# PATH3210

## Visualising Disease

### Course Manual

### Term 1, 2023

School of Biomedical Sciences

Faculty of Medicine & Health

# Table of Contents

# Lecture, Tutorial and Practical Outlines

# Week 1

**Lecture:1 Introduction: Course outline, Assessments and Projects and Cell Biology**

[Professor Peter Gunning, Professor Edna Hardeman and Dr Renee Whan]

**Aim:** This lecture is intended to provide an overview of the course and it structure to students. We will also provide a background to principles of cell biology that underpin imaging of disease.

**Learning Objectives:**

**1.** Breakdown the layout, aims and expectations of the course.

**2.** Examine assessment criteria and articles for assessment 2 and 3.

**3.** Develop and build on core cell biology terminology

**4**. Explain the relationship between cell biology and imaging of disease

**Tutorial 1: Overview of Microscopy** [Dr Michael Carnell]

**Aim:** To provide a high-level overview of the range of imaging techniques you will encounter in this course. Using light microscopy as an example I will illustrate core concepts universal to imaging, such as resolution, contrast, signal-to-noise and sampling.

**Learning Objectives:**

**1.** Understand how the terms resolution, contrast, signal-to-noise and sampling relate to considerations of imaging disease

**2.** Describe the components of a conventional light microscope understanding the problems they solve.

**3.** Be able to justify your choice of a microscope objective lens.

**4.** Distinguish between 3 different methods of introducing contrast into a transparent sample using transmitted light microscopy.

**Lecture 2: Visualising Disease and Treatment** [Professors Peter Gunning and Edna Hardeman]

**Aim:** To connect features of cell and tissue structure and function with information that can be derived from imaging. The core concepts of patient presentation in the clinic and the use of imaging to reach a diagnosis and monitor treatment will illustrate the role of imaging in health and disease.

**Learning Objectives:**

1. Categorise features of cells and tissues used in diagnosis and treatment of disease.

2. Apply the use of imaging to develop new treatment options.

3. Evaluate the limitations of cell and tissue imaging.

**Tutorial 2: Fluorescence Microscopy** [Dr Renee Whan]

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

**Learning Objectives:**

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

2. Calculate resolution for given wavelengths using either the Abbe or Rayleigh Criterion

3. Be able to describe, analyse and apply how fluorescence is harnessed in a microscope; Filters; excitation sources and cameras

4. Determine the different ways to label a specimen with fluorescence

5. Design a solution to separate fluorescence spectra

# Week 2

**Lecture 3: Specimen Preparation for Light Microscopy** [Dr Renee Whan]

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

**Learning Objectives:**

1. Evaluate different techniques available for preparing samples for light microscopy.

2. Be able to predict a specimen preparation technique/s for a given experiment.

3. Be able to modify a specimen preparation technique to improve the visualisation

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

**Practical 1: Labelling Cancer Cells** [Dr Renee Whan]

**Aim:** Be able to apply knowledge of fluorescence spectra and specimen preparation to design an experiment on cancer cells.

**Learning Objectives:**

1. Critically evaluate an experimental design to minimise cross-talk between labels of interest.

2. Compose and model fluorescent labelling of a cell to visualise a disease.
3. Be able to justify your choice of labelling.

**Lecture 4: Electron Microscopy** [Dr Joanna Richmond]

**Aim:** This lecture will introduce students to various electron microscopy techniques that have been developed to investigate the ultrastructure, and organisation of disease (JR)

**Learning Objectives (JR)**

1. Differentiate between scanning and transmission electron microscopy (EM).
2. Evaluate the benefits and limitations of using electron microscopy to visualise disease
3. Determine common EM sample preparations and be able to distinguish between well and poorly fixed biological samples.
4. Be able to identify and contrast various cellular organelles based on their ultrastructural morphology (i,e. mitochondria, ER, endosomes) with a particular focus on normal versus abnormal organelle features.
   Points for discussion:
   How does Electron and Light Microscopy compare? Should you use the merits of both technologies to examine your disease?

**Practical 2: Electron Microscopy** [Dr Joanna Richmond]

**Aim:** In this tutorial you will be introduced to electron microscopy using an online learning platform called MyScope.

1. Perform a realistic operation on the MyScope simulator to understand the fundamental science behind electron microscopy.
2. With focus on good sample preparation, design an experiment using transmission electron microscopy to investigate whether there is uptake of metallic nanoparticles into cells.
3. Create an appropriate experiment using scanning electron microscopy to confirm whether bacteria are colonising on the surface of cells and what cellular morphology would be indicative of cell death?

# Week 3

**Lecture 5: What makes up an Image?** [Dr Michael 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 when drawing conclusions from its contents. 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. Describe the composition of an image including the terms: pixel, bit-depth and look-up-table (LUT)
2. Justify when a processing step is deemed acceptable vs when it is data falsification.

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 and background corrections.

**Practical 3: Processing and Analysing Data** [Dr Michael Carnell]

**Aim:** This practical will involve the use of ImageJ to solve several image processing and image analysis tasks.

**Learning Objectives:**

**1.** Build image processing and analysis pipelines informed by an understanding of preserving data integrity.

**2.** Generate images and results from raw imaging datasets.

**3.** Develop problem solving skills in identifying issues within images and discover solutions to these problems.

**Lecture 6: Confocal and other 3D imaging Techniques** [Dr Renee Whan]

**Aim:** This lecture will introduce you to the third dimension in microscopy. The global aim is to categorize and apply the 3D imaging techniques allow visualisation of disease in cells and tissues.

**Learning Objectives:**

1. Be able to differentiate between different modalities of optical sectioning in light microscopy.

2. Predict the effect of the pinhole on resolution

3. Evaluate the appropriate 3D imaging technique for a given experiment.

4. Understand the fundamentals of 3D reconstruction

5. What 3D imaging allows us to design experimentally when examining a disease

Points for discussion:

What are the advantages and limitations 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.

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

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

# Week 4

**Lecture 7: Co-localisation** [Dr Michael Carnell]

**Aim:** This lecture aims to use co-localisation analysis (measuring the distribution of two channels relative to each other) as an example of considerations that must be addressed when analysing imaging data.

**Learning Objectives:**

**1.** Evaluate different ways of measuring how two signals distribute in a sample relative to each other.

**2.** Compare the difference between co-occurrence and correlation.

**3.** Defend the need to simplify images down to measurements, whilst also critique the problems with describing a set of images by a small subset of measurements.

**4.** Understand the impact of data acquisition, and instrument choice, on colocalisation measurements.

**Practical 5: Image Analysis** [Dr Michael Carnell]

**Aim:** This practical aims to build upon practical 2 diving deeper into image analysis. Various datasets will be supplied with the aim of assessing appropriate methodologies and identifying weaknesses within the datasets themselves.

**Learning Objectives:**

**1.** Create an appropriate experimental design to facilitate meaningful analysis.

**2.** Perform a colocalization analysis and justify the metric(s) you decide to use.

**3.** Generate an analysis method using image filters to quantify scratch wound closure

**4.** Formulate a timelapse analysis measuring cell division.

**Lecture 8: Entering the fourth dimension: Live Imaging** [Dr Renee Whan]

**Aim:** This lecture will outline the specimen preparation, acquisition and analysis methods utilized when performing live cell imaging.

**Learning Objectives:**

1. Determine the necessary environmental conditions for live cell imaging

2. Apply knowledge of different ways of labelling (live cells; genetic encoding and probes) to experimental design.

3. Evaluate issues that effect experimental outcome including, but not limited to; phototoxicity; cell health, contamination

4. Determine common live imaging experiments used to look at key hallmarks of cancer.

Points for discussion:

What are the trade-offs for imaging at high speed? The balancing act between specimen health and image quality.

**Tutorial 3: Live Imaging Analysis of cell division and the effects of chemotherapeutics** [Dr John Lock]

**Aim:** This tutorial aims to connect basic understanding of a dynamic cellular process, mitosis, with considerations for how this process can be analysed via a combination of chemical perturbations and live cell imaging. Quantification of cell mitosis outcomes using imageJ will highlight how chemotherapeutic drugs limit cancer cell division.

**Learning Objectives:**

1. Ability to categorise cell cycle and mitotic phases

2. Capacity to compare and contrast alternate imaging modalities and paramaterisations for mitotic live cell imaging

3. Evaluate the effects of chemotherapeutics on mitotic outcomes using imageJ

# Week 5

**Lecture 9: Intravital imaging of cancer growth and movement** [Professors Peter Gunning and Edna Hardeman]

**Aim:** This lecture will introduce you to intravital microscopy, the live imaging of biological processes in animals at high resolution. Imaging of cancer cell migration will be used to illustrate the power of intravital microscopy.

**Learning Objectives:**

1. Illustrate the principle of intravital microscopy and the design challenges in performing live imaging in animals.

2. Evaluate the advantages and disadvantages of performing live imaging in cell culture compared with a live animal. Based on which considerations would you choose between using one or the other approaches for your live imaging?

3. Contrast the knowledge gained from cancer cell migration in cell culture and the live animal. Develop a strategy to determine the suitability of each modality for different experimental questions.

4. Identify the technical limitations of intravital microscopy and the challenges for the future.

**Tutorial 4: Microscopy of metastasis** [Professors Peter Gunning and Edna Hardeman]

**Aim:** This tutorial will introduce you to intravital microscopy, the live imaging of biological processes in animals at high resolution. You will design an experiment to test the role of branched actin filaments in the migration of tumour cells into the blood.

**Learning Objectives:**

1. Illustrate the experimental procedures used to perform intravital microscopy.

2. Design an intravital experiment to quantify the movement of cells from a tumour into the blood. What will you measure?

3. Expand your experimental design to test the role of branched actin filaments in movement of tumour cells into the blood.

4. Identify the technical limitations of your experiment.

**Lecture 10: Multiplex labelling and systems approaches** [Dr John Lock]

**Aim:** This lecture will introduce the emerging field of multiplexed cellular imaging, and how this is enabling imaging-based systems biology at cellular and sub-cellular scales to better understand complex cellular and disease processes

**Learning Objectives:**

1. Ability to illustrate how different multiplexing techniques overcome classical limitations of immunofluorescence

2. Determine how OPAL multiplexing enhances spatially resolved analysis of cancer

3. Relate how 4i multiplexing permits systems analyses of cellular and subcellular mechanisms

**Practical 6: The use of AI in image analysis QUIZ 4** [Dr John Lock]

**Aim:** This practical will introduce strengths, limitations and some key uses of AI for image analysis. Types of 'AI', from simple multiple regression to support vector machines (SVM) to deep learning networks will be outlined. Live cell mitosis imaging data will be used to apply cell segmentation and classification using an SVM in Ilastik software, and a deep learned image data representation will be 'explained' using custom data visualisation software (BioDive).

**Learning Objectives:**

1. Summarize the general strengths and limitations of AI

2. Contrast key uses and types of AI deployedin image analysis

3. Modify a machine learning workflow to segment and classify mitotic cells using Ilastik

4. Compare your perception of single cell image heterogeneity and that of an AI using BioDive

# Week 7

**Lecture 11: Advanced Microscopy Methods 1** [Dr Alex MacMillan]

**Aim:** To examine imaging methods for visualising molecular dynamics

**Learning Objectives:**

1. To understand the concept of FRET (Förster resonance energy transfer)
2. To understand the concept of FLIM (Fluorescence Lifetime Imaging Microscopy)

3. Determine how FRET and FLIM can be used examine signalling events and spatial localisation
4. Relate the capability of FLIM to examine viscosity, pH and degree of oxidative stress in live cells to different diseases.
5. Compare the capacity of FLIM to examine drug accumulation and localisation, to standard confocal imaging.

**Lecture 12: Advanced Microscopy Methods 2** [Dr Elvis Pandzic]

**Aim:** This lecture is an introduction to the super-resolution microscopy, which resolves beyond the sub-diffraction limit of light microscope, allowing us to see finer details withing cellular nanostructure.

**Learning Objectives:**

1. Learner will be able to compare and classify different super-resolution (SR) microscopy modalities.
2. Contrast key concepts of resolution and single molecule precision and their importance in the implementation of the super-resolution approaches.
3. Predict the technical challenges required to obtain some of these higher resolution images of cells.
4. Evaluate how analysis of the super-resolution approaches relates to the standard microscopy data, and determine whether (SR) can provide useful quantitative data on cells.

# Week 8

**Lecture 13: Overview of Medical Imaging Modalities** [Professor Daniel Moses]

**Aim:** These lectures introduced the basic principle behind medical imaging techniques in generating images for modalities including plain film, fluoroscopy, ultrasound, computed tomography, nuclear medicine and MRI.

**Learning Objectives:**

1. To explain the basic physical principles of image generation in each modality including x-ray and ultrasound generation, attenuation and detection.
2. To explain the general principles of image generation in CT and MRI
3. To describe the common indications of each modality.
4. To list the common advantages and limitations of each modality.

**TUTORIAL 5 – How to image a patient** [Professor Daniel Moses]

**Aim:** This session examines the practicalities of imaging patients using the various modalities including contraindications, preparation for the tests, and how the scans are performed.

**Learning Objectives**

1. To understand the practical aspects of performing radiology tests in each modality including patient positioning and the use of intravenous and oral contrast.

2. To list the contraindications in performing various radiological examinations.
3. To describe the patient preparation required for various radiological examinations.

**Lecture 14: Functional and Interventional Applications of Medical Imaging** [Professor Daniel Moses]

**Aim:** To introduce the basic principles of functional imaging and interventional radiology and show how they are useful in the clinical setting.

**Learning Objectives:**

1. To describe the difference between anatomical and functional imaging
2. To explain the main types of functional imaging.
3. To explain how interventional radiology can assist in the diagnosis and treatment of disease.
4. To give examples of interventional radiology procedures.

**Practical 8: Diagnosing patients using medical imaging** [Professor Daniel Moses]

Aim: Explores how medical imaging is useful in diagnosing and treating patients via a case study of a patient with particular disease.

**Learning Objectives:**

1. To explain how various modalities assist in the diagnosis diseases.
2. To describe how radiological studies help with monitoring and treating diseases.

# Week 9

**Lecture 15 : Preclinical Imaging** [Dr Carl Power and Dr Tzong Hung]

**Aim:** Aim: An introduction to preclinical imaging and applications in research.

**Learning Objectives:**

1. List the key differences between clinical vs preclinical instruments
2. List the challenges of animal imaging
3. Compare the advantages and limitations of specialized pre-clinical imaging methods
4. Outline the key ethical requirements of animal research

# Week 10

**Lecture 16: Personalised Medicine - Emerging imaging approaches for treatment** [Professor Peter Gunning]

**Aim:** To integrate the different approaches to imaging into a holistic strategy that determines the treatment for an individual patient and and monitors the progress of the treatment.

**Learning Objectives:**

1. Illustrate the principles of personalised medicine and how to determine a treatment strategy.

2. Evaluate the place of imaging in deciding a treatment strategy in comparison with OMICS approaches.

3. Determine the imaging approaches best suited to monitoring treatment progress.

4. Critique the assumptions that underpin personalised medicine and the future of imaging approaches.

**TUTORIAL 7: Career Development: Revision with a panel of researchers, industry reps and clinicians**

**Aim:** To provide the class with a practical guide to career paths built on knowledge gained in this course.

**Learning Objectives:**

1. The representatives of the different paths will present a brief overview of their career paths with particular attention to the role of their training in imaging.

2. A question and answer discussion will be facilitated to identify what where the most important decisions and what they would have done differently.

3. We will end with evaluation of the future of these career paths.

**Lecture 17: The visualisation of signalling molecules in cancer** [Professor Paul Timpson]

**Aim:** TBC

**Learning Objectives: TBC**

**Tutorial 8: Course revision**

**Aim:** To prepare the class for the final exam.

**Learning Objectives:**

1. Provide examples of the types of questions in the exam and the approach to marking.

2. Provide an opportunity for the class to ask questions related to the course in general and more specifically the exam.

3. Receive feedback from the class regarding what worked and what could be improved.