COMPARE
CENTRE OF MEMBRANE PROTEINS AND RECEPTORS

# Imaging Capabilities at COMPARE

Below is a summary of the microscopes in COMPARE. For further information including training and costings please contact Dee Kavanagh (d.m.kavanagh@bham.ac.uk) or Joelle Goulding (mailto:joelle.goulding@nottingham.ac.uk).

Please note the equipment is not maintained to GCP in the Laboratory standard and therefore cannot be used in the analysis of clinical trial (CTIMP or non-CTIMP) samples which contribute to the primary, secondary or exploratory outcomes of such trials.

## Birmingham

### The UltramicroscopeII

The UltramicroscopeII is a fluorescent microscope used for 3D imaging of large samples such as whole mouse organs and small tissue samples (essentially 3-D histology). The microscope is suitable for imaging multi-colour fluorescently labelled cell types and vasculature e.g. renal, cardiac and lymphatic, in samples that have been made optically clear using established protocols.

### Marianas diSPIM microscope

The Marianas captures two alternating views of a sample. The microscope is suitable for imaging multi-colour fluorescent cellular structures in spheroids and organoids. These views are then merged together to give an even 3D image without any distortions. The Marianas microscope can be used for long time lapse imaging of living samples (hours) such as formation of platelets from megakaryocytes.

### Lattice Light Sheet

The Lattice microscope is the leading fluorescent microscope for imaging live cellular processes in single cells (hours). Using this microscope you will be able to gain precise information on protein and organelle dynamics, with the highest and most accurate time resolution currently available in a fluorescent imaging system.

### Structured Illumination Microscope (SIM)

SIM allows the observation of live and fixed fluorescent samples at a resolution (100x100nm) greater than a standard fluorescent microscope (300x300nm). Imaging at this resolution can reveal structural organisation that can be otherwise masked.

### Single Molecule Stochastic Optimal Reconstuction Microscope (STORM)

The single molecule microscope is used to identify the position of single fluorescent protein and lipid molecules. The molecule positions can then be used to extract information on protein organisation at a higher level using mathematical algorithms.

### Fast multicolour single-molecule microscope (4-camera TIRF)

This custom 4-camera TIRF is capable of simultaneously imaging up to four different molecules (labelled with different colours) with single-molecule sensitivity, permitting analysis of movement of individual molecules as they diffuse and interact in living cells with a spatial resolution of approximately 20 nm and a temporal resolution of 20 ms. This provides quantitative information on complex populations of molecules, including their nanoscale arrangement, diffusion and interactions.

### Ultrafast FRET/calcium imaging microscope

This custom microscope is capable of performing ultrafast FRET and calcium imaging measurements in living cells with up to sub-millisecond temporal resolution. It is equipped with a fast perfusion system for rapid stimulation, which allows to exchange the solution received by the perfused cell in a few milliseconds. Combined with a series of FRET sensors and indicators, it allows monitoring the kinetics of cell signalling events such as receptor activation or the production of second messengers such as calcium or cyclic AMP in living cells with high spatiotemporal resolution.

**Computational resources and specialist software (Birmingham)**

There are six high-spec dedicated analysis workstations, as well as preferential access to the University's central high performance computing (HPC) resources. The workstations and HPC resources are free to use.

We have in-house expertise in a range of leading open-source software and can provide consultation and advice on image analysis applications. There are two commercial software packages; **Arivis Vision4D** and **SVI Huygens**. Vision4D enables user friendly analysis of large volumetric data and facilities impressive and fast 3D volume rendering for visualisation and inspection of data. Huygens is specialist deconvolution software which improves image quality by removing out of focus light from fluorescent microscopy datasets. Commercial software is chargeable either by annual fee, or at an hourly rate.

**Imaging capabilities at COMPARE (Nottingham)**

**Bioluminescence – Olympus LV200**

The Olympus LV200 is capable of imaging bioluminescence within an environmentally controlled setting. Bioluminescence is the chemically induced release of light which does not need laser or LED excitation light (as with fluorescence) and as such does not suffer from the side-effects of photo-toxicity and photo-bleaching. The microscope can be used to image bioluminescence in cell monolayers, and for proximity assays using bioluminescence as donor (Bioluminescence Resonance Energy Transfer, BRET)

**Ptychography – PhaseFocus Livecyte**

The PhaseFocus Livecyte can measure the change in the phase of transmitted light as it passes through a sample and can reconstruct an image which contains information on cell volume, shape, size etc. Cell movement can be tracked and cell splitting and lineage monitored. No lasers are used and as such photosensitive cells can be imaged for several days, within multi-well plates. The microscope has temperature, humidity and gas control which allows automated imaging long term within the environmentally controlled casing. Potential assays include cell migration, mobility, proliferation, wound healing and angiogenesis (formation of blood vessels).

**Confocal Microscopy – Zeiss 710**

This microscope allows fluorescence confocal microscopy. By the addition of a pinhole within the laser beam path, out-of-focus light from your sample can be discarded allowing the imaging of thin confocal slices and if desired 3D reconstruction of your sample. This is perfect for localisation and quantification of your fluorescent target and can be coupled with more advanced techniques such as FRET (to look at protein-protein interactions) and FRAP (recovery after photo-bleaching to study diffusion and immobile fractions).

**Fluorescence Correlation Spectroscopy (FCS) – Zeiss 880 & Zeiss 510NLO**

These two confocal microscopes are equipped with single photon sensitive detectors which allow fluorescence fluctuation spectroscopy techniques such as FCS. FCS allows quantification of particle

number, diffusion speed and brightness within a very small volume (0.25 femtolitre) and can be applied to samples both in solution or expressed within live cells. Within solution, important pharmacological parameters such as drug binding affinity can be determined. Within live cells the organisation of membrane proteins can be studied and in addition the effects of drug treatment examined. This technique is ideal for low expression systems such as those observed physiologically.

**Total Internal Reflection Fluorescence (TIRF) – Nikon Ti2**

TIRF microscopy produces a very thin field of illumination above the sample chamber (100-200nm) which allows imaging of the lower membrane of a cell or a layer of immobilised protein. Time-lapse capture can be amenable for tracking the movement of membrane proteins and methods can be optimised to enable the study of single molecule dynamics and interactions.

**High-content plate readers – MD Image Express Ultra and Micro**

High-content plate readers enable automated, multi-parameter image capture across various plate formats. Both live and fixed cells can be imaged for fluorescence in 96- or 384-well format. The microscope moves the low-throughput quantification and localisation microscopy into a high-throughput scheme. Analysis packages are available to quantify receptor internalisation, cellular structures and fluorescence intensity/per cell or region. These can subsequently be used to determine important parameters used in screening such as drug binding affinity and potency.