ANAT3212 – MICROSCOPY IN RESEARCH

COURSE OUTLINE

Course Convenor: Dr. Thomas Fath

Contact:

e-mail: t.fath@unsw.edu.au

Ph: 9385 9690

Semester 2, 2014

Lecture:	Tue 10-11 Biomed D	Lab:	Tue 12-2 WW G16/17 and
	Thu 5-6 WW LG02		Fri 1-3 WW G16/G17
	Wks 1-12		Wks 1-12

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 super-resolution microscopy (e.g. PALM and 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.

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 10 and 11, short Projects will be carried out in research laboratories on the UNSW campus. Students will be assigned to the different projects in the first two weeks 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
SUPERVISOR: 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 5NM1 during cell proliferation
SUPERVISOR: 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 Tropomyosin 5NM1 and 2) a key component of the mitogen-activated protein kinase, ERK, known to be stimulated via growth factor stimulation, during initiation of cell proliferation.

PROJECT 3 Fluorescence Calcium Imaging in Neurons
SUPERVISOR: Dr John Power
SUMMARY: The concentration of calcium tightly regulated in cells and is maintained at 50-100 nM, despite being present at mM concentrations in the extracellular environment. Calcium is a key signalling molecule in cells. Rises in cytosolic calcium modulate nearly every cellular process from cell proliferation to apoptosis. Students will load live neurons with calcium sensitive fluorescent dyes. Using a fluorescent microscope equipped with a high speed camera, students will then examine the fluorescent calcium response evoked by application of different neuronal signalling molecules. The acquired fluorescent images will be analysed offline using ImageJ.

PROJECT 4 Live cell imaging of Rho GTPase fluorescent protein biosensors during cell migration

SUPERVISOR: Dr Liz Hinde

SUMMARY: The aim of this research project is to measure how the activity of the small GTPases Rac1 and RhoA cooperate to direct cell migration. By imaging Rac1 and RhoA fluorescent protein biosensors, which employs förster resonance energy transfer (FRET) as a readout of activation, we will detect and then analyse how these two Rho GTPases prepare the cell to move forward or backwards by fluorescence lifetime imaging microscopy (FLIM).

PROJECT 5 Synaptic Vesicle Trafficking

SUPERVISOR: Dr Vladimir Sytnyk

SUMMARY:

During the first session of the project, the students will obtain introduction into the general organization of the work in the lab (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 Cell topography by AFM

SUPERVISOR: Dr Celine Heu

SUMMARY: The cellular shape depends on the origin in the body and different organs will exhibit different cell shape. This project proposes to study by atomic force microscopy the topography of different cell lines. The aim of the project is to understand the strains implied by an AFM experiment on biological sample and to discover the operations and imaging settings for the use of AFM on cells.

This project will be run in the Biomedical Imaging Facility. In the first session the students will be introduced to the lab with an OHS briefing and a short technical training on the equipment. They will then have a discussion about the experimental method and the protocol. During the second session, students will carry out topography images of cells using AFM. The final session will be devoted to image analysis using microscopy software and ImageJ.

PROJECT 7 Cell differentiation

SUPERVISOR: Dr Mark Hill

SUMMARY:

The experiment will involve growing neural cell lines and differentiation of these cells under specific culture conditions. Finally, differentiation specific proteins will be analysed using immunocytochemistry.

PROJECT 8 Intravital Imaging
SUPERVISOR: 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, will have 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.

Assessments:

Assessment activity	Duration	Value	Due Details
Report (Literature Research)	1000 words	10%	Week 6
Oral Presentation (Literature Research)	5 min	10%	Week 6-7
Examination Terminology & Applications of Microscopy Techniques (Format: short answers)	1 hr	30%	Week 8
Oral Presentation on Project (Presentation of project experience; should cover a description of experimental design, data analysis and interpretation)	20 min	20%	Week 11-12
Project Report Individual Projects (aiming for two-three students per project). Students will visit the labs of active research groups. (Format: written report including introduction, methodology, results and discussion/experience)	2500 words	30%	Monday of Week 13

Official Communication by e-mail

All students in the course ANAT2241 Histology: Basic and Systematic 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

* 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.

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.

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% / day 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.

6. It is intended that a supplementary exam for the mid-session exam will be held in the week commencing Monday 22nd September, 2014.

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, 4th Floor, Wallace Wurth Building, Rm 447 (Email: k.ashwell@unsw.edu.au), or the Department of Anatomy's nominated Grievance Resolution Officer, Dr Priti Pandey, 2nd Floor, Wallace Wurth Building, Rm 214 (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.