Faculty of Medicine

School of Medical Sciences

ANAT 3212

Microscopy in Research

Semester 2, 2013

Contents

ANAT3212 Microscopy in Research Course Manual

Course Convenor

Thomas Fath, PhD
Senior Lecturer, Department of Anatomy
Group Leader, Neurodegeneration and Repair Laboratory
School of Medical Sciences
Wallace Wurth Building (C27), G18
e-mail: *t.fath@unsw.edu.au*
Phone: 02 9385 9690

Co-Convenor

Patrick de Permentier
Lecturer, Department of Anatomy
Head of Histology
School of Medical Sciences
Room 112, First Floor
30 Botany St, Randwick
e-mail: p.depermentier@unsw.edu.au
Phone: 02 9385 2465

Units of Credit

ANAT3212 Research Methods in Microscopy is a 6 UoC course. It is offered in the BSc and BMedSc programs, contributing towards a major in Anatomy or a minor in Pathology in the BSc, as well as a specialisation in Anatomy or Pathology in the BMedSc. The pre-requisite for this course is the 2nd year course ANAT2241 Histology: Basic and Systematic.

General Information

ANAT3212 provides both a theoretical and a practical foundation for future researchers who will use microscopy and morphological methods to gather scientific data. Undergraduate teaching of basic histology and histopathology now relies substantially on computer-based virtual microscopy. However, most future researchers in the medical/biological sciences need a thorough grasp of relevant microscopic techniques. This course is targeted towards Year 3 Science and Medical Science students seeking to gain "hands-on" experience with not only conventional light microscopy, including a practical understanding of the preparation of routine sections, but also a range of advanced microscopy techniques..

Aims and Learning Outcomes

This is an advanced course in microscopy, which provides practical, research-oriented experience. The course covers the principles and practice of conventional light microscopy, including an understanding of the preparation of routine paraffin and frozen sections, as well as advanced resin embedding methods and specialised light microscopic techniques such as phase contrast, darkfield and Nomarski differential interference contrast; enzyme

histochemistry; immunostaining techniques; fluorescence and confocal microscopy including principles of quantitative microscopy (morphometry). Furthermore, the course will introduce high-end microscopy techniques such as high-resolution microscopy (e.g. PALM.STED), 2-Photon Microscopy, Atomic Force Microscopy. The course will thus help students to gain a better understanding of the correlation between structure and function.

Format

Teaching will include lectures, laboratory demonstrations and practical sessions, as well as small group discussions. Students will gain experience in an examination of microscopic specimens via a range of different methodologies.

In weeks 11 and 12, short Projects will be carried out in research laboratories on the UNSW campus. Students will be assigned to the different projects during the first half of the course. Students' preferences for individual projects will be taken into consideration. Projects that will be offered are listed on page 36.

Acknowledgement

We would like to thank Prof Rakesh Kumar for the original design, and the previous convenors Dr Maria Sarris and Patrick de Permentier for their work in setting up this course which has been run under the title 'Research Methods in Microscopy' in 2009. This course has formed the basis for the current course which has further evolved to include high-end microscopy applications. We would also like to thank all teaching staff involved in this course and who dedicate their time to make this course an exciting experience.

Lecture:	Tuesday 2-3 Biomed Theatre F
	Wednesday 5-6 Biomed Theatre F
	Weeks 1-12
Laboratory class:	Wednesday 2-4 109/110 and
	Thursday 2-4 109/110
	Weeks 1-13

Teaching Staff

(See for more information and short biographies at the end of the course manual)

Dr Till Böcking [ARC Future Fellow, Centre for Vascular Research, UNSW]

Dr Michael Carnell [Research Associate, Biomedical Imaging Facility, UNSW]

Ms Blathnaid Farrell [School of Medical Sciences Health and Safety Officer, UNSW]

A/Prof Nick Di Girolamo [Department of Pathology, School of Medical Sciences, UNSW]

Dr Celine Heu [Research Associate, Biomedical Imaging Facility (BMIF), UNSW]

Prof Gary Housley [Head, Translational Neuroscience Facility and Department of Physiology, UNSW]

Prof Rakesh Kumar [Department of Pathology, School of Medical Sciences, UNSW]

Ms Toni Le Roux [Academic Services Librarian for the Science, Engineering and Medicine Unit, UNSW]

Dr Gila Moalem-Taylor [Senior Lecturer, Department of Anatomy, School of Medical Sciences, UNSW]

Ms Suzanne Mobbs [Learning Resource Manager, Faculty of Medicine, UNSW]

Dr Steve Palmer [Senior Research Fellow, Department of Anatomy, School of Medical Sciences, UNSW]

Dr Patsie Polly [Senior Lecturer, Honours Coordinator, School of Medical Sciences, UNSW]

Dr Carl Power [Head, Biological Resources Imaging Laboratory, UNSW]

Dr John Power [Senior Lecturer, Translational Neuroscience Facility, School of Medical Sciences, UNSW]

Dr Maria Sarris [Unit Manager, Histology and Microscopy Unit, UNSW]

Dr Galina Schevzov [Research Fellow, Department of Anatomy, School of Medical Sciences, UNSW]

Dr Justine Stehn [OCF-C4 Research Fellow, Department of Anatomy, School of Medical Sciences, UNSW]

Dr Vladimir Sytnyk [Senior Lecturer, School of Biotechnology and Biomolecular Sciences, UNSW]

A/Prof Nicodemus Tedla [Department of Pathology, UNSW]

Dr Pall Thordarson [Senior Lecturer, School of Chemistry, UNSW]

Dr Simone Van Es [Lecturer, School of Medical Sciences, UNSW]

Dr Renee Whan [Lecturer, Head, Biomedical Imaging Facility (BMIF), UNSW]

Prof Rob Yang [ARC Future Fellow, School of Biotechnology and Biomolecular Sciences, UNSW]

Assessments

Assessment activity	Duration	Value	Due Details
Report (Literature Research)	1000 words	10%	Week 5
Oral Presentation (Literature Research)	5 min	10%	Week 6-7
Examination Terminology & Applications of Microscopy Techniques (Format: short answers)	1 hr	30%	Week 8
Project Individual Projects (two-three students per project). Students will visit the labs of active research groups. 10 research groups to choose from. (Format: written report including experience/reflection and evaluation of data)	2000-2500 words	30%	Week 9-10 Report due on Thursday Week 11
Oral Presentation (Presentation of project experience; should cover a description of experimental design, data analysis and interpretation)	20 min	20%	Week 11-12
PLEASE NOTE: YOU MUST PASS ALL 3 COMPONENTS [Literature Research, Exam, Project]			

Details of Assessments

[1} Analysis of a Research Paper

TASK

Find a current (last 3 years) peer reviewed journal article that has used one of the following techniques to answer a research question:

I. Multi-labelling immnuofluorescence

II. Enzyme-based Histochemistry

III. Atomic Force Microscopy

IV. Confocal Microscopy- Biological samples or Live cells

V. Intravital Microscopy

VI. PALM, STORM or STED super high resolution microscopy

VII. Electron Microscopy

PURPOSE

To critically evaluate the method used in the paper and address the following questions:

1. How did this method answer the researcher's question?
2. Was this a valid method for the question been asked?
3. What other methods could have been employed to answer the researcher's question?

Written Report for Research Paper Review

The written report should be 1000 words maximum in length excluding references. Insert relevant images and diagrams to support your evaluation of the paper.

The Due DATE is FRIDAY 30th August, 2013 (the end of week 5) NO LATER THAN 4:30pm. Assignments are to be submitted to Ms Carmen Robinson, G27, Biosciences Building, UNSW

The coversheet (available on Blackboard) should clearly state:

- Your Name
- Your Student Number

PRESENTATION

You will present your findings to the group and a panel of examiners. The presentation will be 5 minutes.

The presentation will be assessed by your peers and the examiners according to the following criteria:

1. CLARITY AND STRUCTURE: Oral presentation was clear, well-structured and easily understood.
2. TIMING: Timing was controlled so that most aspects were covered.

3. UNDERSTANDING: Presenter appeared to have a good understanding of the topic: able to answer audiences' questions clearly.
4. STIMULATED LEARNING: Presentation was interesting; significant issues and answered questions were highlighted.

Your contribution to Peer Assessment worth 25% of the Research Paper Analysis Exercise

Examiners Assessment worth 75% of the Research Paper Analysis Exercise

All students are to complete a peer assessment feedback form for each presenter. Marks will be based on the quality of the feedback provided.

PRESENTATIONS will be in Week 6-7. A projector will be available for Powerpoint presentations

[2} Exam

The exam in the form of short answers will cover material presented in both lectures and practical classes.

[3] Project Report and Presentation

WRITTEN REPORT FOR PROJECT:

Projects will allocated in week one. The students have to contact their respective project supervisor by week 2 to discuss project. The written report should be 2000-2500 words maximum in length excluding references. Insert relevant images and diagrams to support your data.

The report should be in the form of a research paper, divided into Introduction, Aim, Method, Results and Discussion. Projects will be carried out in group format. However, marks will be individually taken into account feedback by project supervisor on individual participation in project.

The Due DATE is Thursday 17th October, 2013 (the end of week 11) NO LATER THAN 4:30pm. Assignments are to be submitted to Ms Carmen Robinson, G27, Biosciences Building, UNSW.

The coversheet (available on Blackboard) should clearly state:

- Your Name
- Your Student Number

Late Submission

Other than in *exceptional circumstances, late submission will attract a penalty of 10% of the total mark per day or part thereof. Thus, submission on Monday 21st October 2013 would attract a 40% penalty. Keeping to a deadline is part of the assessment.

*You have missed at least 3.5 weeks of university during the period of the course AND you have documents to this effect AND you have notified the course convenor (Dr Fath) in writing at least 2 weeks prior to submission that this was likely

PRESENTATION

You will present your findings to the group and a panel of examiners. The presentation will be 15 minutes with 5 minutes question time.

The presentation will be assessed by your peers and the examiners according to the following criteria:

1. CLARITY AND STRUCTURE: Oral presentation was clear, well-structured and easily understood.
2. TIMING: Timing was controlled so that most aspects were covered.
3. UNDERSTANDING: Presenter appeared to have a good understanding of the topic: able to answer audiences' questions clearly.
4. STIMULATED LEARNING: Presentation was interesting; significant issues and answered questions were highlighted.

Your contribution to Peer Assessment worth 25% of the Project component

Examiners Assessment worth 75% of the Project component

All students are to complete a peer assessment feedback form for each presenter. Marks will be based on the quality of the feedback provided. Presentations will be in group format, however marks will be issued individually.

PRESENTATIONS will be in Week 11 and 12. A projector will be available for Powerpoint presentations

Structure of the assignment adapted from PATH2201/PATH2202, Semester II, 2009 Course Manual

Textbooks

This is a research-oriented course and textbooks will not be prescribed. Students will be provided lists of relevant journal articles and chapters in research monographs as a starting point for their reading of the literature.

Recommended reference sources for this course are

Basic Methods in Microscopy.D.L. Spector, R.D. Goldman, Cold Spring Harbor Laboratory Press 2005, ISBN 978-0-879-69751-8

Theory and Practice of Histological Techniques, 6th ed. J.D. Bancroft, M. Gamble. Churchill Livingstone 2007, ISBN 978-0-443-10279-0.

Attendance

In accordance with University regulations, students must attend at least 80% of all scheduled learning activities (80% of lectures and 80% of practicals).

Late Assessment Items will be penalized by 5% for every day late.

There will be an attendance role taken for both lectures and practicals. If you are coming later than 5 minutes after the start of the full hour (e.g. for a 2:00pm lecture/practical you need to be present in the lecture theatre or laboratory no later than 2:05pm) you will be deemed absent).

OFFICIAL COMMUNICATION BY EMAIL

All students in this course are advised that email is now the official means by which the School of Medical

Sciences at UNSW will communicate with you.

All email messages will be sent to your official UNSW email address (e.g., z1234567@student.unsw.edu.au) and, if you do not wish to use the University email system, you MUST arrange for your official mail to be forwarded to your chosen address.

The University recommends that you check your mail at least every other day. Facilities for checking email are available in the School of Medical Sciences and in the University library.

Further information and assistance is available from DIS-Connect, Tel: 9385 1777. Free email courses are run by the UNSW Library.

Academic Honesty and Plagiarism

The School of Medical Sciences will not tolerate plagiarism in submitted written work. The University regards this as academic misconduct and imposes severe penalties. Evidence of plagiarism in submitted assignments, etc. will be thoroughly investigated and may be penalized by the award of a score of zero for the assessable work. Flagrant plagiarism will be directly referred to the Division of the Registrar for disciplinary action under UNSW rules.

What is plagiarism?

Plagiarism is the presentation of the thoughts or work of another as one's own* Examples include:

- direct duplication of the thoughts or work of another, including by copying work, or knowingly permitting it to be copied. This includes copying material, ideas or concepts from a book, article, report or other written document (whether published or unpublished), composition, artwork, design, drawing, circuitry, computer program or software, web site, Internet, other electronic resource, or another person's assignment without appropriate acknowledgement;
- paraphrasing another person's work with very minor changes keeping the meaning, form and/or progression of ideas of the original;
- piecing together sections of the work of others into a new whole;
- presenting an assessment item as independent work when it has been produced in whole or part in collusion with other people, for example, another student or a tutor; and,
- claiming credit for a proportion a work contributed to a group assessment item that is greater than that actually contributed.† Submitting an assessment item that has already been submitted for academic credit elsewhere may also be considered plagiarism.

The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does *not* amount to plagiarism. Students are reminded of their

Rights and Responsibilities in respect of plagiarism, as set out in the University Undergraduate and Postgraduate Handbooks, and are encouraged to seek advice from academic staff whenever necessary to ensure they avoid plagiarism in all its forms. The Learning Centre website is the central University online resource for staff and student information on plagiarism and academic honesty. It can be located at: www.lc.unsw.edu.au/plagiarism

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:

- correct referencing practices;
- paraphrasing, summarizing, essay writing, and time management;
- appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre. Students are also reminded that careful time management is an important part of study and one of the

identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.

* Based on that proposed to the University of Newcastle by the St James Ethics Centre. Used with kind permission from the University of Newcastle.

† Adapted with kind permission from the University of Melbourne.

Appropriate citation of sources therefore includes surrounding any directly quoted text with quotation marks, with block indentation for larger segments of directly quoted text. The preferred format for citation of references is an author-date (APL) format with an alphabetically arranged bibliography at the end of the assignment. Note that merely citing textbooks or website URLs is unlikely to yield a bibliography of satisfactory standard. The Internet should be avoided as a primary source of information. Inclusion of appropriate journal articles, both primary research publications and reviews, is usually expected.

Applications for Special Consideration

The School of Medical Sciences follows UNSW guidelines when you apply for special consideration on the basis of sickness, misadventure or other circumstances beyond your control. For further information, see:

https://my.unsw.edu.au/student/atoz/SpecialConsideration.html

Please note the following

1. Applications must be submitted via UNSW Student Central. It would also be appropriate for you to inform the course convenor that you have lodged an application.

2. You must submit the application as soon as possible and certainly **within three working days** of the assessment to which it refers.

3. Submitting a request for Special Consideration does ***not*** automatically mean that you will be granted additional assessment or awarded an amended result.

4. Your application will be assessed by the course convenor on an individual basis. Note that UNSW Guidelines state that special consideration will not be granted unless academic work has been hampered to a substantial degree (usually not applicable to a problem involving only three consecutive days or a total of five days within the teaching period of a semester). Under such circumstances, the School of Medical Sciences reserves the right to determine your result on the basis of completed assessments.

5. You should note that if you are granted additional assessment or a supplementary examination (which is ***not*** guaranteed), that assessment may take a different form from the original assessment. Furthermore, the results of the original assessment may then be overridden by the results of the additional assessment, at the discretion of the course convenor. Also be aware that a revised mark based on additional assessment may be greater or less than the original mark.

Health and Safety Guidelines

Generic Safety rules for the School of Medical Sciences can be found at the following URL: http://medicalsciences.med.unsw.edu.au/SOMSWeb.nsf/page/Health+and+Safety.

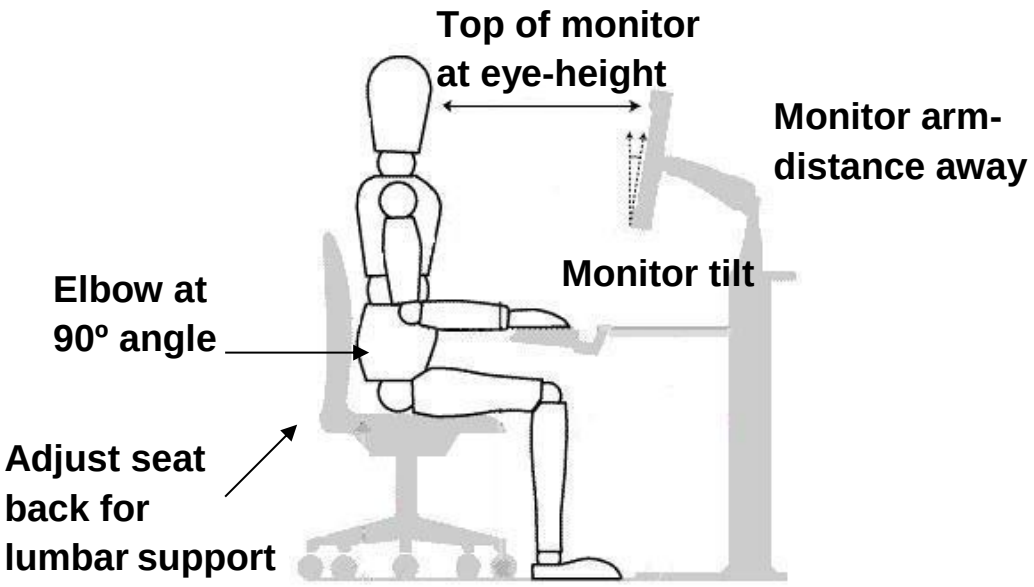
For practicals carried out in Rm 109/110 read and sign the Risk Assessment form on page 14 in the course manual. In research laboratories, everyone must wear a lab coat and closed footware and comply at all times with SoMS health and safety requirements (see above).

Practical labs carried out in individual research laboratories will have additional H&S information and requirements. Information about any additional requirements will be provided by the respective lab managers or online prior to the practical.

ScienceTeaching Laboratory Student Risk Assessment	UNSW THE UNIVERSITY OF NEW SOUTH WALES	ANAT3212 in 109/110 Wallace Wurth building Practicals from weeks 1 to 13 in Semester 2, 2012.

Hazards	Risks	Controls
Ergonomics	Musculoskeletal pain.	Correct workstation set-up.
Electrical	Shock/fire	Check electrical equipment in good condition before use. All portable electrical equipment tested and tagged.

Workstation set-up



Personal Protective Equipment

Not necessary in these practicals.

Emergency Procedures

In the event of an alarm, follow the instructions of the demonstrator. The initial sound is advising you to prepare for evacuation and during this time start packing up your things. The second sound gives instruction to leave. The Wallace Wurth assembly point is the lawn in front of the Chancellery. In the event of an injury, inform the demonstrator. First aiders and contact details are on display by the lifts. There is a first aid kit in the laboratory and the Wallace Wurth security office.

Clean up and waste disposal

No apparatus or chemicals used in these practicals.

Declaration

I have read and understand the safety requirements for these practical classes and I will observe these requirements.

Signature:……Date:……
Student Number:……

Equity and Diversity Issues

Those students who have a disability that requires some adjustment in their teaching or learning environment are encouraged to discuss their study needs with the course convener prior to, or at the commencement of, their course, or with the Equity Officer (Disability) in the EADU 9385 4734 or http://www.studentequity.unsw.edu.au/. Issues to be discussed may include access to materials, signers or note-takers, the provision of services and additional exam and assessment arrangements. Early notification is essential to enable any necessary adjustments to be made.

Grievance Officer

If you have any problems or grievances with the course you should, in the first instance, consult the Course Organiser. If you are unable to resolve the difficulty, you can consult the Head of Teaching in the Department of Anatomy, Professor Ken Ashwell, First Floor, 30 Botany Street, Randwick (Room 113), or the Department of Anatomy's nominated Grievance Resolution Officer, Dr Priti Pandey, Ground Floor, 32 Botany Street, Randwick (Email: p.pandey@unsw.edu.au).

ANAT3212 - TIMETABLE: LECTURES & PRACTICALS

Week	Lecture	Date	Time	Title	Lecturer	Prac.	Date	Time	Location	Title	Lecturer
1	1	Wednesday 31/07/2013	13:00-14:00	*Introduction: Course outline, assessments, projects*	Dr T. Fath Mr P. de Permentier						
1	2	Friday 02/08/2013	9:00-10:00	*H&S: School of Medical Sciences*	Ms B. Farrell	1	Friday 02/08/2013	10:00-12:00	WW G16/17	*Risk Assessments,MSDS & Safe Work Procedures*	Ms B. Farrell
2	3	Wednesday 07/08/2013	13:00-14:00	*Sections: Paraffin & Frozen*	Dr M. Sarris	2	Wednesday 07/08/2013	14:00-16:00	WW G16/17	*Introduction to a Histology Laboratory*	Dr M. Sarris
2	4	Friday 09/08/2013	9:00-10:00	*Standard and Special Stains*	Mr P. de Permentier	3	Friday 09/08/2013	10:00-12:00	WW Rm116	*Standard and Special Stains*	Mr P. de Permentier
3	5	Wednesday 14/08/2013	13:00-14:00	*Immunofluorescence*	Dr T. Fath	4	Wednesday 14/08/2013	14:00-16:00	Museum for Human Disease	*Brightfield Imaging*	A/Prof N. Tedla
3	6	Friday 16/08/2013	9:00-10:00	*How to perform a library search*	Ms T. Gifford	5	Friday 16/08/2013	10:00-12:00	G16/17 Library	Tutorial: Job application documents *How to perform a*	Dr Jia-Lin Yang Ms S. Mobbs
4	7	Wednesday 21/08/2013	13:00-14:00	*Introduction to Histochemistry and Immunohistochemistry*	Dr M. Sarris	6	Wednesday 21/08/2013	14:00-16:00	WW G16/17	*Virtual Lab*	Dr T. Fath Mr P. de Permentier
4	8	Friday 23/08/2013	9:00-10:00	*Using Light Microscopy Techniques*	Mr P. de Permentier	7	Friday 23/08/2013	10:00-12:00	WW Rm116	*Immunofluorescence Cell Culture*	Dr T. Fath Mr P. de Permentier A/Prof N. Tedla
5	9	Wednesday 28/08/2013	13:00-14:00	*Introduction to Confocal Microscopy*	Dr R. Whan	8	Wednesday 28/08/2013	14:00-16:00	WW G16/17	*Applications of Confocal Microscopy, Live Cell Imaging, FRAP and Photoactivation*	Dr R. Whan
5	10	Friday 30/08/2013	9:00-10:00	*STM and AFM Microscopy*	Dr P. Thordarson	9	Friday 30/08/2013	10:00-12:00	WW G16/17	*Immunohistochemical Analysis (Group 1)* *BMIF Tour (Group 2)*	Dr T. Fath Dr C. Heu Dr R. Whan
6	11	Wednesday 04/09/2013	13:00-14:00	*Intravital Imaging*	Prof G. Housley	10	Wednesday 04/09/2013	14:00-16:00	WW G16/17	*Immunohistochemical Analysis (Group 2)* *BMIF Tour (Group 1)*	Dr T. Fath Dr C. Heu Dr R. Whan
6	12	Friday 06/09/2013	9:00-10:00	*Single-molecule and super-resolution fluorescence microscopy (Part 1)*	Dr T. Böcking	11	Friday 06/09/2013	10:00-12:00	WW G16/17	*Presentations of* Literature Report	Dr T. Fath Mr P. de Permentier

Week	Lecture	Date	Time	Title	Lecturer	Prac.	Date	Time	Location	Title	Lecturer
7	13	Wednesday 11/09/2013	13:00-14:00	*Single-molecule and super-resolution fluorescence microscopy (Part 2)*	Dr T. Böcking	12	Wednesday 11/09/2013	14:00-16:00	WW G16/17	Tutorial: Job interview *Presentations of* Literature Report	Dr Jia-Lin Yang Dr T. Fath Mr P. de Permentier Dr P. Polly
7	14	Friday 13/09/2013	9:00-10:00	*Immunohistochemistry Applications – Eye Research*	Dr N. Di Girolamo	13	Friday 13/09/2013	10:00-12:00	EMU	Visit of EMU	Dr P. Polly
8	15	Wednesday 18/09/2013	13:00-14:00	*Neurohistology*	Dr G. Moalem-Taylor	14	Wednesday 18/09/2013	14:00-16:00	WW G16/17	*Digital Pathology*	Dr Maria Sarris
8	16	Friday 20/09/2013	9:00-10:00	*Applying Microscopy Techniques: Data Quantification*	Prof R. Kumar	15	Friday 20/09/2013	10:00-12:00	WW G16/17	*Exam*	Dr T. Fath
9	17	Wednesday 25/09/2013	13:00-14:00	*Pathological Diagnosis in Cancer*	Dr S. Van Es	16	Wednesday 25/09/2013	14:00-16:00	WW G16/17	*Image Analysis*	Dr M. Carnell
9	18	Friday 27/09/2013	9:00-10:00	*Project*	Contribution by various academics	17	Friday 27/09/2013	10:00-12:00	N/A	*Project*	Contribution by various academics
					Mid-Session Break 30/09-06/10						
10	19	Wednesday 09/10/2013	13:00-14:00	*Project*	Contribution by various academics	18	Wednesday 09/10/2013	14:00-16:00	N/A	*Project*	Contribution by various academics
10	20	Friday 11/10/2013	9:00-10:00	*Project*	Contribution by various academics	19	Friday 11/10/2013	10:00-12:00	N/A	*Project*	Contribution by various academics
11	21	Wednesday 16/10/2013	13:00-14:00	*Imaging in live animals*	Dr C. Power	20	Wednesday 16/10/2013	14:00-16:00	N/A	*Visit of the Animal Imaging Facility*	Dr C. Power
11	22	Friday 18/10/2013	9:00-10:00	*Presentations*	Dr T. Fath Mr P. de Permentier	21	Friday 18/10/2013	10:00-12:00	WW G16/	*Presentations*	Dr T. Fath Mr P. de Permentier
12	23	Wednesday 23/10/2013	13:00-14:00	*Presentations*	Dr T. Fath Mr P. de Permentier	22	Wednesday 23/10/2013	14:00-16:00	WW G16/	*Presentations*	Dr T. Fath Mr P. de Permentier
12	24	Friday 25/10/2013	9:00-10:00	*Presentations*	Dr T. Fath Mr P. de Permentier	23	Friday 25/10/2013	10:00-12:00	WW G16/	*Presentations*	Dr T. Fath Mr P. de Permentier
						23	Friday 01/11/2013	14:00-16:00	WW G16/	*Presentations*	Dr T. Fath Mr P. de Permentier

Wednesday lectures are held in Biomed F / Friday lectures are held in Biomed E

Lecture Outlines

Health and Safety [Ms B. Farrell]

The lecture will provide a brief introduction to health and safety in research laboratories. This will include

- How accidents and incidents happen and how to prevent them
- The legal consequences of accidents and incidents
- Laboratory safety:
    - Chemical safety
    - Biological safety
    - Sharps
    - Ergonomics
- Laboratory compliance:
    - Personal protective clothing and equipment
    - Inductions
    - Training, etc
- Emergency arrangements:
    - Hazardous substance spills
    - Fires etc
- The theory of risk assessment and safe work procedures

This is followed by a practical class on how to complete a risk assessment and Safe Work Procedure (SWP) to a standard that is acceptable in a research laboratory.

Sections: Paraffin vs Frozen [Dr M. Sarris]

Most microscopic preparations in Anatomy and Pathology are tissue sections. How the tissue is preserved has a criticial influence on the technique that can be performed and the quality of the results that will be obtained. Tissues may be chemically fixed or frozen.

Paraffin sections

Chemical fixation is the process whereby the cells and the extracellular structures of tissue are preserved in a state which is both chemically and structurally as close as possible to that of living tissue. For this process to occur, the fixative used must be able to stabilise the proteins, nucleic acids and mucosubstances of the tissue by making them insoluble.

The following will be discussed in the lecture:

- Types of Chemical Fixatives
- Dehydration Factors that affect fixation
- Tissue Processing

Frozen sections

Frozen section morphology is inferior to the morphology of paraffin embedded tissue sections. However frozen sections have an important role in research through the demonstration of antigens / enzymes which maybe lost during subsequent fixation and processing schedules and demonstration of lipids in tissue.

Resin

Resin-embedded tissues are mainly used for electron microscopy and for bone pathology.

Standard and Special Histological Stains & Their Applications [P. de Permentier]

This lecture will cover the general principles of staining employed to elucidate morphological cellular and tissue details. The most common staining procedure in histopathology, namely haematoxylin (visualizes nuclei "blue") and eosin (visualizes cytoplasm "shades of pink"), will be described. Other staining methods, which highlight various cellular and tissue components e.g. collagen, muscle, mucopolysaccharides, elastic fibres, bone, blood, macrophages, mast cells, etc will be presented. Examples are Verhoeff's elastic stain, PAS, Trichrome stains which include Masson's and Van Gieson, Romanowsky, Oil Red O, Congo Red, Toluidine Blue, Methenamine silver and Perls' reaction.

Using Light Microscopy Techniques [P. de Permentier]

The main topics covered in this lecture are a brief description of the principles of the light microscope and the use of bright field microscopy, where contrast is enhanced with various histological stains that depend on light absorption. Polarizing microscopy, employed in studies such as the examination of microtubules and membranes, will be described. Phase contrast microscopy will also be discussed as it

can be used in the studies of live cells or unstained fixed material. Finally, the main principles surrounding fluorescence microscopy will be covered as it has a number of applications such as immunofluorescence.

How to Perform a Library Search [Ms T. Le Roux]

This lecture will focus on providing an in-depth understanding of the varied resources (databases, peer-reviewed journals, eBooks etc) relevant to ANAT3212.

Introduction to Histochemistry and Immunohistochemistry [Dr M. Sarris]

This lecture will focus on the use of Histochemistry as a research tool and the data that such techniques can yield.

Histochemistry refers to the methods used to localise different substances in tissue sections. Most commonly used histochemical techniques are:

- Enzyme Histochemistry, which reflects the intensity of enzyme activity in tissue samples e.g. acid phosphatase, dehydrogenases, peroxidase.
- Immunohistochemistry, used to localise antigens in tissue by either single or double labelling.
- Immunofluorescence

Interpretation of staining methods will be discussed.

Immunofluorescence and cell culture [Dr T. Fath]

Immunofluorescence techniques are widely used to analyse protein localisation in combination with the analysis of cell morphology. A good example for a particularly complex cell type with regards to morphology is a neuron which can easily be examined in a culture dish. Besides the culturing of cell lines and primary cells directly derived from an organism, cultures of tissue slices are used to understand intracellular processes as well as mechanisms of cell-cell interaction and communication. In this lecture, I will briefly discuss the use of these different systems in experimental approaches to study protein function and morphogenesis in the nervous system. This includes a brief overview on the strengths and disadvantages of three different culture systems: (1) neuroblastoma cell lines (e.g. SHSY5Y, B35, N2a, PC12, P19 and NT2N); (2) primary dissociated cells (e.g. primary hippocampal and cortical cells); (3) tissue slice cultures (e.g. organotypic hippocampal slice cultures).

We will focus on the use of immunocytochemical techniques using fluorophore-tagged antibodies and fluorescenct probes in order to analyse protein localisation on the cellular level in fixed specimen. Compared to immunocytochemical staining methods using enzymatic reactions, the use of multiple fluorophores that are coupled to antibodies of different antigen specificity opens up considerable flexibility in visualising different sub-cellular structures in the same specimen.

In the first part of the lecture, the principles of fluorophore excitation and emission will be discussed as well as giving a general overview on the absorption and emittance spectra of commonly used fluorophores. This will also include a closer look at the light sources and filter systems used in epi-fluorescence microscopy.

In the second part of the lecture, practical aspects with regards to sample preparation (including the appropriate choice of fixatives and detergents) and choice of antibody reagents will be discussed in more detail.

Strategies for troubleshooting unsatisfactory staining results will be discussed. In this section, I will address e.g. problems arising from high-fluorescence background (low signal to noise or bleed- through in specimens stained with multiple fluorophores.

Confocal Microscopy – Practical applications (Prof. P. Gunning)

The principle of confocal microscopy will be covered with an emphasis on the practical application of this technology. The ability of the confocal microscope to optically section a sample allows the construction of a three dimensional image of the cell with elimination of out of focus background. Co-localisation of multiple signals provides much more accurate information than standard epifluorescense imaging. Live imaging with the confocal microscope gives a much greater appreciation of movement within the z axis. Comparison of confocal microscopy with epifluorescense and super resolution imaging will consider the advantages and disadvantages of each system.

Fluorescence and Confocal Microscopy –Live cell Imaging [Dr R. Whan]

This lecture will outline the specimen preparation, acquisition and analysis methods utilized when performing live cell imaging. Key concepts such as resolution, the theory of confocal, optical sectioning, environmental control, choice of labeling will be discussed. Furthermore advanced light and optical techniques that often are used with live cells such as FRAP, FLIM, FRET and photoactviation will be examined.

STM and AFM Microscopy [Dr P. Thordarson]

TBA {check update on Blackboard]

Intravital Imaging [Prof G. Housley]

This lecture will provide an introduction to intravital imaging. The lecture will commence with a description of the theoretical basis for (infrared) multi-photon excitation of fluorescence reporter molecules within living tissue and indicate how this is utilized to image physiological processes at the cellular and molecular level *in vivo*, in real-time. The material will contrast the features and limitations of conventional confocal laser scanning microscopy (LSM) using single-photon visible light excitation, against LSM imaging via two/multi-photon infrared excitation of fluorescent molecular probes, for intravital imaging applications.

Pathological Diagnosis in Cancer [Prof N. Hawkins]

This lecture will examine the role of microscopy in the diagnosis of disease within anatomical pathology and cytopathology laboratories.

It will review the nature of materials collected for the diagnosis of disease, the handing and processing of tissues and cytology specimens, the decision making of pathologists in arriving at a diagnosis, and some of the particular microscopy techniques that are useful in assisting diagnostic decision making.

Double/multiple labelling – Axonal Transport [Dr G. Moalem-Taylor]

Various histological techniques have been developed over the past 150 years to probe the structure, anatomical organisation and connectivity of the nervous system.

1. Morphology (soma, dendrites, axons)

- *The Golgi technique*: random but complete staining of the neurons
- *Intracellular or juxtacellular labelling*: selected neuron can stain the entire neuron and can be combined with cellular recording and immunohistochemical techniques

Intracellular labelling: Lucifer yellow, biocytin, neurobiotin

Juxtacellular labelling: biocytin, neurobiotin

2. Ultrastructure (synapses and organelles)

- Electron microscopy: only method to verify synaptic contacts. Can be combined with immunohistochemical techniques. Very tedious.

3. Cytoarchitecture (nuclei and tracts)

- *Cationic dyes=Nissl stains.* Stain Nissl bodies (stack of rough endoplasmic reticulum) will reveal soma of neurons and nuclei of glia. *Cresyl Violet*, *Neutral red*, *toluidine blue, thionines*
- *Myelin stains* stains protein bounds phospholipids to reveal myelin sheath and myelinated fibber pathways, eg *Weigert stain*.
- *Reduced silver methods.* Deposits of reduced sliver on proteins of neurofibrils (neurofilaments +microtubules in soma, dendrites and axons).

4. Chemoarchitecture (chemical contents)

- *Enzymatic stains.* Stain neurones by activating endogenous enzymes: eg Acetylcholinesterase and NADPH-diaphorase (Nitric oxide synthase).
- *Immunohistochemical staining* of enzymes and neurotransmitters, eg, *serotonin, tyrosine hydroxylase.*

Fluorescent or opaque tag.

- *In situ hybridization.* Reveals specific mRNA. Use labelled cDNA sequences.
- *Gene knock-in*. Reporter gene like GFP is linked to a particular gene promotor
- *Receptor binding.* Reveals receptors. Uses radioactive ligands. Fresh tissue

5. Functional anatomy (neuronal activity)

- *Immediate early genes such as Fos, CREB, Jun, etc...* Immunohistochemical or in situ hybridization.

6. Tract tracing (neuronal connections).

- *Based on axonal transport.* Uses retrograde and anterograde transport systems along the axon. Tracers are taken up and transported from one end to the other.

Retrograde tracers. Reveal afferents to a brain region**.** Label soma. Fluorescent ones: *Fluorogold*, *red beads*, *green beads*, *Fast blue*, *Diamidino Yellow*. Non fluorescent ones: *Horseradish peroxidase (histochemical)*, *Cholera toxin B (immunohistochemical)*

Anterograde tracers. Reveal efferents of a brain region. Label axonal terminals and bouttons. Fluorescent ones: *dextran (red, green, blue)*. Non fluorescent ones: *Phaeseolus Leucoagglutinin* (PHA-L), *dextran* (biotinylated), *biocytin*, *3[H] leucine* (by autoradiography)

Transynaptic transport with virus. A living tracer! Neurotropic viruses, eg, *Herpes Simplex 1, Pseudorabies* will go transsynaptically (up to 2 or 3 synapses).

- *Based on diffusion (Lipophilic dyes).* Slowly diffuse along axonal membranes. eg *DiI* (red) and *DiA* (green). Only on very short distance (up to 5 mm). Ideal for embryos.

Imaging in Live Animals – [Dr C. Power]

The lecture will provide an overview of a number of imaging techniques including positron emission tomography (PET), micro-computed tomography (microCT), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and optical imaging systems including bioluminescence, fluorescence and intravital microscopy. The theory and application of each imaging technology will be discussed with emphasise on preclinical systems and research. When possible, examples of specific experiments and experimental results generated within the Biological Resources Imaging Lab at UNSW will be provided.

Immunohistochemistry Applications – Eye Research [A/Prof N. Di Girolamo]

This lecture will cover the basic principles of immunohistochemistry, a technique that is routinely used in diagnostic and investigative laboratories to identify different cells or the proteins they produce under either physiological or pathological conditions. The lecture focuses on a class of matrix dissolving enzymes known as the matrix metalloproteinases, the cells that produce them and how immunohistochemistry is used to detect their tissue location. Examples of their expression will be shown in ocular cancer and in rheumatoid arthritis. The advantages and disadvantages of this technique and other comparable techniques will be discussed. The second part of the lecture focuses on adult stem cells of the cornea, the diseases caused by their absence, how immunohistochemistry is used to detect corneal stem cells and a novel therapeutic strategy to treat conditions that develop in their absence.

Single –molecule and Super-resolution Fluorescence Microscopy (Part 1 and 2) [Dr T. Böcking]

The lectures will introduce the students to the concept, instrumentation and application of super-resolution and single-molecule fluorescence microscopy. After being chosen as method of the year by the journal Nature in 2008, super-resolution techniques have been developing rapidly and are set to make a major impact in cell biology and related disciplines as commercial instruments become available. The first lecture will present the physical principles underlying fluorescence microscopy, approaches to enable the imaging of single molecules (such as total internal reflection fluorescence microscopy) and how to use single molecule imaging to break the diffraction limit. Several different modes of super-resolution microscopy will be introduced and compared. The second lecture will be centred on the applications of these techniques with appropriate recent example to highlight how these techniques can illuminate questions that are not accessible with traditional approaches.

Applying Microscopy Techniques: Data Quantification [Prof. R. Kumar]

Seeing is believing, but most experimental research needs quantitative data and statistical analysis. In this lecture, research papers will be used as the basis for discussion of approaches to quantify microscopic findings. We will examine applications of morphometry in pulmonary research: for example, how the severity of inflammation can be quantified (both in H&E and immunostained sections); how cellular responses (such as goblet cell metaplasia in the airways) can be stratified; and how changes in lung structure (such as emphysema or fibrosis) can be assessed. Approaches to interpretation of data and some basic statistical concepts will also be reviewed.