ANAT3212 – MICROSCOPY IN RESEARCH

COURSE OUTLINE

Course Convenor: Dr. Thomas Fath

Contact:

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Semester 2, 2012

Lectures:

Location:

Central Lecture Block (CLB2)

Time:

Tuesdays 2pm-3pm

and

Wednesdays 5pm-6pm

Weeks 1-12

Laboratory Sessions:

Location:

Wallace Wurth Building, 1st Floor Rooms 109/110

Time:

Wednesdays 2 pm-4pm

and

Thursdays 2pm-4pm

Weeks 2-13

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.

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, and Atomic Force Microscopy. The course will thus help students to gain a better understanding of the correlation between structure and function.

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.

Format

Teaching will include lectures, laboratory demonstrations and practical sessions, as well as small group discussions. Students will gain experience in 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 different projects during the first half of the course. Students' preferences for individual projects will be taken into consideration. The following provides an overview on the projects that will be offered:

PROJECT 1: Cellular dynamics of sub-cellular compartments in neurons.
LECTURER: Dr Thomas Fath
SUMMARY: The motility of cellular regions in nerve cells such as growth cones at the tips of cellular processes is dependent on the dynamics of the underlying actin cytoskeleton. The motile behaviour of a neuronal growth cone is critical to allow for establishing of complex networks between nerve cells. The aim of this project is to visualise changes in growth cone motility in response to manipulation of the actin cytoskeleton.

PROJECT 2: Investigating the role of Tropomyosin during cell division.
LECTURER: Dr Galina Schevzov
SUMMARY: The actin cytoskeleton plays a critical role in regulating the progression of cells through the cell cycle. An important regulator of the structural organisation and dynamics of actin filaments is the actin-associated protein, Tropomyosin. This project proposal aims to 1) visualise the subcellular localisation of the fluorescently tagged Tropomyosin during cell division by live cell imaging and 2) evaluate the specificity of a Tropomyosin drug.

PROJECT 3: Using live cell imaging to characterize the anti-angiogenic properties of novel anti-cancer drugs.
LECTURER: Eddy Pasquier
SUMMARY: Angiogenesis, the formation of new blood vessels from pre-existing ones, is a crucial step in tumour growth and metastasis, which has become a major therapeutic target in the fight against cancer. Live cell imaging technology can be used to model tumour angiogenesis *in vitro* and study the effects of pro- or anti-angiogenic molecules. Here, we will use time-lapse videomicroscopy to characterize the anti-angiogenic properties of a novel anti-cancer agent. This will provide important insights into the mechanisms of action of this promising drug.

PROJECT 4: Using live cell imaging to characterize the anti-angiogenic properties of novel anti-cancer drugs.
LECTURER: Dr Till Böcking
SUMMARY: The laboratory session will be run in the Biomedical Imaging Facility (BMIF) utilising the state-of-the-art total internal reflection microscope. The students will investigate the dynamics and structure of protein complexes at the plasma membrane, eg clathrin-mediated endocytosis. In the first session, the students will be introduced to the BMIF with an OHS briefing and a tour of the instruments. The facilitator of the laboratory will then lead a discussion with the students about the experimental methods and aims involved in the lab. The students will proceed to prepare samples (culturing cells of cover-slips and fixation/staining of cells). In the second session the

students will examine the samples in different modes of fluorescence microscopy: bright-field (live cell imaging), TIRF illumination (live cell imaging), super-resolution (fixed samples). The final session will be devoted to quantitative image analysis using microscopy software and ImageJ.

PROJECT 5: Synaptic Vesicle Trafficking.
LECTURER: Dr Vladimir Sytnyk
SUMMARY: During the first session of the project, the students will obtain an introduction into the general organization of the work in the laboratory (including OHS issues) and the equipment that they will use. The students will conduct the preparatory work for the experiments in Session 2 & 3, including plating of neuronal cell line cells. In Session 2, students will load living neuronal cells with a vital stain of synaptic vesicles and observe labelling of organelles and unloading of the dye under the microscope.
In Session 3, students will repeat the experiment, and quantify the rate of dye unloading in the absence or presence of stimulation of synaptic vesicle recycling in cells.

PROJECT 6: Fluorescence microscopy in studying cellular lipid storage and trafficking.
LECTURER: Dr Rob Yang
SUMMARY: Session 1 (3hrs): specific OHS intro to our laboratory / background and introduction to cholesterol trafficking/lipid droplet dynamics. Session 2 (3hrs): setting up and performing the experiment: Nile red staining of lipid droplets, filipin staining of cholesterol, observation of GFP/mCherry tagged proteins, and immuno-fluorescence. Session 3 (3hrs): continuing experiment if necessary and analysis of data.

PROJECT 7: Cell differentiation.
LECTURER: Dr Mark Hill
SUMMARY: The experiment will involve growing neural cell lines. Differentiation of these cell lines under specific culture conditions. Finally, analysis using immunocytochemistry for a differentiation specific protein.

PROJECT 8: Intravital Imaging.
LECTURER: Prof Gary Housley
SUMMARY: Students will undertake real-time imaging of living neurons within the cerebellar region of the adult mouse brain. The imaging will be achieved using multi-photon excitation of green fluorescence protein expressed in GABAergic neurons in the cerebellum of a GAD67-GFP transgenic reporter mouse. The purpose of the project will be to initially contrast the (limited) performance of conventional visible light (single-photon excitation) confocal laser scanning microscopy (LSM) against multi-photon IR excitation for

imaging. Once proficiency is established, the work will proceed to determine of the fine structure of the dendrites in Purkinje neurons and determine the effect of hypoxia on that cytoarchitecture (mimicking the acute effect of stroke). This experiment, using gaseous anaesthesia in transgenic mice, has the approval of the UNSW Animal Care and Ethics Committee (ACEC) and will be undertaken in the Translational Neuroscience Facility (TNF), 3rd floor Wallace Wurth - south. The students will be inducted into the TNF and receive training on the Zeiss 710 NLO multiphoton microscope which utilizes a Spectraphysics MaiTai femtosecond pulsed IR laser system for deep tissue intravital imaging.

PROJECT 9: Chemotherapeutic drug screening using a High Content cell imager
LECTURER: Dr Justine Stehn
SUMMARY: In this project, we will demonstrate how a high content imaging microscope can be utilized for chemotherapeutic drug screening. Images acquired from the BD pathway will be used to quantitate cellular changes such as cell size, morphology and integrity of the actin microfilament system in tumour cells upon drug treatment.

Assessments:

Assessment activity	Duration	Value	Due Date
Report (Literature Research)	500 words	10%	Week 9
Oral Presentation (Literature Research)	5 min	10%	Week 9
Examination Terminology & Applications of Microscopy Techniques (Format: short answers)	1 hr	30%	Week 10
Project Individual Projects (two students per project). Students will visit the labs of active research groups. 10 research groups to choose from. (Format: written report including experience/reflection & evaluation of data)	2000-2500 words	30%	Week 12
Oral Presentation (Presentation of project experience; should cover a description of experimental design, data analysis and interpretation)	20 min	20%	Week 12
PLEASE NOTE: YOU MUST PASS ALL 3 COMPONENTS.			

Official Communication by e-mail

All students in the course ANAT3212 Microscopy in Research 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.

Attendance

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

Late Assessment Items will be penalized by 5% each day the assessment is late.

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.

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 www.equity.unsw.edu.au/disabil.html. 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, 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).

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/OHS.

Students must wear a lab coat and closed footware in research laboratories and comply at all times with SoMS health and safety requirements (see above).

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