Faculty of Medicine

School of Medical Sciences

ANAT 3212

Microscopy in Research

Semester 2, 2015
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ANAT3212 Microscopy in Research Course Manual

Course Convenor
Thomas Fath, PhD
Senior Lecturer, Department of Anatomy
Group Leader, Neurodegeneration and Repair Unit
School of Medical Sciences
Wallace Wurth Building East, Level 2, Rm233
e-mail: t.fath@unsw.edu.au
Phone: 02 9385 9690

Co-Convenor
Renee Whan, PhD
Lecturer, Head of Biomedical Imaging Facility (BMIF)
Mark Wainwright Analytical Centre
Room 411A, Lowy Cancer Research Centre, (C25)
e-mail: r.whan@unsw.edu.au
Phone: 02 9385 9342

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

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

Aims and Learning Outcomes
This is an advanced course in microscopy, which provides practical, research-oriented experience. The course covers the principles and practice of conventional light microscopy, including an understanding of the preparation of routine paraffin and frozen sections, as well as advanced resin embedding methods and specialised light microscopic techniques such as phase contrast, darkfield and Nomarski differential interference contrast; enzyme histochemistry; immunostaining techniques; fluorescence and confocal microscopy.
including principles of quantitative microscopy (morphometry). Furthermore the course will introduce high-end microscopy techniques such as super-resolution microscopy (e.g. PALM and STED) and Intravital Microscopy. The course will thus help students to gain a better understanding of the correlation between structure and function.

**Format**

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

In weeks 10 and 11, short Projects will be carried out in research laboratories on the UNSW campus. Students will be assigned to the different projects in the first two weeks of the course. Students’ preferences for individual projects will be taken into consideration. Projects that will be offered are listed on page 44.

**Lecture:**
- Tue 9-10 WW LG02
- Wed 10-11 WW LG02
- Wks 1-12

**Lab:**
- Tue 10-12 WW G16/17 and
- Wed 11-12 WW G16/G17
- Wks 1-12
Teaching Staff
(See for more information and short biographies at the end of the course manual)

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

Dr Till Böcking [ARC Future Fellow, School of Medical Sciences, UNSW]

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

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

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

Dr Thomas Fath [Senior Lecturer, CONVENOR, School of Medical Sciences, UNSW]

Dr Richard Francis [Research Associate, Biomedical Imaging Facility (BMIF), UNSW]

Dr Sandra Fok [Research Associate, Biomedical Imaging Facility (BMIF), UNSW]

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

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

Dr Elizabeth Hinde [Vice Chancellor’s Postdoctoral Fellow, School of Medical Sciences, UNSW]

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

Dr Enrico Klotzch [Research Associate, School of Medical Sciences, UNSW]

Dr Rhiannon Kuchel [Microscopist, Electron Microscope Unit, UNSW]

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

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

Prof Fred Meunier [Queensland Brain Institute, University of Queensland]

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

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

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

A/Prof Patsie Polly [Department of Pathology, School of Medical Sciences, UNSW]

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

Dr John Power [Senior Lecturer, Translational Neuroscience Facility, School of Medical Sciences, UNSW]
Dr Jeremie Rossy [ARC DECRA Research Fellow, EMBL Australia Node in Single Molecule Science, School of Medical Sciences, UNSW]

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

Dr Renee Whan [Lecturer, CO-CONVENOR Head of Biomedical Imaging Facility (BMIF), UNSW]

A/Prof Jia Lin Yang [Prince of Wales Clinical School, UNSW]

Assessments

<table>
<thead>
<tr>
<th>Assessment activity</th>
<th>Duration</th>
<th>Value</th>
<th>Due</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report (Literature Research)</td>
<td>1000 words</td>
<td>10%</td>
<td>Week 9</td>
</tr>
<tr>
<td>Oral Presentation (Literature Research)</td>
<td>5 min</td>
<td>10%</td>
<td>Week 9</td>
</tr>
<tr>
<td>Examination Terminology &amp; Applications of Microscopy Techniques (Format: short answers)</td>
<td>1 hr</td>
<td>35%</td>
<td>Week 10</td>
</tr>
<tr>
<td>Oral Presentation on Project (Presentation of project experience; should cover a description of experimental design, data analysis and interpretation)</td>
<td>20 min</td>
<td>15%</td>
<td>Week 12</td>
</tr>
<tr>
<td>Project Part I: Individual Projects (aiming for two-three students per project). Students will visit the labs of active research groups. (Format: written report including Introduction/Methods/Results/Discussion/References)</td>
<td>2000-2500 words</td>
<td>25%</td>
<td>Monday of Week 13</td>
</tr>
<tr>
<td></td>
<td>1500 words</td>
<td>5%</td>
<td>Monday of Week 13</td>
</tr>
<tr>
<td>Part II: Part II includes Reflection on Lit Report, project report and oral presentation I and oral presentation II</td>
<td></td>
<td></td>
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</tbody>
</table>
Details of Assessments


TASK
You will be provided with a list of 12 papers. These papers include methods for;

I. Multi-labelling immuno-fluorescence
II. Enzyme-based histochemistry
III. Confocal Microscopy- Biological samples or Live cells
IV. Intravital Microscopy
V. PALM, STORM or STED super resolution microscopy
VI. Electron Microscopy

Choose one of the journal articles and notify the convenor and co-convenor by beginning of week 3. Note that only 2 students per article will be permitted.

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?

Written Report for Research Paper Review
The written report should be 1000 words maximum in length excluding references. Insert relevant images and diagrams to support your evaluation of the paper.
The Due DATE is FRIDAY 25th September, 2015 (the end of week 9) NO LATER THAN 4:30pm.
Assignments are to be submitted to Ms Carmen Robinson, G27, Biosciences Building, UNSW

The coversheet (available on Moodle) should clearly state:
- Your Name
- Your Student Number

PRESENTATION
You will present your findings to the group and a panel of examiners. The presentation will be 5 minutes.
The presentation will be assessed by your peers and the examiners according to the following criteria:
1. CLARITY AND STRUCTURE: Oral presentation was clear, well-structured and easily understood.
2. TIMING: Timing was controlled so that most aspects were covered.
3. UNDERSTANDING: Presenter appeared to have a good understanding of the topic: able to answer audiences’ questions clearly.
4. STIMULATED LEARNING: Presentation was interesting; significant issues and answered questions were highlighted.

Your contribution to Peer Assessment worth 25% of the Research Paper Analysis Exercise
Examiners Assessment worth 75% of the Research Paper Analysis Exercise
All students are to complete a peer assessment feedback form for each presenter. Marks will be based on the quality of the feedback provided.
PRESENTATIONS will be in Week 9. A projector will be available for Powerpoint presentations

[2] Exam

The exam held in week 10 will be in the form of short answers and will cover material presented in both lectures and practical classes.
For exceptional circumstances, a supplementary exam can be scheduled on Tuesday, 13th October, 2015 at 9am.


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

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

The Due DATE is Monday 26th October, 2015 (the start of week 13) NO LATER THAN 4:30pm. Assignments are to be submitted to Ms Carmen Robinson, G27, Biosciences Building, UNSW.

The coversheet (available on Moodle) should clearly state:
• Your Name
• Your Student Number
Late Submission

Other than in *exceptional circumstances, late submission will attract a penalty of 10% of the total mark per day or part thereof. Thus, submission on Thursday 30\textsuperscript{th} October 2014 would attract a 30% penalty. Keeping to a deadline is part of the assessment.

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

PRESENTATION

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

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

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

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

Examiners Assessment worth 75% of the Project component

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

PRESENTATIONS will be in week 12. A projector will be available for Powerpoint presentations
Textbooks

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

Recommended reference sources for this course are


Further recommended reference sources for this course will be provided throughout the course.

Helpful library search resources

- *Getting Started* and *Smart Searching* videos, providing quick overviews of library services and finding resources.
- *ELISE*, offering advice and assistance with developing your research skills.
- UNSW Library *Subject Guides*, designed to help you get started finding information using key resources for your topic. The *Medicine subject guide* is a relevant starting point for these students.
- *Library Search* is a discovery tool that helps you get started. You can find print books and journals, e-books, High Use Collection items, exam papers, DVDs and music, maps books, ebooks and online resources in one easy search. Library Search has different search features that allow you to refine your searching and limit results by available and full-text online resources.
- *How to read a paper: the basics of evidence based medicine* is available as an e-book through the Library or as a set of articles on the BMJ website.
- The *Medicine Information Skills Tutorial* is provided as part of the Foundation program for Medicine students and covers much of the material needed by this group.
- PubMed itself provides a set of self-help guides and tutorials:
  - *Quick Start guide*
  - *YouTube tutorials*
  - *Online training*
  - *Tutorial*
Attendance

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

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

OFFICIAL COMMUNICATION BY EMAIL

All students in this course are advised that email is now the official means by which the School of Medical Sciences at UNSW will communicate with you.

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

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

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

Academic Honesty and Plagiarism

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

What is plagiarism?

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

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

The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does not amount to plagiarism. Students are reminded of their Rights and Responsibilities in respect of plagiarism, as set out in the University Undergraduate and Postgraduate Handbooks, and are encouraged to seek advice from academic staff whenever necessary to ensure they avoid plagiarism in all its forms. The Learning Centre website is the central University online resource for staff and student information on plagiarism and academic honesty. It can be located at: https://student.unsw.edu.au/plagiarism.

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:
• correct referencing practices;
• paraphrasing, summarizing, essay writing, and time management;
• appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre. Students are also reminded that careful time management is an important part of study and one of the identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.

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

† Adapted with kind permission from the University of Melbourne.

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

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

https://student.unsw.edu.au/special-consideration

Please note the following

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

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

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

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

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

Health and Safety Guidelines

Generic Safety rules for the School of Medical Sciences can be found at the following URL:

For practicals carried out in Rm 109/110 read and sign the Risk Assessment form on page 14 in the course manual. In research laboratories, everyone must wear a lab coat and closed footwear 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.
Important note: Practical class and research laboratory specific SWPs and RAs will be provided by academic staff or supervisors at the time of the activity. Practicals from weeks 1 to 13 in S2, 2014.

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Risks</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergonomics</td>
<td>Musculoskeletal pain.</td>
<td>Correct workstation set-up.</td>
</tr>
<tr>
<td>Electrical</td>
<td>Shock/fire</td>
<td>Check electrical equipment in good condition before use. All portable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>electrical equipment tested and tagged.</td>
</tr>
<tr>
<td>Practical class specific Hazards as outlined in practical class specific SWPs and RAs</td>
<td>Practical class specific risks as outlined in practical class specific SWPs and RAs</td>
<td>Practical class specific controls as outlined in practical class specific SWPs and RAs</td>
</tr>
</tbody>
</table>

General comments
The Hazards, Risks and Controls listed above are only referring to teaching activities carried out in G16/17 in the Wallace Wurth building. Prior to each laboratory activity, activity specific inductions, including SWPs and RAs have to be provided by the academic staff running the activity and/or the allocated supervisor (in the case of research laboratory based projects).

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 indicated in practical disposal.

Declaration
I have
A) attended the ANAT3212 H&S lecture on 29th July  
B) I have carefully read the lecture material of the H&S lecture given on 29th July

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

Signature:..........................................................Date:...........................................
Student Number:........................................
Equity and Diversity Issues

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

Grievance Officer

If you have any problems or grievances with the course you should, in the first instance, consult the Course Organiser. If you are unable to resolve the difficulty, you can consult the Head of Teaching in the Department, Professor Ken Ashwell, 4th Floor, Wallace Wurth Building, Rm 447 (Email: k.ashwell@unsw.edu.au), or the Department of Anatomy’s nominated Grievance Resolution Officer, Dr Priti Pandey, 2nd Floor, Wallace Wurth Building, Rm 214 (Email: p.pandey@unsw.edu.au).
<table>
<thead>
<tr>
<th>Week</th>
<th>Date</th>
<th>Time</th>
<th>Title</th>
<th>Lecturer</th>
<th>Prac. Date</th>
<th>Time</th>
<th>Location</th>
<th>Title</th>
<th>Lecturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 Tuesday 28/07/2015</td>
<td>9:00-10:00</td>
<td>Introduction: Course outline, assessments, projects</td>
<td>Dr T. Fath</td>
<td>Tuesday 28/07/2015</td>
<td>10:00-12:00</td>
<td>WW G16/17</td>
<td>Dispelling Misconceptions about Imaging</td>
<td>Dr M. Carnell</td>
</tr>
<tr>
<td></td>
<td>2 Wednesday 29/07/2015</td>
<td>10:00-11:00</td>
<td>How to perform a literature search - NB held in WG16/17</td>
<td>S. Mobbs</td>
<td>Wednesday 29/07/2015</td>
<td>12:00-13:00</td>
<td>WW G16/17</td>
<td>e-Portfolio</td>
<td>A/Prof J.L. Yang</td>
</tr>
<tr>
<td></td>
<td>3 Tuesday 04/08/2015</td>
<td>9:00-10:00</td>
<td>Introduction to Light Microscopy</td>
<td>Dr M. Carnell</td>
<td>Tuesday 04/08/2015</td>
<td>10:00-12:00</td>
<td>WW G16/17</td>
<td>Principles of Fluorescence Introduction to Fluorescence Microscopy</td>
<td>Dr R. Whan</td>
</tr>
<tr>
<td></td>
<td>4 Wednesday 05/08/2015</td>
<td>10:00-11:00</td>
<td>Specimen Preparation for light microscopy 1</td>
<td>Dr R. Francis</td>
<td>Wednesday 05/08/2015</td>
<td>11:00-13:00</td>
<td>WW G16/17</td>
<td>Tutorial 1: Know your Spectra Tutorial 2: Filter blocks and PMTs</td>
<td>Dr A. MacMillan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Specimen Preparation for light microscopy 2</td>
<td>Dr R. Francis</td>
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<td></td>
<td>Dr S. Fok</td>
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<tr>
<td></td>
<td>5 Tuesday 11/08/2015</td>
<td>9:00-10:00</td>
<td>Virtual Laboratory</td>
<td>Dr T. Fath</td>
<td>Tuesday 11/08/2015</td>
<td>10:00-12:00</td>
<td>WW G16/17</td>
<td>Practical: Standard Stains</td>
<td>Dr T. Fath</td>
</tr>
<tr>
<td></td>
<td>6 Wednesday 12/08/2015</td>
<td>10:00-11:00</td>
<td>Immunocytochemistry</td>
<td>Dr T. Fath</td>
<td>Wednesday 12/08/2015</td>
<td>11:00-12:00</td>
<td>WW G16/17</td>
<td>Virtual laboratory</td>
<td>Dr T. Fath</td>
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<tr>
<td></td>
<td>7 Tuesday 18/08/2015</td>
<td>9:00-10:00</td>
<td>Practical: Viewing standard stains and Immunocytochemistry</td>
<td>Dr T. Fath</td>
<td>Tuesday 18/08/2015</td>
<td>10:00-12:00</td>
<td>WW G16/17</td>
<td>Practical: Viewing standard stains and Immunocytochemistry</td>
<td>Dr T. Fath</td>
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<tr>
<td></td>
<td>8 Wednesday 19/08/2015</td>
<td>10:00-11:00</td>
<td>Practical: Viewing standard stains and Immunocytochemistry</td>
<td>Various</td>
<td>Wednesday 19/08/2015</td>
<td>11:00-13:00</td>
<td>WW G16/17</td>
<td>Practical: Viewing standard stains and Immunocytochemistry</td>
<td>Various</td>
</tr>
<tr>
<td>No.</td>
<td>Date</td>
<td>Time</td>
<td>Event</td>
<td>Speaker</td>
<td>Time</td>
<td>Location</td>
<td>Speaker</td>
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<td>5</td>
<td>Tuesday</td>
<td>9:00-10:00</td>
<td>Summary and Q and A</td>
<td>Dr R Whan, Dr R. Francis, Dr M. Carnell</td>
<td>Tuesday 25/08/2015 10:00-12:00</td>
<td>WW G16/17</td>
<td>Dr R. Whan, Dr R. Francis, Dr M. Carnell</td>
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<td>5</td>
<td>Wednesday</td>
<td>10:00-11:00</td>
<td>The third Dimension: Optical Sectioning Techniques;</td>
<td>Dr J. Rossy</td>
<td>Wednesday 26/08/2015 11:00-13:00</td>
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<td>Entering the fourth Dimension Dynamic imaging</td>
<td>Dr R. Whan</td>
<td>Tuesday 1/09/2015 10:00-12:00</td>
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<td>6</td>
<td>Wednesday</td>
<td>10:00-11:00</td>
<td>FRET and FLIM</td>
<td>Dr E. Hinde</td>
<td>Wednesday 2/09/2015 11:00-13:00</td>
<td>WW G16/17</td>
<td>Dr. M. Carnell</td>
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<td>7</td>
<td>Tuesday</td>
<td>9:00-10:00</td>
<td>Single Molecule and Super resolution (part 1)</td>
<td>Dr T. Boecking</td>
<td>Tuesday 8/09/2015 10:00-12:00</td>
<td>WW G16/17</td>
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<td>7</td>
<td>Wednesday</td>
<td>10:00-11:00</td>
<td>Electron Microscopy for Biomedical Research</td>
<td>Dr R. Kuchel</td>
<td>Wednesday 9/09/2015 11:00-13:00</td>
<td>WW G16/17</td>
<td>EMU staff</td>
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<td>8</td>
<td>Tuesday</td>
<td>9:00-10:00</td>
<td>Imaging in Live Animals: Pre-Clinical Imaging</td>
<td>Dr C. Power</td>
<td>Tuesday 15/09/2015 10:00-12:00</td>
<td>WW G16/17</td>
<td>Dr C. Power</td>
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<td>8</td>
<td>Wednesday</td>
<td>10:00-11:00</td>
<td>Application of single molecule imaging in neuronal cells.</td>
<td>Prof F. Meunier</td>
<td>Wednesday 16/09/2015 11:00-12:00</td>
<td>WW G16/17</td>
<td>Prof R. Kumar</td>
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<td>9</td>
<td>Tuesday</td>
<td>9:00-10:00</td>
<td>Intravital Microscopy</td>
<td>Dr A. Masedunskas</td>
<td>Tuesday 22/09/2015 10:00-12:00</td>
<td>WW G16/17</td>
<td>A/Prof N. DiGirolamo, Dr G. Moalem-Taylor</td>
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<td>18</td>
<td>Wednesday</td>
<td>10:00-11:00</td>
<td>Presentations of Literature Report</td>
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**Mid-Session Break**

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<td>Tuesday</td>
<td>9:00-10:00</td>
<td>EXAM</td>
<td>WW G16/17</td>
<td>Tutorial: e-profile and interview</td>
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**ALL Lecturers Tuesday 9am and Wednesday 10am lectures will be held in WWLG02**
Dispelling Common Misconceptions about Imaging. [Dr M. Carnell]

**Aim:** This tutorial will introduce some of the misconceptions made by people as they embark on learning imaging techniques. It is intended to prevent confusion, and remove some barriers that hinder people from fully understanding the concepts being conveyed in later lectures.

In an open discussion we will address primarily 1) confusion surrounding words that have varying meanings and scopes, especially between technical applications vs colloquial usage, and 2) provide an overview, and some advice, on how to set out understanding and organizing the various information that will be coming your way.

**Learning Objectives:**

1. To understand the primary reasons why imaging is undertaken by scientists. Magnification is often presumed to be the main reason, and although it does play its part it is rarely the primary reason. It is important to appreciate the difference between resolution and magnification, as well as the necessity of introducing contrast into specimens.

2. To broaden your understanding of the terms resolution and contrast. Many students enter imaging with the notion of resolution relating to the number of pixels in an image, and contrast being settings that you can change to make an image brighter or darker. Whereas these are true they are special cases of specific ‘types’ of resolution and contrast.

3. To learn to approach imaging by understanding the problem it is attempting to address. ‘Imaging Techniques’ is a term that covers a range of methods from the type of instrument used, how it is used, possibly the method of preparing samples, or how the data is processed once captured, or a combination of any of these. Although these will be addressed later, here we will give an overview of how to go about organizing these ideas.

4. To appreciate that imaging requires a careful consideration of compromise.

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.
Health and Safety [Dr M. Hill]

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

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

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

Introduction to Light Microscopy [Dr M. Carnell]

Aim: This lecture will introduce you to the fundamentals of light microscopy. Beginning with some of the core properties of light that underpin many of the optical techniques mentioned throughout this course. We will then progress onto the core components of a basic modern day compound microscope. Finally, finishing with a few examples of transmitted light techniques and explaining how they utilize the aforementioned properties of light to add contrast to otherwise transparent samples.

Learning Objectives:

1. 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.
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. Identify basic components of the compound microscope.
4. 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.
5. Appreciate how contrast can be introduced into otherwise transparent samples without the use of probes by utilizing refraction, phase shifts or polarization of light.

Additional Resources
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:

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

**Aim:** This lecture will introduce you to Histology/Histochemistry

**Learning Objectives:**

1. To describe and understand some common histology techniques for mounting and sectioning biological samples.
2. To be able to discuss the advantages and disadvantages of each histology technique.
3. To describe and understand some common histochemistry stains.
4. To 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:**


Immunocytochemistry [Dr T. Fath]

Immunofluorescence techniques are widely used to analyse protein localisation in combination with the analysis of cell morphology. A good example for a particularly complex cell type with regards to morphology is a neuron which can easily be examined in a culture dish. Besides the culturing of cell lines and primary cells directly derived from an organism, cultures of tissue slices are used to understand intracellular processes as well as mechanisms of cell-cell interaction and communication. In this lecture, I will 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).
The third Dimension: Optical Sectioning Techniques [Dr J. Rossy]

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. To know the different approaches that can be used to perform optical sectioning in light microscopy.
2. To understand the concepts defining confocal microscopy
3. To know and understand the basic components of a confocal microscope
4. To understand the principles of light sheet illumination
5. To 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 light sheet illumination imaging.

Additional Resources:

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

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. To be able to describe the necessary environmental conditions for live cell imaging
2. To understand the different ways of labelling live cells; genetic encoding and probes
3. To be aware of problems that can be encountered when performing live cell imaging; drift; cell health, contamination
4. To be familiar with concepts of FRAP and photoactivation
Förster Resonance Energy Transfer (FRET) and Fluorescence Lifetime Imaging Microscopy (FLIM): [Dr E. Hinde]

The development of fluorescent protein biosensors coupled with live-cell imaging has enabled the visualization of intracellular molecular dynamics with high spatiotemporal resolution. Fluorescent biosensors designed to probe GTPase activity, second messenger dynamics and metabolites such as glucose are just a few examples of the continuously expanding collection currently available. 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.

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

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

Visualise protein interactions [Dr J. Rossy]

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

Learning Objectives:
1. To understand why it is crucial to be able to visualise interactions between molecules
2. To know which microscopy techniques can be used to investigate if two proteins interact
3. To be 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?
Electron Microscopy for Biomedical Research [Dr R. Kuchel]

Aim: This lecture will introduce you to the concepts of electron microscopy in the field of biomedical science and key properties that underpin electron microscopy.

Learning Objectives:

1. To describe and understand the principles of both transmission and scanning electron microscopy; electrons, vacuum, specimen preparation/trials and tribulations.

2. To understand concepts such as resolution, fixation and contrast.

3. To be able to discuss the difference between scanning and transmission electron microscopy and the different ways they can be used i.e. semi-thin staining, immuno-gold labelling etc.

4. To understand the principles behind biological specimen preparation.

Points for discussion:

Why is electron microscopy so important within the research community? What are it’s advantages/disadvantages compared with other microscopy techniques?

Additional Resource:


Imaging in Live Animals – [Dr C. Power]

The lecture will provide an overview of a number of imaging techniques including positron emission tomography (PET), micro-computed tomography (microCT), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and optical imaging systems including bioluminescence, fluorescence and intravital microscopy. The theory and application of each imaging technology will be discussed with emphasis 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.

Application of single molecule imaging in neurons [Prof F. Meunier]

Guest Lecture. TBA (check update on Moodle)

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.

**Multiphoton and Intravital Microscopy [Dr John Power]**

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

**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. To describe and understand the process of immunohistochemistry and immunofluorescence
2. To understand where stem cells reside in the cornea and their importance to vision
3. To understand how to mark these cells with antibodies and report on their location
4. To understand how to genetically mark cells with fluorescent proteins and how to visualise their dynamics under fluorescence and intravital 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. To describe the morphology of neurons (soma, dendrites, axons) as manifested by the Golgi technique and cellular labelling.
2. To describe the ultrastructure of neurons (synapses and organelles) as revealed by electron microscopy.
3. To describe the cytoarchitecture of neurons (nuclei and tracts) and the chemoarchitecture (chemical contents) of neurons as shown by different stains and immunohistochemistry.
4. To understand the principles of tract tracing (neuronal connections) based on axonal transport including the use of retrograde and anterograde labelling, and transsynaptic transport using viruses.
5. To discuss newly developed neurohistology methods in the past couple of years.
Practical Notes

Integrative ePortfolio 1 - [A/Prof Jia-Lin Yang] (Tutorial 1)

Aim: To provide theory and examples of the individual student ePortfolios and how we will assess them.

Points for discussion: In what ways could an ePortfolio be of use over the course of a degree? In what ways is an ePortfolio better than other social media tools available on the web?

The ePortfolio is a student self-created and self-managed digital framework where a student will present learning information, achievement and evidence, as well as reflective learning during the session within the course and across courses, in and out of the campus.

ePortfolio assessment

The purpose of the ePortfolio is for you to reflect on your learning progress, collect evidence of learning, and integrate your learning into your wider degree program and your professional and personal aspirations. While the mark allocated is small, this activity will help you to evaluate your learning achievements, identify your learning needs, and collect evidence of learning for future use.

ePortfolio instructions

- Please set up or access your personal ePortfolio in Mahara (via Moodle) or a self-selected software such as Google Sites or WordPress.
- Review your portfolio, upload new content from your learning activities, and write a reflection on your learning progress at least once a fortnight. Reflections are for your own benefit, not for assessment per se. For your final ePortfolio submission you should provide all recordings (evidence of learning progress) together with a 1,500-word summary of your reflections for your courses, our university, and your life in and out of the university campus.
- Make collection of pages to represent your course and life learning and submit as required.
Know your Spectra and Filter blocks [Dr A. MacMillan and Dr S. Fok]

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


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

<table>
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<tr>
<th>Fluorophore</th>
<th>Excitation maximum (nm)</th>
<th>Excitation spectrum range</th>
<th>Emission maximum (nm)</th>
<th>Emission spectrum range</th>
<th>Normalised fluorescence with Standard lasers</th>
<th>Corresponding Emission collection</th>
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<tr>
<td>Rhodamine 123</td>
<td>507</td>
<td>425-547</td>
<td>529</td>
<td>500-650</td>
<td>488nm =13%</td>
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<td>LysoTracker Blue</td>
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<td>488nm =45% 514nm =83%</td>
<td>500-650, 525-650</td>
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<td>Hoechst 33342</td>
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<td>WLL = 507nm =100%</td>
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<td>Texas Red</td>
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Notes:
Part 2: Filters

We will examine in detail some of the filter sets that are commonly used in epifluorescent 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.

<table>
<thead>
<tr>
<th>Filter Block</th>
<th>Excitation Filter</th>
<th>Dichroic Filter</th>
<th>Emission Filter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2A</td>
<td>450-490</td>
<td>505LP</td>
<td>520LP</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2A</td>
<td>510-560</td>
<td>575LP</td>
<td>590LP</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy5.5</td>
<td>665/45</td>
<td>695LP</td>
<td>725/50</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFP</td>
<td>435/20</td>
<td>455LP</td>
<td>480/40</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas Red</td>
<td>540-580</td>
<td>595LP</td>
<td>600-660</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMUI</td>
<td>540/50</td>
<td>560LP</td>
<td>590SP</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V2A</td>
<td>380-420</td>
<td>430LP</td>
<td>450LP</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YFP</td>
<td>500/20</td>
<td>515LP</td>
<td>535/30</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RW1404</td>
<td>380-420</td>
<td>400LP</td>
<td>515SP</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XF104</td>
<td>500/25</td>
<td>525LP</td>
<td>545/35</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EK2903</td>
<td>590/30</td>
<td>625LP</td>
<td>650LP</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narrow Blue</td>
<td>480/20</td>
<td>505LP</td>
<td>535/40</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FMT07</td>
<td>450LP</td>
<td>525LP</td>
<td>580LP</td>
<td>300-800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**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 compound’s 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**

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

**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. Xyol  ................................................................................... 2mins
2. Xyol  ................................................................................... 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. Xyol  ................................................................................. 2min
14. Xyol  ................................................................................. 1min
15. Xyol  ................................................................................. 1min
Mount with Ultramount

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

REFERENCES:


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


Brightfield Imaging – Microscopic examination of stained tissue samples [Dr T. Fath, Mr P. de Permentier]

**AIM:**
1. Understand the use of various fixatives which are available
2. Understand the principles of tissue processing
3. Recognise fixation, sectioning and embedding tissue and artefacts
4. 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 REQUIRED:**

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

**REFERENCES:**

## Fixative Observations

<table>
<thead>
<tr>
<th>Fixative Type</th>
<th>Tissue fixed for 1 hr</th>
<th>Tissue left on bench before fixation</th>
<th>Tissue fixed for 24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bouin’s Fixative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carnoy’s Fixative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**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-fluorescence 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 REQUIRED:**

*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
- TUJ-1 (β3 neuronal tubulin isoform) and Tropomyosin isoform specific antibodies
- Fluorophor-tagged Phalloidin (probe to detect filamentous actin)
- Fluorophor-tagged anti mouse and anti-rabbit secondary antibodies
- Nuclear Dye DAPI (4',6-diamidino-2-phenylindole)

READINGS

**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 Sessions 1 and 2** [Dr R. Whan]

In these 2 x two hour practicals you will view a hands-on demonstration 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.

**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.

**Visit of the Animal Imaging Facility** [Dr C. Power]

TBA {check update on Blackboard}

**Visit of Electron microscopy Unit** [Dr R. Kuchel]

TBA {check update on Blackboard}
Project Descriptions

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

**PROJECT**: Control of the Inflammatory Response by a Lipid Kinase  
SUPERVISOR: Dr Anthony Don  
SUMMARY: Inflammatory responses are regulated largely through the metabolism of membrane lipids to produce inflammatory signalling metabolites. The production of these inflammatory metabolites is blocked by the most common anti-inflammatory drugs, such as Ibuprofen and Aspirin. One condition under which inflammatory mediators are produced is cell death, and our current research suggests that this is controlled by the enzyme ceramide kinase. In this project, the student group will use live cell microscopy to investigate whether the intracellular localisation of the enzyme ceramide kinase changes upon induction of cell death, resulting in its re-location from the cytosol to specific intracellular membranes.

**PROJECT**: Intravital Imaging  
SUPERVISOR: Prof Gary Housley  
SUMMARY: Students will undertake real-time imaging of living neurons within the cerebellar region of the adult mouse brain. The imaging will be achieved using multi-photon excitation of green fluorescence protein expressed in GABAergic neurons in the cerebellum of a GAD67-GFP transgenic reporter mouse. The purpose of the project will be to initially contrast the (limited) performance of conventional visible light (single-photon excitation) confocal laser scanning microscopy (LSM) against multi-photon IR excitation for imaging. Once proficiency is established, the work will proceed to determine of the fine structure of the dendrites in Purkinje neurons and determine the effect of hypoxia on that cytoarchitecture (mimicking the acute effect of stroke). This experiment, using gaseous anaesthesia in transgenic mice, will have the approval of the UNSW Animal Care and Ethics Committee (ACEC) and will be undertaken in the Translational Neuroscience Facility (TNF), 3rd floor Wallace Wurth - south. The students will be inducted into the TNF and receive training on the Zeiss 710 NLO multiphoton microscope which utilizes a Spectraphysics MaiTai femtosecond pulsed IR laser system for deep tissue intravital imaging.
PROJECT: Live cell imaging of Rho GTPase fluorescent protein biosensors during cell migration
SUPERVISOR: Dr Liz Hinde
SUMMARY: The aim of this research project is to measure how the activity of the small GTPases Rac1 and RhoA cooperate to direct cell migration. By imaging Rac1 and RhoA fluorescent protein biosensors, which employs fürster resonance energy transfer (FRET) as a readout of activation, we will detect and then analyse how these two Rho GTPases prepare the cell to move forward or backwards by fluorescence lifetime imaging microscopy (FLIM).

PROJECT: Synaptic Vesicle Trafficking
SUPERVISOR: Dr Vladimir Sytnyk
SUMMARY: During the first session of the project, the students will obtain introduction into the general organization of the work in the lab (including OHS issues) and the equipment that they will use. The students will conduct the preparatory work for the experiments in Session 2&3, including plating of the mammalian cells. In Session 2, students will use live cell imaging to analyse formation and trafficking of organelles using vital stains of the membranes and different fluorescent reporters of the protein components of the vesicles. In Session 3, students will continue the experiment, and learn how to quantify the rate of organelle formation and transport in living cells.

PROJECT: Intravital intracellular microscopy of exocytosis of secretory granules in living mice.
SUPERVISOR: Dr Andrius Masedunskas
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: Using super resolution microscopy to understand how calcium regulates protein clustering at the plasma membrane
SUPERVISOR: Dr Jeremie Rossy
SUMMARY: The spatial organisation of proteins – such as receptors or ion channels – within cell membranes greatly determines how cell work and react to their environment. Quite intuitively, two proteins have a higher chance to interact if they are close to each other or happen to sit in the same cluster. Many mechanisms regulate protein distribution, including the lipid composition of the membrane, protein-protein interactions or membrane charge. Here we will use single
molecule imaging to determine how changes in the charge of the plasma membrane triggered by calcium can lead to clustering of membrane proteins.

**PROJECT:** Single-molecule imaging of membrane pore formation  
**SUPERVISOR:** Dr Till Boecking  
**SUMMARY:** Cytolytic proteins insert into the plasma membrane of a target cell to form a pore. This mechanism is exploited by cytotoxic T cells and natural killer cells to deliver proteases to cells that have been infected with viruses or bacteria. The dynamics of membrane insertion and pore formation are not understood at the molecular level. The aim of the project is to reconstitute pore formation and visualise the process using TIRF microscopy. The students will acquire movies of pore opening and analyse images using ImageJ.

**PROJECT:** Using single molecule FRET for the analysis of T-cell activation  
**SUPERVISOR:** Dr Enrico Klotzsch  
**SUMMARY:** smFRET Modern molecular biology, with its emphasis on analysis of entire genomes, has provided a ‘parts list’ of cellular proteins as well as an enumeration of many of their associations, including receptor- ligand interactions, cell migration and cell – matrix interaction. It appears surprising that molecular forces in this context until recently have been largely overlooked. As there are mechanical forces being involved in decision-making processes such as T-cell activation, the project aims to measure them on the pico-newton level. For that we will use sensors that change their fluorescence behaviour upon force exposure. The participants will learn how to prepare samples in the first step, measure single molecule FRET and later analyse the results with custom written Matlab code.

**PROJECT:** Identifying novel anti-cancer compounds that target the actin cytoskeleton  
**SUPERVISOR:** Dr Nicole Bryce  
**SUMMARY:** This project will use automated high content imaging to examine the effects of several different classes of drugs on the actin cytoskeleton in neuroblastoma cells. Session 1: OHS induction, introduction to the project, cell plating and drug treatment of cells. Session 2: Fixation and staining of cells, tour of automated imaging facility. Session 3: Image and data analysis.

**PROJECT:** Electron Microscopy of Nanoparticles  
**SUPERVISOR:** Prof Richard Tilley/ Dr Rhiannon Kuchel  
**SUMMARY:** Magnetic nanoparticles are currently the subject of intense research worldwide and hold a strong interest in the field of nanoscience for their numerous applications including drug delivery and contrast enhancement in magnetic resonance imaging, leading to potential detection of smaller tumours resulting in earlier cancer diagnosis and more enhanced treatment. However for successful application, strict control on nanoparticle size needs to be achieved, in order to obtain magnetic nanoparticles that are
superparamagnetic; that is they do not retain magnetization once an applied field is removed and hence do not aggregate. The project will focus on the shape, size, and crystallography characterisation of novel, cutting edge nanoparticle materials using the instruments available in UNSW’s state-of-the-art electron microscopy unit, in particular high resolution electron microscopy.

**PROJECT**: The role of Yes Associated Protein (YAP) in epidermal homeostasis  
**SUPERVISOR**: Dr Annemiek Beverdam  
**SUMMARY**: We recently found that Yes-associated protein activates epidermal stem/progenitor cell proliferation. To do this, we generated a transgenic mouse model in which a constitutively active YAP mutant protein was overexpressed in the basal keratinocytes of the mouse epidermis. This resulted in a dramatic thickening of the epidermis, and the hair follicles transformed into stem cell masses. Eventually this resulted in complete baldness. In this project we will investigate events downstream of YAP activation in the regulation of epidermal stem cell proliferation. We will investigate the transgenic phenotype by H&E staining and light microscopy. In addition, we will look into expression of proteins that may be regulated by YAP, and we will assess epidermal stem cell proliferation rates by confocal microscopy. Session 1: Introduction into project, and H&E and antibody staining. Session 2: Analysis of H&E staining, completion of antibody staining and imbedding. Session 3: Imaging and analysis.

**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**

f Practice, practice, practice
f Obtain feedback, and use it
f Be ruthless - delete unnecessary information
f Speak loudly & clearly
f Use short simple sentences
f Avoid jargon & abbreviations
f Vary pitch, tone, volume, speed and pauses
f Avoid distracting mannerisms
f Relax, be enthusiastic
f Make eye contact
f Keep an eye on the time remaining
f Explain figures, and point to important aspects
f Give a clear and concise summary, then stop
f Don’t go overtime. Ever.

**Tips on handling questions**

f Anticipate likely questions and prepare extra slides with the answers
f Maybe even plant a stooge
f Paraphrase questions:
  f so that other people hear the question.
to check your understanding 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