Loss
July 2014 through June 2015

	Jul '14 - Jun 15
Income	
Advertising	7,700.00
Event Income	33,633.85
Other income	1,006.98
Sales	2,477.70
Sponsorship	1,650.00
Total Income	46,468.53
Expense	
Bank fees	50.00
Event expenses	30,059.74
Expenses	
Bank fees	72.50
Insurance	2,494.24
Web Hosting	1,672.02
Total Expenses	4,238.76
Fees and permits	53.00
Newsletter	1,974.99
Refund	506.38
Scientific meeting	1,139.00
Total Expense	38,021.87
Net Income	**8,446.66**

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Case Study-by Kerrie Scott-Dowell

History:

53 year old male presents with suspected sebaceous cyst on the neck.

Microscopy:

Round-to-polygonal neoplastic cells that are compactly arranged. Rare fusiform cells are also observed. A grenz zone separates the tumor from the epidermis. Areas of focal necrosis and lymphocytic invasion are present. Cytoplasm is moderate, nuclei are vesicular, and mitoses are abundant. Immunochemistry staining shows CK20 and NSE strongly positive and weakly positive for Chromogranin and negative for S100.

Diagnosis:

Merkel Cell Carcinoma

Background:

More than half of Merkel cell carcinomas (MCCs) occur in the head and neck of elderly people in areas of actinically damaged skin. Merkel-cell carcinoma is a rare and highly aggressive skin cancer, which, in most cases, is caused by the Merkel cell polyomavirus (MCV) . Approximately 80% of Merkel-cell carcinomas are caused by MCV. The epidermis is commonly spared because the Merkel cell carcinoma (MCC) alternatively extends into the subcutaneous tissues, vessels, and lymphatics. This cancer is considered to be a form of neuroendocrine tumor with cytoplasmic, dense-core neuroendocrine granules and keratin filaments

Immunohistochemistry is often used to confirm Merkel cell carcinoma (MCC). Merkel cell tumors stain positively for NSE, as would any APUD cell tumor. They also demonstrate perinuclear staining with antikeratin antibodies to low-molecular-weight cytokeratins 8. These 2 markers are the most constant immunohistochemical markers and are often said to be present in 100% of Merkel cell carcinomas (MCCs). A third marker, neurofilament protein, is used to distinguish Merkel cell carcinoma (MCC) from oat cell carcinoma. Neurofilament protein is seen in nearly all Merkel cell carcinomas (MCCs) but few oat cell carcinomas

Merkel cell carcinoma (MCC) has a propensity to recur and to cause local and distant metastases. Distant metastases indicate a condition that is nearly always fatal. Current treatment consists of wide local excision with adjuvant irradiation. Neck dissection is used for clinically positive nodes, and chemotherapy is given for advanced disease.

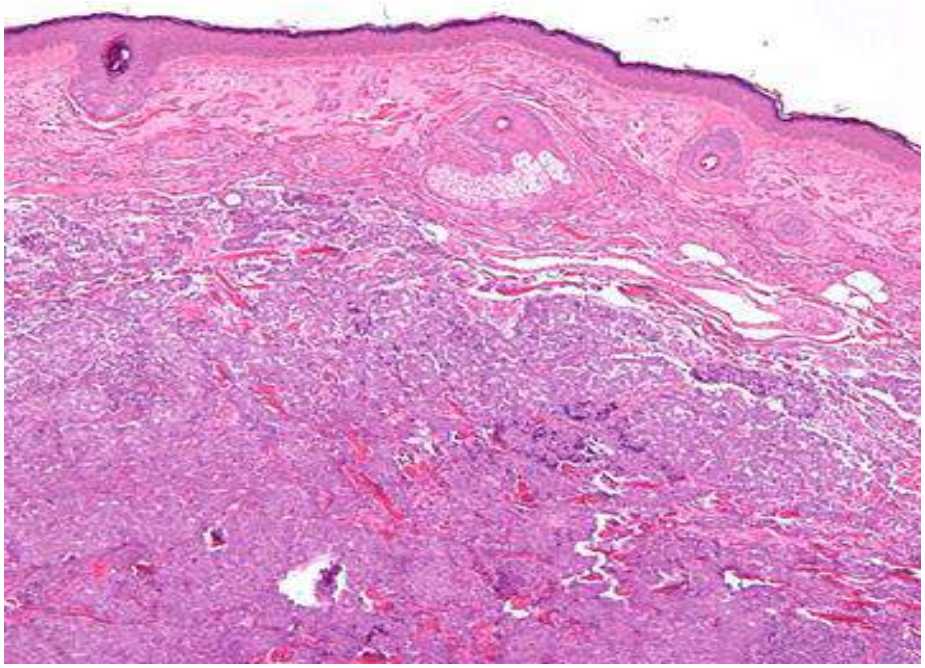

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Figure 1: Low power field of the Merckel Cell Tumour showing a clear grenz zone between epithelium and tumour

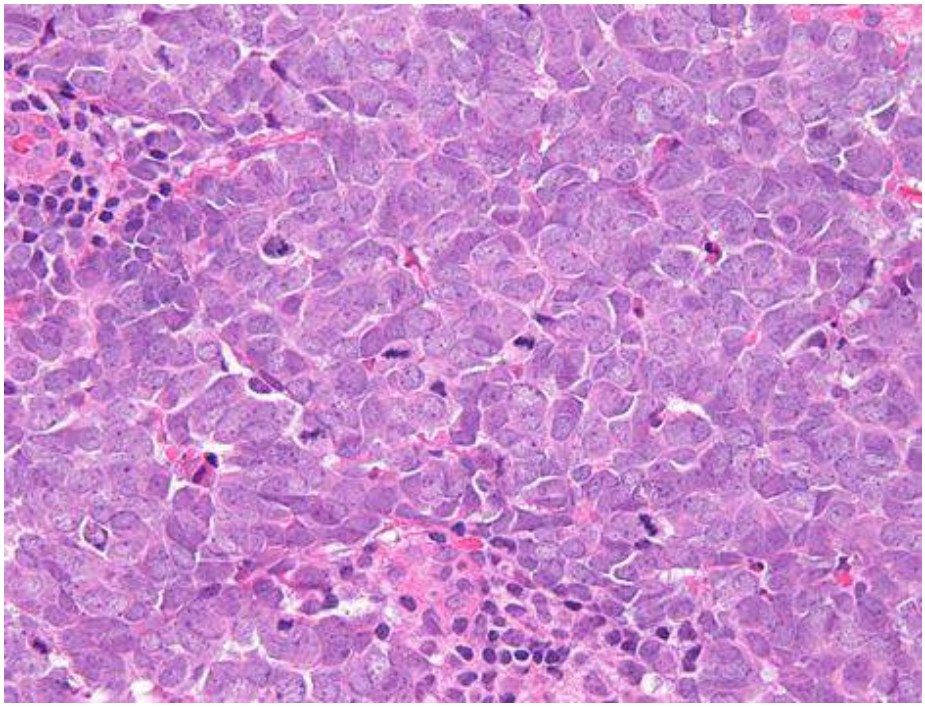

Figure 2: High power field showing large tumour cells with many mitotic figures and lymphocytic infiltrate

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JOURNAL REVIEW
BY KELLIE VUKOVIC

Study in ER/PR and Her2 receptor IHC

Journal of Histotechnology 2015 Volume 38 No. 3

Caitlin Ann Routhier

The journal published by the Massachusetts General Hospital in Boston compares different immunohistochemistry staining protocols for Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor 2 (Her2) in an aim to develop a standardised procedure for each breast marker. The introduction of these antibodies provides an inexpensive and widely available testing protocol which yields vital pathological information, however there is still a lack of standardisation in routine practice. Through manipulation of antigen retrieval methods and dilutions, highly accurate and reproducible staining results were achieved.

The introduction of IHC based stains for the detection of hormone receptor (ER/PR) positivity and Her2 glycoprotein amplification has provided crucial patient predictive and prognostic information in breast carcinoma cases. ER/PR tumour diagnosis through IHC aids in the determination of endocrine therapy usefulness for individual patients. Similarly, Her2 amplification shows resistance to most endocrine based therapy and as such is used to determine a patient's need for Herceptin as an alternative targeted therapy. One of the most pronounced drawbacks of the ER/PR/Her2 IHC transition is seen at the laboratory level where a pervasive lack of standardisation in the field can cause concern relating to quality, reproducibility and accuracy between both individual cases and the laboratory.

Pre-analytical factors including fixation time and processing technique, an overflow of available antibodies and detection systems and a general difference between laboratory ability for compliance makes standardisation an extremely difficult prospect. This study demonstrates how the manipulation of varying retrieval solutions for ER and PR pre diluted antibodies from Leica Microsystems as well as an extensive 6 month validation and comparison of three different Her2 antibodies addressed these concerns. Highly reproducible ER/PR/Her2 IHC based tests have been developed that are accurate and easy to interpret by a breast pathologist.

All slides were run using the Leica Bond III polymer based automated staining platform. Heat-induced epitope retrieval (HIER) was performed using Epitope Retrieval Solution 1 (citrate based) and Epitope Retrieval Solution 2 (EDTA based) from Leica. ER/PR antibodies were both pre-diluted with a protein concentration of 10mg/ml in a 7ml solution from Leica. Three Her2 clones were tested over a 6 month period from Dako, Thermo Fisher and Ventana at differing dilution ranges.

All optimisation tests were narrowed to validated protocols based on staining quality, accuracy of staining, reproducibility and ease of pathologist interpretation. Estrogen receptor protocols using EDTA based retrievals revealed strong nuclear staining but had background present at all retrieval times. ER runs with citrate buffer showed very weak staining for times between 2-10 minutes. Optimal staining was seen using citrate for 15 minutes. Optimal

staining for progesterone receptor was seen using EDTA for 20 minutes, producing a slightly cleaner stain. Her2 staining with Ventana's clone held the most promise at a dilution of 1:8 with a retrieval of citrate for 30 minutes. The addition of a haematoxylin 560 counterstain increased staining clarity and interpretation.

The translation of quantitative Estrogen, Progesterone and Her2 receptors to an IHC based platform is overall a faster and more cost effective method without sacrifice of result accuracy and more importantly patient care. Through successful standardisation of receptor panel protocols, laboratories can greatly improve testing results through enhanced turnaround time, staining reproducibility and ease of pathologist interpretation.

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(Bush-mite burrowing into skin – Paraffinalia August 2015)

and

HISTO-OGRAPHY

(Alien hiding in breast tumour cells -- Paraffinalia August 2015)

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……

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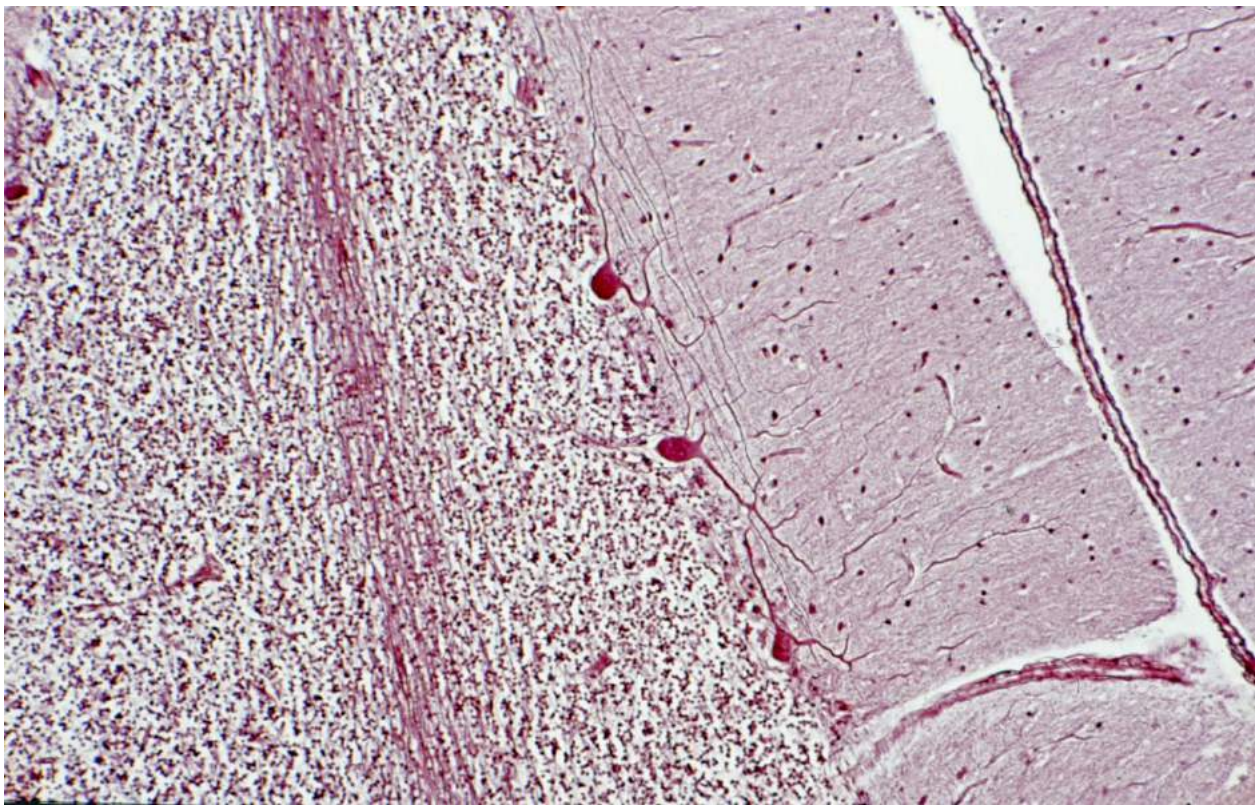

Cerebellum, Bodian stain

Submitted by Elizabeth Baranyai

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Speakers: **Savreet Kaur – Movat's project**
Alex Johnston – RNA extraction from FFPE
Cristina Bitzilis – 3D printing

Date: Thursday18 February, 2016

Time: 6:00 – 6.45 Refreshments
6.45 – 7.45 Presentations

Venue: Brockhoff Lecture Theatre
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Creating integrated partnerships to enable analysis of the world we live in.

Trajan Scientific and Medical is a privately-owned Australian company providing a wide range of pathology consumables in Australia and New Zealand to gain deeper insights into clinical and research settings by working closely with customers. With this knowledge, we innovate, develop, manufacture and commercialize products, and also work in collaboration with academia and industry.

Our experience in supplying Australian laboratories within this space encompasses more than 30 years with the acquisitions of organizations including Grale Scientific in Melbourne and HD Scientific in Sydney. With a global presence and a wide range of pathology consumables together with the SGE Analytical Science product portfolio, our strength is to be a reliable resource to support your work.

Future Events:

2016

Org. No. A0035235F

12-14 February
Fifth International Workshop in Diagnostic Immunohistochemistry
Venue: Mantra Twin Towns, Coolangatta-Tweed Heads Qld/NSW

Thursday 18thst February
Scientific Meeting-RIMT Student Project Presentations
Venue: Peter Mac

Thursday 14th April
Scientific Meeting: Basic Molecular
Venue: Peter Mac

Thursday 16th June
Scientific Meeting: Haematoxylin
Venue: St. Vincent's

Friday 29th July
Trivia night
Venue: Metropolitan Hotel
263 William St.
Melbourne VIC 3000

Thursday 15th September
Scientific Meeting: The New Peter Mac tour
Venue: Peter Mac

September 16-21
NSH Symposium/Convention
Longbeach, California
USA

September 30- October 2
Histotechnology Society of NSW
Histotechnology Group of Queensland
Joint State Conference
Port Macquarie Panthers

Thursday 10th November
Scientific Meeting/AGM: New Antibodies
Venue: Peter Mac