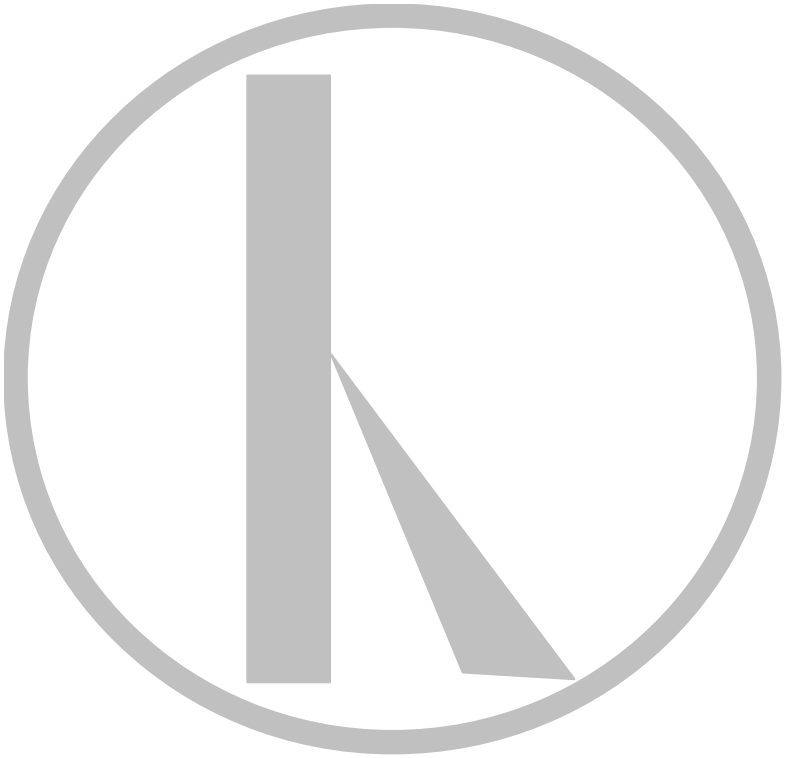
Histograph

www.histonsw.org.au

ISSUE 2
July 2014



Newsletter of the Histotechnology Society of NSW

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Committee Members

Chairman	Trevor Hinwood	tdhinwood@hotmail.com	Product Specialist
Secretary	Bharathi Cheerala	bcheerala@dhm.com.au	In charge IHC Lab. DHM
Assistant Secretary	Katherine Wells	kwells@dhm.com.au	Histology Lab, DHM
Treasurer	Allison Morgan	amorgan@dhm.com.au	
Trade Rep	Natalie Fennell	Natalie.Fennell@dako.com	NSW & ACT Account Manager
Country Rep	Penelope Whippy	Penelope.Whippy@act.gov.au	Quality Manager ACT Pathology
Country Rep	Anne Prins	anne.prins@anu.edu.au	John Curtin School of Medical Research, ANU
Country Rep	Dominic Singh		
Country Rep	George Youssef		
Assistant Editor Histograph	Tony Henwood	tony.henwood@health.nsw.gov.au	Histopathology Lab Dept Head CHW
Editor Histograph	Linda Prasad	linda.prasad@health.nsw.gov.au	Scientist, Histopathology CHW
Student Rep	Hani Norouzi		
Committee	Bill Sinai	snai_family3@bigpond.com	Life Member
	Dianne Reader	dreader@nssccahs.health.nsw.gov.au	Histology, RNSH
	Margaret James	megajam@optusnet.com.au	Laverty Pathology
	Grant Taggart	gtaggart@dhm.com.au	Department Manager Histology DHM
	Simon Flecknoe		Histopathology Lab CHW
	Leah Simmons	leah.simmons5@tafensw.edu.au	Histology teacher, Granville TAFE

Editorial

We have reached mid-year now. This is the Second issue for 2014. If ANYONE is interested in publishing any articles in the Histograph please email me anytime. I am happy to encourage anyone - Student, Technical Aide, Technical Officer or Scientist to send me any articles, histology art or technical issues, and I can publish it on your behalf.

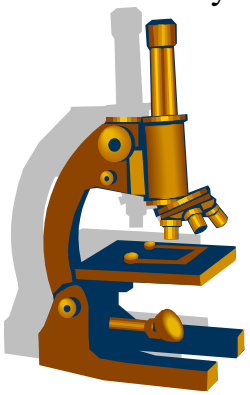
Firstly I would like to thank all the committee members in organising this year's State Conference which was held on the 16th-18th of May at the Ex-Services Club, Orange. Also a Big thank you to all the guest speakers and trade sponsors that made this event such a success. The Trade sponsors were generous as usual with donations towards food, wine and novelty gifts. The conference in Orange is now a fading memory, but was an impressive weekend for its notable speakers with a wide variety of content and the workshop on double labelling. Inside you will find a summary of the workshop and abstracts of the posters and speakers.

Anne and Penny have started their Surgical Cut-Up Workshop in Canberra. The first workshop was held on April the 9th-12th. The second workshop will take place in September. If anyone is interested in attending this workshop please see details inside.

Hope everyone enjoys reading the Histograph and please feel free to email me anytime as I would love everyone's feedback.

We now have a Facebook page called 'Histotechnology Society of NSW'. Please like and share this page to colleagues and friends.

The next educational event is to be held at North Ryde RSL on the 1st of August featuring Dr Esther Myint will concentrate on 'the zoo of the body and the magic of Special Stains'. Watch



out for the flyer for more details.

Cheers
Linda Prasad,
Editor
linda.prasad@health.nsw.gov.au

Chairman's Report

Our State Conference in Orange in May has received many favourable comments. A success on many fronts. The venue was ideal with good staff support and food. The practical immunohistochemistry workshop had 25 attendees who all managed to end up with results and gave positive feedback. Speakers were excellent and we really appreciated them giving up their weekend to be involved. The conference dinner in a winery restaurant had 112 people booked and everybody had a great night, Tony Henwood and Anne Prins winning prizes for the best Autumn Colours attire, something that helped make the night. It was great to see the number and quality of the posters from delegates and students, congratulations to the winners of prizes. It was pleasing to see the number of companies in attendance, 13. Thank you for your support and involvement. We had 109 total registrations which is more than expected for a country conference. We owe a lot of people a "Big Thankyou" for their assistance in making this the success it was.

Following many requests we are already looking into possible areas for the next Conference to be held in 2016.

At the time we are going to press with this "Histograph" we have received information on the next National Conference in Brisbane. The "Histotechnology Group of Queensland" in conjunction with the 9th Asia Pacific IAP Congress will be holding the Conference in the Brisbane Convention Centre the 5th to the 7th of June 2015. This will be a Conference not to be missed so add it into your diaries and start making plans to attend.

The date and venue for our AGM night has been set and is on Friday the 1st of August, 6.30pm at our usual venue of "North Ryde RSL". Finger food will be provided and a bar will be open. The evening presenters are Dr Esther Myint and Amirazian Asrafy with an interesting presentation "The Zoo of the Body and the Magic of special stains". Put this in your diaries and come along for an enjoyable night.

Information has been mailed out on the "Annual General Meeting", committee nomination forms and membership renewal forms. We are always looking for new committee members so consider being part of a great group of people. Note that nomination forms need to be with the Secretary by the 23rd of July 2014. Memberships are due for renewal from the 1st of July 2014. We have been able to keep the membership cost at $38.50 including GST.

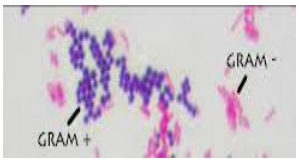
Cheers,

Trevor Hinwood.
Chairperson.
Histotechnology Society of NSW.

HISTOTECHNOLOGY SOCIETY OF NSW

ANNUAL GENERAL MEETING (AGM)

1st AUGUST FRIDAY 2014 AT 6.30PM



The Zoo of the Body & The Magic of Special Stains

By Dr Esther Myint, Douglass Hanly Moir Pathology

We live in the sea of organisms and our body is exposed to all of them at all times.
The skin, GIT from mouth to anus, airways from nostrils to lungs, ears, eyes, and the urogenital tract; although with innate defense by the immune system can be infiltrated and infected by them.
They give little to big problems- from irritations to malignancies.
Some organisms can be diagnosed with H&E but most of them can be seen only with the magic of special stains and immunohistochemistry stains.
This talk is on the common and uncommon organisms that we see mostly in our daily work and the importance of special stains.

WHERE: **North Ryde RSL, 27/41 Magdala Rd**
Cnr of Pittwater and Magdala Rds, North Ryde
Finger food provided at the conclusion of the meeting.
RSVP:
23rd JULY WEDNESDAY 2014

Contact: Bharathi Cheerala, Douglass Hanly Moir Pathology

Ph: 02 9855 6271 **Email:** bcheerala@dhm.com.au

2015 National Histotechnology Conference

Hosted by: Histotechnology Group of Queensland

When: Friday 5th June 2015 - Sunday 7th June 2015

Where: Brisbane Convention & Exhibition Centre, Southbank, QLD 4101

** ***Conference information & registration forms*** will be available mid 2014

"The National Conference will be proudly run in conjunction with the International Academy of Pathology (IAP) International Conference"

Location : Brisbane Convention & Exhibition Centre, Southbank, QLD 4101

Contact : admin@hgq.org.au

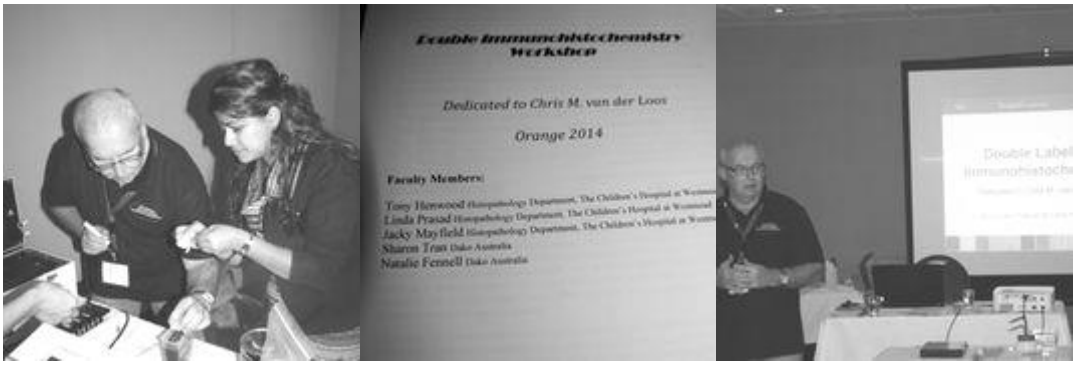
For more info & latest updates, please check the website regularly - www.hgq.org.au

HISTOTECHNOLOGY SOCIETY OF NSW
POSTAL ADDRESS

PO BOX 871
SEVEN HILLS, NSW 1730

DOUBLE LABELLING IMMUNOHISTOCHEMISTRY WORKSHOP

Tony Henwood (Children's Hospital at Westmead), Linda Prasad (Children's Hospital at Westmead)
Jacky Mayfield (Children's Hospital at Westmead, Natalie Fennell (DAKO) and Sharon Tran (DAKO)



This workshop gave an overview of xylene-free heated detergent de-waxing and basic double labelling immunohistochemistry for histotechnologist. The study of co-localization, the presence of two antigens in one cell, is one of the main reasons for performing double staining. Sometimes pathologists need to detect two or more antigens in the same tissue. The objective of this workshop was to provide a theoretical background and hands on experience on double staining. The workshop involved an in-depth and hands on practical experience on Sequential double immunohistochemical system (for same species antibodies) using the Envision G|2 Double stain System. Combined immunohistochemistry using the DuoFLEX Cocktail Anti-AMACR Anti-CKHMW Anti-CK5/6 and the Envision DuoFLEXDoublestain System. Antigen retrieval using the DAKO* PT Link (Pre-Treatment Module for Tissue Specimens) and Xylene-free Heated Detergent de-waxing and dry-mounting using the DAKO* Cover slipper.

Tony Henwood explained the basic and complicated steps involved in double staining. Tony's presentation had a good sense of humour, making it not just an interesting workshop but an enjoyable one also! The presentation included interesting case studies and photographs of stained slides. Tony also explained using examples when it would be necessary to do double staining. He explained in detail the benefits and pitfalls of double staining and heat retrieval.

This workshop was a great opportunity for histotechnologist from all knowledge backgrounds to get hands on experience, meet and talk to other histotechnologist in a fun and relaxed environment. It was a great day.

Abstract

Tony Henwood (Children's Hospital at Westmead), Linda Prasad (Children's Hospital at Westmead)
Jacky Mayfield (Children's Hospital at Westmead, Natalie Fennell (DAKO) and Sharon Tran (DAKO)

Double immunohistochemical labelling can be a very useful technique in histopathology. In order to achieve confident and accurate results, scientific attention must be paid to test design. This workshop, using the wide range and excellent quality reagents from DAKO* will introduce histologists to double labeling techniques and present the design parameters that need to be considered in (hopefully) achieving success.

The following techniques will be done:

- Combined histochemical and immunohistochemical staining
- Combined immunohistochemistry using the DuoFLEX Cocktail Anti-AMACR Anti-CKHMW Anti-CK5/6 and the Envision DuoFLEXDoublestain System.

- Sequential double immunohistochemical system (for same species antibodies) using the Envision G|2 Double stain System.
- Xylene-free Heated Detergent de-waxing and dry-mounting using the DAKO* Cover slipper.
- Antigen retrieval using the DAKO* PT Link (Pre-Treatment Module for Tissue Specimens).

Thank you to all who attended the workshop.

DAB plus Anything

Routine Histochemistry can be combined with immunohistochemistry giving double labelling of antigens and cell and tissue substances in the same section. Poletti et al (1992) have demonstrated that several histochemical techniques can be combined with immunohistochemistry to allow double staining on same paraffin sections (PAS, Alcian Blue pH2.5, Alcian Blue-PAS, Van Gieson, Gomori silver reticulum, and Giemsa).

It is important to consider the histochemical treatments used and their effect on antigens. Poletti et al (1992) found that strong acids (eg HCl) and strong oxidizing agents such as potassium permanganate and silver nitrate would denature some antigens. They found that the best procedure was to perform immunostaining before the histochemical reaction though some minor modifications of the histological staining methods were necessary (Poletti et al 1992).
Some specific examples include:

- Differentiating DAB from hemochromatosis
- CD3 immunohistochemistry with PAS basement membrane staining in renal transplant biopsies
- Myelinated Axons Demonstrated in the central and peripheral vervous systems using neurofilament Immunohistochemistry and Luxol Fast Blue Counterstaining (Yao et al 1994).

Too Much Iron

There are several endogenous and exogenous brown to black pigments that can occur in tissues including carbon, formalin, melanin and hemosiderin. If present in excess, then morphology can be obscured. Most histopathology laboratories use immunoperoxidase histochemistry with diaminobenzidine as one of the substrates. In some instances, excess pigmentation can mask the brown DAB product.

Angiomatoid malignant fibrous histiocytoma is a rare soft tissue tumour whose typical clinical presentation in the superficial soft tissues of children and young adults is associated with a good prognosis. Smith et al (1991) found that the tumours often contained fine dust-like hemosiderin granules. They noted that the colour and refractile qualities of the hemosiderin enabled it to be

distinguished from the brown immunoperoxidase label. The case presented in the current study was extensively pigmented and as shown in the accompanying figures, differentiation of the immuno-product from hemosiderin was quite difficult. A case of Secondary Haemophagocytic Lymphohistiocytosis in a bone marrow trephine was similarly affected.

There are three tactics that can be used. The pigment can be bleached or removed, either before or after immunostaining. An alternate chromogen, of contrasting colour, can be used, or the obscuring pigment can be coloured a contrasting colour. The appropriate technique used will depend on the antigen being localised and the type of obscuring pigment present.

Hemosiderin can be removed by (Morton 1978):

- 15 min in 1% sodium dithionite in 0.1M acetate-hydrochloric acid buffer (pH 4.5)
- 3 hours in 2.4N hydrochloric acid
- 30 min in 3.7N sulphuric acid
- 15 min in 5% oxalic acid

Heavily pigmented tissues may need to have these times extended. Unfortunately several investigators have found that the use of similar bleaching solutions can have an adverse effect on immunoreactivity (Poletti et al 1992, McGovern & Crocker 1986).

Using an immunohistochemical technique that uses a non-brown chromogen (blue, red or green) would allow the differentiation of the immuno-product from brown hemosiderin. However, these labels are not available in many histopathology laboratories.

Lindop & Fleming (1984) and Nickols (1984) initially recognised the value of staining hemosiderin-rich tissues with the Perl's reaction following immunoperoxidase staining. Nichols (1984) found that "the addition of this simple but old technique (Perls stain) to the modern one could further increase its specificity".

Lascano and Berria (1988) used the Perl's reaction following osmium tetroxide treatment to enhance the DAB product following immunoperoxidase staining. They found that the staining colour changed from the original golden-brown, through transient grey, to a very dark brown that was almost black. They improved the intensity of weak immunostaining without an increase in background. Interestingly, no comment was made as to whether hemosiderin was demonstrated with this technique.

It is important that the Perl's reaction is done following the immunohistochemical technique and not prior. Otherwise, extensive, variable sized, brown granular background staining may result. This was especially seen in liver sections. The intensification of Perl's stained iron with hydrogen peroxide – DAB is a common procedure for the detection of small amounts of iron. The method depends on the oxidative/reductive degradation of Prussian blue coupled with the oxidative polymerisation of DAB (Meguro et al 2007).

Renal Transplant Biopsies (CD3-PAS)

Using the Banff classification scheme for acute rejection in kidney transplants, pathologists score the intensity and distribution of infiltrating cells to assess the type and severity of a rejection episode, specifically noting the extent of interstitial mononuclear cell infiltration, the translocation of lymphocytes across the tubular basement membrane (tubulitis) and the invasion of arterial walls by lymphocytes (intimal arteritis). Interstitial infiltration by mononuclear inflammatory cells in isolation is not sufficient to justify a diagnosis of acute rejection. It is only when interstitial inflammation is accompanied by other changes, notably tubulitis and intimal arteritis, that classification as acute rejection is considered. The accurate and consistent identification of these changes is therefore central to transplant biopsy interpretation (Elshafie & Furness 2012).

It has long been recognized that tubulitis can be seen in transplants showing stable graft function. Grade 1 tubulitis (up to four lymphocytes per tubular cross section) is described as 'borderline' or 'suspicious for acute rejection', while more severe tubulitis in a stable graft is classified as 'subclinical acute rejection' and may imply an impaired prognosis. Intimal arteritis is a much rarer lesion, but even when mild, it is regarded as pathognomonic of acute rejection; just one lymphocyte beneath arterial endothelium can justify treatment (Elshafie & Furness 2012).

It can be very difficult to distinguish between apoptotic tubular epithelial cells and infiltrating lymphocytes (Elshafie & Furness 2012).

The T-PAS stain has two components that are superimposed. Immunoperoxidase staining for CD3 permits the specific identification of T-cells, the hallmark of allograft rejection. The PAS stain is a histochemical stain that highlights the tubular basement membrane as well as the brush border on proximal convoluted tubular epithelial cells (Resch et al 2002).

The T-PAS stain easily allows the transplant pathologist to determine whether a tubule-interstitial infiltrate is purely interstitial (ie, T cells are only outside of the PAS positive tubular basement membrane) or whether there is a component of tubulitis (ie, 1 or more T cells are within the confines of the PAS positive tubular basement membrane). Furthermore, it also allows the pathologist to more easily and precisely count the numbers of T cells within the confines of the tubular basement membrane (ie, a measure of the severity of tubulitis in both Banff classification systems) (Resch et al 2002).

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Surgical Cut Up Workshops, 2014 and beyond

From 2014, we are planning a different format for our Surgical Cut Up Workshops. Instead of running the Workshops on four separate Saturdays as we have done since 2008, we are going to run them over three consecutive days (Wednesday to Friday), still to be held at the CIT in Bruce, ACT. This will mean one trip to Canberra – it will make the Workshops more feasible for interstate attendees.

The format would be similar – commencing at 9 am and running til 5 pm, with morning tea, lunch & afternoon tea provided. We envisage that the fees will remain the same at $400.00 per Workshop for those who pre-pay and $440.00 for those who pay on attendance. Payment is by cash, cheque or direct debit as we **do not** have credit card facilities.

There is no accommodation within walking distance of the CIT but you can find information on www.visitcanberra.com.au – Belconnen Premier Inn (3.3 km), Canberra Motor Inn (4.2 km) and Belconnen Way Motel (6.4 km) are three suggestions.

There would be a minimum of 6 attendees and a maximum of 12 per Workshop. We envision running 2 perhaps 3 Workshops next year, depending on interest and suggested times are April, July and October. We are seeking expressions of interest in attending on this basis and this will not be considered binding.

If you are interested, please write your name, workplace and contact details on the sheet indicating which, if any, time of year would suit you best.

Many thanks,

Penny & Anne

penelope.whippy@act.gov.au and anne.prins@anu.edu.au

HISTOTECHNOLOGY SOCIETY of NSW

(ABN 63 128 868 343)

Postal address:
P.O. Box 871
SEVEN HILLS NSW 1730

NOMINATION FOR COMMITTEE POSITIONS – "2014-2015"

Nominations for committee positions will be received by mail up to "***23rd July 2014***". Nominations will be received from the floor of the meeting only for positions with no nominations received by 23rd July. Persons making nominations must have the agreement of the nominees.

The current positions are: Chairperson, Secretary, Assistant Secretary, Treasurer, 5 Committee members (minimum), 2 country representatives, 2 student representatives, 2 advisors and 2 company representatives.

I…… hereby nominate……for

the position of ……..at the election of the

committee of the Histotechnology Society of NSW to be held on "***1st AUGUST 2014***"

Signature Proposer……

Signature Seconder……..

I……being a financial member hereby accept nomination for the

above position.

Signature of nominee……..

Under the Constitution as an association there is NO Proxy voting allowed.

nswhistogroup@bigpond.com

Speaker Abstracts

Pediatric blue cell core biopsies, triage of the blue cell tumours

Dr Amanda Charlton. The Children's Hospital at Westmead, Sydney, Australia.

Children are not little adults especially when it comes to cancer. Adults get carcinomas. Children get sarcomas and lymphomas; collectively called 'malignant blue cell tumours'. The Histopathology department is central in the triage of a pediatric tumour biopsy. We will follow the journey of several common pediatric malignant blue cell tumours from arrival of a fresh biopsy, through immediate assessment including smears, imprints, and frozen section; to triage for FISH, cytogenetics, PCR, flow cytometry; and then IHC panels in order to synthesize a diagnosis.

Cervical Lymph node metastases from unknown primary: Role of histopathology and ancillary techniques in diagnosis and management.

Dr Ruta Gupta RPAH

Metastatic malignancies without known primary site at the time of presentation account for approximately 0.5-10% of all malignancies. Recent data suggest that unknown primary malignancy presenting with cervical lymph node metastases account for 2-9% of all head and neck malignancies and include a highly diverse group of tumours. While a vast majority are squamous cell carcinoma and its variants, melanoma, salivary gland tumours and tumours from sites below the clavicle may also be seen.

The management of these patients rests on accurate cytologic or histopathologic diagnosis including identification of the primary site if possible. Accurate histologic diagnosis is the key

as the treatment plan can vary from surgical resection of the primary site with neck dissection to radiation of the primary site and metastases or chemotherapy depending upon the tumour type. Thus histology, special stains and immunohistochemistry play a very critical role in these cases. Newer molecular techniques such as fluorescent insitu hybridization help augment the accuracy of the diagnosis and genetic sequencing can help in identification of targetable genetic changes.

Illustrative case studies are used in the presentation to demonstrate several critical issues including the influence of pre-analytical factors, reagents and technical aspects on histopathologic evaluation, immunohistochemical analysis and other ancillary prognostic and predictive tests.

Natural & Unnatural Hair Loss in the Forensic Context

Elizabeth brooks[1], and James Robertson[2]

1. *Australian Federal Police, PO Box 401, Canberra ACT 26012*
2. *National Centre for Forensic Studies, University of Canberra, Canberra ACT 2601*

Hair forms a distinctive characteristic of all mammals, and while animals probably remain relatively indifferent to their hair the same cannot be said of humans. Whole industries are devoted to hair, its grooming, its loss or its unwanted presence. Forensic hair examiners are equally interested in hair for its probative value as it forms associative evidence of a crime, a suspect or a victim. On average, human scalp hair is lost at the rate of 100 – 200 hairs per day – this is normal hair loss and occurs through brushing, washing etc. The innate structure and physiology governing human hair growth can tell the forensic scientist many facts. Such facts include whether the hair was lost naturally or was forcibly removed; whether nuclear DNA analysis can be successfully performed; the treatment the hair has undergone and any disease condition of a particular hair. Based on research involving transfer and persistence of hair lost naturally, hair evidence enables the scientist to establish a possible timeline connection with the crime committed. Using a simple microscope, the questioned hair is viewed and is sufficient to indicate if the item is 'actually hair'; if it is animal or human hair; the growth stage of the hair and the best approach to analyzing the hair from a forensic perspective. This is a simple step that takes only a few minutes and requires simple equipment.

QAP Slides & Assessment

Grant Taggart (DHM) & Bill Sinai (Retired Lab Manager)

This will be an open discussion of the slides presented. There is often confusion and ambiguity with the QAP results and hopefully some of these questions can be answered.
The committee consists of a group of experienced histology scientists sitting around a multiheader microscope and examines up to 300 slides at a time and enters their individual responses into a data base. Each examiner gives a score out of 5 per slide (no half numbers) 5 being the top

score. The technical programs examine H&E, special stains, frozen sections and IHC. The QAP program is run in Australia, Asia, Middle East, India and Canada. The slides shown are from numerous QAP participants over the past few years.

Photography for Anatomical Pathology; from macro to PowerPoint

Dr Amanda Charlton. The Childrens's Hospital at Westmead, Sydney, Australia.

Aim: To equip Histology technical staff and Scientists with the knowledge to photograph macroscopic specimens. How to get the best images, identify and troubleshoot common problems. How to manage photos in PowerPoint using batch processing and image editing.

Input: How to take a gross specimen photo or video using '**SLE**' mnemonic for **S**tage, **Li**ghting, **E**xposure. Settings for Exposure being AIS mnemonic for **A**perture, **I**SO and **S**hutter release. Save as jpeg for screen output, tiff for print output.

Output: For screen (Powerpoint), how to automatically insert multiple images using Create Album, and reduce all image sizes using Compress Pictures. Directly insert parts of images using Screen clipping. Link to videos. Correct colour and brightness using Format.

Pancreatic Tumours

Dr Kasim Ismail. Liverpool Hospital

The pancreas is a complex organ deep in the abdomen with several functions. Like all tissues, they are subject to abnormalities in function as a result of pathology such as inflammation and neoplasia.

This talk aims to provide a structured overview as to function and some of the common abnormalities it is subject to, including inflammation and neoplasia. The talk will focus on clinical and histological features, expanding a little on ancillary testing using immunophenotyping. Coverage of an emerging entity, IgG4 related disease will be included.

Fungi Demonstration in Histopathology

Tony Henwood, HistopathologyDepartment, The Children's Hospital at Westmead.

Yeasts and Moulds can cause disease in humans. These fungi are often difficult to isolate, hard to grow and a definitive microbiological diagnosis can take several days to weeks. Fortunately histopathology has a high success rate in the detection of these pathogenic organisms. In this presentation, using interesting case studies, fungi identification including histochemistry will be reviewed and the issue of pseudo-fungi will be presented.

The Histology of Human Hair Shaft

Elizabeth Brooks, Eric Hines, Anne Prins

1 Australian Federal Police, PO Box 401, Canberra ACT 2601.
2 CSIRO Ecosystem Sciences, Canberra ACT, 2601
3 John Curtin School of Medical Science, Australian National University, Canberra ACT 2601

Human scalp hair represents a good source of trace evidence to the investigator or the forensic biologist where the growth stage of the hair essentially determines the type of analysis. Human hair has three growth stages - anagen that is actively growing, catagen that is quiescent and finally telogen that is at a resting stage, and effectively waiting to fall out. Due to the propensity of telogen hairs to fall out easily (for example during brushing or washing hair) telogen stage hairs are the most common growth stage found at crime scenes or persons associated with crime. However, telogen hairs have challenged forensic biologists since STR typing was routinely applied to nearly all samples examined in the laboratory. Extracting a nDNA template from the telogen stage hair has been investigated extensively, and apart from some success with staining nuclei in remnant sheath material, obtaining nDNA from the telogen shaft has remained elusive. This preliminary study focused on nuclear staining of longitudinal sections through the hair shafts and roots of both anagen and telogen hairs. Using the anagen hair as a positive control, Trypan Blue staining (Trypan Blue stains for necrotic or apoptotic cells) was applied to both hair growth stages, with Nuclear Fast Red counter staining to indicate nuclear viability. Other staining techniques have been applied to the 'acquired' features observed in hair such as bleaching and are used in the process of forensic hair examination.

Interesting Case Studies

Dr Garry Simmons & Dr Greg Rhodes (DHM Orange)

The pathology they see arises from larger rural cities such as Dubbo, Bathurst and Orange as well as many of the smaller towns. The practice includes a mix of Specialist and General Practitioner referrals ranging from endoscopic and core biopsies to complex cases from gastro-intestinal, gynaecologic, urologic and breast surgeons.

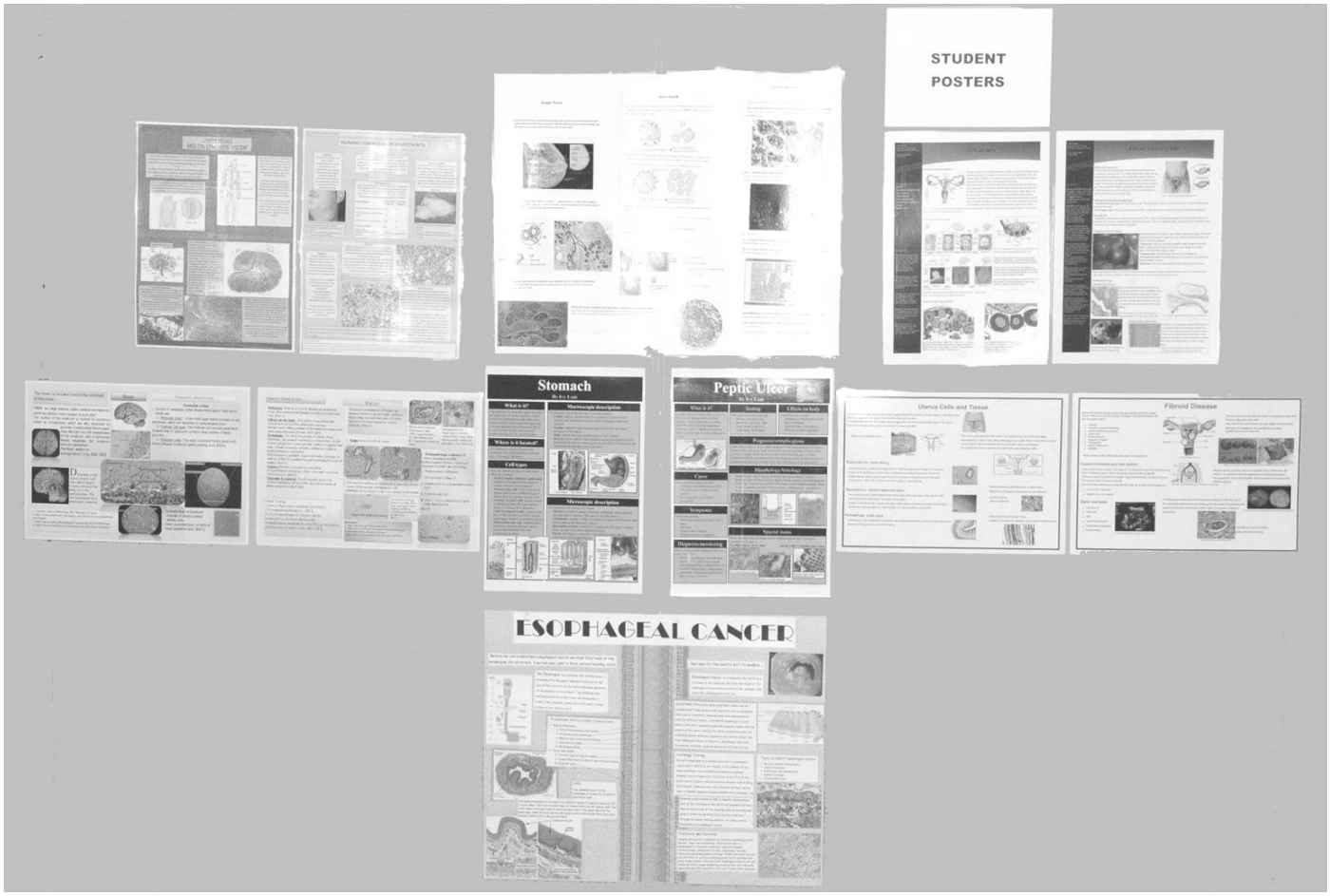
They will present some interesting cases and discuss various facets of rural pathology."

Optimization of Rentsch's Haematoxylin

Penny Whippy (A/Prof Uc & CIT)

While attending the National Histology Conference in Melbourne in 2013, Penny encountered a new haematoxylin that had been developed locally by Mr Mike Rentsch. Mike's slides looked extremely promising – all the clarity and specificity of a regressive haematoxylin, but with the ease of use and flexibility of a progressive…. is this too good to be true?
Mike supplied Penny with several bottles of the new product (Rentsch's Haematoxylin) to run validation and optimisation studies with the students from UC and CIT back in Canberra and the results are produced in this presentation.

Poster Abstracts



The Application of Fluorescent Immunohistochemistry in Traumatic Spinal Cord Injury Research

Yilin Mao[1], Catherine Gorrie[1]
[1]University of Technology, Sydney

A pool of endogenous neural progenitor cells (NPCs) found in the ependymal area of the spinal cord is reported to proliferate in response to traumatic spinal cord injury (SCI).These cells can potentially be manipulated within a critical time period offering one innovative approach to the repair of SCI. In this study, the fluorescent immunohistochemistry techniques were employed to investigate the responses of for endogenous NPCs, inflammatory cells and astrogliosis following SCI.

Paraformaldehyde-fixed frozen sections at 15μm were produced from rat spinal cord tissue to assess the general morphology of injury by Mayer's Haematoxylin and Eosin

(H&E) staining. Meanwhile, the fluorescent immunohistochemistry was utilised to indicate the responses of endogenous NPCs (anti-Nestin, Abcam), macrophages (anti-ED1, Serotec) and astrogliosis (anti-GFAP, Dako), which were visualised by fluorescence-conjugated secondary antibodies (Alexa Fluor 488 and 568).

The development of lesion cavity in the spinal cord over 6 weeks post-injury time period was evaluated in the H&E stained sections. The double labelling of Nestin and GFAP illustrated endogenous NPCs (Nestin+/GFAP$^-$) in the ependymal area, reactive astrocytes (Nestin$^+$/GFAP$^+$) at the lesion edge and endothelium cells in blood vessels. The formation of astrogliosis was demonstrated by the accumulation of astrocytes (GFAP$^+$) at the lesion edge. Moreover, the macrophages (ED1$^+$) cells were distributed mainly at the ventral grey matter initially and then gradually migrated to the lesion site over time.

Further studies are required to detect the neuronal differentiation of endogenous NPCs by double labelling of Nestin and NeuN. In addition, anti-BrdU will also be recruited to assess the proliferation and the migration of endogenous NPCsin conjunction with anti-Nestin.

Histopathological stages of atherosclerosis

Sana Ahmed UTS

Cardiovascular disease is the leading and most ubiquitous cause of death in Australia in spite of advancements in cardiovascular research, the development of cholesterol-lowering pharmaceuticals and the promotion of lifestyle amelioration. The pathological progression attributable to the preponderance of mortalities is atherosclerosis. Former concepts of atherosclerosis were established on the singular idea of gradual calcification, though contemporary investigation portrays the disease as rather more chronic and convoluted. Broadly, the process of lipid aggregation, scarring and vascular wall irritation, specifically in the sub endothelial region of arteries, produces the stiffening of the vascular wall, constriction of the lumen, calcification and, circumstantially, thrombosis. Intricate connections exist between endothelial malfunction and immune response, as well as the behavior of lipid and smooth muscle in response to these systemic failures. This review endeavours to elucidate the stages of atherosclerosis with respect to histological features and provide a concise evaluation of the course of future atherosclerotic research.

Clastogenic effects of Methidathion Pesticide

Mohammed Ali Alshehri, King Khalid Hospital, ABHA

Methidathion is a non-systemic organophosphorus insecticide. Genotoxicity potential of Methidathion was evaluated in rat bone marrow cells (*in vivo*) using different doses based on LD50 by means of micronucleus test. MNNCE (Micronucleatednormocromatic erythrocytes) and MNPCE (Micronucleated polychromatic erythrocytes), NDI (Nuclear division index) and NDCI (nuclear division cytotoxicity index), necrotic and apoptotic cells were recorded in rat's bone marrow samples. Results showed that there was not significant increase in the frequency of micro nucleated in bone marrow cells. However, it showed significant increase in necrotic and apoptotic cells following Methidathion administration in a dose-dependent manner comparing to positive and negative control groups.

In light of these results, Methidathion can be considered unsafe to use as insecticide especially human water or food resources control.

Key words: Methidathion, Micronucleus, NDI, NDCI, Toxicity, Clastogenic effect.

Integration of ImmunoHistoChemistry with Digital Pathology

Chua Yong Quan (Ken), Singapore General Hospital

ImmunoHistoChemistry (IHC)stains are carried out upon request submitted by the individual pathologist and does not link directly with the main laboratory workflow. With the integration of Digital Pathology (DP) to the laboratory workflow, cases with IHC requests will need to be entered into the Laboratory Information System (LIS) for flow of IHC stain information to DP. In view of this, the list of IHC stains would have to be made available in LIS in order to create the IHC stain information (including IHC double stain naming method) on the 2D bar-coded slide label which will be subsequently captured by the DP system. With this integration, the digitalised IHC stained slides will be made available to the pathologist and collaborators within or outside of the campus which may improve the turnaround time. These digitalised slides can also be archive for a very long period of time without subjecting to the physical vulnerability of the glass slide.

Single and Dual Immunohistochemistry staining.

Sharifah Athirah, Singapore General Hospital

Immunochemistry is the identification of a certain antigen in a histological tissue section or cytological preparation by employing the specificity of antigen and antibody reaction. The significant differences exist between Immunohistochemistry (IHC) and immunocytochemistry (ICC) in the nature of the biological sample that is analyzed and

the processing of the sample in preparation of antibody staining. This will be illustrated. In addition, various methods for staining such as the direct and indirect methods can be used for detection and visualization. Double staining in immunohistochemistry (IHC) allows for the detection and localization of two antigens in situ. This will be illustrated.

Patterns of HER2 Status Heterogeneity

Belinda Brown, St Vincent's Hospital

Aims: Data review of a large, single-institution cohort of HER2 ISH-tested breast and gastric carcinomas to determine incidence and patterns of HER2 status heterogeneity. This study has the potential to influence HER2 testing practices and clinical decision-making.

Methods: The database of 21 655 diagnostic patients referred for HER2 ISH testing to St Vincent's Hospital Sydney (Nov 2000 – July 2013) was screened to find all cases demonstrating HER2 heterogeneity/clonality defined as discrete aggregated populations of tumour with dissimilar HER2 status. H&E, IHC and ISH (FISH, CISH or SISH) slides for the 124 clonal patients identified were re-analysed to determine the percentage, distribution and morphological characteristics of the HER2 amplified cell population.

Results: Heterogeneity for HER2 amplification status by ISH was detected in 0.58% of invasive breast cancers (124/21260) and 4.81% of gastric cancers (19/395) tested. The proportion of amplified tumour cells ranged from 1% to 95%. Correlation of heterogeneous HER2 populations was observed between IHC and ISH results in all cases where slides were available for analysis. Where DCIS was present in breast cancer cases, 68.4% of the clonal cases also demonstrated heterogeneity in DCIS HER2 status. Twelve patients with paired primary and metastatic breast cancer samples referred for HER2 ISH testing showed variation in presence of heterogeneity between samples.

Conclusions: HER2 status heterogeneity is rare in breast cancer as opposed to gastric cancer; IHC remains a useful triage in detecting such cases. Heterogeneous DCIS may be a clue to the presence of an occult amplified invasive clone. Heterogeneity may underlie changes HER2 status between primary and metastatic tumours.

Tissue microarray construction using the stack method

Aysen Yuksel[1], Michael Krivanek[2], Nicole Graf[2], Tony Henwood[2], Daniel Catchpoole[1]
1. Children's Cancer Research Unit, The Children's Hospital at Westmead, NSW, Australia.
2. Histopathology Department, The Children's Hospital at Westmead, NSW, Australia.

This is a review of an approach developed by The Tumour Bank at the Children's Hospital at Westmead to resolve an issue involved in tissue microarray (TMA) construction and use. A major challenge in using formalin fixed paraffin embedded cases in TMA construction is the variable thickness of the sample donor blocks. A uniform depth of 3-

4mm is needed to construct a TMA to obtain 100 or more consecutive sections that avoids sample drop out and maintains consistent representation of samples across the array. The ideal TMA should produce maximum numbers of usable sections which include all cores. Hence, we revised the conventional technique in constructing TMA's and accustomed it to the special need of tissue blocks of varying thickness. In brief, routinely processed cases (block, slide and report) were retrieved from the archives of Histopathology Department at The Children's Hospital at Westmead. A Histopathologist marked areas of interest on the H&E stained slide, keeping in mind homogeneity and tumour architecture of sample. A core was taken from the donor block, ejected from the stylus onto a clean surface, excess paraffin wax cut using a scalpel blade and the core than placed into the recipient block with a pair of forceps. This allowed us to stack up to three tissue cores obtained from one donor block on top of each other into one single hole in the recipient block. Using this "stack method" a final thickness of tissue in the recipient block of up to 4 mm was achieved. This technique leads to a reduction of depletion of TMA representation. Uniform depth of almost all the cores in a TMA block is particularly desirable, since it avoids the well-known problem of cores being not yet or not any more cut from a TMA. This method can easily be performed by anyone experienced in the construction of conventional TMAs.However, a minor "loss of tissue" might be observed in sections of stack junctions. Being gentle when stacking is essential to minimise introduction of crush artefact. These minor concerns lead the way to alternative methods e.g. using gauged corers.

Built for Learning Histology:

Developing effective pedagogies to continue the finest histological traditions.

Leah Simmons CSU, SWSI TAFE, Novartis Pre-clinical Safety and Built for Learning

Traditional teaching methods and the needs of industry

Traditionally laboratory based education was limited to practical demonstrations and activities strictly confined to the laboratory, black and white printed theory notes, and paper based examinations. The relevance and effectiveness of these teaching strategies are questionable considering the development in technology and current work trends that favor operation in teams and the collaborative efforts of whole departments to create a synergistic effect. Not only have the pedagogies failed to keep pace with technology but the theories of how adults learn in the workplace are largely ignored in most educational settings. As a result, there exists a gap between the needs of industry for work ready technicians and the ability for educational institutions to fulfill these needs. In addition, the constraints of changing educational policies which are driven by tightening fiscal measures have added further pressure on the TAFE system. Developing effective pedagogies are a crucial step in ensuring that the finest histological traditions are passed onto the next generation of histotechnologists.

The effective use of web technology to enhance learning

With the death of the overhead projector and the birth of 'SMART' technologies the teaching laboratory is no longer restricted by the physical containment levels of educational institutions or the color of the ink in the copier. Development in technology has made possible new strategies for teaching and learning that have previously been unavailable. The enormous potential for improved pedagogies has only just begun to be explored. In particular, learning through web mediated technologies that offer the flexibility and convenience for learning anytime anywhere on mobile devices. This enables the teacher's role to be shifted from the more labor intensive duties of tuition to tasks that resemble that of a workplace supervisor, one who oversees the interactions of the work team and provides stewardship to the learning. Through this, higher levels of engagement and responsibility lay with the learner, as is expected in the workplace.

Engagement with learners

Connectivist theories applied by educational elite from US universities such as Stanford in massive open online courses have been trialed and used with great success by several cohorts within a vocational education setting in Sydney. The learning design reflects the work environment and promotes collaborative cultures, autonomous learning and problem solving. Key skills and attitudes desired in the workplace. The learning platform enables a student centered approach that values student feedback for continuous improvement. The learning management system used is able to provide valuable data on the learning behaviors of course participants in order to refine the teaching strategies applied in the learning environment.

Benefits for industry and enterprise

The broader implications of a learning strategy that offers flexibility and convenience without compromising the quality of learning outcomes is that the pedagogical model can be applied to other learning and development needs of an organization. Learning through web mediated technology can be integrated with face2face delivery to offer blended learning options. Designing learning solutions based on contemporary pedagogical underpinnings can provide organizations with professional development options that are able to be integrated with the operational demands of the workplace.

Histology Quiz

Bill Sinai (Retired Lab Manager)

The presentation of `The quiz' will be a test of general knowledge of several aspects of Histotechnology. Questions and discussion after the presentation.

Introduction to Correlative Light and Electron Microscopy

Filip Braet[1, 2]*

[1]Australian Centre for Microscopy and Microanalysis, The University of Sydney, NSW 2006, Australia;

[2]School of Medical Sciences (Discipline of Anatomy and Histology) — The Bosch Institute, The University of Sydney, NSW 2006, Australia

**Corresponding author: Email filip.braet@sydney.edu.au*

To genuinely understand how complex biological structures function, we must integrate knowledge of their dynamic behavior and of their molecular machinery[1]. The combined use of light or laser microscopy and electron microscopy has become increasingly important to our understanding of the structure and function of cells and tissues at the molecular level. Such a combination of two or more different microscopy techniques, preferably with different spatial- and temporal-resolution limits, often is referred to as 'correlative microscopy'[2, 3].

Correlative imaging allows researchers to gain additional novel structure–function information and this provides a greater degree of confidence about the structures of interest because observations from one method can be compared to those from the other method(s)[4]. This is the strength of correlative microscopy, especially when it is combined with combinatorial or non-combinatorial labeling approaches (Figure 1).

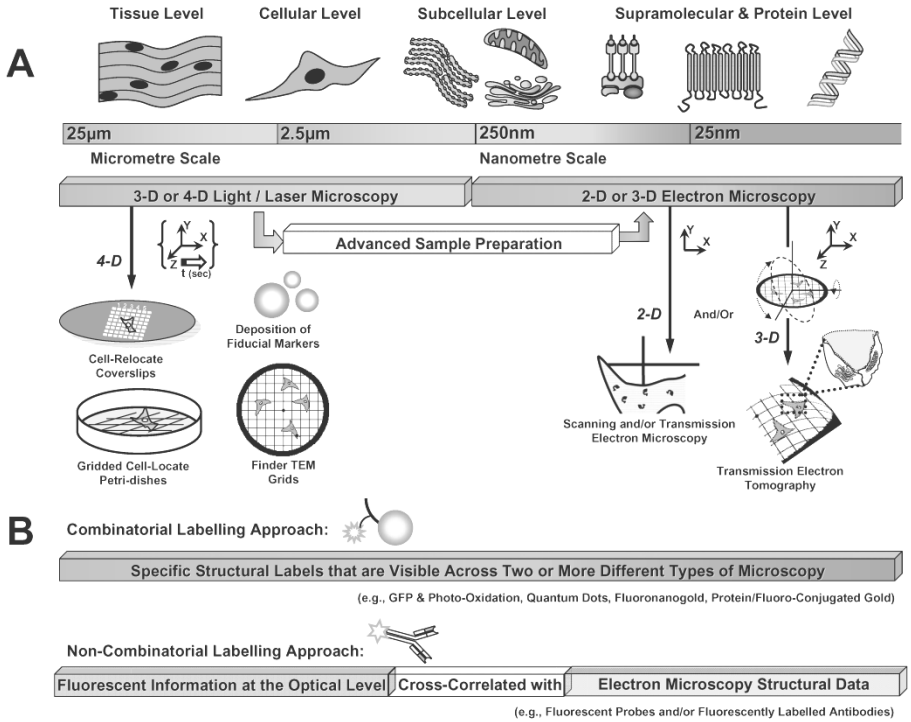


Fig. 1. Scheme illustrating the concept of correlative multidimensional light (3-D) or confocal laser optical imaging (4-D) with electron microscopy (2-D or 3-D) imaging (Reprinted with permission from, [5]). **A**: Depending on the sample source (e.g., tissue

slices, organoid-like cell systems, cell cultures) and the advanced sample preparation approaches applied (i.e., chemical or physical fixation), researchers can collect dynamic structural and molecular information on the cells' behaviour down to the supramolecular level. High-end multidimensional live-cell imaging systems also allow the collection of large datasets over multiple positions (i.e., different cells within the same experiment), thereby increasing the statistical rigour of the observations. Full CLEM information is obtained when multidimensional real-time optical imaging data (left, green) are integrated with electron microscopy imaging (right, blue), via advanced sample preparation and relocation approaches (middle, grey box). **B**: Schematic drawing of the two mainstream labeling approaches presently available to perform CLEM studies. A choice must be made at the start of the experimental design as to whether the same markers or labels will be visually identified across the different microscopy platforms (i.e. combinatorial labeling, top), or whether different types of markers or labels will be used for each imaging platform (i.e. non-combinatorial labeling, bottom). In the combinatorial approach, the probe is followed at both the light microscopy and electron microscopy levels, but often this requires the use of post-labeling procedures. Such methods typically involve enhancement of one molecular label (e.g., GFP-photoxidation) after the dynamic experiments are completed to allow the detection of the enhanced label by TEM. In contrast, non-combinatorial labels are only used for during the light or laser microscopy, after which samples are prepared directly for subsequent electron microscopy studies. The ability to use double- or even multiple-labeling approaches for one or both types of microscopy is the major advantage of the non-combinatorial approach. For more information on the available correlative-labeling approaches, we refer interested readers to expert review papers [4, 6].

In this contribution, an overview of correlative sample-preparation and imaging methods currently available will be presented, including recent developments on correlative optical nanoscopy and electron microscopy; and the trend towards integrative microscopy and microanalysis. This all will be illustrated by practical examples applied on our colorectal cancer and transcellular transport studies.

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