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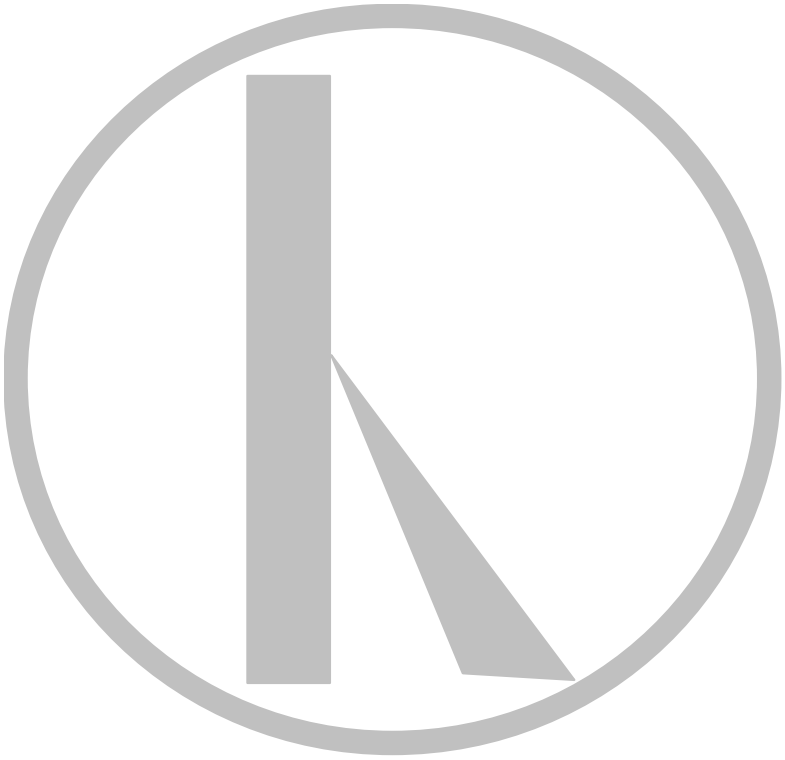

Newsletter of the Histotechnology Society of NSW

Contents

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Editorial

This will be our final issue of Histograph for the year (a little obvious since it is December already!). The National scientific meeting in Hobart went really well with more than expected registrants. Well done Tasmania.

In this issue, we have been blessed with several excellent scientific articles. Grant Taggart from Douglas Hanly Moir pathology has shared with us their Tissue Reprocessing method for us Real World Histotechs who can be sidewinded when our processors decide not to play ball. Sarah Tarrant and Penny Whippy share with us a rapid plant stain that shows great promise in being a routine microscopic botanical stain. Caroline Eddy from Sysmex reviews Automated Electronic Specimen Tracking in Histopathology. Microscopic staining for Nanobots and comparing immunostochemistry platforms are my contributions to this issue. We hope you find these of interest.

This will have to be my last Histograph as editor since I have agreed to be the editor of Cytoletter, the journal of the Australian Society of Cytology. After 15 years of being the Editor of Histograph, I thought it wise to hand on the reigns to our younger members. This will allow me to indulge in my other professional passion, cytopathology. Histograph will now be looked after by a committee of Histotechs and this is exciting. I have been proud of our society and its journal and I have tried to ensure that each issue has value for our members. Hopefully, you will still see my contributions in up-coming issues as I will continue to submit articles of interest to our editorial committee for consideration.

So we hope you all have a happy and restful Christmas and bring on 2018!

Tony Henwood,
Acting Editor
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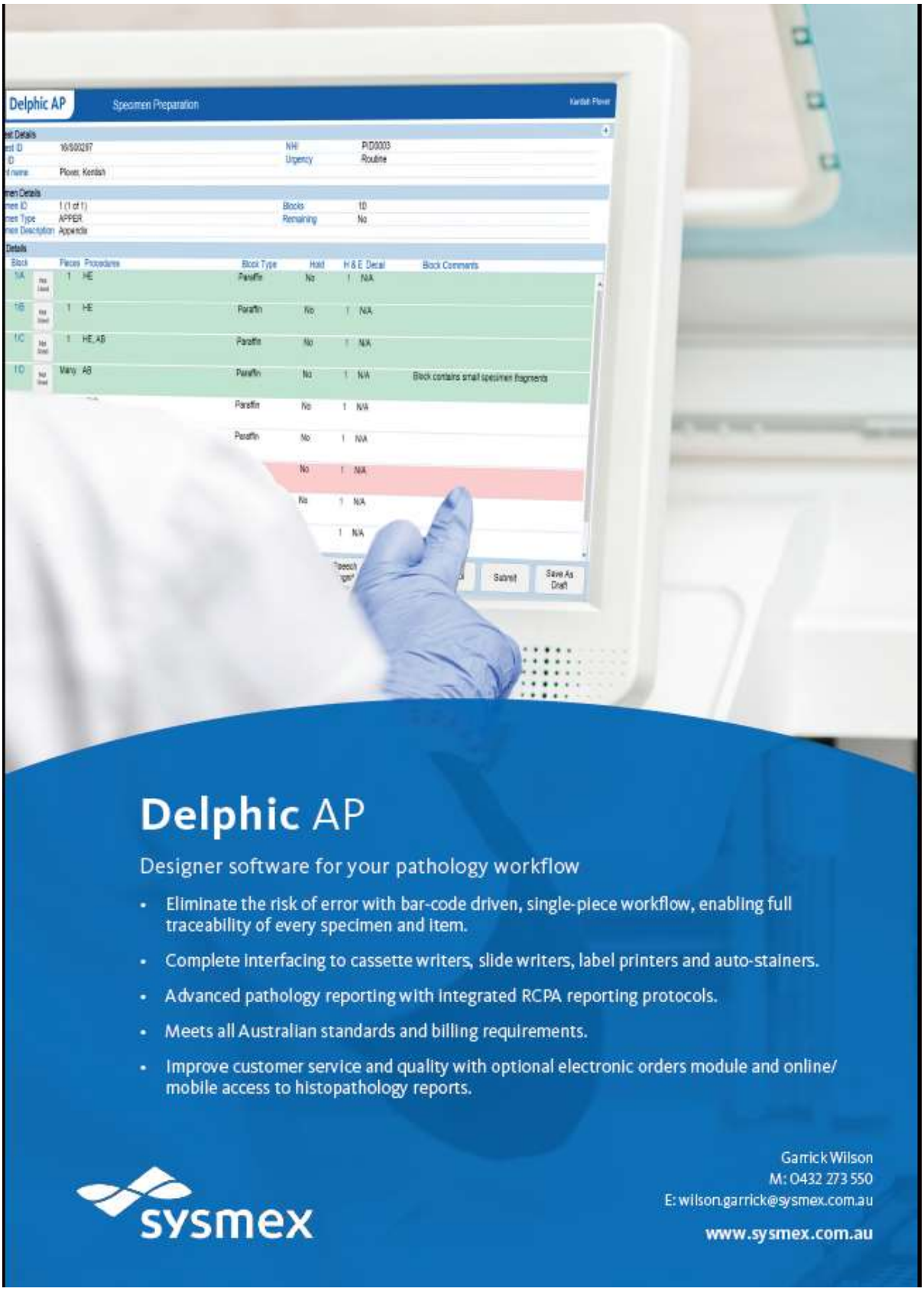
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Chairman's Report

Our Annual General Meeting was held at the Douglass Hanly Moir Pathology facility on the 22nd of September which was sponsored by Leica. Anne and Penny were asked to do a presentation on their very successful "Surgical Cut Up" workshops. Penny gave us a very interesting and informative review which highlighted the work that both these ladies have put into these courses. Anne and Penny were presented with our Societies first Merit awards, for the many years of organising and running their successful course. A new committee was dually elected with all committee positions filled.

Our National Histology Conference was held in Hobart on the 17th to 19th of November. This was a very successful Conference with some 300 attendees, 21 companies involved, 6 workshops which were heavily booked and 12 posters. There was a good variety of topics and speakers. Our Conference convenor was Alistair Townsend from Hobart who was kept very busy coordinating the workshops and Conference. The workshops have received very positive comments. The Conference was opened by her Excellency, Professor, the Honourable Kate Warner, AM, Governor of Tasmania. Some attendees were fortunate to be invited by the Governor to Government House, a magnificent building with an interesting history. This Conference reinforced the need for National meetings and the State bodies working together to produce a meeting such as this. The next National Conference will be held Adelaide in 2019.

Anne Prins and Penny Whippy's last "Surgical Cut Up" workshop was held on the 27th to 29th of September. As mentioned previously they have put a tremendous amount of work into these workshops over a number of years with the support of some very dedicated sponsors. A great effort and well-deserved retirement.

So what is the future of the Surgical Cut Up Workshop? One of our committee members, Rick Farquharson, implemented a survey which received 17 responses which our committee is now analysing. The RCPA is reviewing the situation and several Universities are looking at or are already involved in courses to cover this area. The situation was raised at the National committee meeting in Hobart and we all agreed to work together on this important topic. 2018 will see a lot happen in this area.

Another committee member, Leah Simmons, is a team member on "Innovation + Business Skills Australia, Manufacturing, SSO Team". This team is looking into National Laboratory Operating Training packages. This would involve teaching institutes such as TAFE which our Society feels is important in the training of potential Histology Laboratory staff. We are therefore supporting this initiative.

Planning is moving forward for our next State Conference to be held at Rooty Hill RSL, the 5th to the 7th of October 2018. More information early in the New Year.

We have also finalised arrangements with AIMS for their joint National Conference with the AACB in September, 2018, Darling Harbour, Sydney. We have arranged two speakers and a workshop panel in the Cancer section.

With best wishes for the Holiday break and have a happy New Year, Safe Travelling.
Cheers,

Trevor Hinwood.
Chairperson.
Histotechnology Society of NSW.

Reprocessing Tissue

Grant Taggart
Senior Clinical Scientist
Anatomical Pathology, Douglass Hanly Moir Pathology

In a perfect world, poor processing should never happen, but if it does, you will need a strategy to resolve the situation.
In my early days in histology labs, when the tissue was badly processed, the process was to place the tissue back in xylene to remove the wax, then into alcohol to remove the xylene and then into formalin to remove the alcohol (reverse processing). People might still do this - but this technique is a disaster. The resultant tissue is often brittle and the cells appear severely shrunken, sometimes unrecognisable.
There are many reasons why tissue fails to process adequately:

A. The tissue is too thick for the processing run time.
B. The reagents have exceeded their threshold of use.
C. The processing reagents are wrongly labelled or configured in the processor.
D. Tissue processor malfunctions.

If the tissue has been 'cooked' by the processor (processing temperature above 80^{o}C and the tissue is hard and brittle – it is near impossible to retrieve. When tissue is processed to wax and placed in the embedding centre, it is noted that the blocks are floating and on examination the tissue appears soft, then there has been a processing failure.
First of all don't panic. That's when you make your next mistake by rushing into another protocol that doesn't work or was unnecessary.
Leave the cassettes in the embedding centre and find the cause of the soft tissue. Check the processor for any of the reasons given above (A, B, C or D). You will find that It will be one or more which caused the problem.
If the tissue was too thick for the processor run time (sometimes it is found that the surface of the tissue is processed and the centre is soft or unprocessed) and the specimen has already been embedded then the following is suggested:

1. Trim the block to within 2mm of the tissue. Use a scalpel and cut away as much surrounding wax as possible from the tissue.
2. Place the cassette in a mould in the warming tray of the embedding centre. Leave for a minimum of one hour. (What we are trying to do is 'burn off' any solvents still in the tissue; it may take longer than one hour).
3. Dispose of the melted wax in the mould (it will be contaminated with solvent/s)
4. Once the wax has melted and stood for an hour, re-embed the tissue in fresh wax.

If the specimen is still unable to be cut it will have to be re-processed.

At DHM, reprocessing is done as follows:

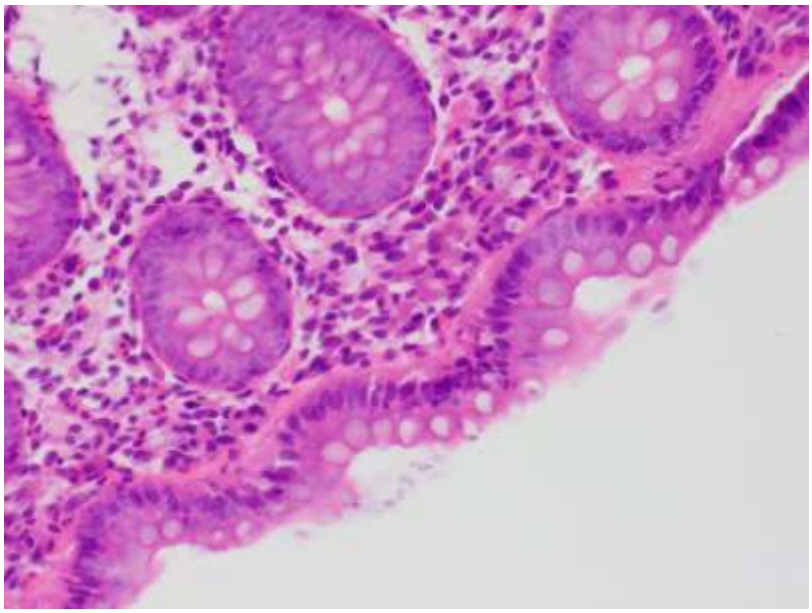
1. The wax needs to be removed from the tissue in the cassettes, this is done by placing in warm saline (65 to 70°C) - enough to melt the wax.
2. To warm the saline - place normal saline (saline is 0.9% - prepared by weighing 9g NaCl and making up to 1 litre) in a plastic tray. Place the tray in the microwave, using power level "High" to heat to 65 to 70°C, this will take about 6 to 7 minutes. Test the temperature. Important, never microwave the saline with cassettes at the same time (the microwave is for heating the saline only).

3. Place the cassettes in the warm saline. At this temperature the wax will melt. Leave up to 10 minutes, agitating the cassettes every 2 minutes. The temperature at this stage is critical and should not exceed 70°C.
4. Remove the cassettes from the warm saline one by one and examine the contents. If biopsy pads are used on the small tissue they will need to be replaced with new pads, and may require new cassettes and clean lids if the wax has not completely melted. The tissue without biopsy pads usually requires no further treatment.
5. If the saline begins to cool and the wax begins to solidify around the cassettes and in the saline tray they can be reheated in the microwave as above. Remember to immerse the cassette entirely in saline.
6. Note – if you need to reheat the saline – only do so until the wax re-melts. Remember – reheating tissue for long periods will “cook” and harden the tissue.
7. Once the cassettes are cleared of wax, they can be placed in 10% formalin and are ready to be processed.

The processing time should be determined by the size of the tissue as per their initial run times.
If possible do not use the same processor or at least change all the reagents including the wax.

If the tissue has been ‘cooked’ and brittle, it is virtually impossible to revive. You can try removing the wax from the tissue using warm saline as previously stated and then leave the cassettes in normal saline at room temperature for 4 hours or more. This process is designed to rehydrate the tissue. However, it only has limited success. You will find the blocks cut better but the morphology will be compromised.

Any tissue which has undergone reprocessing needs to be recorded in the macroscopic description and pathologists notified.



An example of Reprocessed Intestine Tissue H&E

Showing Nanobots in Histopathology

Tony henwood, Principal Scientist, Histopathology, The Children's Hospital at Westmead.

Nanoparticles are very small materials with overall dimensions under 100nm (there are 1000 nanometres in 1 micrometre). This puts nanoparticles in the same size domain as proteins. Some types of nanoparticles have already been in existence for a long time in the environment as, e.g. air pollution, but some are newly engineered for various purposes like microelectronics, drug delivery and imaging technology[1]. There are several types of nanoparticles including metallic nanoparticles and liposomes. Metallic nanoparticles, including silver and gold, have been used in drug delivery, especially in treatment of cancer and also in biosensors. Nanoparticles have an outer shell that can be hydrophobic and contain an aqueous phase that can contain chemotherapy drugs. Such an arrangement, theoretically, retards the release and breakdown of these drugs, allowing time for the nanoparticles to reach the tumour and thus have more effective action on cancer cells and not on the "innocent bystander" cells.

We are probably aware of Quantum dots which are nanoparticles composed of inorganic semiconductor molecules. These nanoparticles emit strong fluorescent light under ultraviolet illumination, and the wavelength (colour) of the fluorescent light emitted depends sensitively on particle size. This size dependence is a unique characteristic of these materials. Quantum dots can emit light that is far more intense and significantly more stable against photobleaching compared with conventional organic dyes such as fluorescein.

Awaad et al[2] studied the toxicity of magnetic nanoparticles (MNPs) after intra-testicular injection, and the protective effect of Echinacea purpurea (EP) extract as antioxidant and immune cells activator. The intra-testicular injection of MNPs caused spermatogenic apoptosis, cellular necrosis, and interstitial fibrosis. Simultaneous EP extract administration reduced the toxicity of MNPs. They prepared MNPs from iron (III) 3-acetylacetonate. They used transmission electron microscopy to estimate the crystal shape and formation as well as the size distribution of the MNPs. Interestingly; they also used the Perls stain to investigate the distribution of iron particles in tissues.

Myllynen et al[1] described the demonstration of nanoparticles that contain gold using classical histochemical techniques. They were investigating the capability of nanoparticles to cross tissue barriers. They used a commercial silver enhancement kit (Molecular Probes Inc). Gold particles in the presence of silver (I) ions and a reducing agent will act as catalysts to reduce silver (I) ions to metallic silver. The silver is deposited onto the gold, enlarging the particles to between 30 and 100 nm in diameter. Tissue or blots stained with colloidal gold are "developed" by this autometallographic procedure to give black staining which can be seen in the light microscope.

Claudia et al[3] described the histopathology of exenatide-Induced panniculitis (inflammation of subcutaneous adipose tissue). Exenatide is administered as a subcutaneous injection and is sometimes encapsulated in 0.06mm nanoparticles of poly (DL-lactic-co-glycolic acid) (PLGA), where the drug is loaded onto the surface of or into the nanoparticle. The drug is then released as the nanoparticle matrix hydrolyses into lactic acid and glycolic acid. PLGA nanoparticles are spherical with a smooth surface. Pharmacokinetic studies have shown an initial burst of the drug in the first 2 days that is believed to be due to the loosely bound exenatide on the surface with a slower release of the drug over a 2-week period as the nanoparticle degrades. Histologically,

there were multinucleate giant cells, some of sometimes containing foreign material in the form of small rounded structures that were birefringent but non-polarizable. Interestingly, these small foreign-rounded structures were positive with both AFB and Fite stains. The ability of a structure to resist decolourisation by acids defines the property "acid fast". In mycobacteria, this property is related to the lipid-rich nature of the organisms' cell wall. Other notable acid-fast organisms include the oocysts of Cryptosporidium, Isospora, and Cyclospora. Sperm and nuclear inclusion bodies seen in lead poisoning have also been reported to be acid fast[3]. Claudia et al[3] suggest that the acid fast positivity observed is a result of the lipid quality of vehicle PLGA used in the sustained release formulation of exenatide.

References

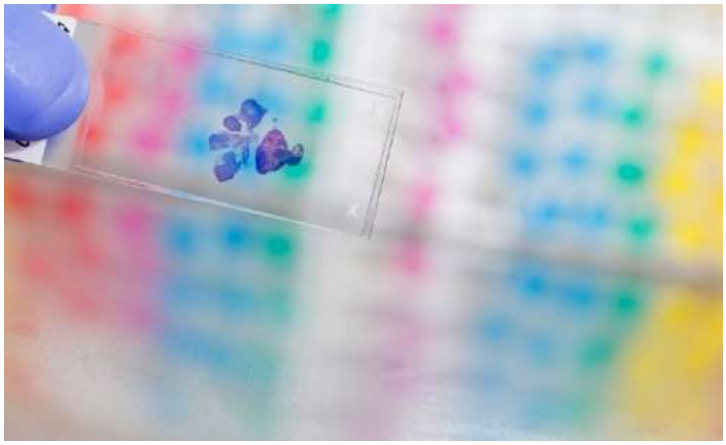
1. Myllynen, P. K., Loughran, M. J., Howard, C. V., Sormunen, R., Walsh, A. A., & Vähäkangas, K. H. (2008). Kinetics of gold nanoparticles in the human placenta. Reproductive Toxicology, 26(2), 130-137.
2. Awaad, A., Adly, M. A., & Hosny, D. (2017). Histological and histopathological studies on the protective role of Echinacea purpurea extract after intra-testicular injection of magnetic nanoparticles in male albino rats. Journal of Histotechnology, 40(4), 100-114.
3. Claudia, V. I., Sofia, C., & Nicole, B. M. (2017). Exenatide-Induced Panniculitis: Utility of the Acid-Fast Stain to Identify Injected Microspheres. The American Journal of Dermatopathology. DOI: 10.1097/DAD.0000000000000952

Automated Electronic Specimen Tracking Central to Increased Quality and Patient Safety

Caroline Eddy, Marketing Communication Specialist at Sysmex

The processing of tissue samples through the anatomical pathology laboratory has typically been a very manual process, due to its complex nature and the level of skill required to prepare and review tissue specimens. A patient's anatomical pathology test request is often associated with several specimens, blocks and slides. The gross tissue description and pathologist review of multiple slides create the patient report, which then goes on to form the basis for diagnosis and treatment plans. One error at any step of this process can result in incorrect reporting, creating the potential for misdiagnosis and incorrect treatment.

However, while the work may be complex, the most common errors in the histology lab are often simple. For example, the accidental transposition of patient specimens, where a label is applied to the wrong sample, or where a label is misread due to illegible hand writing.



Lab staff take great care to ensure that such errors do not occur, or at least are quickly rectified when they do, but the manual tracking of samples through the anatomical pathology lab is a time-consuming process. It requires reconciliation of blocks and worklists, and the manual logging of each step and action taken on every specimen.

Over recent years, histology labs have also been dealing with an increase in volume and complexity of work due to an aging population, new cancer treatment protocols and the expansion of cancer screening programmes. Increasingly, labs are looking for ways to improve workflow efficiency to absorb their increasing workload, and reduce the risk of error.

Specimen tracking technology, which has been in use in the clinical laboratory for a number of years, has now become available for the more complex AP workflow. A fully electronic specimen tracking system enables labs to move towards a leaner, automated workflow, and at the same time improve patient safety by ensuring accurate sample identification throughout the entire laboratory workflow.

With a specimen tracking system, every item belonging to a patient case including blocks, cassettes and slides is uniquely identified using barcode labelling so that it can be traced at any point throughout the laboratory workflow. Only one patient request is processed at a time, eliminating the chance of

accidental transposition of patients' samples at the lab work bench.

As labs move away from batch-based and manual processing, including practices such as the pre-printing or hand-writing of labels, the opportunity for labelling errors is also greatly reduced. Instead, with single piece processing, cassette writers and slide printers are positioned at the appropriate points on the workbench. These are interfaced to the tracking system at each step so the correct number of legible, permanent labels are printed, just-in-time.

For example, at the specimen preparation stage, the barcode on the specimen pot is scanned, and the appropriate number of cassettes for the specimen type are automatically printed right then and there, with the unique case and specimen details embedded into the barcode. At the cutting stage, the barcoded cassette is scanned, triggering the slide writer to print the appropriate number of slides, uniquely labelled for the procedures / stains to be performed.

When it comes to staining, information is retrieved from the system via an autostainer interface, directing the instrument as to the type of staining to perform, removing the need for manual staining work lists and setup in the staining instrument.

Introducing this type of technology also offers labs the opportunity to improve ergonomics at the workbench. With an automated, barcode driven workflow, printed worklists are no longer required. Instead, these are replaced by barcode scanners and wall-mountable touch screens at each step, saving valuable space at the work bench.

With the addition of request form imaging the lab can go completely paperless, as the scanned request form is available in the system for everyone to view, at any point in the process.

With a single source for specimen traceability it is easier to check up on progress of a particular patient request, or locate a specimen if further lab work is required. In addition, an audit trail of all user activity makes it easier and quicker to meet accreditation requirements.

When choosing and implementing an automated electronic specimen tracking system there are a number of things to consider, both in terms of technology and impact on staff.

New hardware investments will be required, including touch screen computers, barcode readers, and slide or label printers as well as cassette writer instruments. However, some tracking systems may only work with particular instrument makes and models. More flexible systems can be interfaced to different auto stainers, cassette writers and slide printers so that the lab doesn't get locked into using particular instruments.

In addition, there may be costs associated with developing an interface between the specimen tracking software and the LIS, unless it is a fully integrated system.

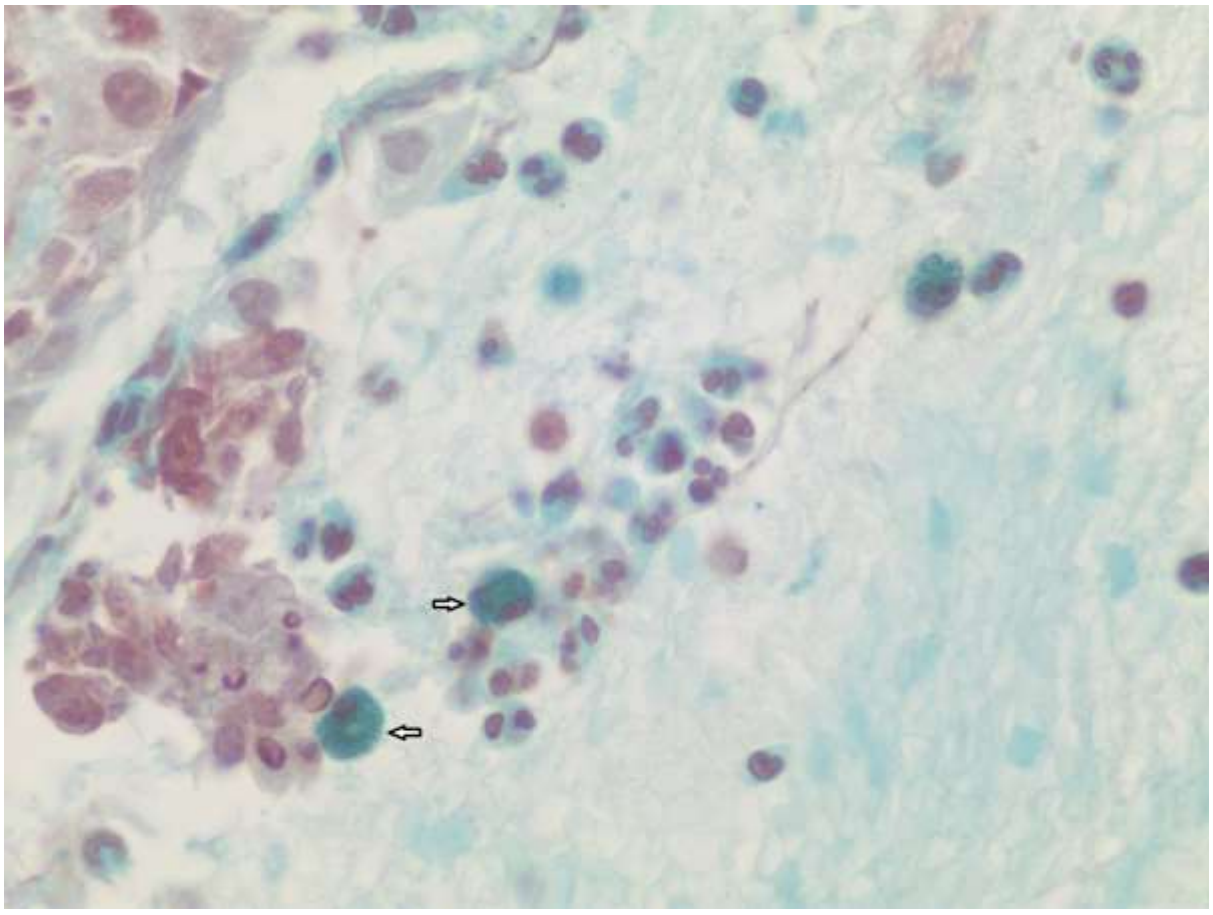
The introduction of an automated tracking system and single piece processing will likely result in considerable processing changes with implications for staff functions, so it is important to conduct a workflow analysis to assess the likely impact. While most labs report that overall turn-around time for sample processing is maintained or reduced, some processes may in fact become more time-consuming (at least initially while the new system is bedding-in) and some staff may end up with new responsibilities. When staff are already busy with their day-to-day jobs and are facing the prospect of a change to

their role, the can often be resistant to the introduction of a new system. In these cases, it often helps to obtain their buy-in to the quality and patient safety objectives for the new system at the outset, and continue to keep them informed and involved throughout the process.

While anatomical pathology labs using automated electronic tracking technology are still in the minority, it is a technology whose time has come, and labs that go down this path say they wouldn't go back.

Test and Teach

What are these cells and what staining technique was used?



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Development of the Tarrant and Whippy Safranin and Picromethyl Green (SPG) Rapid Plant Stain

Sarah Tarrant, and Penny Whippy, School of Laboratory and Forensic Science, Canberra Institute of Technology, Bruce, ACT

Advances and discoveries in the development of specialised staining seen in human and animal tissue are not reflected in the study of plant histological stains. The initial aim of this study was to use existing and available plant stains to examine the development of the sections of metabolically active/inactive plant tissue in flower ovaries. However, in researching no direct specialised plant stains were found, apart from general stains using Safranin, Methyl Green and Orange G [1].

Plants are composed of cells like other living organisms. However, many plants cells are mostly metabolically inactive and more structural in nature. The majority of plant proteins that makeup the structural components of their cells do not show detail and differences on Haematoxylin and Eosin[2] (H&E) staining which is the basic stain for all animal tissue. Alcian Blue/Safranin[2] (ABS) is a primary plant stain that stains for acid mucopolysaccharides which visualises the cell wall and membrane structure of plants including vascular structures such as xylem and phloem but does not define any other properties. In order to histologically explore the metabolic activity of cells/proteins in the developing ovary of the rose flower, the existing stains (H&E and ABS) have been used as a baseline. Modified Safranin, Methyl Green and Orange G [1] and other common histological stains were trialled to produce a result that allows metabolic differentiation across cells to be visualised. Rose ovary was the chosen tissue as it is known to have clearly defined areas of metabolic activity[2] (figs 4&5). Rose petal was also stained to illustrate metabolically inactive tissue without the possible distortions of xylem and phloem activity (figs 1, 2, & 3).

Safranin is a popular stain used in histology for animal and bacterial staining, and is also used for plants. It is a basophilic dye which stains cell walls and membranes, cytoplasm and other non-acidic components of plant cells. Counterstains used with safranin include Methyl Green, Fast Green and Orange G. The literature makes scant reference to concentrations and timings of all these reagents when staining plant tissue, and tends to be “historic” or allegorical rather than factual, however Thompson, L, K. (2000)[3] provided some guidelines

In the experimental phase of the development of a metabolic activity indicator (MAI) stain the first stains completed were the H&E[2] and ABS[2]. These stains were used for control and comparison purposes. A number of different stains were trialled as indicated at the concentrations described and in the table below. The strongest definition of metabolic activity came from the trial using Safranin and Picromethyl Green (SPG). We then modified this stain, changing the timing ratios to determine which ratios gave us the strongest contrast and differentiation (orange and red) across the cell types (metabolically active/inactive).

All slides taken to water before staining and dehydrated, cleared, and mounted after staining.

Reagent Concentrations:

Alcian Blue – 1% in 3% acetic acid
Safranin – 0.5% in 50% ethanol
Methyl Green – 0.5% in 70% ethanol
Picrosirius red/fast green – commercial product – Australian Biostain P/L
Orange G – commercial product – Australian Biostain P/L
Picro-methyl Green – 0.5% Methyl Green in Saturated Aqueous Picric Acid
Celestine Blue – Australian Biostain P/L
Martius Yellow – Australian Biostain P/L

The H&E and ABS did not show metabolically active areas (MAA) in the ovarian cells. Picrosirius red/fast green showed some highlighting of the MAA. The Celestine Blue and Martius Yellow showed similar results to the H&E and ABS.

Orange G appeared to act as a differentiator for the Safranin, removing Safranin from all cell components other than cell walls and membranes. It is thought that it is the 90% alcohol in Orange G was the actual differentiating agent, but this was not verified experimentally. Safranin stains were overstained after 10 minutes, requiring differentiation, but could be under stained if stained for less than 2 minutes, resulting in pale uninterpretable slides. Counterstains after 10 minutes had to be differentiated with Orange G, or left to sit in the Picro-methyl green for nearly an hour

Picric Acid and Picromethyl green both showed a stronger MAA staining than the Picrosirius red/fast green with the Safranin and Picromethyl green have the strongest contrast.

The modifications of Safranin and Picro-methyl green timing ratio best suited was 2:5 minutes of Safranin and Picromethyl Green respectively. The slides did not overstain with the safranin, and allowed sufficient time for the Picromethyl Green to counterstain.

In experimentation with different stains, and then different timing ratios it is apparent that with the final combination with Safranin and Picro-methyl Green and the 2:5 minute ratio that the MAA of cells can be determined from the stain.

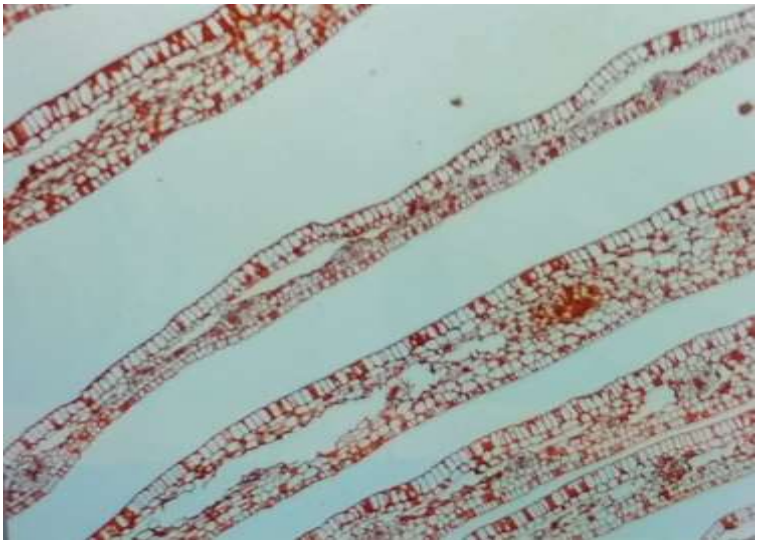

Figure 3: SPG 2:5

Alcian Blue/Safranin[2] 1. 3% acetic acid ……..3 mins 2. Alcian Blue ……..30 mins 3. Wash in running water ……10 mins 4. Rinse in distilled water 5. Counterstain Safranin ……..1 min	Trial 6 1. Picro-methyl green……..10 mins 2. Orange G ……..30 secs Safranin …….. 10 mins 3. Picrosirius red/fast green ……..5 mins
Trial 1 1. Picrosirius red/fast green ……5 min 2. Methyl green……..5 min 3. Orange G ……..30 secs 4. Safranin ……..1 min	Trial 7 1. Safranin ……..10 mins 2. Picro-methyl green……..10 mins 3. Picrosirius red fast green……..10 mins 4. Methyl Green ……5 mins
Trial 2 1. Picro-methyl Green……10 mins 2. 0.5% Safranin ……..1 minute 3. Picrosirius red/fast green ……5 mins	Celestine Blue and Martius Yellow[2] 1. Celestine Blue ……..5 mins 2. Martius Yellow ……..5 mins
Trial 3 1. Safranin ……..10 mins	Trial 8 1. Picric Acid …….. 1 min 2. Methyl Green ……..30 secs 3. Safranin ……..30 secs
Trial 4[3] 1. Safranin ……..overnight 2. Methyl Green ……..10 mins	Trial 9 1. Picrosirius red/fast green ……. overnight 2. Safranin ……..1 min
Trial 5 1. Safranin …….. 10 mins 2. Picro-methyl Green ……..30 mins	Trials 10 - 16 1. Safranin …….. X mins 2. Picro-methyl Green …….. Y mins. In ratios of X:Y – 1:1, 2:2, 5:5, 2:5, 5:1 and 5:2

References

1. *Staining*, http://microscopy.berkeley.edu/Resources/instruction/staining/htm
2. Whippy, P (2017) *Histology Tests Practical Manual*, Canberra Institute Technology
3. Thompson, L, K. (2000) *Introduction to Plant Vascular Systems* in *Tested studies for Laboratory Teaching* Volume 21 pp 180-194 (Karcher, S, J (Ed.)). Proceedings for the 21st Workshop/Conference of the Association for Biology Laboratory Education.

Consistency across Immunohistochemistry Platforms

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There are now several main suppliers of Automated Immunohistochemistry Systems: Leica (Bond), Roche (Ventana) and recently Agilent (Omnis). Others, not as well known, are Biogenix's Xmatrx Elite and Biocare's Valent. The question often arises as to how staining results with these platforms compare. If you review the RCPAQAP Anatomical Pathology Immunohistochemistry Technical Module Generic Reports, you will observe that there is often a difference in average scores across the platforms when both the same antibody clone and different clones are used. Interestingly, even though there are often reports in the literature regarding comparisons of different antibodies to the same antigen, differences in results due to differing platforms are rarely presented.

Years ago, immunostaining was a manual and complex process with many steps, typically entrusted only to the most highly focused and skilled staff. The discovery of heat-induced epitope retrieval methods in the early 1990s opened up another source of problems with consistent super-heating of sections an issue. To ensure quality, reproducibility, and speed with increasing volumes, there arose a need for automation[1].

Tan et al[2] described the occurrence of positive HMB45 and MelanA melanophages. These melanophages were confirmed by positivity for CD163. The HMB45 and MelanA staining occurred when the Ventana platform was used and did not occur when the Bond platform was used. Tan et al[2] suggest several possibilities to account for the positive expression of melanocytic markers in lymph node biopsies of patients without a history of melanoma.

1. There may be aberrant expression by non-melanocytic cells, owing to similar or shared antigenic determinants.
2. Alternatively, the positively stained cells may represent benign melanocytic cells/inclusions or macrophages containing melanocytic compounds.

In support of the latter hypothesis, expression of melanocyte-specific genes has also been detected by reverse transcription polymerase chain reaction in lymph node biopsies, and this may be attributable to the phagocytosis of cell remnants with sufficiently intact mRNA in macrophages. The absence of expression of melanocytic markers in non-pigmented macrophages in their cases further supports the hypothesis that the expression is attributable to phagocytosis of melanocytic compounds by melanophages. Tan et al[2] state that nuclear expression of SOX10 is the most reliable marker, as it is consistently negative in melanophages, regardless of the staining platform. Furthermore, nuclear expression of SOX10 is easier to

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interpret, as it is not masked by cytoplasmic deposits of melanin. They propose the use of CD163 and SOX10 in the differentiation of melanophages and melanocytic cells.

Anaplastic lymphoma receptor tyrosine kinase gene (ALK) rearrangements have been described in 3% to 5% of cases of non–small cell lung cancer (NSCLC), and their identification is mandatory to select patients for treatment with anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitors. Different technologies are available to assess ALK gene rearrangements. Fluorescence in situ hybridization (FISH) is the accepted standard because it has been used as a reference method in clinical trials; however, it is an expensive, time-consuming, and labour-intensive assay. In addition, result interpretation is often operator dependent. An alternative diagnostic method based on the detection of ALK fusion protein expression is immunohistochemical (IHC) analysis. This method is widely used in pathology laboratories, faster, cheaper, and particularly useful in patients with advanced-stage carcinoma, for whom small biopsy specimens with a limited number of neoplastic cells are often available [3]. Marchetti et al[3] compared two commonly used immunohistochemical assays on a large series of lung adenocarcinomas: the ALK (D5F3) CDx Assay on the BenchMark XT platform with the Optiview Amplification Kit and an assay based on the use of the clone 5A4 on the BONDMAX platform.

The results obtained indicated that the two tests had the same level of sensitivity, whereas the specificity was higher with the Ventana system (99.8% versus 98.3%). In the same way, the Negative Predictive Value was similar for the two tests, whereas a significant difference was observed for the Positive Predictive Value (93.7% with the Ventana system versus 63.8% with the Leica system)[3].

Kornaga et al[4] directly compared platforms from Dako, Leica and Ventana in the demonstration of oestrogen receptor. They found all assays showed good intra-observer agreement. Inter-observer pathological scoring showed some variability: Ventana had the strongest agreement followed closely by Dako, whereas Leica only showed substantial agreement. They also analysed each oestrogen receptor assay with respect to 5-year disease-free survival, and found that all performed similarly in univariate and multivariate models. Determination of measures of test performance found that the Leica assay had a lower negative predictive value than Dako or Ventana, compared with the original ligand-binding assay, while other measures—sensitivity, specificity, positive predictive value, and accuracy— were comparable between the three ready-to-use assays. When comparing against disease-free survival, the difference in negative predictive value between the vendor assays were not as extreme, but Dako and Ventana still performed slightly better than Leica.[4]

Rimm et al[5] compared platforms and antibodies for the assessment of PD-L1

expression in non-small cell lung cancers. They looked at the 28-8 antibody on the Dako Link 48 platform, 22c3 antibody on the Dako Link 48 platform, E1L3N antibody on the Leica Bond platform, and SP142 antibody on the Ventana Benchmark platform and found that there was concordance between the first three platforms but the assay on the Ventana platform detected significantly less PD-L1 expression in tumour cells and immune cells.

So, where to from here? All we can do is watch the literature and the QAP results and hopefully any platform issues can be detected and appropriate action can then be taken.

References

1. Prichard, J. W. (2014). Overview of automated immunohistochemistry. Archives of pathology & laboratory medicine, 138(12), 1578-1582.
2. Tan, C. L., Maheshwari, P., Choo, S. N., Chan, Y. H., & Ng, S. B. (2017). Expression of melanocytic markers in melanophages across platforms: a potential diagnostic pitfall. Histopathology, 70(3), 501-504.
3. Marchetti, A., Di Lorito, A., Pace, M. V., Iezzi, M., Felicioni, L., D'Antuono, T., ... & Buttitta, F. (2016). ALK protein analysis by IHC staining after recent regulatory changes: a comparison of two widely used approaches, revision of the literature, and a new testing algorithm. Journal of Thoracic Oncology, 11(4), 487-495.
4. Kornaga, E. N., Klimowicz, A. C., Guggisberg, N., Ogilvie, T., Morris, D. G., Webster, M., & Magliocco, A. M. (2016). A systematic comparison of three commercial estrogen receptor assays in a single clinical outcome breast cancer cohort. Modern Pathology, 29(8), 799-809.
5. Rimm, D. L., Han, G., Taube, J. M., Eunhee, S. Y., Bridge, J. A., Flieder, D. B., ... & Fujimoto, J. (2017). A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for pd-l1 expression in non–small cell lung cancer. JAMA oncology.

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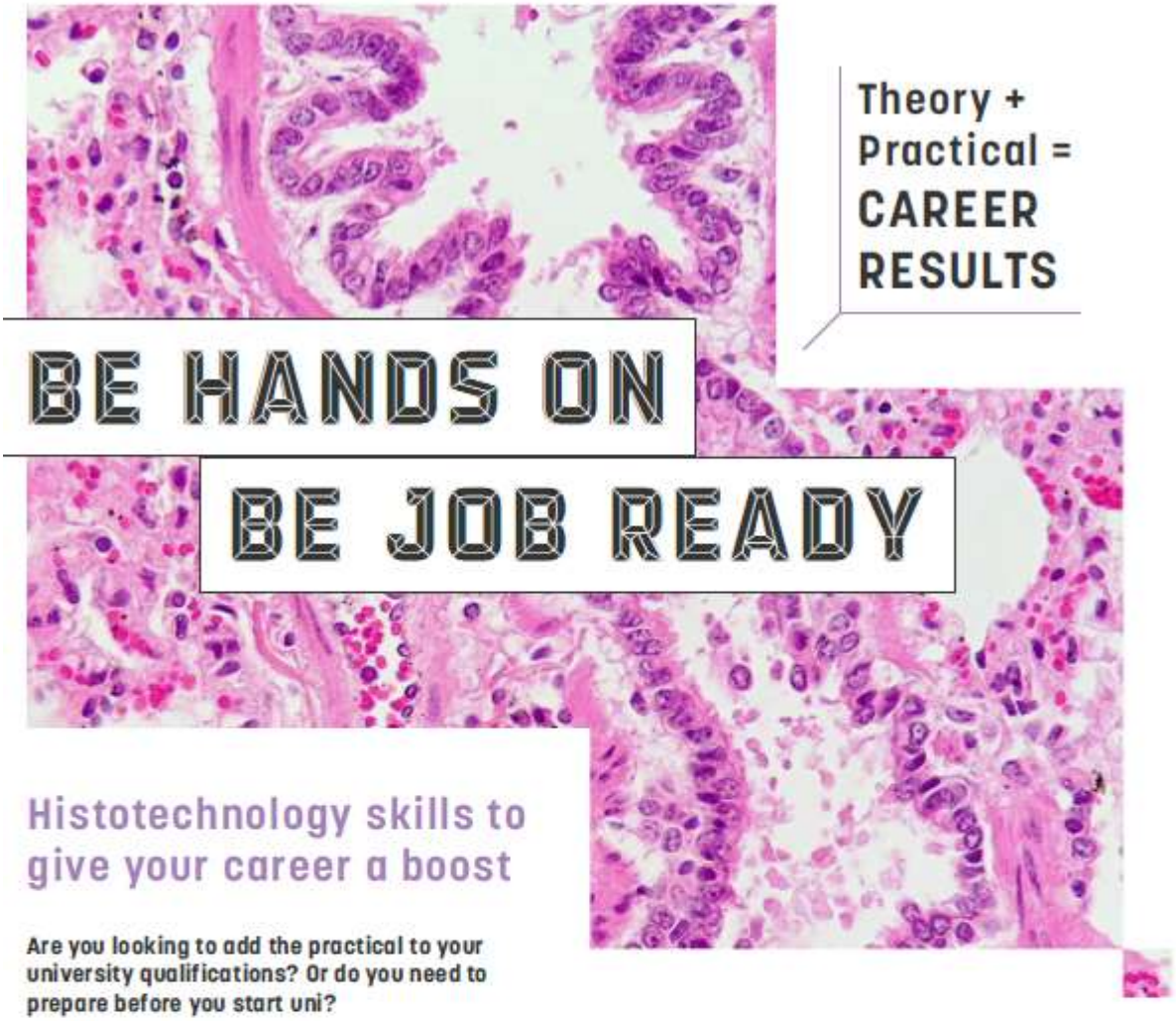

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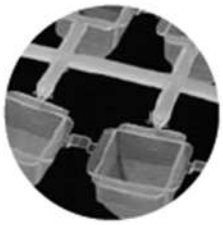

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