UNSW AUSTRALIA | Medical Sciences Medicine

Department of Anatomy

ANAT 3212

MICROSCOPY IN RESEARCH

Semester 2, 2015

CRICOS Provider Code 00098G

ANAT3212 – MICROSCOPY IN RESEARCH
COURSE OUTLINE

Course Convenor: Dr. Thomas Fath
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Lecture:	Tue 9-10 WW LG02 Wed 10-11 WW LG02 Wks 1-12	Lab:	Tue 10-12 WW G16/17 and Wed 11-13 WW G16/G17 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.

Please read this manual/outline in conjunction with the following pages on the School of Medical Sciences website:

- Advice for Students
- Learning Resources

(or see "STUDENTS" tab at medicalsciences.med.unsw.edu.au)

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 of some of 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 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 9
Oral Presentation (Literature Research)	5 min	10%	Week 9
Examination Terminology & Applications of Microscopy Techniques (Format: short answers)	1 hr	35%	Week 10
Oral Presentation on Project (Presentation of project experience; should cover a description of experimental design, data analysis and interpretation)	20 min	15%	Week 12
Project Part I: Individual Projects (aiming for two-three students per project). Students will visit the labs of active research groups. (Format: written report including Introduction/Methods/Results/Discussion/References) Part II: Part II includes Reflection on Lit Report, project report and oral presentation I and oral presentation II	2000-2500 words 1500 words	25% 5%	Monday of Week 13 Monday of Week 13

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.