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# PARAFFINALIA

**Volume 21 Number 2**

**April, 2016**

**Contents**:



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*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 |
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| 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

Our first scientific meeting of the year was well attended. We all heard some very interesting presentations from final year Histology students from 2015. Our forthcoming April scientific meeting delves into the mysteries of molecular pathology from a technical perspective. Molecular pathology as everyone knows is becoming the keystone to diagnosis and is ever increasing in our laboratories across the state. As part of the evening we will be announcing and presenting awards for the top 2015 students in Histology subjects from a range of tertiary institutions around Victoria. Not only are we recognising the traditional top RMIT student, but also students from Federation University, Holmesglen Institute of TAFE and The Gordon Institute of TAFE. We hope to increase this list where other institutions demonstrate a significant component of Histology within their course.

The website for the National meeting 2017 is under construction and should be to a point where we can point members to it within the next two months. The dates for the 2017 National Meeting have been locked in as November 17-19th at the Grand Chancellor Hotel in Hobart. A suite of workshops will precede this and social events will filter throughout. The committee is very excited about the event, with more details to be released as they come to hand.

Adrian Warmington

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# Review of The Fifth International Workshop in Diagnostic Immunohistochemistry-Part 1

## Antibody Selection and Protocol Optimization

The Fifth International workshop in Diagnostic Immunohistochemistry was held at the Mantra Twin Towers , Coolongatta.

International speaker Søren Nielsen gave a series of five talks on Antibody selection and Protocol Optimization, highlighting the need for appropriate control tissue when aiming for an optimal staining protocol. He also focused on being mindful of the platform you are working with when selecting the best Clone for your protocol.

Søren is based at the Aalborg University Hospital, Denmark, where he is Project Co-ordinator and scheme manager of NordiQC.

www.nordiqc.com is a website that provides an extensive amount of information regarding their comprehensive list of assessed antibodies, and has been my go-to site whenever a new antibody has been introduced into our lab as an alternative to the usual starting point of optimization using the accompanying data sheet. As often found, and pointed out by Søren, these can be misleading and incorrect when it comes to protocol selection, with the information regarding use of controls either very limited or non-existent. Recommended epitope retrieval methods should also be scrutinized. In their assessments the use of enzyme retrieval is limited. Proteolysis has never produced optimal staining results, and it should not be assumed that HIER is always required.

Ready to Use antibodies (RTU's) also come with a data sheet and suggested protocol, however optimization is still required. Søren emphasized that the RTU may have been optimized on a different platform and using tissue subjected to very different pre-analytical factors than those in your laboratory. NordiQC assessments have experienced RTU products having optimal staining on one platform, yet when used on a different platform with the same protocol, may produce insufficient staining.

Optimizing an IHC protocol using only a strong positive control will give you limited information on the stain's sensitivity and specificity. Regardless of how strong and distinct the stain may be, a protocol that has not been properly optimized may result in either false positive or false negative staining patterns. It is therefore crucial during optimization to include controls that exhibit tissues that demonstrate both strong and moderate staining patterns as well as tissue that demonstrates what should NOT be staining.

One of Søren's many examples was the optimization of CD45. NordiQC have a range of IHC Critical Assay Performance Controls (iCAPs), that they use when assessing staining protocols. For CD45 they recommend the use of appendix and liver as control tissue.

Optimal staining results will demonstrate strong and distinct membranous staining of the intra-epithelial lymphocytes in the appendix, with no staining of the epithelial cells. The liver control should show a distinct weak to moderate membranous stain of Kupffer cells and sinusoidal endothelial cells, while the hepatocytes remain negative.

A sub-optimal protocol may produce definite staining of the intra-epithelial lymphocytes in the appendix, however not be sensitive enough to stain the Kupffer cells in the liver. Had both controls not been used in the optimization process, the stain would appear adequate, but the risk of false negatives when using the protocol would be high.

Confidence that the protocol is sensitive enough to demonstrate all targeted cells, and specific enough to avoid false positive staining can only be achieved when adequate control tissue is used in the optimization process.

Søren addressed the crucial role IHC plays in the detection of Her2 glycoprotein and its consequence, and therefore the necessity to have optimal staining protocols with regard to ER / PR hormone receptors and Her 2 glycoprotein amplification. Her 2 amplification indicates resistance to endocrine therapy and therefore the need for an alternative in the form of Tamoxifen. A patient who expresses a 2+ amplification or higher will have access to Tamoxifen, a 1+ amplification will not. It is therefore critical that the protocols are properly optimized, sensitive enough to pick up the true 2+ cases. A sub-optimal protocol that is not sensitive enough, may result in these cases being interpreted as 1+, and the difference between a patient receiving adequate treatment or not. On the other hand, a protocol that is not specific enough could result in a patient receiving unnecessary and costly treatment. You must have a protocol optimised to detect the low expressers without resulting in false positive staining in other tissues.

The take home message of Søren's presentations was the importance of adequate controls when optimizing, with more emphasis on using the low expressing controls. The Pathologist viewing the slides should have confidence that the staining protocol will give accurate results regardless of tissue type, and robust enough to cope with specimens subjected to a range of pre-analytical factors.

Time for this antibody to move to the next workshop, aka cocktail party: crank up the amp to 2+, get some red wine stains happening, and look for compatible sites!

Sandra Van Brummelen

Anatpath

# Pictures from IHC conference dinner

# Under the Microscope with Kerrie Scott-Dowell

**Leica Biosystems**
**Dorevitch Pathology**
**Anatomical Pathology**

1. **What was your first part-time job?**
   I was a milk bar attendant at a corner shop in Morwell. This was back in the day when you sold most of your cigarettes to children (they said it was for the parents)

2. **How long have you worked in histology?**
   33 interesting years- 10 years full-time at the Alfred hospital, 23 years part-time at Dorevitch and 14 years part-time at Vision Biosystems/Leica

3. **When people ask, "So, what do you do?" How do you explain Histology?**
   I usually say "I am part of a team that helps with tissue diagnosis, like when you get an odd mole taken off and it gets sent away" I may sometimes mention breasts or prostates depending on the audience.

4. **What is a skill you'd like to learn and why?**
   I would like to learn gymnastics, because very early on in my life I perfected the Nadia Comaneci dismount and 10/10 celebrations and have the Romanian leotard. Unfortunately, due to size, lack of balance and without the strict Eastern Block coaching, I have been largely unfulfilled in my gymnastic aspirations.

5. **If you won the lottery, what would you do?**
   Travel and then travel some more. I would like to think I might do good philanthropy work along the way.

6. **Who do you most admire in life?**
   Probably my Pop, as he had seemingly endless time for people and his mind was always inventing. He had a great sense of humour and incredible loyalty to family.

7. **If you could witness any event of the past, present or future, what would it be?**
   I would have loved to have been in the mosh pit with the screaming masses at an AC/DC concert

8. **What is the best conference you have ever attended?**
   The last one in Hobart was such fun. After a long break from conferences due to children, it was nice to get out

9. **If you could only keep five possessions, what would they be?**
   Do kids count as possessions? If so there is 2 of my 5. I need my purse, lip balm and glasses. Perhaps I will dump one of the kids for a bottle of Bombay Sapphire Gin.

10. **What is your dream holiday destination and why?**
    I can never pass up a holiday in Disneyland, preferably in the US, as I love the rides, daggy as well as heart thumping and have the perfect head for mouse ears.

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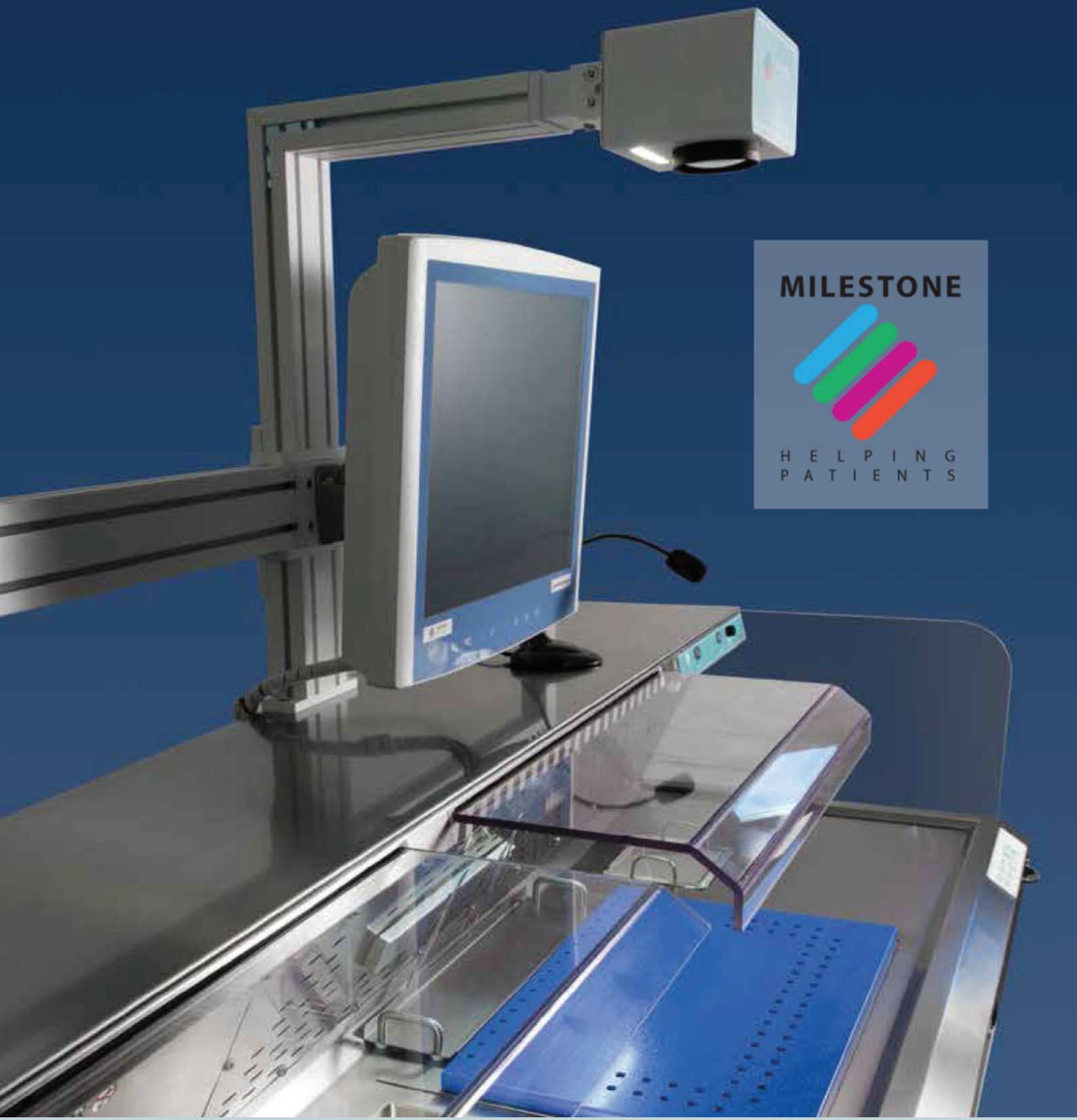


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**PHOTO**

Reported by: Kellie Vukovic

# Review of 18th February Scientific meeting – RMIT Student Project Presentations

## Reviewed by Meghan Leo

For the first scientific meeting of the 2016 we had the pleasure of listening to the top three RMIT student project presentations of 2015.

Savreet Kaur delivered the first presentation for the evening. The aim of Savreet's project was to see if performing a single Movat's pentachrome stain could be used as a replacement to performing a Martius Scarlet Blue, Alcian Blue, Verhoff-van Gieson and Masson Trichrome.

As Savreet completed her project at the Victorian Institute of Forensic Medicine, specimens used were autopsy specimens and the method was modified to be more relevant to autopsy specimens.

The result was a visually stunning stain with very clear representation of connective tissue that would enable connective tissue differentiation in many different tissues. A downside of the stain though is that even though it has been modified to take less time, the stain still takes 2.5 hours and 12 steps to complete.

The second presentation of the evening was delivered by Alex Johnson. The aim of Alex's project was to optimise the method for extracting RNA from Formalin-Fixed, Paraffin-embedded pancreas tissue sections using the Qiagen manual isolation kit.

Insulin gene expression levels in both human and porcine specimens were tested and compared against each other. The results had great variability and there was no established link found between islet numbers and gene expression and Alex found that the age of the specimen was the major factor in degradation of RNA in FFPE tissue that markedly affected all the downstream analyses.

The third and final presentation of the night was delivered by Christina Bitzilis. The aim of Christina's project was to manufacture 3D printed models of pathological conditions from real patient CT data as a surgical cup-up teaching tool for students.

The various 3D models that were printed ended up looking relatively similar to the original models but unfortunately the softest polymer that is currently on the market is still too hard to replicate the feel and process of cutting up real tissue.

The three presentations were all delivered excellently and each project was fascinating to hear about and the possibilities that could eventuate with further research and time kept the audience enthralled.

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**Date:** Friday 29th July

**Time:** 6.30pm-10.30pm

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**Price:** $25 per person
(Tables of 10)


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(Menu to follow at a later date)

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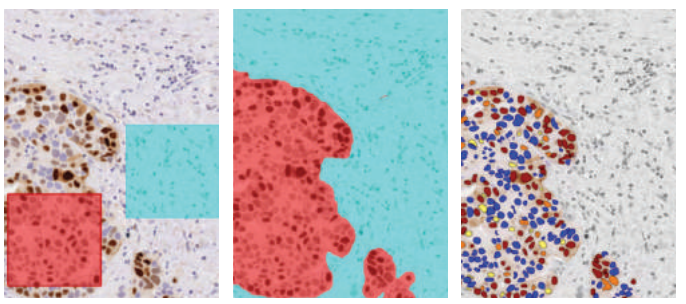

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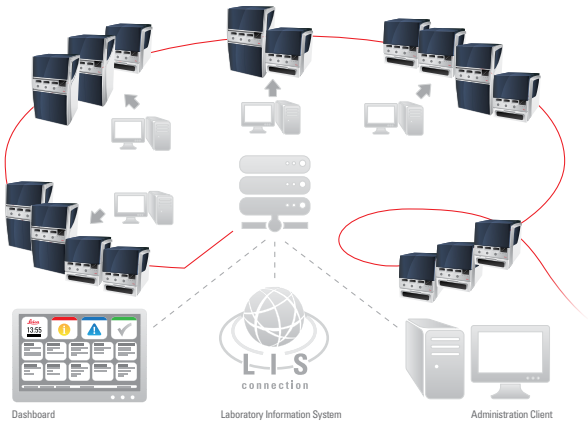


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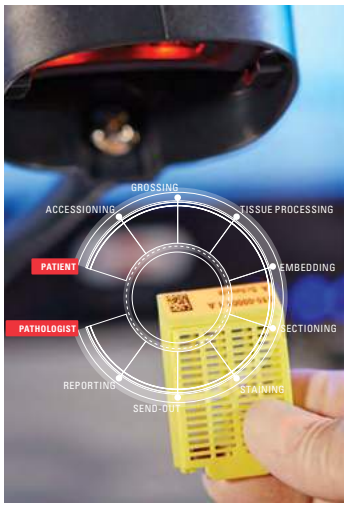


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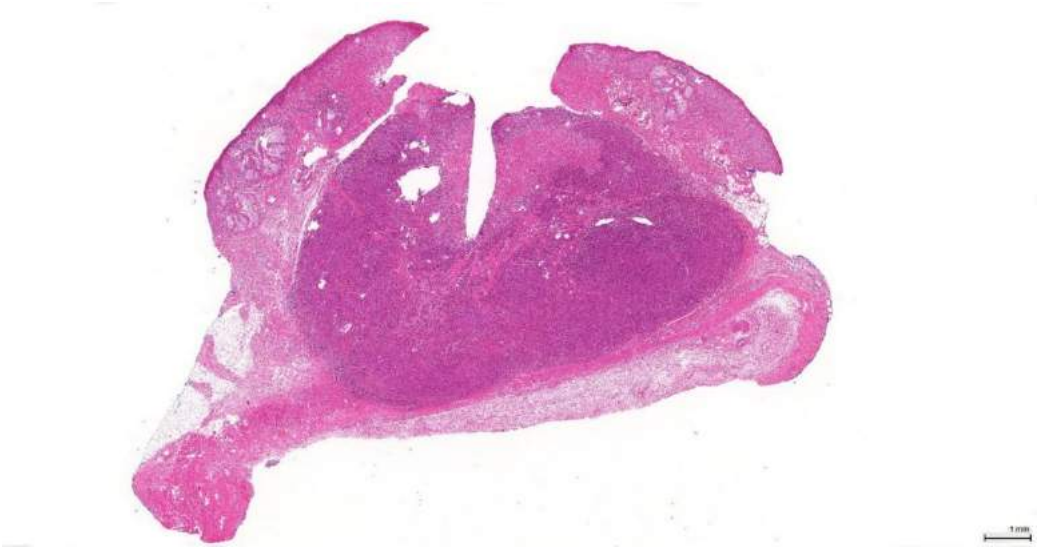
# Case Study-by Kellie Madigan

## Appearances  Can Be Deceiving

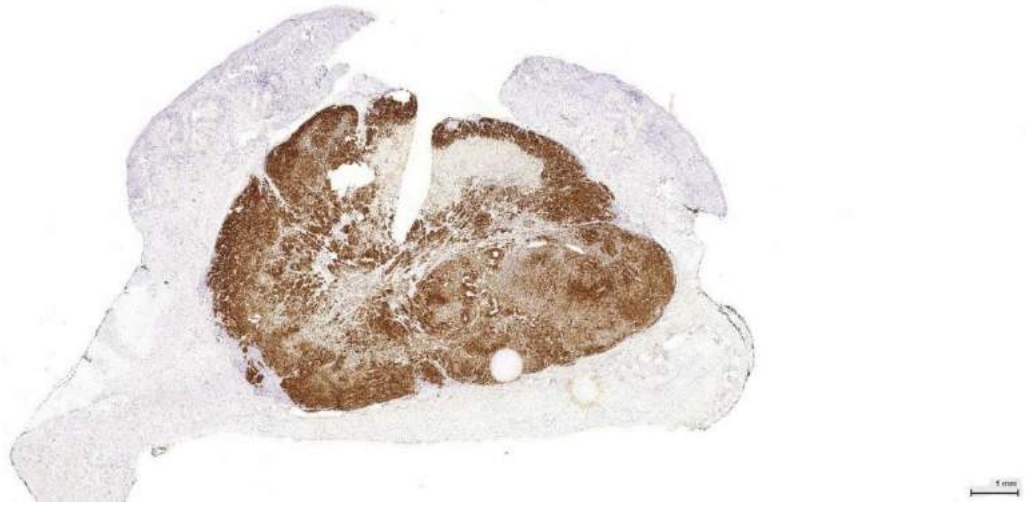
A 10mm melanoma lesion of the scalp, which appeared as a 3mm lesion.

HISTORY – Previous biopsy of 3mm brown lesion with underlying nodule, melanoma. Now wider excision of nodule.

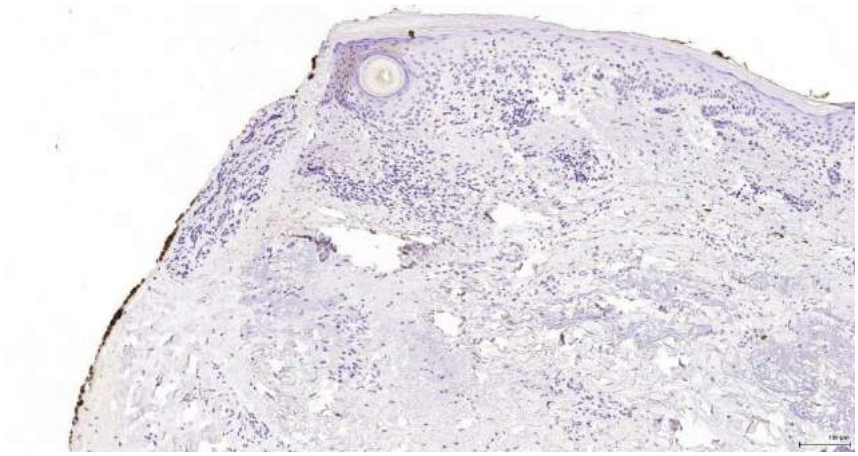
MACROSCOPIC DESCRIPTION – Pale tan portion of skin 15x15mm, and 12mm deep. The skin surface shows a 4mm diameter defect. Within the deep tissue a firm nodule can be felt and slicing reveals a mottled tan firm area 10x7mm. The lesion extends to within 1mm of the deep margin.



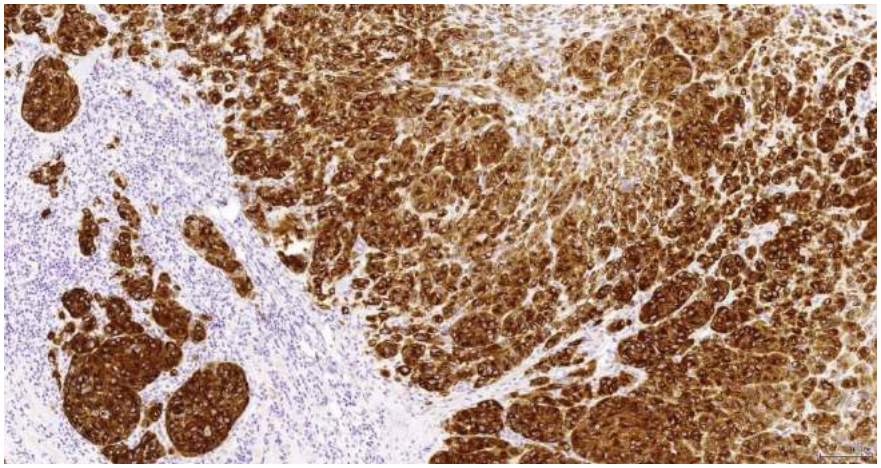
Melanoma from the Scalp @ 2x magnification, H&E stain. Lesion measures 10x7mm, with a 4 mm punch bx site centrally.



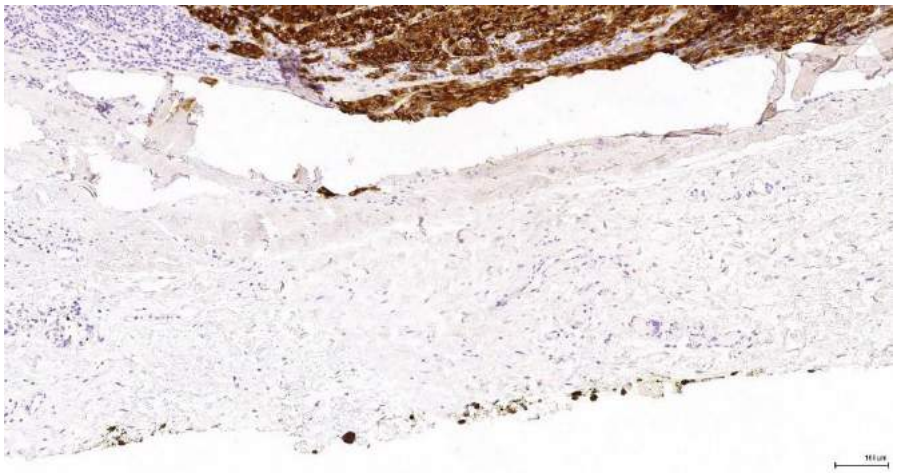
Melanoma from the Scalp @ 2x magnification, Melan A IHC stain. Note how remaining the skin epithelium shows no evidence of the lesion.



Melanoma from the Scalp @ 10x magnification, Melan A IHC stain. Showing one cell layer of positive cells running along the bx site.



Melanoma from the Scalp @ 10x magnification, Melan A IHC stain.



Melanoma from the Scalp @ 10x magnification, Melan A IHC stain, showing positive cells along deep margin.

Kellie Madigan – Scientist,  Leica Biosystems Melbourne

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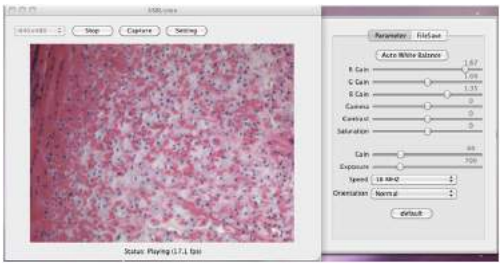

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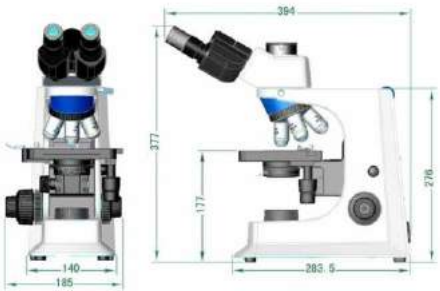


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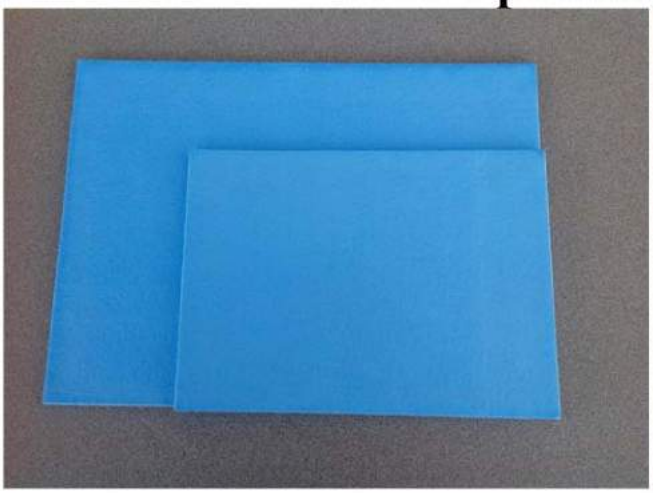

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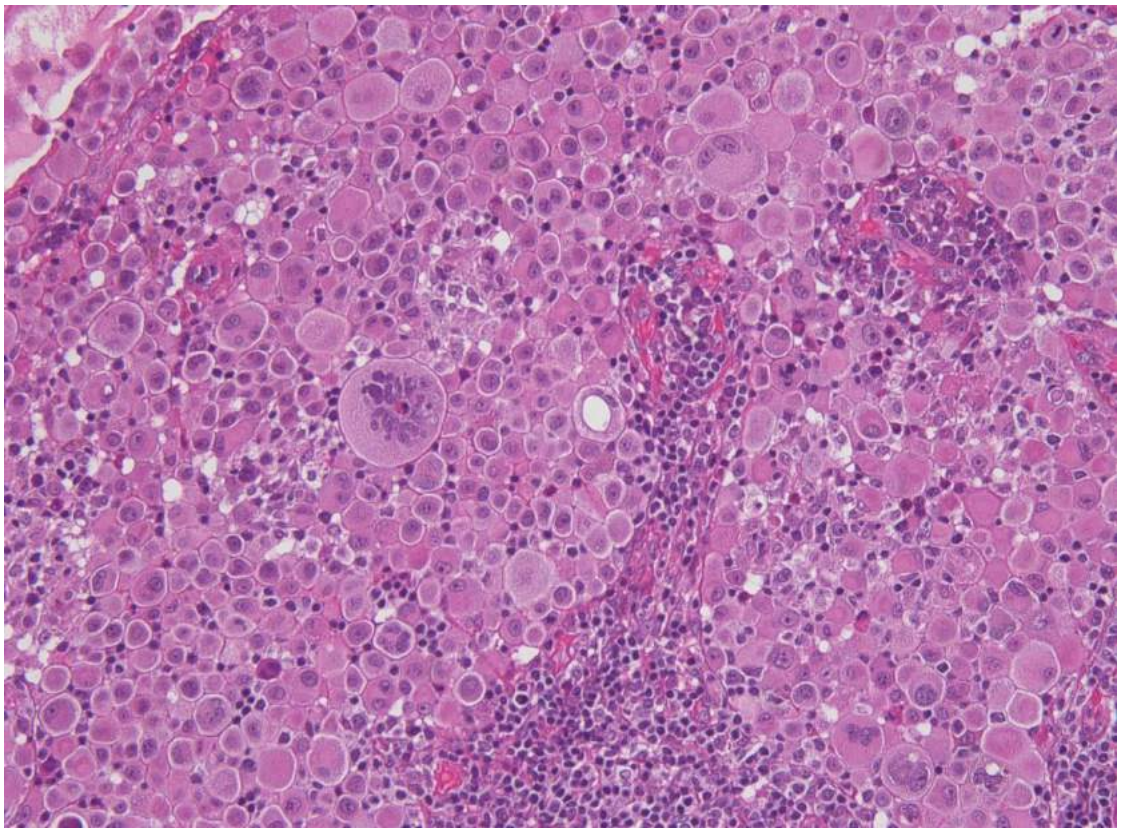

Metastatic Melanoma
Pleomorphic nuclei

Submitted by Kerrie Scott-Dowell
Leica Biosystems

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# Mysteries of Molecular Pathology Revealed!

**Speaker:** **Suzanne Svobodova – AustinHealth**

**Date:** Thursday14th April, 2016

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

**Venue:** Brockhoff Lecture Theatre
Level 3, Smorgon Family Building

Peter MacCallum Cancer Centre
St. Andrew's Place, East Melbourne

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Attendance at this meeting contributes to APACE points

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# 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 18th st February**~~
~~Scientific Meeting-RIMT Student Project Presentations~~
~~Venue: Peter Mac~~

**Thursday 14th April**
Scientific Meeting: Mysteries of Molecular Pathology Revealed
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 8th 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