UNSW AUSTRALIA | Medical Sciences
Medicine

Department of Anatomy

ANAT 3212

MICROSCOPY IN RESEARCH

Semester 2, 2017

CRICOS Provider Code 00098G

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ANAT3212 – MICROSCOPY IN RESEARCH

Course Introduction

Microscopy in Research (ANAT3212) is an undergraduate course for 3rd year students. It is run by the School of Medical Sciences (SoMS) in conjunction with the Mark Wainwright Analytical Centre (MWAC).

Light and Optical Microscopy has undergone rapid transformation over the last two decades and in turn it has become an vital tool in the method toolbox of researchers. This course aims to provide a theoretical and practical foundation to the suite of microscopy techniques so that they can be applied to a particular research project or hypothesis. This course is well suited for future researchers in the medical/biological sciences.

Course Administration

Course Convenors

Course Convenor: A/Prof Thomas Fath

Head, Neurodegeneration and Repair Unit

School of Medical Sciences

Wallace Wurth Building East, Level 2, Rm 233

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

Ph: 9385 9690

Course Co-Convenor: Dr Renee Whan

Head, Biomedical Imaging Facility (BMIF), Senior Lecturer

Mark Wainwright Analytical Centre

Lowy Cancer Research Centre, Level 4, Rm 411A

e-mail: r.whan@unsw.edu.au

Ph: 93859342

A/Prof Fath and Dr Whan share responsibility for academic and administrative matters regarding this course. Please approach them for any questions or problems concerning the course. It is best to arrange an appointment in advance by email, copying both into the email.

Ms Nadia Ghafoorzada and Mr Dylan Lewis are available to help with administrative problems such as enrolment or scheduling. They can be found in the Student office, G27, Ground floor of the Biosciences Building (D26). Ph 93852426.

Email: SOMSenquiries@unsw.edu.au

If students are having difficulties of a personal nature, they should contact the School of Medical Sciences Grievance Officer, Dr Nick Di Girolamo, n.digirolamo@unsw.edu.au

Units of Credit

ANAT3212 Microscopy for Research 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.

Course Objectives

This is an advanced course in microscopy, which provides practical, research-oriented experience. There are four key objectives of this course;

1. Promote understanding of the principles and practice of core and advanced microscopy techniques for biomedical research

2. For students to develop skills in using microscopy to examine molecules, cells, tissue and organs to create relationships with structure and function.

3. Provide an excellent basis of core microscopy experiments for utilization in further research studies.

4. Develop oral and written communication skills, which underpin the dissemination of research findings.

Student Learning Outcomes

By the end of this course students are expected to have gained

- A demonstrable knowledge of microscopy, including specimen preparation, acquisition and image analysis.
- An understanding of the relationship between microscopy techniques and the data they produce.
- Be familiar with the application of microscopy for fields of research including but not limited to; cancer sciences, anatomy, neuroscience, physiology, immunology and biology.
- Experience and expertise in critical enquiry by designing and executing microscopy experiments to answer a scientific hypothesis.
- Work in collaborative teams to communicate concepts of microscopy use in state- of- the- art biomedical research in an oral presentation
- Work independently to communicate report and evaluate the microscopy within specialist scientific journals.
- Demonstrate reflective practice and teamwork in your development of professional skills.

Format

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

In weeks 10 to 12, short Projects will be carried out in research laboratories on the UNSW campus. Students will be assigned to the different projects in week 5 of the course. Students' preferences for individual projects will be taken into consideration. The summaries of the projects that will be offered in 2017 will be provided in the first week.

Timetable in brief:

Tuesday	11am-12pm	WW LG02
Tuesday	1-3pm	WWG16/17
Wednesday	3-4pm	WWLG02
Wednesday	4-6pm	WWG16/17

Attendance

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

There will be an attendance role taken in all classes.

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)

Timetable

Week	Date	Time	Venue	Lecturer	Title
1	Tues 25/7	11am-12pm	WW LG02	Thomas Fath & Renee Whan	Introduction: Course outline, Assessments, Projects
	Tues 25/7	1-3pm	WWG16/17	1. Michael Carnell 2. Mark Hill	1. Introduction to Light Microscopy (part 1) 2. Health and Safety in SoMS
	Wed 26/7	3-4pm	WW LG02	Suzanne Mobbs	How to Perfrom a Library Search
	Wed 26/7	4-6pm	WWG16/17	1. Jia Lin Yang 2. Michael Carnell	1. E-portfolio and statistics 2. Introduction ot Light Microscopy (part 2)
2	Tues 1/8	11am-12pm	WW LG02	Renee Whan	Fundamentals of Fluorescence
	Tues 1/8	1-3pm	WWG16/17	1. Renee Whan 2. Richard Francis	1. Principles of Fluorescence Microscopy 2. Core specimen preparation for microscopy and fixatives
	Wed 2/8	3-4pm	WW LG02	Richard Francis	Histology and Histochemistry
	Wed 2/8	4-6pm	WWG16/17	Renee Whan & Alex Macmillan	Tutorial 1: Know your Spectra and Filter Blocks
3	Tues 8/8	11am-12pm	WW LG02	Gila Moalem-Taylor	Neurohistology
	Tues 8/8	1-3pm	WWG16/17	Thomas Fath	1. Immunocytochemistry 2. Virtual Laboratory
	Wed 9/8	3-6pm	WW R116	Patrick de Permentier & Thomas Fath	Practical 1: Immunocytochemistry
4	Tues 15/8	11am-12pm	WW LG02	Patrick de Permentier	Overview of Practical 2
	Tues 15/8	1-3pm	WW R116	Patrick de Permentier & Thomas Fath	Practical 2: Standard and Special Stains for Light Microscopy
	Wed 16/8	2-3pm	LG02	Rakesh Kumar	Applying Microscopy Techniques: Data Quantification
	Wed 16/8	4-6pm	WW R116	Patrick de Permentier & Thomas Fath	Practical 3: Immunohistochemistry
5	Tues 22/8	11am-12pm	WW LG02	Thomas Fath & Renee Whan	Overview of Practical 4
	Tues 22/8	1-3pm	Lowy LG22-24	BMIF staff	Practical 4: Viewing Standard Stains and Immunocytochemistry
	Wed 23/8	3-4pm	WW LG02	Renee Whan	The 3rd Dimension: Optical Sectioning Techniques- confocal
	Wed 23/8	4-6pm	WWG16/17	Renee Whan and Sandra Fok	Tutorial 3: Myscope -Virtual Microscopy
6	Tues 29/8	11am-12pm	WW LG02	Sandra Fok	The 3rd Dimension: Optical Sectioning Techniques- lightshee
	Tues 29/8	1-3pm	WWG16/17	Renee Whan	The 4th Dimension : Dynamic imaging
	Wed 30/8	3-6pm	Lowy LG 22-24	BMIF staff	Practical : Confocal and Live cell imaging

Timetable cont.

Week	Date	Time	Venue	Lecturer	Title
7	Tues 5/9	11am-12pm	WW LG02	Nicole Bryce	Fluorescent Proteins for Live Imaging
	Tues 5/9	1-3pm	WWG16/17	1. Kate Poole 2. Alex MacMillan	1. Optogenetics and Voltage Sensing 2. FRET and FLIM
	Wed 6/9	3-4pm	WW LG02	Michael Carnell	Image Analysis
	Wed 6/9	4-6pm	WWG16/17	Michael Carnell	Practical: Image Analysis
8	Tues 12/9	11am-12pm	WW LG02	Senthil Arumugam	Superresolution and Single Molecule
	Tues 12/9	1-3pm	WWG16/17	1.Till Boecking 2. Gregory Redpath	1. Single molecule 2. Measuring Protein Interactions
	Wed 13/9	3-4pm	WW LG02	Paul Timpson	Molecular Biosensors for understanding cancer
	Wed 13/9	4-6pm	WWG16/17	1. John Power 2. Olivier Friedrich	1. Multiphoton Microscopy 2.TBC
9	Tues 19/9	11am-12pm	WW LG02	Annemiek Beverdam	Imaging of Protein Expression in Mice
	Tues 19/9	1-3pm	WWG16/17	Tzong Tyng Hung	Imaging in Live Animals: Preclinical Imaging
	Wed 20/9	3-4pm	WW LG02	Nick DiGirolamo	Applications for Eye Research
	Wed 20/9	4-6pm	WWG16/17	Presentations of Literature reports	
Mid Session Break 25/9-2/10					
10	Tues 3/10	11am-12pm	WW LG02	Thomas Fath	EXAM
	Tues 3/10	1-3pm	WWG16/17	1. Jia Lin Yang 2. Thomas Fath	1. E-portfolio and reflection checkin 2. Q & A-feeedback
	Wed 4/10	3-4pm	WW LG02	Project	
	Wed 4/10	4-6pm	WWG16/17	Project	
11	Tues 10/10	11am-12pm	WW LG02	Project	
	Tues 10/10	1-3pm	WWG16/17	Project	
	Wed 12/10	3-4pm	WW LG02	Project	
	Wed 12/10	4-6pm	WWG16/17	Project	
12	Tues 17/10	11am-12pm	WW LG02	Project	
	Tues 17/10	1-3pm	WWG6/7	Project	
	Wed 18/10	3-4pm	WW LG02	Project	
	Wed 18/10	4-6pm	WWG16/17	Project	
13	Tues 24/10	1-3pm	WWG16/17	Project Presentations	

NB: The practicals on Wednesday of week 3, as well as, Tuesday and Wednesday of week 4 will be held in a different location: WWR116, which is a wet lab. Please ensure

you are wearing close toed shoes and long hair is tied back. Please meet outside WWR116 prior to commencement of the practical

For practicals Tuesday of Week 5 and Wednesday of week 6. You will also be in a wetlab. On both days, please ensure you are wearing close toed shoes and long hair is tied back. Please meet in the normal allocated places (WWG16/17 and LG02 respectively) where you will then be taken to the microscope labs.

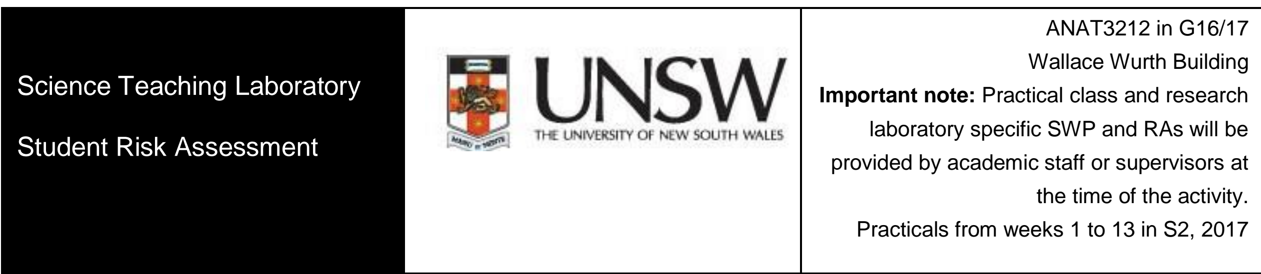
Health and Safety Guidelines

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 116 read and sign the Risk Assessment form on page 7 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.



Science Teaching Laboratory Student Risk Assessment	UNSW THE UNIVERSITY OF NEW SOUTH WALES	ANAT3212 in G16/17 Wallace Wurth Building **Important note:** Practical class and research laboratory specific SWP and RAs will be provided by academic staff or supervisors at the time of the activity. Practicals from weeks 1 to 13 in S2, 2017

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.
Practical class specific Hazards as outlined in practical class specific SWPs and RAs	Practical class specific Hazards as outlined in practical class specific SWPs and RAs	Practical class specific Hazards as outlined in practical class specific SWPs and RAs

Personal Protective Equipment

As indicated in practical class specific SWPs and RAs.

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

As indicate in practical class specific SWPs and RAs

Declaration

I have

A) Attended the ANAT3212 H and S lecture on 25th July ☐

B) I have read the lecture material of the H&S lecture given on 25th July ☐

And understand the safety requirements for the practical classes in ANAT3212, and I will observe these requirements

Signature:…………………………………………………………………..Date:………………………

Student Number:…………………………………………

Assessments- Summary

Assessment activity	Duration	Value	Due Details
Literature Research (*Report*) - Individual	1000 words	10%	Week 9
Literature Research (*Oral Presentation*) - Individual	5 min	10%	Week 9
Exam Terminology & Applications of Microscopy Techniques (Format: short answers) - Individual	1 hr	35%	Week 10
Research Project (*Oral Presentation*; Presentation of project experience; should cover a description of experimental design, data analysis and interpretation) - Group	20 min	15%	Week 13
Research Project (*Project Report*; Format: written report including Introduction, Methods, Results, Discussion, References) - Individual	2000-2500 words	25%	Week 13
Reflection Essay (Reflection on Lit Report, project report and oral presentation I and oral presentation II) - Individual	1500 words	5%	Week 13

Late Submission of assignments/reports:

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 the Monday afternoon after a Friday due date would attract a 30% penalty. Keeping to a deadline is part of 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 (A/Prof Fath) in writing at least 2 weeks prior to submission that this was likely.

Details of Assessments

1. Analysis of a Research Paper

You will be provided with a list of 10 papers which can be found on Moodle at the end of week 1. These papers include methods for;

I. Multi-labelling immunofluorescence

II. Enzyme-based Histochemistry

III. Confocal Microscopy - Biological samples or Live cells

IV. Intravital Microscopy

V. PALM, STORM or STED super resolution microscopy

Provide your first three preferences for the journal articles to the convenors **by the end of week 2 via Moodle**. Note that only 2 students per article will be permitted. You will receive a confirmation at the beginning of week 3 as to the research paper that forms the basis of your assessment.

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. What other microscopy methods could have been employed to answer the researcher's question

1a. Written Report for Research Paper Review

Your individual written report (10%) should be 1000 words maximum in length excluding references. Insert relevant images and diagrams to support your evaluation of the paper. The marking criteria for the literature review can be seen as APPENDIX 2

The Due Date is FRIDAY 22nd September 2017 (the end of week 9) NO LATER THAN 4 pm. Assignments are to be submitted to the Student office, G27, Ground floor of the Biosciences Building (D26), UNSW

For all submitted reports a coversheet (available on Moodle) should clearly state:

- Your Name
- Your Student Number

1b. Presentation

You will present your findings to the group and a panel of examiners (10%). The presentation will be 5 minutes.

All students are to complete a peer assessment feedback form for a fellow student. This will be randomly delegated by the examiners at the time of the presentations. Marks will be based on the quality of the feedback provided.

NOTE: The contribution of your peers' assessment is worth 25% of the Research Paper Presentation. The examiners assessment is worth 75% of the Research Paper Presentation

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.

The marking scheme for the presentation can be seen as APPENDIX 3.

Presentations will be on Wednesday 20th September 2017 in the scheduled class times shown in the timetable. A projector will be available for Powerpoint presentations

2. Exam

In week 10, a 1 hour exam (35%) will be held in the form of **short** answers. It will cover material presented in both lectures and practical classes. It will possibly include some calculations, so please bring a scientific calculator (not a phone). Smart watches are not allowed. The exam will be held on Tuesday 3rd October 2017 at 11am. Please ensure you arrive 5 minutes early.

For exceptional circumstances, a supplementary exam can be scheduled on Tuesday, 10th October 2017 at 11am. Please liaise with the course convenors.

3. Research Project

Following the programme of scientific study (weeks 1-9) you will have the opportunity to transition this knowledge into practise in the research environment. A number of short research projects utilizing microscopy can be found described on page 26 and others

will be added by the end of week 2. The projects will run from Wed week 10 to the end of week 12. It is expected that you shall carry out the program over at least 3 x 3 hours.

To choose you project you should submit to the convenors at the **end of Week 4 (via Moodle)** a preference 1-3 of the potential projects you would like to do. Note that only 2-3 students per project will be permitted. You will be notified at the beginning of week 5 as to your allocated project.

The students must contact their respective project supervisor via email by **Tuesday of week 6** to discuss project.

3a. Written Report for 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 marking criteria for the report can be seen in APPENDIX 4

The Due DATE is Friday 27th October, 2017 (the end of week 13) NO LATER THAN 4 pm. Assignments are to be submitted to Assignments are to be submitted to the Student office, G27, Ground floor of the Biosciences Building (D26), UNSW

For all submitted reports a coversheet (available on Moodle) should clearly state:

- Your Name
- Your Student Number

3b. Presentation of outcomes from the project

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 (if more than one person in a project), however marks will be issued individually.

PRESENTATIONS will be in week 13. A projector will be available for Powerpoint presentations

The marking scheme for the presentation can be seen as APPENDIX 5.

4. Reflection Essay - ePortfolio

The ePortfolio is part of your course assessment and encourages you to reflect on different aspects of their learning journey in this course within Moodle. Throughout the semester you will be asked to make 7 entries into the blog by answering a prompt question. Your response should be approximately 200 to 250 words. The entries are spaced throughout the semester and link to different aspects of the course. This will include Reflections on learning activities and assessment tasks carried out in the course.

You are required to post the reflection entries to OU Blog and submit the same blog to Turnitin for originality checks and marking. Instructions on how to submit blog entries to OU Blog and Turnitin are available within the ANAT3212 Moodle site.

When reflecting on your research experience within your ePortfolio, you should **NOTE** the following: The ePortfolio should be used as a reflective space, rather than discussing research content and any data or unpublished methods that may be presented in the research lectures and laboratories. You should **NOT** upload research data into your ePortfolio. If using WordPress as a personal blog, always set your ePortfolio to ‘private’ in order to avoid general visibility.

The seven entries are to be compiled into a single document which forms the basis of the reflective essay assessment task to be submitted at the end of the course.

Assessment of ePortfolio will be based on the following criteria: demonstration of engagement with ePortfolio, reflective practice, building an awareness of skills, including subject/course related skills, professional development and related skills, transferrable skills, development of career awareness and skills for future employability or post-graduate programs, work experience, personal values, strengths and weaknesses.

Key Dates for the Calendar

Date	Action	Who	Research Article Assessment	EXAM	Research Project Assessment	E-portfolio /reflection essay
Week 1- By Friday 28/7 5pm	Check Moddle for scientific articles for literature report	Students				
Week 2- By Friday 4/8 5pm	Provide your 3 top preferences of the research articles on Moodle	Students				
Week 3- Tuesday 7/8 11am	Provision of article for report and presentation	Convenors				
Week 4- By Friday 18/8	Examine the reseach projects offered and provide your 3 top preferences on Moodle	Students				
Week 5- Tuesday 22/8 11am	Provision of reseach project for report and presentation	Convenors				
Week 6- Monday 28/8 5pm	Make contact with the project leader via email to set up a meeting to discuss project	Students				
Week 7- By Friday 8/9 5pm	Make sure you are keeping up to date with your e-portfolio/reflection task.	Students				
Week 8- By Friday 15/09	You should be nearing completion of your presentations and reports for rsearch paper	Students				
Week 9- Wed 20/9 4-6pm	Presentations of Literature reports	Students				
Week 9- Friday 22/9 4pm	Submission of report on research paper review	Students				
Week 10- Tuesday 3/10	Marks from literature review and presentation on Moodle EXAM Feedback session	Students and convenors				
Week 10- Wed 4/10	Projects	Students				
Week 11	Projects	Students				
Week 12	1. Projects / Working on assessments 2. Exam marks online	1. Students 2. Convenors				
Week 13-Tues 24/10 1-3pm	Project Presentations	Students				
Week 13 - Friday 27/10 5pm	Submission of report and reflection essay	Students				
Week 14- Friday 3/11 5pm	Marks from Project online	Convenors				

Teaching Staff

Dr Senthil Arumugam [EMBL Australia Node in Single Molecule Science, School of Medical Sciences]

Dr Annemiek Beverdam [Senior Lecturer, Department of Anatomy, School of Medical Sciences]

Dr Nicole Bryce [Research Fellow, Department of Anatomy, School of Medical Sciences]

Associate Professor Till Böcking [EMBL Australia Node in Single Molecule Science, School of Medical Sciences]

Dr Michael Carnell [Research Associate, Biomedical Imaging Facility]

Mr Patrick De Permentier [Lecturer, School of Medical Sciences]

Professor Nick Di Girolamo [Department of Pathology, School of Medical Sciences]

Associate Professor Thomas Fath [CONVENOR, Department of Anatomy, School of Medical Sciences]

Dr Sandra Fok [Research Associate, Biomedical Imaging Facility]

Dr Richard Francis [Research Associate, Biomedical Imaging Facility]

Professor Olivier Friedrich [Chair and Head of Institute of Medical Biotechnology, University of Erlangen-Nuremberg]

Dr Celine Heu [Research Associate, Biomedical Imaging Facility]

Dr Mark Hill [Senior Lecturer, Department of Anatomy, School of Medical Sciences]

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

Dr Alexander MacMillan [Senior Research Scientist, Biomedical Imaging Facility]

Dr Gila Moalem-Taylor [Senior Lecturer, Translational Neuroscience Facility, School of Medical Sciences]

Dr Paul Timpson [Lab Head- Invasion and Metastasis, Garvan Institute for Medical Rsearch]

Dr Andrius Masedunskas [Research Associate, Department of Anatomy, School of Medical Sciences]

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

Dr Tzong Tyng Hung [National Imaging Fellow, Biological Resources Imaging Laboratory]

Dr Kate Poole [Senior Lecturer, EMBL Australia Node in Single Molecule Science, School of Medical Sciences]

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

Dr Gregory Redpath [Postdoctoral Fellow, EMBL Australia Node in Single Molecule Science, School of Medical Sciences]

Dr Renee Whan [Senior Lecturer, CO-CONVENOR, Head of Biomedical Imaging Facility]

Associate Professor Jia Lin Yang [Prince of Wales Clinical School]

Lecture Outlines

Introduction: Course outline, Assessments and Projects [Dr T. Fath & Dr R. Whan]

Aim: This lecture is intended to provide an overview of the course and it structure to students

Learning Objectives:

1. Understand the layout of the course and have knowledge of the timetable

2. Be aware of the assessment tasks and important dates

3. Know the purpose of short research projects in the second half of semester and key dates to be aware of

4. Understand the key aims of the course.

Introductory Microscopy: part 1 [Dr Michael Carnell]

Aim: In this first lecture on optical microscopy we will aim to lay some foundations for the course to come. Building on the laypersons view of a microscope past that of a magnification machine, highlighting that its primary function is more accurately described as a resolving and sampling machine. Secondary to this it is often utilized to engineer contrast into samples that innately have very little. We shall also introduce some basic properties of light and how it interacts with materials as this will help with understanding techniques and concepts addressed throughout this course.

Learning objectives:

1. To understand the primary reasons why imaging is undertaken by scientists, enhance resolution, introduce contrast and adequately sample the specimen.

2. Understand the properties of light relevant to basic light microscopy. Including light as a particle and a wave, refraction, diffraction and the resolution limit of light.

3. Clearly distinguish the difference between resolution and magnification.

4. Understand the optical aspects that determine the resolving power of an optical system

Health and Safety [Dr M. Hill]

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

1. How accidents and incidents happen and how to prevent them

2. The legal consequences of accidents and incidents

3. Laboratory safety: Chemical safety, Biological safety, Sharps, Ergonomics

4. Laboratory compliance: Personal protective clothing and equipment, Inductions, Training, etc

5. Emergency arrangements: Hazardous substance spills, Fires etc

6. 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.

How to Perform a Library Search [Ms S. Mobbs]

The class provide an overview of the services offered by the UNSW Library and will introduce students to effective research methods when using online bibliographic databases. Students will gain hands-on experience in searching Medline using medical subject headings (MESH) and using the Limits, Explode and Focus functions of the OVID search software. The class will also cover use of bibliographic management software such as RefWorks and Endnote to help students organise their research and assist in writing up their papers.

ePortfolio Enhances Professional and Career Development [A/Prof Jia Lin Yang]

Aim: The lecture series will introduce you to eportfolio learning and how it will enhance your professional and career development.

Learning Objectives:

1. Understand the significance and function of eportfolio as a learning tool and beyond.
2. Use eportfolio software for writing career goals, regular eportfolios and reflection.
3. Use eportfolio as resources for job/scholarship application.
4. Use eportfolio to enhance professional and career development.
5. Participate in career survey (5 min each) at both lectures.
6. Submit regular eportfolios and final (1000 words) summary at the 2nd lecture.

Points for discussion:

How eportfolios can improve your self-directed learning towards your career aspirations?

Additional Resources:

Yang, Jia-Lin, Patsie Polly, Thomas Fath, Nicole Jones, John Power. 2016. 'ISA model and integrative career development learning in year 3 science courses'. International

Journal of Science, Mathematics and Technology Learning. Accepted for publication on 7 June.

Introductory Microscopy: part 2 [Dr Michael Carnell]

Aim: In this second lecture on introductory microscopy we will cover some of the basic components of a modern compound optical microscope. We will discuss many of the factors that are important to consider in choosing microscope objectives, and highlight some common aberrations that can occur if care is not taken. We will conclude with some transmitted light based contrast enhancement techniques, covering the mechanisms by which they function, and how they should be setup.

Learning Objectives:

1. Identify basic components of the compound microscope.
2. Understand how refraction is utilized throughout the optical path in image formation, and how this can at times go wrong (chromatic and spherical aberrations).
3. Understand the types of objective lenses available and from its labelling identify both the conditions it specifies for its use, and the purpose it is fit for.
4. Identify images captured using Differential Interference Contrast (DIC), Phase Contrast and Dark Field microscopy, and be able to explain how these techniques introduce contrast into low contrast samples.
5. Learn the steps required for Kohler illumination to ensure correct illumination of specimens during transmitted light microscopy.

Additional Resources:

https://www.microscopyu.com/articles/optics/components.html

https://www.microscopyu.com/articles/optics/objectivespecs.html

https://www.microscopyu.com/articles/phasecontrast/index.html

Principles of Fluorescence Microscopy [Dr R. Whan]

This lecture outlines how we harness fluorescence properties in the microscope and then how we label specimens for fluorescence microscope techniques

Learning Objectives:

1. Describe the photophysical properties of fluorescence; absorption, excitation and emission and the Jablonski diagram.

2. Understand the concepts of photobleaching and quenching.

4. Be able to calculate resolution for given wavelengths using either the Abbe or Rayleigh Criterion

5. Be able to describe how fluorescence is harnessed in a microscope; Filters; excitation sources and cameras

6. Understand the different ways to label a specimen with fluorescence

Specimen Preparation for Light Microscopy (Part 1) [Dr R. Francis]

Aim: This lecture will introduce you to a range of techniques available for preparing samples for light microscopy

Learning Objectives:

1. Describe and understand the range of different techniques available for preparing samples for light microscopy.

2. Understand the advantages and disadvantages of each technique.

3. Be able to choose the best specimen preparation technique based on your experimental requirement.

Points for discussion:

Why are there so many ways for preparing samples for light microscopy? How would you choose the best technique? Is there one?

Additional Resources:

1. https://www.microscopyu.com/articles/confocal/confocalintropreparation.html

Specimen Preparation for Light Microscopy (Part 2) [Dr R. Francis]

Aim: This lecture will introduce you to Histology/Histochemistry

Learning Objectives:

1. Describe and understand some common histology techniques for mounting and sectioning biological samples.

2. Be able to discuss the advantages and disadvantages of each histology technique.

3. Describe and understand some common histochemistry stains.

4. Be able to choose the best histochemistry stain for different cells/tissues.

Points for discussion:

Why is Histology/Histochemistry still a popular specimen preparation technique? What are its advantages over other specimen preparation techniques?

Additional Resources:

1. http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/
2. http://www.jove.com/science-education/5039/histological-sample-preparation-for-light-microscopy

Neurohistology [Dr G. Moalem-Taylor]

Aim: This lecture will introduce students to various histological techniques that have been developed to investigate the structure, anatomical organisation and connectivity of the nervous system.

Learning Objectives:

1. Describe the morphology of neurons (soma, dendrites, axons) as manifested by the Golgi technique and cellular labelling.

2. Describe the ultrastructure of neurons (synapses and organelles) as revealed by electron microscopy.

3. Describe the cytoarchitecture of neurons (nuclei and tracts) and the chemoarchitecture (chemical contents) of neurons as shown by different stains and immunohistochemistry.

4. Understand the principles of tract tracing (neuronal connections) based on axonal transport including the use of retrograde and anterograde labelling, and trans-synaptic transport using viruses.

5. Discuss newly developed neurohistology methods in the past couple of years.

Immunocytochemistry [A/Prof 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 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).

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.

The Third Dimension: Optical Sectioning Techniques-Confocal [Dr R. Whan]

Aim: This lecture will introduce you to the third dimension in microscopy. The global aim is to understand the imaging techniques that allow optical sectioning of samples (cells and tissues)

Learning Objectives:

1. Know the different approaches that can be used to perform optical sectioning in light microscopy.
2. Understand the concepts defining confocal microscopy
3. Know and understand the basic components of a confocal microscope
4. Know the effect of the pinhole on axial resolution
5. Understand the idea of 3D reconstruction

Points for discussion:

What are the advantage and limitation of a confocal microscope, in other words for what kind of imaging would you use a confocal microscope? Compare confocal and wide field fluorescence imaging.

Additional Resources:

1. http://olympus.magnet.fsu.edu/primer/techniques/confocal/confocaljava.html
2. http://zeiss-campus.magnet.fsu.edu/articles/opticalsectioning/index.html
3. https://en.wikipedia.org/wiki/Light_sheet_fluorescence_microscopy

The Third Dimension: Optical Sectioning Techniques-Lightsheet [Dr S. Fok]

Aim: This lecture will introduce you to ligthsheet microscopy. The global aim is to understand how the imaging technique allows optical sectioning of samples (cells and tissues)

Learning Objectives:

1. Understand the concepts defining ligthsheet

2. Know and understand the advantages of lightsheet imaging over confocal

3. Be familiar with methods of specimen preparation for ligthsheet; including clearing and for live imaging.

4. Be familiar with Gaussian and Bessel beam lightsheet properties

Points for discussion:

What are the disadvantages of lightsheet microscopy as compared to confocal? What is the major challenge encounter with the data?

Entering the fourth dimension: Dynamic Imaging [Dr R. Whan]

Aim: This lecture will outline the specimen preparation, acquisition and analysis methods utilized when performing live cell imaging. Furthermore advanced light and optical techniques that often are used with live cells such as FRAP and photoactivation will be examined.

Learning Objectives:

1. Be able to describe the necessary environmental conditions for live cell imaging

2. Understand the different ways of labelling live cells; genetic encoding and probes

3. Be aware of problems that can be encountered when performing live cell imaging; drift; cell health, contamination

4. Be familiar with concepts of FRAP and photoactivation

Fluorescent proteins for live imaging [Dr N.Bryce]

Aim: This lecture will introduce you to fluorescent proteins and their use in cell biology. The global aim is to understand the principles of protein tagging and how fluorescent proteins can be used experimentally.

Learning Objectives:

1. Understand how GFP was discovered and developed.

2. Know how to attach a fluorescent protein to a protein of interest or target the fluorescent protein to a specific organelle

3. Know the different types of fluorescent proteins and their use in cell biology

Points for discussion:

What are the advantages and limitations of attaching a fluorescent tag to a protein? What types of experimental questions can you address with fluorescent proteins? What are the benefits/detriments of live cell microscopy with fluorescent proteins?

Additional Resources:

1. http://www.microscopyu.com/articles/livecellimaging/fpintro.html
2. https://en.wikipedia.org/wiki/Green_fluorescent_protein

Optogenetics and Voltage Sensing **[Dr K. Poole]**

Aim: This lecture will discuss the use of light-based techniques to investigate ion channel-mediated signalling in cells. The aim is to understand how light can be used to trigger electrical signalling in cells and how channel activity can be monitored using voltage sensitive proteins.

1) Understand the development of optogenetic tools and how they function

2) Understand the broad types of processes that can be addressed using optogenetics

3) Understand how ion channel activity can be measured using voltage sensing dyes/proteins

Points for discussion:

What are the advantages and limitations of light-based methods for investigating cellular electrophysiology? What improvements are needed in the field to extend the capabilities of these research tools?

Förster Resonance Energy Transfer (FRET) and Fluorescence Lifetime Imaging Microscopy (FLIM): [Dr A. MacMillan]

The development of fluorescent protein biosensors coupled with live-cell imaging has enabled the visualization of intracellular molecular dynamics with high spatiotemporal resolution. The great majority of biosensors based on fluorescent proteins employ a FRET (Förster resonance energy transfer) interaction to respond to the level of cellular activity being probed. Given that these molecular tools are designed to report on the spatial localization of specific signalling events, detection and quantitation of FRET as a function of time and space in cells is a matter of great interest. In this lecture we will cover what is FRET, how is a FRET biosensor constructed and how FRET is detected in live cells.

FLIM (Fluorescence Lifetime Imaging Microscopy) is a technique in cell biology to map the lifetime within living cells, tissues and whole organisms. The fluorescence lifetime of a molecule is sensitive to the physical and chemical properties of its environment and thus FLIM can be used to detect the viscosity, pH and degree of oxidative stress in live cells. Another major application of FLIM is to quantitate fluorescent protein interaction

by FRET. In this lecture we will cover the theory behind FLIM, how lifetime measurements are performed and applications to studying intracellular dynamics.

Image processing and analysis [Dr M. Carnell]

Aim: When performed correctly microscopy is a powerful scientific and analytical technique. The image itself is deemed data and great care must be taken when processing it for presentation and also when drawing conclusions from its content. Here we aim to cover what makes up a digital image as well as many of the common processes and operations that are carried out on them.

Learning Objectives:

1. Understand the composition of an image including the terms: pixel, bit-depth and look-up-table (LUT)
2. Distinguish between common non-destructive operations (e.g. adjusting contrast, merging 3 8bit channels into an RGB image) and destructive operations (e.g. applying contrast, merging 3 16bit channels into an RGB image).
3. Understand and perform common image processing tasks: Contrast Enhancements, Merging channels, adding annotations and time stamps, altering look-up-tables.
4. Understand and perform common image analysis tasks: Manual measurements, threshold based object counting, threshold based measurements, background correction.

Superresolution Microscopy [Dr S. Arumugam]

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.

Single molecule Microscopy [A/Prof T. Böcking]

The lecture will introduce the students to the concept, instrumentation and application of single-molecule fluorescence microscopy. The 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.

Visualise protein interactions **[Dr G. Redpath]**

Aim: The purpose of this lecture is to demonstrate the many ways imaging can be used to probe for molecule interactions

Learning Objectives:

1. Understand why it is crucial to be able to visualise interactions between molecules

2. Know which microscopy techniques can be used to investigate if two proteins interact

3. Being able to identify which techniques should be apply to a given experimental question

Point for discussion: Why would we choose imaging to investigate protein interaction rather than other experimental approaches?

Multiphoton and Intravital Microscopy [Dr John Power]

Aim: The aim of this lecture is provide an introduction to microscopy techniques used to visualise biological processes in live animals. The lecture will discuss the challenges associated with cellular imaging in live tissue and the microscopy techniques used to overcome them. The lecture will explain the theoretical basis for multi-photon fluorescence microscopy and how the technique is used to image physiological processes at the cellular and molecular level *in vivo*, in real-time.

Learning Objectives:

1. Understand the challenges associated with cellular imaging of live tissue.

2. Understand the theoretical basis of two-photon fluorescent microscopy.

3. 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.

Points for discussion:

Compare theoretical spatial and temporal resolution of wide field, confocal, and multiphoton fluorescent microscopy. How do tissue depth and fluorophore intensity impact each imaging technique. Why is a pulsed laser used for multiphoton excitation?

Additional Resources:

1. Helmchen, F and Denk, W. (2005) Deep tissue two-photon microscopy Nature Methods. 2, 932 – 40. http://dx.doi.org/10.1038/nmeth818

2. Svoboda, K and Yasuda, R. (2006) Principles of Two-Photon Excitation Microscopy and Its Applications to Neuroscience. Neuron 50(6):823-39. http://dx.doi.org/10.1016/j.neuron.2006.05.019
3. http://www.jove.com/video/50885/two-photon-calcium-imaging-mice-navigating-virtual-reality
4. https://micro.magnet.fsu.edu/primer/techniques/fluorescence/multiphoton/multiphotonhome.html

Intracellular Intravital Microscopy [Dr A. Masedunskas]

Aim: This session will introduce you intravital microscopy and will focus primarily on intracellular intravital microscopy in mice and rats. The main goal is to cover the available imaging modalities and to understand technical considerations that make such microscopy possible.

Learning Objectives:

1. Know what intravital microscopy is and what it can do for us in our quest to understand how living things work. Why would we want to do such technically challenging experiments?

2. Know what the modalities of microscopy are that can be applied to intravital imaging (two broad categories: single-photon and multi-photon). Based on which considerations would you choose a given modality over another for your intravital work?

3. What are the benefits of intracellular intravital microscopy and what type of quantitative assays can be performed?

4. Understand technical considerations that make intracellular intravital microscopy possible and current limitations. What are the best suited imaging modalities for this?

Points for discussion:

Can you think of new microscopy approaches / techniques that can be adapted for intravital? For example, do you think single molecule detection and tracking is possible in live animals? How can we achieve this?

Imaging in Live Animals – [Dr T. Hung]

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.

Imaging of Protein Expression in Mice [Dr A. Beverdam]

TBA

Immunohistochemistry and Immunofluorescence in Eye Research [A/Prof N. Di Girolamo]

Aim: This lecture will introduce you to the basic principles that underpin IHC and IF

Learning Objectives:

1. Describe and understand the process of immunohistochemistry and immunofluorescence

2. Understand where stem cells reside in the cornea and their importance to vision

3. Understand how to mark these cells with antibodies and report on their location

4. Understand how to genetically mark cells with fluorescent proteins and how to visualise their dynamics under fluorescence and intravital microscopy.

Project Outlines

PROJECT: Cellular dynamics of sub-cellular compartments in neurons

SUPERVISOR(S): Dr Thomas Fath and Sandra Fok

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: Intravital intracellular microscopy of exocytosis of secretory granules in living mice.

SUPERVISOR(S): Dr Andrius Masedunskas and Marco Heydecker

SUMMARY: Exocrine glands (such as pancreas, salivary glands) accomplish secretion of enzymes by delivery and fusion of large secretory granules at the apical pole of polarized epithelial cells. After membrane fusion step, actin coat is assembled around the granule that is crucial in facilitating the completion of granule exocytosis. In this project, we will investigate the assembly kinetics of actin cytoskeleton machinery on the secretory granule and its role in exocytosis in live mice by using high resolution intravital

microscopy. We will utilize transgenic mice and targeted drug delivery as well as image processing and quantitative analysis to achieve the aims of this project.

PROJECT: Detection of protein expression and cell proliferation in tissue sections of wildtype and genetically manipulated mice

SUPERVISOR(S): Dr Annemiek Beverdam

SUMMARY: Yes-associated protein activates epidermal stem/progenitor cell proliferation and is thought to be a key molecular player in skin cancer and eczema. This project seeks to examine protein expression of other key molecules that are associated with YAP in tissue sections from transgenic mouse models. Immunolabeling, confocal microscopy and analysis of the protein expressing

PROJECT: Imaging mechano-sensing complexes in melanoma cells

SUPERVISOR(S): Dr Kate Poole

SUMMARY: The migration of melanoma cells is influenced by the cell's ability to sense their mechanical surroundings. Recent data has identified a novel mechanically-gated ion channel that is required for directional migration of melanoma cells in response to extracellular matrix cues. What is not known is how the activity of this channel can influence migration. This aim of this project is to visualise focal adhesion proteins to determine if channel activity modulates focal adhesion structures in melanoma cells.

PROJECT: Live imaging of migrating T cells in an in vitro tumour model system

SUPERVISOR(S): Dr Sophie Pageon

SUMMARY: TBA

Practicals and Tutorials

Know your Spectra and Filter blocks [Dr R. Whan and Dr A. MacMillan]

Part 1: Fluorescence and Common Fluorophores

The importance of knowing and understanding fluorescence profile of the dyes cannot be overstated. It underpins all fluorescence microscopy techniques. Not knowing them leads to many of the common artifacts and errors in acquiring and interpreting microscopy data.

In this tutorial we will practise and exercise using single and multiple fluorophores together accurately. There are a number of online tools you can utilise to find the spectra of a given fluorophore

1. Semrock Search light: http://searchlight.semrock.com/

2. Invitrogen/Life Technologies Fluorescence spectraviewer: http://www.lifetechnologies.com/au/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html

3. Chroma Spectra Viewer: http://www.chroma.com/spectra-viewer

Each has its own advantages and disadvantages: in this tutorial we will utilise the Life technologies site

Part 1: Identify the spectral properties

1. Fill in the spectra properties of the given fluorophores in the following table

2. Utilise common laser lines on the confocal microscope to show the corresponding normalised spectra:

The common lasers in the BMIF are: 405, 440, 458, 470, 488, 514, 532, 543, 561, 594, 633, 640, 647. We also have a White light laser (WLL) that allows you to pick any wavelength between 470-670nm

3. When you excite a fluorophore to emit, the collection of the emission MUST be 10nm away from the excitation source. Tabulate the values you would choose for the emission collection.

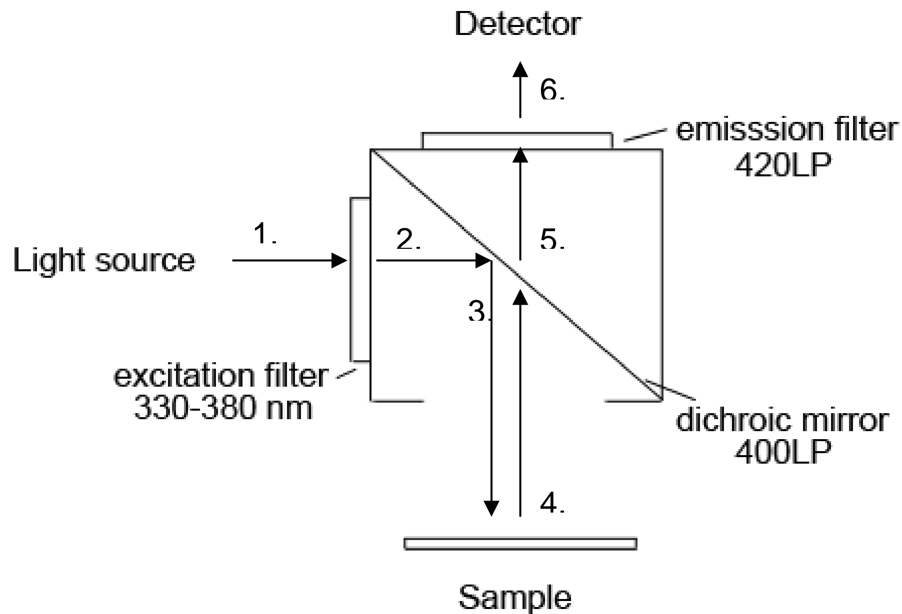
TABLE 1: Properties of Common Fluorophores

Fluorophore	Excitation maximum (nm)	Excitation spectrum range	Emission maximum (nm)	Emmision spectrum range	normalised fluorescence with Standard lasers	Corresponding Emission collection
Rhodamine 123	507	425-547	529	500-650	458nm =13%	500-650
					488nm =45% 514nm = 83%	500-650, 525 - 650
					WLL = 507nm =100%	520-650
LysoTracker Blue						
Hoechst 33342						
Propidium Iodide						
FITC						
GFP						
AlexaFluor 568						
Texas Red						

Notes:

Part 2: Filters

We will examine in detail some of the filter sets that are commonly used in epifluorescence microscopy. Below is the scheme of the filter block UV-2A.



1. Light source is a Hg Lamp, light is wavelengths of ~300-800nm

2. The excitation filter allows light of wavelengths 330-380nm through to the dichroic filter

3. The dichroic mirror reflects light of wavelengths of 330-380nm to the sample

4. The emitted light from the sample is returned to the filter block

5. The dichroic filter allows light of wavelengths of > 400nm through to the excitation filter.

6. The emission filter allows light of wavelengths > 420 nm through to the detector.

Table 2 shows a number of common filter blocks (plus a couple of imaginary ones). Given the excitation, dichroic and emission filters, fill in the wavelengths of light for paths 1-6 as shown in the above scheme for UV2A.

Filter Block	Excitation Filter	Dichroic Filter	Emission Filter	1	2	3	4	5	6
B2A	450-490	505LP	520LP	300-800			emitted light from sample		
G2A	510-560	575LP	590LP	300-800					
Cy5.5	665/45	695LP	725/50	300-800					
CFP	435/20	455LP	480/40	300-800					
Texas Red	540-580	595LP	600-660	300-800					
EMU1	540/50	560LP	590SP	300-800					
V2A	380-420	430LP	450LP	300-800					
YFP	500/20	515LP	535/30	300-800					
RW1404	380-420	400LP	515SP	300-800					
XF104	500/25	525LP	545/35	300-800					
EK2903	590/30	625LP	650LP	300-800					
Narrow Blue	480/20	505LP	535/40	300-800					
FMT07	450LP	525LP	580LP	300-800					

Part C: **Multiple fluorophores**

The table below shows a number of combinations of fluorophores. Some are good combinations and others are poor. Make sure you look at each compounds use and fluorescent properties to decide whether they are a good match. The goal is to be able to separate the fluorophores through a sequential acquisition. Utilise common laser lines on the confocal microscope to show the corresponding normalised spectra:405, 440, 458, 470, 488, 514, 532, 543, 561, 594, 633, 640, 647. Assume the confocal has spectral detectors

Table 3: Multiple fluorophore separation

Fluorophores	optimal excitation with Standard lasers	Transmission of fluorescence	Does this laser also excite the other fluorphore?	IF they cannot be separated by exciation can they be separted by emission?	What sequential collections would you use? Or is the experiment problematic	Which filters from Table 2 could be used to isolate the emissions of the dual labelled
Propidium Iodide	532	17%	no	NA	532ex 550-730nm em	TEXas Red
Hoechst 33342	405	5%	yes (~4%)	yes	405ex 415-540nm em	RW1404
FITC						
TRITC						
Cy3						
Cy5						
CFP						
YFP						
LysoTraker Blue						
ER Traker Green						
DAPI						
SYTO 59						

TIPS:

Load all spectra on.

Utilise the normalisation tool

Look at the excitation first

Then if there is multiple excitations look to the emission profiles.

Look at each emission separately under the influence of each laser

Then look to restrict the collection

Standard and Special Stains [Dr T. Fath, Mr P. de Permentier]

AIM:

1. To perform H&E, Toluidine Blue and PAS/Alcian Blue staining
2. Interpret the results obtained from each staining procedure
3. Identify artifacts in stained sections

METHOD:

Students will each perform the following stains

1. ***Haematoxylin & Eosin***
2. ***Alcian Blue Pas***
3. ***Toluidine Blue***

TECHNIQUE:

SOLUTIONS

1% ALCIAN BLUE (pH 2.5)

Alcian Blue 8GX 1g

Distilled Water 97mls

Acetic Acid (glacial) 3mls

1% PERIODIC ACID

50% Periodic Acid (Frig) 10mls

Distilled Water 490mls

SCHIFFS REAGENT

(COMMERCIAL Fronine)

HAEMATOXYLIN

(COMMERCIAL Fronine)

1% ACID ALCOHOL

(COMMERCIAL Fronine)

0.1% TOLUIDINE BLUE

Toluidine Blue 0.5g Distilled Water 500mls

HAEMATOXYLIN & EOSIN

STAINING PROCEDURE

1. Xylol 2mins
2. Xylol 2mins
3. Absolute Alcohol 1min
4. Absolute Alcohol 1min
5. 70% Alcohol 1min
6. Wash in running water 1min
7. Wash in running water 1min
8. Haematoxylin -.. 5mins
9. Wash in running water 2mins
10. 1% Acid Alcohol - .... 3 dips
11. Wash in running water 1min
12. Scott's Blue Solution 1min
13. Wash in running water 1min
14. Eosin 4mins
15. 70% Absolute Alcohol .... rinse
16. Absolute Alcohol 2mins
17. Absolute Alcohol 1min
18. Absolute Alcohol 1min
19. Xylol 2min
20. Xylol 1min
21. Xylol 1min
22. Mount with Ultramount

ALCIAN BLUE/PAS STAIN

1. Xylol 2mins
2. Xylol 2mins
3. Absolute Alcohol 1min
4. Absolute Alcohol 1min
5. 70% Alcohol 1min
6. Wash in running water 1min
7. Wash in running water 1min
8. Alcian Blue .... 20 min (filter stain)
9. Distilled Water .... Wash
10. Periodic acid .... 10 min
11. Distilled Water .... Wash
12. Schiff's reagent .... 10 min
13. Running Water .... 10 min
14. Haematoxylin (Shandon Instant) .... 3 min
15, Distilled Water .... Wash
16. 1% Acid Alcohol .... 3-4 dips
Differentiate
17. Running Water .... Wash
18. Scotts Blue .... 1 min
19. Distilled Water .... Wash
20. 70% Alcohol .... Wash
21. 90% Alcohol .... Wash
22. Absolute Alcohol x2 .... Wash
23. Xylol 2min
24. Xylol 1min
25. Xylol 1min

Mount with Ultramount

TOLUIDINE BLUE STAIN

SOLUTIONS

STAINING PROCEDURE

1. Xylol 2mins
2. Xylol 2mins
3. Absolute Alcohol 1min
4. Absolute Alcohol 1min
5. 70% Alcohol 1min
6. Wash in running water 1min
7. Wash in running water 1min
8. Toluidine Blue .... ½ - 1 minute (45 seconds)
9. Distilled Water .... Rinse rapidly
10. 70% Alcohol .... Wash
11. 90% Alcohol... Wash
12. Absolute Alcohol x2.. Wash
13. Xylol 2min
14. Xylol 1min
15. Xylol 1min

Mount with Ultramount

MATERIAL REQUIIRED: Unstained Slides: Intestine- PAS/Alcian Blue Skin-Toluidine Blue

REFERENCES:

Theory and practice of Histotechnology, 2nd ed. Sheehan & Hrapchak. The CV Mosby Co. 1980.

Theory and practice of Histological Techniques, 4th ed. Bancroft & Stevens. Churchill Livingstone 1996.

Virtual Laboratory [Dr T. Fath, Mr P. de Permentier]

Fluorescence-based immunohistochemistry allows the localisation of proteins to specific areas and specific cell types in animal tissues. Commonly, tissues can be prepared by paraffin embedding or cryopreservation.

Aims:

1. To become familiar with the critical steps of tissue preparation
2. To learn Immunohistochemical staining techniques that are used to analyse protein expression in animal tissue
3. To understand the use of fluorescence-based double-immuno labelling to analyse co-localisation of proteins => testing of cell- and tissue-specific expression of proteins

Method/technique

The virtual laboratory is a computer-based learning exercise and includes a theoretical introduction to immunohistochemical staining procedures, short video sequences of the activities and an interactive module in which you can carry out immunohistochemical staining.

SUGGESTED READINGS

Fallini B & Taylor CR. (1983). New developments in immunoperoxidase techniques and their application. Arch Pathol Lab Med, 107, 105-117.

Harlow E & Lane D. (1999). Using Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press: New York.

Taylor CR. (1978). Immunoperoxidase techniques. Practical and theoretical aspects. Arch Pathology Lab Med, 102, 113-121.

Taylor CR, Phil D & Kledzik G. (1981). Immunohistologic techniques in surgical pathology - A spectrum of "new" special stains. Human Pathology, 12, 590-596.

DAKO, Educational Guide, Immunohistochemical Staining Methods, Fourth Edition, 2006.

Immunofluorescence: Cell Cultures [Dr T. Fath, Mr P. de Permentier]

AIM: To acquire skills in designing and carrying out experiments employing cell culture and immunofluorescence based techniques. To understand the importance and role of cytoskeletal elements in neuronal cells.

METHOD:

Immunocytochemical analysis of cultured differentiated and undifferentiated mouse neuroblastoma cells using epi-fluoresecence microscopy.

TECHNIQUE:

Fixation and immunostaining of cells

- Remove coverslips from the wells of culture plates and place into plastic dishes prepared for staining
- Aspirate off any PBS and apply a drop of MeOH (sufficient to cover the whole of the coverslip) OR 0.2% NP40 (detergent)
- Incubate for 5 min
- Wash 5 x 2 min with PBS
- Wash 5 x 2 min with blocking solution
- Remove washing solution and apply primary antibodies (diluted in blocking solution)
- After 45 min wash 5 x 2 min with PBS
- Remove washing solution and apply secondary antibodies and the nuclear stain DAPI and fluorochrome tagged Phalloidin (diluted in blocking solution)
- After 30 min incubation wash 5 x 2 min with PBS
- Mount coverslips on glass slides

A figure for the application of primary antibodies will be provided on the day of the course

MATERIAL REQUIIRED:

Media and Solutions

- FBS (Fetal Bovine Serum)
- PBS (Phosphate Buffered Saline) Glass- and Plasticware
- Glass coverslips
- 4-well plastic dishes
- 10 cm culture dish (used as staining chamber)

- Parafilm
- 2x fine tipped forceps

Reagents

- 2 X Primary antibodies-TBA
- Fluorophor-tagged Phalloidin (probe to detect filamentous actin)
- 2 x secondary antibodies-TBA
- Nuclear Dye DAPI (4',6-diamidino-2-phenylindole)

READINGS

- B. Alberts, Molecular Biology of the Cell, 2008, 5th Edition, Chapter 16, pp.965-1052. (Extensive coverage on the Cytoskeleton)
- Dehmelt et al.: Journal of Neuroscience, (2003), Vol. 23(29), pp.9479-9490. (The use of N2a neuroblastoma cells in the analysis of the neuronal cytoskeleton)
- Gunning et al.: TRENDS in Cell Biology, (2005), Vol. 15(6) 2005 pp. 333-341. (The Biology of Tropomyosins, a family of actin-filament associated proteins)

Brightfield Imaging – Microscopic examination of stained tissue samples

[Dr T. Fath, Mr P. de Permentier]

AIM:

1 Understand the principles of tissue processing

2 Familiarise yourself with brightfield examination of stained tissues

METHOD:

Station 1: Students will observe H&E stained tissue sections [fixed with different fixatives and for different times] using brightfield illumination.

Station 2: Tissue sections stained with H&E, Alcian Blue/PAS and Toluidine Blue in the previous practical class will be examined using brightfield illumination.

TECHNIQUE:

Students are to examine stained slides provided in the class and slides that were stained in the previous practical class. For the H&E stained tissue sections that were fixed with different methods, comment on the type of tissue and the cell morphology ie size of the nucleus, cytoplasmic detail etc. Overall, which fixatives give clearer results? Why? What is the impact time has on fixation?

Insert observations in the worksheet contained in your manual.

MATERIAL REQUIIRED:

H&E, Alcian Blue/PAS and Toluidine Blue stained slides

REFERENCES:

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

Analysis of Neuronal, Morphology [Dr T. Fath, Mr P. de Permentier]

Continued from previous practical class. Examination of stained cultures

The focus in examining the stained cultures will be with regards to:

- cell density (using 10x or 20x air objective)
- cell morphology (using 40x air objective)
- sub-cellular localisation of examined proteins (using 60x oil objective)

At the end of the lecture and practical, you should be able to answer the following:

1. What effect does the application of retinoic acid have on cell proliferation?
2. What effect does the induction of differentiation have on cell morphology?
3. Where does the protein of interest localises to in the cell and what biological significance does this localisation suggest for the function of the protein (segregation of different tropomyosin isoforms to distinct sub-cellular compartments)?

Biomedical Imaging Facility Session [Dr R. Whan]

In this 3 hour practical you will have a hands-on session of live cell imaging, confocal microscopy, TIRFM and multiphoton microscopy. The hardware and software on the microscopes will be shown and we will highlight how to use them to obtain an image.

More details to be posted on Moodle

Integrative ePortfolio 2 [A/Prof Jia-Lin Yang]

Aim: To engage student learning and improve personal reflection, the tutorial will focus on how career and reflective ePortfolios to deliver integrative career development learning.

Points for discussion: What is the relationship between Integrative eportfolio and integrative career development learning? What is a new model of integrative eportfolio learning talking about (an eportfolio-DOTS-CDMSE model, Yang et al 2014)? What other knowledge and skills are required to improve employability apart from obtaining a Bachelor of Science degree?

Contents: This tutorial will introduce evidence that integrative eportfolio (career and reflective eportfolios) can improve student engagement and reflection in integrative career development learning as well as improving student confidence in their ability to make decisions about their own careers.

Image Analysis Practical [Dr M. Carnell]

In this practical you will gain the basic image analysis skills necessary for a career in science. This will involve basic image processing for presentations and publications. As it is also important to know how not to process images, you will take on the role of a scientific journal publisher and analyse a collection of images to identify a range of bad practices and deliberate attempts to falsify data.

By the end of this practical you should understand what makes up a digital image and be familiar with the terms bit-depth, dynamic range and look-up tables. We will also cover the differences between some of the most common file types, and which are the most appropriate to use. The processing of images will be carried out using ImageJ, a freely available image processing software commonly used by scientists. It will conclude with a quick glimpse into the range of image analysis techniques used to quantify data acquired with light microscopy.

APPENDIX 1: What makes a good presentation?

Some tips for using PowerPoint slides

- Generally 1-2 min per slide
- Use white or light colour backgrounds with dark text
- Avoid distracting backgrounds
- Minimise text (no essays!)
- 6 words per line, 6 lines per slide MAXIMUM
- Use large font, graphics
- Use pictures instead of text where you can

Some tips on structuring a presentation

- Title Slide: Title, author, affiliation.
- Rationale: Why this is interesting.
- Aim: Why are you doing this.
- Methods: What you did.
- Results: What did you find and what does it mean.
- Summary: One thing you want them to remember.
- Acknowledgements/References.

Some tips on structuring the introduction and body of a presentation

- Introduce yourself
- Capture the attention of the audience and draw them into the topic (agenda)
- Establish rapport with the audience and motivate them to listen
- In the body of the presentation make sure you include the Aim, Methods, Results
- Provide a logical framework which addresses the aim of the presentation
- Break the content into understandable parts (usually no more than five)
- Develop these main points through appropriate supporting material

Some tips on structuring the conclusion of a presentation

- Summarise the main points
- Examine implications (if any)
- End on a positive and engaging note

Some tips on presenting

ƒ Practice, practice, practice

ƒ Obtain feedback, and use it

ƒ Be ruthless - delete unnecessary information

ƒ Speak loudly & clearly

- Use short simple sentences
- Avoid jargon & abbreviations
- Vary pitch, tone, volume, speed and pauses
- Avoid distracting mannerisms
- Relax, be enthusiastic
- Make eye contact
- Keep an eye on the time remaining
- Explain figures, and point to important aspects
- Give a clear and concise summary, then stop
- Don’t go overtime. Ever.

Tips on handling questions

- Anticipate likely questions and prepare extra slides with the answers
- Maybe even plant a stooge
- Paraphrase questions:
- so that other people hear the question.
- to check your understand of the question.
- to stall while you think about an answer.
- If you don’t know the answer, say so
- Offer to find out
- Ask the audience

APPENDIX 2: Marking Criteria for Research Article Report

Criteria	Review of Research Article (Mark out of 10 for each marking criteria)						
	10-9.0 Outstanding	8.9-8.5 Excellent	8.4-8.0 Very Good	7.9-7.5 Good / Average	7.4-6.5 Fair	6.4-5.0 Poor	4.9-0 Very Poor
Introduction of Research Article **___/10 x 1.5**	Very comprehensive, detailed and focused introduction.	Highly detailed and focused introduction.	Detailed and focused introduction	Detailed introduction.	Some key basic information missing in introduction.	Major lack of key basic information in introduction.	Lack of significant detail in introduction.
Critical Analysis* **____/10 x 3.5**	Comprehensive critical analysis of strengths and limitations of the imaging technique(s) used in the research article.	Critical analysis of strengths and limitations of the imaging technique(s) used in the research article	Some critical analysis of strengths and limitations of the imaging technique(s) used in the research article	Some critical analysis of strengths and limitations of the imaging technique(s) used in the research article but mostly descriptive.	Limited critical analysis of strengths and limitations of the imaging technique(s) used in the research article but mostly descriptive.	Very limited critical analysis of strengths and limitations of the imaging technique(s) used in the research article	No critical analysis of strengths and limitations of the imaging technique(s) used in the research article
Hypotheses & Aims **____/10 x 1.5**	Original hypotheses and aims clearly and concisely outlined and comprehensively justified.	Original hypotheses and aims clearly outlined and justified.	Original hypotheses and aims outlined and justified. Bit long or some aspects less clear.	Original hypotheses, Aims partly outlined and justified. Some aspects unclear or excessive.	Original hypotheses unclear. Aims outlined but not justified.	Original hypotheses and aims not at all clear.	No original hypotheses or aims apparent.
References **1-4 references** **____/10 x 1.5**	Clear use of reference(s) to compare and contrast the imaging techniques used, Chosen reference highly relevant to reviewed research article	Clear use of reference(s) to compare and contrast the imaging techniques used, Chosen reference relevant to reviewed research article	Mostly clear use of reference(s) to compare and contrast the imaging techniques used, Chosen reference relevant to reviewed research article	Mostly clear use of reference(s) to compare and contrast the imaging techniques used, Chosen reference has some relevance to reviewed research article	Partially unclear use of reference(s) to compare and contrast the imaging techniques used, Chosen reference has little relevance to reviewed research article	Unclear use of reference(s) to compare and contrast the imaging techniques used, Chosen reference has little relevance to reviewed research article	Poor use of reference(s) to compare and contrast the imaging techniques used, Chosen reference has no relevance to reviewed research article

APPENDIX 2a : Marking Criteria for Research Paper Report continued

Citaions **____/10 x 0.5**	Citation style correct and consistent throughout. Reference list completely accurate with no errors.	Citation style correct and consistent. Reference list complete, but a few minor errors.	Citation style consistent. Reference list incomplete and some minor errors.	Some references inconsistent between text and list with many minor errors. Citation style mostly consistent.	Many references inconsistent between text and list with many minor errors. Citation style incorrect / inconsistent.	Many inconsistencies between text and list. Some major errors. Inappropriate citation style used.	Many references inconsistent between text and list. Many major errors.
Presentation **____/10 x 1.5**	No grammatical or spelling errors. Professional expression and style used consistently. All figures accurate, focussed and informative. Word count: 1000+/-10%	No grammatical or spelling errors. Professional expression and style mostly used. All figures accurate, focussed and informative. Word count: 1000+/-10%	No grammatical errors and minor spelling errors. Professional expression and style used. All figures accurate, focussed and informative. Word count: 1000+/-10%	Minor grammatical errors and minor spelling errors. Professional expression and style used. Most figures accurate and informative. Word count: 1000+/-10%	Minor grammatical errors and minor spelling errors. Professional expression used. Most figures accurate, but not so relevant. Word count: 1000+/-20%	Major grammatical and spelling errors. Professional expression used. Numerous errors in figures or largely irrelevant. Word count: 1000+/-30%	Major grammatical and spelling errors. Language used not professional. No relevant figures. Word count: 1000+/-50%

*The critical analysis should be a detailed discussion that addresses primarily the following two questions:

1) How did the method answer the researcher(s)'s question

2) What other methods could have been employed to answer the researchers' question, explain why

APPENDIX 3: Marking Criteria for Research Article presentation

Criteria	**Presentation of Research Article** (Mark out of 10 for each marking criteria)				**Examiner**		**Student name**
	10-9.0 **Outstanding**	**8.9-8.5** **Excellent**	**8.4-8.0** **Very Good**	**7.9-7.5** **Good / Average**	**7.4-6.5** **Fair**	**6.4-5.0** **Poor**	**4.9-0** **Very Poor**
Introduction of Research Article **___/10 x 1.5**	Very comprehensive, detailed and focused introduction.	Highly detailed and focused introduction.	Detailed and focused introduction	Detailed introduction.	Some key basic information missing in introduction.	Major lack of key basic information in introduction.	Lack of significant detail in introduction.
Hypotheses & Aims **____/10 x 1**	Original hypotheses and aims clearly and concisely outlined and comprehensively justified.	Original hypotheses and aims clearly outlined and justified.	Original hypotheses and aims outlined and justified. Bit long or some aspects less clear.	Original hypotheses, Aims partly outlined and justified. Some aspects unclear or excessive.	Original hypotheses unclear. Aims outlined but not justified.	Original hypotheses and aims not at all clear.	No original hypotheses or aims apparent.
Critical Analysis* **____/10 x 2.5**	Comprehensive critical analysis of strengths and limitations of the imaging technique(s) used in the research article.	Critical analysis of strengths and limitations of the imaging technique(s) used in the research article	Some critical analysis of strengths and limitations of the imaging technique(s) used in the research article	Some critical analysis of strengths and limitations of the imaging technique(s) used in the research article but mostly descriptive.	Limited critical analysis of strengths and limitations of the imaging technique(s) used in the research article but mostly descriptive.	Very limited critical analysis of strengths and limitations of the imaging technique(s) used in the research article	No critical analysis of strengths and limitations of the imaging technique(s) used in the research article
Presentation: **Structure & material** **____/10 x 1.5**	The font, colour, graphics and slide layout used greatly enhances the presentation. Figures clearly labelled. No errors. Clear and logical structure throughout	The font, colour, graphics and slide layout used enhances the presentation. Figures clearly labelled. Minor errors. Clear and logical structure throughout	The font, colour, graphics and slide layout used enhances the presentation. Figures labelled. Minor errors. Mostly clear and logical structure throughout	The font, colour, graphics and slide layout used sometimes distracts from the presentation. Figures labelled with some errors. Mostly clear and logical structure throughout	The font, colour, graphics and slide layout used sometimes distracts from the presentation. Not all figures used are labelled or have errors. Lacking clear and logical structure	The font, colour, graphics and slide layout used distracts from the presentation. Poor use of figures including lack of labels or errors. Lacking clear and logical structure	The font, colour, graphics and slide layout used distracts from the presentation. Figures used not labelled. No logical structure to presentation

APPENDIX 3a: Marking Criteria for Research Article presentation continued

Presentation: **Engagement** **/10 x 1.5**	Delivery clear, articulate and professional. Well-paced and timing perfect. Confident stance and body language. Enthusiastic	Delivery clear, articulate and professional. Well-paced. Confident stance and body language. Enthusiastic.	Delivery mostly clear, articulate and professional. Well-paced. Mostly confident stance and body language. Enthusiastic.	Delivery mostly clear, occasional lapses in clarity and/or speed. OK stance and body language, some lapses.	Delivery mostly clear, occasional lapses in clarity and/or speed. Some major lapses in body language.	Delivery largely unclear / inaudible. Poorly paced. Major lapses in body language	Delivery largely unclear / inaudible. Poorly paced. Didn't engage with audience at all.
Questions **/10 x 2**	All responses to Qs demonstrated clear understanding of the project and its relevance. Consistently strongly argued and accurate answers to questions drawing from related literature.	Responses to Qs demonstrated clear understanding of the project and its relevance. Strongly argued and accurate answers to questions drawing from related literature.	Responses to Qs demonstrated understanding of the project and its relevance. Mostly accurate answers to questions, drew from related literature	Responses to Qs demonstrated some understanding of the project and its relevance. Average but seemingly accurate answers to questions.	Responses to Qs demonstrated some understanding of the project or its relevance. Multiple minor errors made in responses to questions	Responses demonstrated little understanding the project and its relevance. Major errors made in responses to questions.	Responses did not demonstrate any understanding of the project. Significant errors made in responses to questions.

*The critical analysis should be a detailed discussion that addresses primarily the following two questions:

1) How did the method answer the researcher(s)'s question?

2) What other methods could have been employed to answer the researchers' question, explain why?

<u>Comments:</u>

APPENDIX 4: Marking scheme for Project presentation

	Project Presentation (Mark out of 10 for each marking criteria)						
Criteria	**10-9.0 Outstanding**	**8.9-8.5 Excellent**	**8.4-8.0 Very Good**	**7.9-7.5 Good / Average**	**7.4-6.5 Fair**	**6.4-5.0 Poor**	**4.9-0 Very Poor**
Background /10 x 3	• Very focused introduction with sufficient detail and all concepts and terminology described to allow for understanding by a non-expert audience.	• Focused introduction with sufficient detail and most concepts and terminology described to allow for understanding by a non-expert audience	• Somewhat focused introduction with satisfactory detail and most concepts and terminology described to allow for understanding by a non-expert audience	• Introduction lacks some focus, has adequate detail and most concepts and terminology described to allow for understanding by a non-expert audience	• Introduction lacks focus and detail. Many concepts and terminology not described to allow for understanding by a non-expert audience	• Introduction lacks focus and has inadequate detail. Most concepts and terminology not described to allow for understanding by a non-expert audience	• Cannot be understood by a non-expert audience
Hypotheses & Aims /10 x 1	• Very clear and well developed links between hypotheses, aims and literature.	• Very clear links between hypotheses, aims and literature.	• Clear links between hypotheses, aims and literature.	• Links between hypotheses, aims and literature.	• Some links between hypotheses, aims and literature.	• No clear links between hypotheses, aims and literature.	• No hypotheses or aims
Methods /10 x 1	• Clear and detailed description of proposed experiments. • Linked clearly to aims and literature	• Clear description of proposed experiments. • Mostly linked well to aims and literature	• Clear description of experiments, but minor detail lacking. • Linked to aims and literature	• Descriptions of experiments mostly clear, some minor aspects or linking to aims lacking..	• Description of experiments lacked some major detail or poorly linked to aims.	• Description of experiments lacked some major detail and poorly linked to aims.	• Experiments not described.
Presentation: Structure & material /10 x 1.5	• The font, colour, graphics and slide layout used greatly enhances the presentation. Figures clearly labelled. No errors. • Clear and logical structure throughout	• The font, colour, graphics and slide layout used enhances the presentation. Figures clearly labelled. Minor errors. • Clear and logical structure throughout	• The font, colour, graphics and slide layout used enhances the presentation. Figures labelled. Minor errors. • Mostly clear and logical structure throughout	• The font, colour, graphics and slide layout used sometimes distracts from the presentation. Figures labelled with some errors. • Mostly clear and logical structure throughout	• The font, colour, graphics and slide layout used sometimes distracts from the presentation. Not all figures used are labelled or have errors. • Lacking clear and logical structure	• The font, colour, graphics and slide layout used distracts from the presentation. Poor use of figures including lack of labels or errors. • Lacking clear and logical structure	• The font, colour, graphics and slide layout used distracts from the presentation. • Figures used not labelled. • No logical structure to presentation
Presentation: Engagement /10 x 1.5	• Delivery clear, articulate and professional. Well paced and timing perfect. Confident stance and body language. Enthusiastic.	• Delivery clear, articulate and professional. Well paced. Confident stance and body language. Enthusiastic.	• Delivery mostly clear, articulate and professional. Well paced. Mostly confident stance and body language. Enthusiastic.	• Delivery mostly clear, occasional lapses in clarity and/or speed. OK stance and body language, some lapses.	• Delivery mostly clear, occasional lapses in clarity and/or speed. Some major lapses in body language.	• Delivery largely unclear / inaudible. Poorly paced. Major lapses in body language.	• Delivery largely unclear / inaudible. Poorly paced. Didn't engage with audience at all.
Questions /10 x 2	• All responses to Qs demonstrated clear understanding of the project and its relevance. • Consistently strongly argued and accurate answers to questions drawing from related literature.	• Responses to Qs demonstrated clear understanding of the project and its relevance. • Strongly argued and accurate answers to questions drawing from related literature.	• Responses to Qs demonstrated understanding of the project and its relevance. • Mostly accurate answers to questions, drew from related literature	• Responses to Qs demonstrated some understanding of the project and its relevance. • Average but seemingly accurate answers to questions.	• Responses to Qs demonstrated some understanding of the project or its relevance. • Multiple minor errors made in responses to questions	• Responses demonstrated little understanding the project and its relevance. • Major errors made in responses to questions.	• Responses did not demonstrate any understanding of the project. • Significant errors made in responses to questions.

APPENDIX 5: Marking scheme for Project Report

Criteria	Project Report (Mark out of 10 for each marking criteria)						
	10-9.0 Outstanding	8.9-8.5 Excellent	8.4-8.0 Very Good	7.9-7.5 Good / Average	7.4-6.5 Fair	6.4-5.0 Poor	4.9-0 Very Poor
Abstract _____/10 X 0.5	• Concise and informative summary of project rationale, results and relevance	• Concise and informative summary of project rationale, results and relevance. Minor aspect unclear/missing	• Nice summary of project rationale, results and/or relevance. Some key aspect potentially missing	• Nice summary of project rationale, results and/or relevance. Some aspect missing and/or some error(s).	• Fair summary of project, some aspect missing, and/or some error(s). Potentially inconsistent with main text.	• Significant inaccuracies in the summary of project.	• Significant inaccuracies in the summary of project.
Introduction, Hypothesis & Aims ______/10 X 1	• Concise and clear account of the scientific background and the rationale of the experiment. Very clear links between hypotheses / aims and literature.	• Concise and clear account of the scientific background and the rationale of the experiment. Clear links between hypotheses / aims and literature.	• Clear account of the scientific background and the rationale of the experiment. Clear links between hypotheses / aims and literature. Minor errors.	• Clear account of the scientific background and the rationale of the experiment. Minor omissions or errors. Links between hypotheses / aims and literature	• A good introduction of the scientific background and the rationale of the experiment. Some factual error or omissions. Some links between hypotheses / aims and literature	• Some introduction to the scientific background and the rationale of the experiment. More detail needed. Some links between hypotheses / aims and literature. Factual errors or omissions in text.	• Lacking detail of the rationale of the experiment and scientific background. No links between hypotheses / aims and literature. Factual errors or omissions in text.
Materials & Methods ______/10 X 1	• Clear and detailed description of experiments and data analysis (including statistical analysis).	• Clear description of experiments and data analysis (including statistical analysis).	• Good description of experiments and data analysis (including statistical analysis), with minor errors.	• Description of experiments and data analysis (including statistical analysis) mostly clear but significant detail lacking. Minor errors present in methods.	• Description of experiments and data analysis (including statistical analysis) lacking major details. Minor errors present methods.	• Description of experiments and data analysis (including statistical analysis) lacking major details. Major errors in methods.	• Description of experiments and data analysis (including statistical analysis) absent or unclear.
Results: Description & Content ___/10 X 1.5	• Logical and clear description of the experimental results with reference to tables and figures. No conclusions or interpretation of results presented. • Sufficient controls and replicates with appropriate data analysis (including statistics) performed correctly. Represents an extensive body of work	• Clear description of the experimental results with reference to tables and figures. No conclusions or interpretation of results presented. • Sufficient controls and replicates with minor errors in data analysis (including statistics). Represents an large body of work	• Clear description of the experimental results with reference to tables and figures. No conclusions or interpretation of results presented. • Sufficient controls and replicates with minor miscalculations in data analysis (including statistics) or inaccurate presentation of data. Represents an large body of work	• Good description of the experimental results with reference to tables and figures in most instances. Generally no conclusions or interpretation of results presented. • Sufficient controls and replicates with significant minor miscalculations in data analysis (including statistics) or inaccurate presentation of data. Represents an adequate body of work	• Description of the experimental results lacks required detail and appropriate reference to figures and tables. Some conclusions or interpretation of results presented. • Sufficient controls and replicates. Inappropriate data analysis, including statistics, used in some parts or inaccurate presentation of data. Represents an adequate body of work	• Description of the experimental results lacks required detail. Some conclusions or interpretation of results presented. • Insufficient controls and replicates used. Major errors or omissions in data analysis. Represents an inadequate body of work	• No description of the experimental results given. Lack of controls and replicates with appropriate data analysis (including statistics) performed. Represents an inadequate body of work

APPENDIX 5a: Marking scheme for Project Report continued.

(Cont.)	10-9.0 Outstanding	8.9-8.5 Excellent	8.4-.8.0 Very Good	7.9-7.5 Good / Average	7.4-6.5 Fair	6.4-5.0 Poor	4.9-0 Very Poor
Results: Presentation ____/10 X 1	• Graph axes labelled and units of measurement given in parentheses. Legends explain the figures in sufficient detail that they can be understood without reference to the text. Tables clearly labelled with clear footnotes if necessary so self-explanatory. • No errors in presentation.	• Graph axes labelled and units of measurement given in parentheses. Legends explain the figures in sufficient detail that they can be understood without reference to the text. Tables clearly labelled with footnotes if necessary so self-explanatory.. • A few minor errors in data presentation.	• Graph axes labelled and units of measurement given in parentheses. Not all legends explain the figures in sufficient detail. Most tables clearly labelled with footnotes if necessary so self-explanatory. • Some minor errors in data presentation.	• Most graph axes labelled and units of measurement given in parentheses. Not all legends explain the figures in sufficient detail to be understood without reference to the text. Most tables clearly labelled with footnotes if necessary so self-explanatory. • Some significant errors in data presentation.	• Results are poorly presented, most graph axes labelled and units of measurement given in parentheses. Not all legends explain the figures in sufficient detail that they can be understood without reference to the text. Most tables are self-explanatory, some errors in description or labels. • Some significant errors in data presentation	• Results are poorly presented. Most graph axes not labelled or missing units of measurement. Most legends do not explain the figures in sufficient detail that they can be understood without reference to the text. Most tables are not self-explanatory and/or poorly labelled. • Major errors in data presentation	• Results poorly presented or missing. Graph axes not labelled and units of measurement absent. Legends do not explain the figures in sufficient detail that they can be understood without reference to the text. Tables are not self-explanatory and/or poorly labelled. • Major errors in data presentation
Discussion ____/10 X 3.0	• Discussion is insightful, clear and logical. Extensive interpretation of the results with reference to previous scientific studies. Significance of findings extensively placed within the broader context of the field. • Comprehensive critical analysis of strengths and limitations of experiments. Future directions identified and clearly justified.	• Discussion is clear and logical. Appropriate interpretation of the results with reference to previous scientific studies. Significance of findings well placed within the broader context of the field. • Significant critical analysis of strengths and limitations of experiments. Future directions identified and justified	• Discussion is clear. Appropriate interpretation of results, some reference to previous studies, but not always. Significance of findings placed within the broader context of the field. • Critical analysis of strengths and limitations of experiments. Future directions identified and justified	• Discussion is mostly clear. Appropriate interpretation of the results with a few minor errors. Reference to previous scientific studies in most cases. Significance of some findings placed within the broader context of the field. • Some critical analysis of strengths and limitations of experiments. Future directions identified and mostly justified	• Discussion is unclear in many areas. Some inappropriate interpretation of the results. Lacking reference to previous scientific studies. Significance of findings not placed within the broader context of the field. • Lacking some critical analysis of strengths and limitations of experiments. Future directions identified.	• Results are restated with little interpretation or reference to previous scientific studies. Major findings not placed within the broader context of the field. • No critical analysis of strengths and limitations of experiments. No future directions identified. • Misunderstanding of some major concepts.	• Results are restated with no interpretation or reference to previous scientific studies. Findings not place within the broader context of the field. • No critical analysis of strengths and limitations of experiments. No future directions identified. • Little understanding of most major concepts.
References _____/10 X 1	• Predominant and comprehensive use of primary articles. Many articles presented from recent or seminal publications. • Citation style correct and consistent throughout. Reference list completely accurate with no errors.	• Predominant use of primary articles. Many articles presented from recent or seminal publications. • Citation style correct and consistent. Reference list complete, but a few minor errors.	• Predominant use of primary articles. Could have used more articles from recent or seminal publications. • Citation style consistent. Reference list incomplete, and some minor errors.	• Some over reliance on reviews or texts. Could have used more articles from recent or seminal publications. • Some references inconsistent between text and list with many minor errors. Citation style mostly consistent	• Some over reliance on reviews or texts. Many articles not from recent or seminal publications. • Many references inconsistent between text and list with many minor errors. Citation style incorrect / inconsistent.	• Significant over reliance on reviews or texts. Limited number of recent or seminal articles used. • Many inconsistencies between text and list. Some major errors. Inappropriate citation style used.	• Use of literature limited to a few articles and reviews. Poor attempt to explore literature. • Many references inconsistent between text and list. Many major errors.
Overall Presentation _____/10 X 1	• No grammatical or spelling errors. Professional expression and style used consistently. All figures accurate, focussed and informative • Word count 2250±250	• No grammatical or spelling errors. Professional expression and style used. All figures accurate, focussed and informative. Word count 2250±250	• No grammatical errors but some spelling errors. Professional expression and style used. All figures accurate, focussed and informative • Word count 2250±250	• Some grammatical and spelling errors. Professional expression and style used. Most figures accurate and informative. • Word count 2250±350	• Some grammatical and spelling errors. Professional expression used. Most figures accurate, but not so relevant. • Word count: 2250±400	• Major grammatical and spelling errors. Professional expression used. Numerous errors in figures or largely irrelevant • Word count: : 2250±500	• Major grammatical and spelling errors. Language used not professional. Numerous errors in figures or largely irrelevant • Word count: 2250±600