

The Microscopy Unit Handbook

The microscopy unit at the UCL Institute of Ophthalmology provides training and access to a number of microscopy techniques.

We also provide microscopy services, many of which are outlined briefly in the following pages.

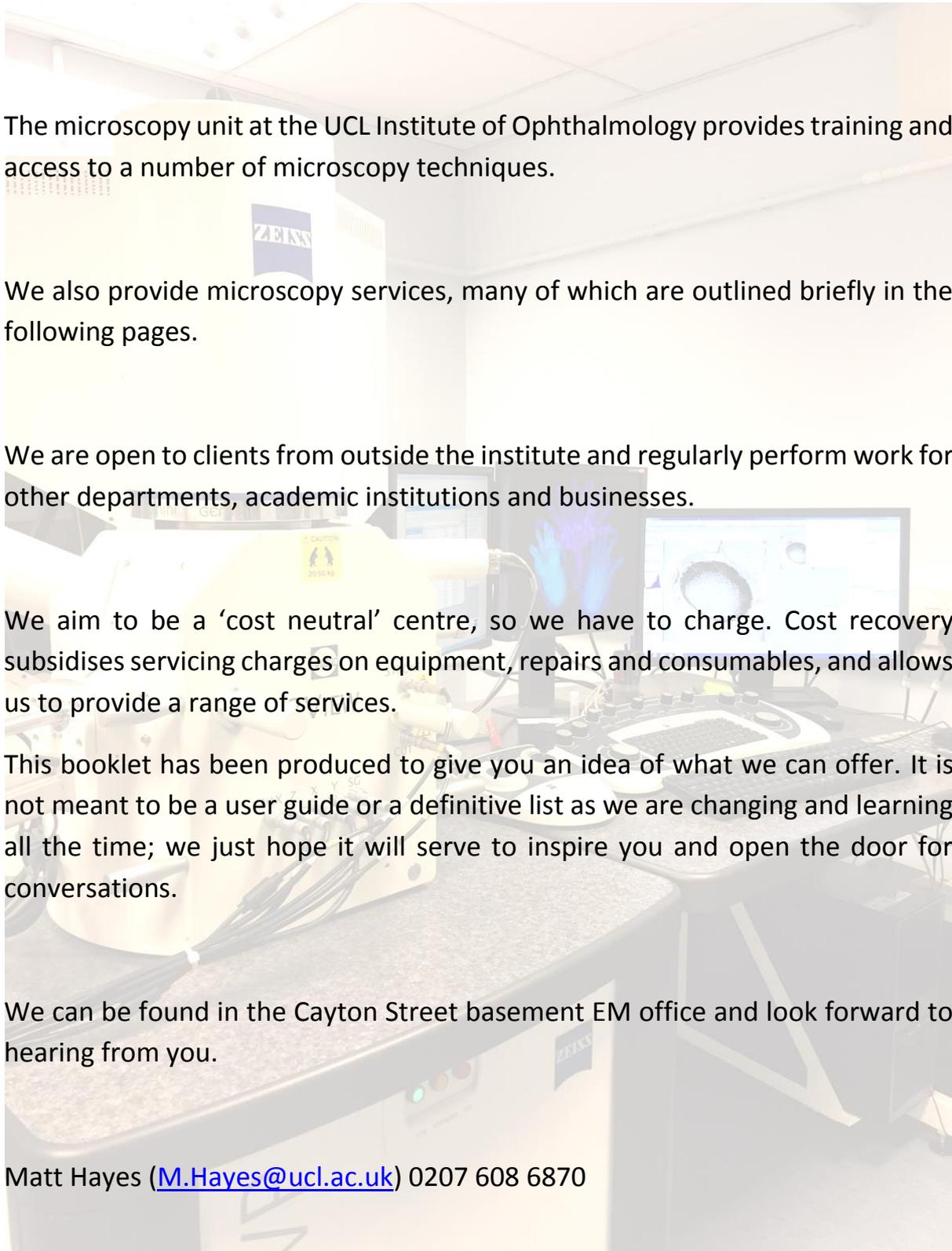
We are open to clients from outside the institute and regularly perform work for other departments, academic institutions and businesses.

We aim to be a 'cost neutral' centre, so we have to charge. Cost recovery subsidises servicing charges on equipment, repairs and consumables, and allows us to provide a range of services.

This booklet has been produced to give you an idea of what we can offer. It is not meant to be a user guide or a definitive list as we are changing and learning all the time; we just hope it will serve to inspire you and open the door for conversations.

We can be found in the Cayton Street basement EM office and look forward to hearing from you.

Matt Hayes (M.Hayes@ucl.ac.uk) 0207 608 6870



The Rules

I would just like to take this opportunity to mention a few basic rules for our unit. We are a small facility with significant demand, with limited time, physical and personnel resources. In order that we are able to continue to support everyone; we need our users to be mindful of other people and considerate when booking and using equipment.

Bookings are currently made through *Schoolbooking* or *Gmail* systems. Only users added by us may use the equipment. Health and safety considerations require everyone to be inducted into our facility. Anyone found using equipment without training and consent from us will be banned.

Do not try to train your colleagues to use equipment.

A particular confocal can only be booked for up to **4 hours per day** by a particular person and no more than **12 hours in any one week**. Please only book time you need.

Note the hours of use in the record sheets provided.

Tidy up after you have finished and clean lenses appropriately. Please report any problems or concerns.

If you are at all uncertain how to use something **PLEASE ASK**. We are happy to help and this can avoid damage to expensive machinery.

Prices:

TEM	Up to 10 specimens Embedded in resin. £300*	+ semithin sections (650nm on slides with/out Toluidine blue staining) £700@	+ semithin sections + ultrathin sections (50-90nm) on grids. £800@
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* Additional specimens will double the cost of embedding as no more than 10 can be processed safely at any one time.

@ Additional labour charges may be made for complex specimens that require repeat sectioning and examination at £70 per hour.

SEM	Up to 5 Specimens Fixation/dehydration/HMDS/Critical-point drying/mounting/coating £200
SBF-SEM 3-View	£600 to embed up to 10 specimens plus £600 per specimen mounted/run

MICROSCOPE PRICES hourly (Training/Support)	
Nikon Live Cell	£10 (£100)
Micron III	£10 (£100)
Bioptigen OCT	£10 (£100)
Zeiss 510 Axio	£10 (£100)
Zeiss 700	£28 (£250)
Zeiss 710	£33 (£250)
Leica SP8	£40 (£250) £20 for overnight time-lapses (after 6pm)
PALM	£30 (NA)
TEM	£30 (£100)
SEM (Zeiss Sigma)	£30 (£100)
SBF-SEM 3-View	£600 overnight run
CRYOULTRAMICROTOME plus liquid nitrogen	£50 (half day) (£200)
AMIRA segmentation	£10 (£200)

These are only estimated prices. Individual projects that are atypical and require additional labour or consumables may attract additional charges.

When using equipment we ask everyone to fill in details of their name (in capitals), hourly usage, their supervisor, and the grant to which invoices should be made out. The latter, in particular, is necessary as many grants are short-lived and change rapidly. Failure to provide relevant information wastes a great deal of time.

Invoices are generated quarterly. Principle investigators are given 10 days to read through them and request clarification if there are any questions arising. After this period, they are sent to the finance department. Failure to honour a bill in three successive quarters will result in a ban.

We hope in the near future to introduce an invoicing system based on hours booked rather than hours used.

Acknowledgement

Most of our equipment has been purchased via charitable donation; some has been provided by collaboration with industry. In order to raise further funds (we are engaged in grant writing), we need to demonstrate that our services are useful, used and support your research. We would ask that if you make use of our equipment you acknowledge that in any publications that arise. A simple **'Thanks to the Institute of Ophthalmology Microscopy Unit'** will suffice. This also alerts other people to our services.

Imaging Protocol Analysis

Artefacts and issues:

We can provide advice on the design of experiments and in particular the necessary 'imaging controls' that may need to be done to prevent the generation of artefacts.

Much of the image data published (even in very reputable journals) is artefactual and based on inadequate understanding of the pitfalls of sample staining, image acquisition and image processing.

We hope to run a number of short seminars on these issues.

We have quite a bit of experience at looking at the typical specimens generated in this department. This means we know some of the problems other people have experienced.

It may make sense to come and see us before embarking on an extended period of imaging, in case there are things you might want to do before generating a huge body of work. It is not uncommon for people to come and see us rather late in the day, when it is harder to help.

If you have issues with overlapping dye spectra (in fluorescence) we may be able to help by using *spectral unmixing* to separate them. These kinds of problems are always best avoided by careful planning.

What next?

Once you have generated your images you need to work out how to extract data from them. A picture may say a thousand words; but a statistic may require a thousand pictures.

We have experience in a number of analysis programs. ImageJ (aka Fiji) is free online and incredibly useful. Many 'add-ons' are available, short programs written by the imaging community to perform automated measurement and segmentation (finding objects within images).

There are many useful algorithms in Matlab and Mathematica if you are familiar with those programming environments.

We have licences to:

Huygens (a high-level deconvolution software program), which is currently only available on the Leica SP8 (we hope to purchase additional licences soon). On the SP8 it is embedded in the acquisition hardware (as Hyvolution) which means it is very easy to use.

Amira (version 6.5). A state-of-the-art segmentation and rendering tool. This software is available to use on a dedicated work-station in the EM basement with a WACOM touch screen to allow rapid segmentation. We can provide basic instruction on how to use this.

Zen Black/ Zen Blue (Zeiss). We have an offline work-station which allows basic analysis of data, stitching of large tile scans, colocalisation etc.

Molecular Imaging Browser (MIB). This is freeware run in a Matlab environment. We have this loaded onto a workstation with WACOM touch screen. It works on different principles to Amira which make it useful for the automatic segmentation of certain types of images.

Digital Micrograph (dm4). Used for the acquisition and image manipulation of EM images.

NIS Elements AR. We have off line workstations which allow for basic and advanced analysis of data including deconvolution (Autoquant Blind deconvolution module).

Data storage.

We do not currently offer a long-term data storage solution. We ask that as soon as data is generated it is taken away. Extra time should be booked on equipment to perform this. We recommend that users *save data as they work*. Sometimes equipment and computers fail during use and it can be frustrating to lose lots of data at the end of a session. Large data sets can also take a very long time to save or export. A large tile-scan of z-stacks or a complex render may take hours.

Saving your hard-won data ought to be a major priority, not left to the last minute, whilst the next user is crossly waiting to get on the machine. Data should immediately be backed up by the user, preferably in an off-site data centre. UCL provide individual and group-wide, networked data solutions which are more than sufficient for most users. Our *Drobo* data systems connected to several of our microscopes automatically back-up and cross-correct large data sets. If one component unit fails they can easily be replaced without loss of data. These are NOT for long term data storage, however. **We reserve the right to delete all data stored on our machines without notice.**

Microscope Unit equipment

Our microscopes are broadly grouped as Light Microscopes (widefield and laser scanning confocal microscopes) and Electron Microscopes (transmission TEM and scanning SEM microscopes).

They include:

Widefield

Zeiss 510 Axio transmission light with colour camera

Nikon Ti-E transmission and fluorescence with mono camera (live cell imaging ready)

Confocal

Zeiss LSM700

Zeiss LSM710 with Airyscan detector

Leica SP8 with optional fast resonant scanning and hybrid detectors (live cell imaging ready)

TEM

Jeol 1400

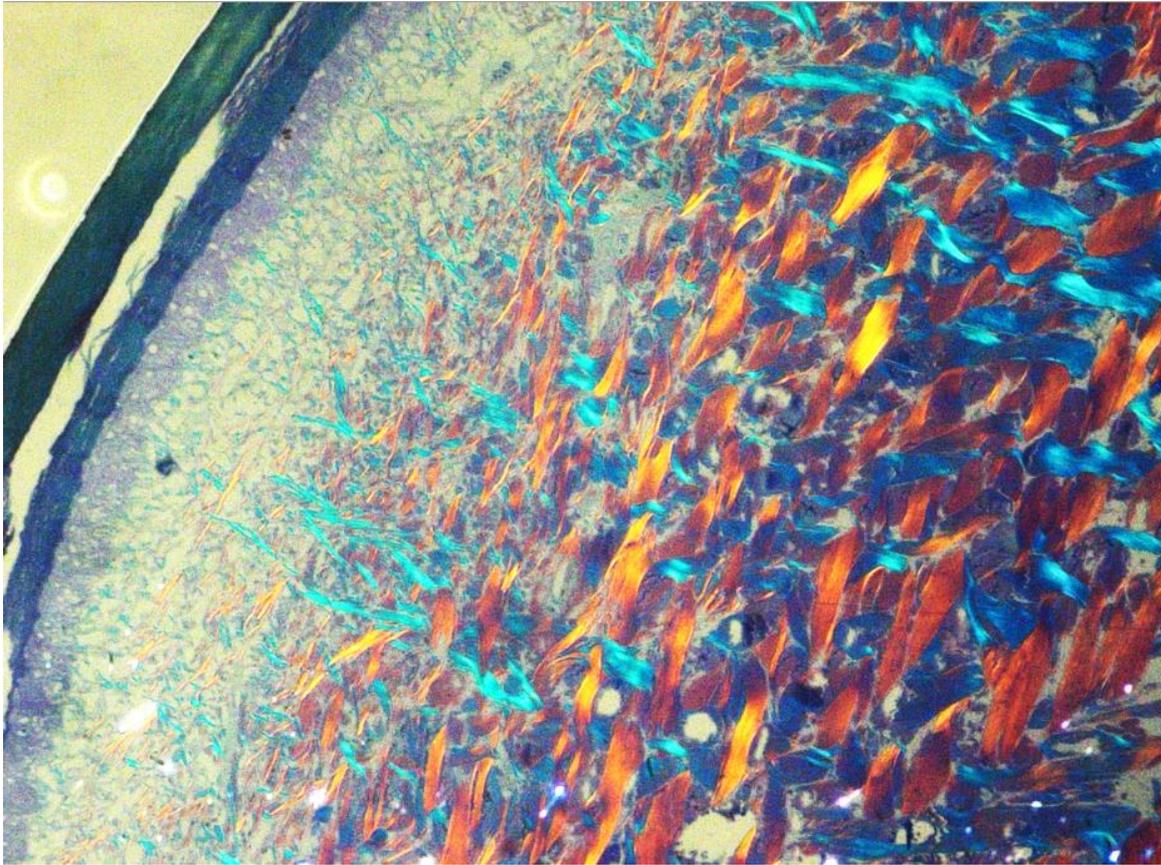
SEM

Zeiss Sigma VP SEM including 3-view (scanning block face)

The following pages aim to provide you with an overview of imaging techniques you can do using our equipment.

Light Microscopy: Colour Camera

Exploring tissue structure in semi-thin sections



Colour photography still has a place in imaging. Certain dyes and stains produce a range of colours under various conditions and these cannot always be easily predicted.

Sections can be generated by various means:

Cryostat (-20 degrees) sectioning of OCT blocks - a material that infiltrates the sample and freezes into a cuttable, relatively soft, water-soluble block ([we can do this for you](#)). [Such sections can be stained with antibodies for histology or fluorescence studies]

Paraffin sectioning (usually performed by the pathology department) [Usually used for histological stains such hematoxylin and eosin (H and E).

Plastic sectioning.

The image above is a resin-embedded section of lizard skin stained with toluidine blue.

This generates an image in a range of blues and pinks. The sample was then viewed using polarised light. The highly-structured collagen filaments in the sample cause the toluidine blue to form para-crystalline arrays, giving rise to 'metachromatic staining'. This generates the beautiful gold and purple colours we see above, reflecting the alignment of the fibres.

(Thanks to [A. Kirby](#) and [S. Bartazzo](#) for the tissue).

We have a colour camera fitted on our [Zeiss 510 Axio](#) microscope. This is also quite good for taking quick images of sections of tissues to see if there are gross changes in ultra-structure.

Live Cell Imaging

Life in motion.

The challenge of working with live cells is to stably maintain optimal environmental conditions during the imaging sessions. The dedicated live cell microscope systems are equipped to control the optimal temperature, humidity and CO₂ conditions.

In addition, these systems are fully motorised to allow creation of imaging protocols which can be used for recording dynamic processes (FRAP, Ca⁺ imaging) or slower time-lapse observations.

The full motorisation allows for a higher throughput imaging within a session, combining multi-channel imaging with z-stack imaging, multiple locations within a specimen or a well plate, increasing a field of view by tiling images - all under controlled time-lapse acquisition parameters.

Furthermore, we have means of correcting any focus drifts which can occur due to temperature changes or vibrations.

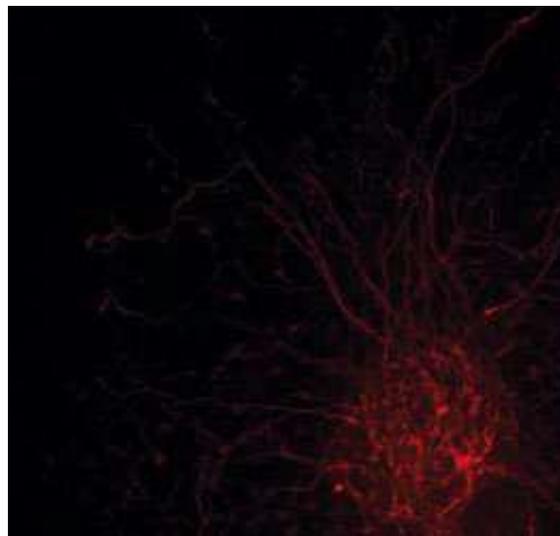
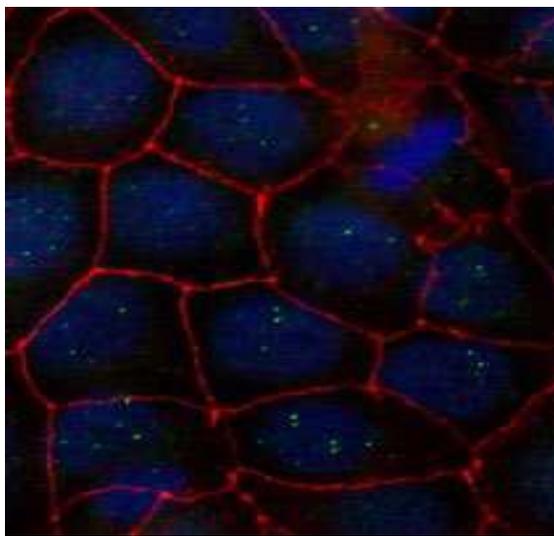
Live cell imaging also requires sensitive detectors (cameras or PMT detectors), to help minimise the exposure to light and avoid photobleaching or phototoxicity within the specimen.

Widefield microscope (Nikon Ti-E)

The objectives 10x-60x are long working-distance ones with focus correction collar allowing adjustment between varying specimen mount types. The 60x oil immersion objective offers maximum resolution through a glass coverslip. Our camera has a large dynamic range for low intensity signals.

The fluorescence channel combinations available are: DAPI, FITC/GFP/Alexa488, TRITC/Cy3/RFP/Alexa555, Cy5/Alexa647, CFP, YFP, mCherry/Texas Red.

The images taken on a widefield microscope do record out-of-focus haze, especially with higher magnification lenses. We offer access to a workstation on which you can deconvolve your 3D datasets using 'blind' deconvolution algorithms.



We can train you how to use our widefield live cell system ([£100 for the 2-hour session](#))
Use is [£10ph during the day](#) and [£5ph overnight, after 6pm](#).

Confocal Microscopy I: Leica SP8 (with HyVolution)

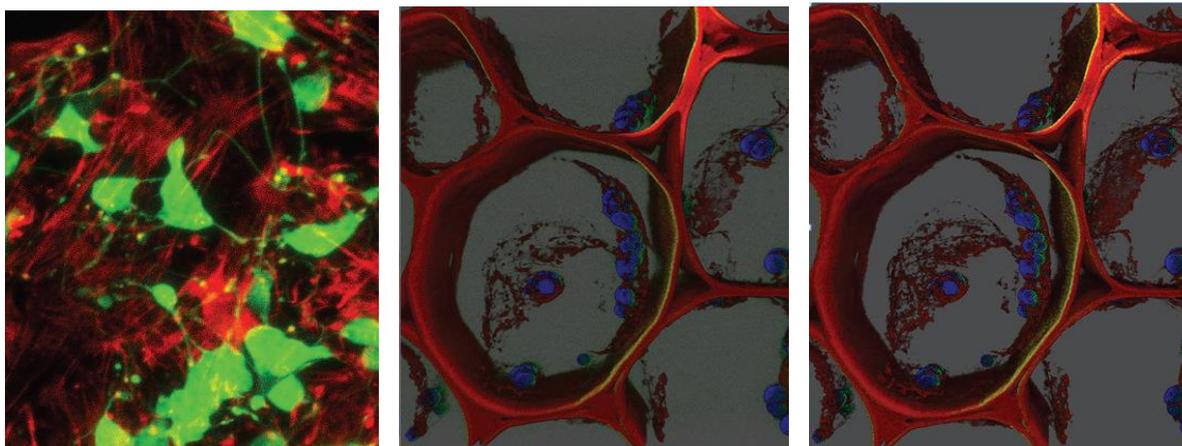
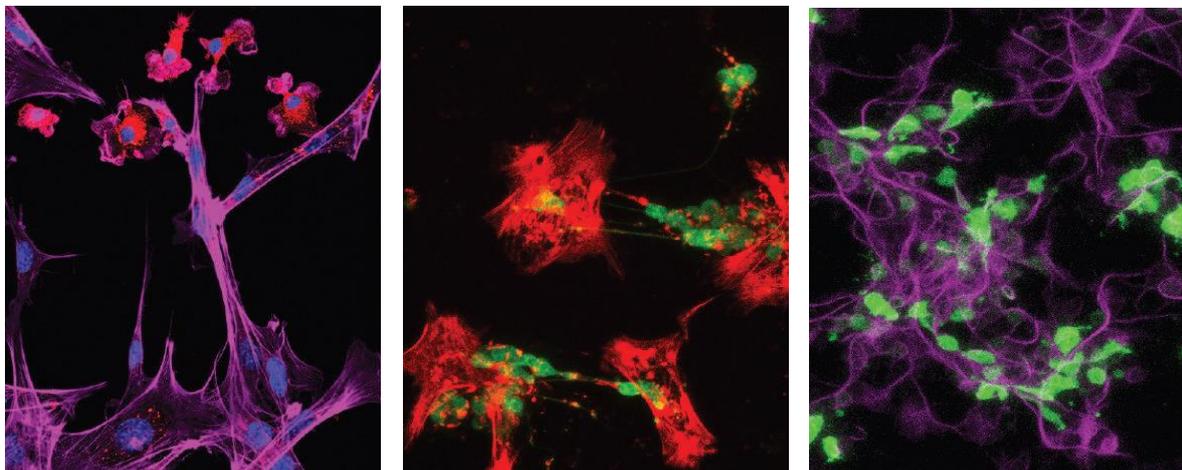
Live Cell imaging on confocal

Our newest addition is the live cell confocal, Leica SP8. It comprises HyVolution (deconvolution software), has a fully motorised, programmable stage system, and full environmental control. It boasts a [white light laser](#) (470-670nm wavelength) with acousto-optic beam splitter and a new generation of highly sensitive hybrid detectors enabling precise match of many fluorophores with excitation and emission ranges.

Apart from additional FRAP and FRET modules, the software includes the [Huygens deconvolution module](#) driving the acquisition of super resolution images with detail down to 140nm.

The Spectral Unmixing option allows full spectral characterisation of the sample and unmixing channels with overlapping spectra.

The AFS (adaptive focus system) will insure that your timelapse experiments will remain in focus by dynamically tracking and rapidly correcting for z-shifts between the specimen coverslip and objective lens. Thanks to Katerina Kalargyrou and Rachael Pearson for live cell images



Raw image

Deconvolved image

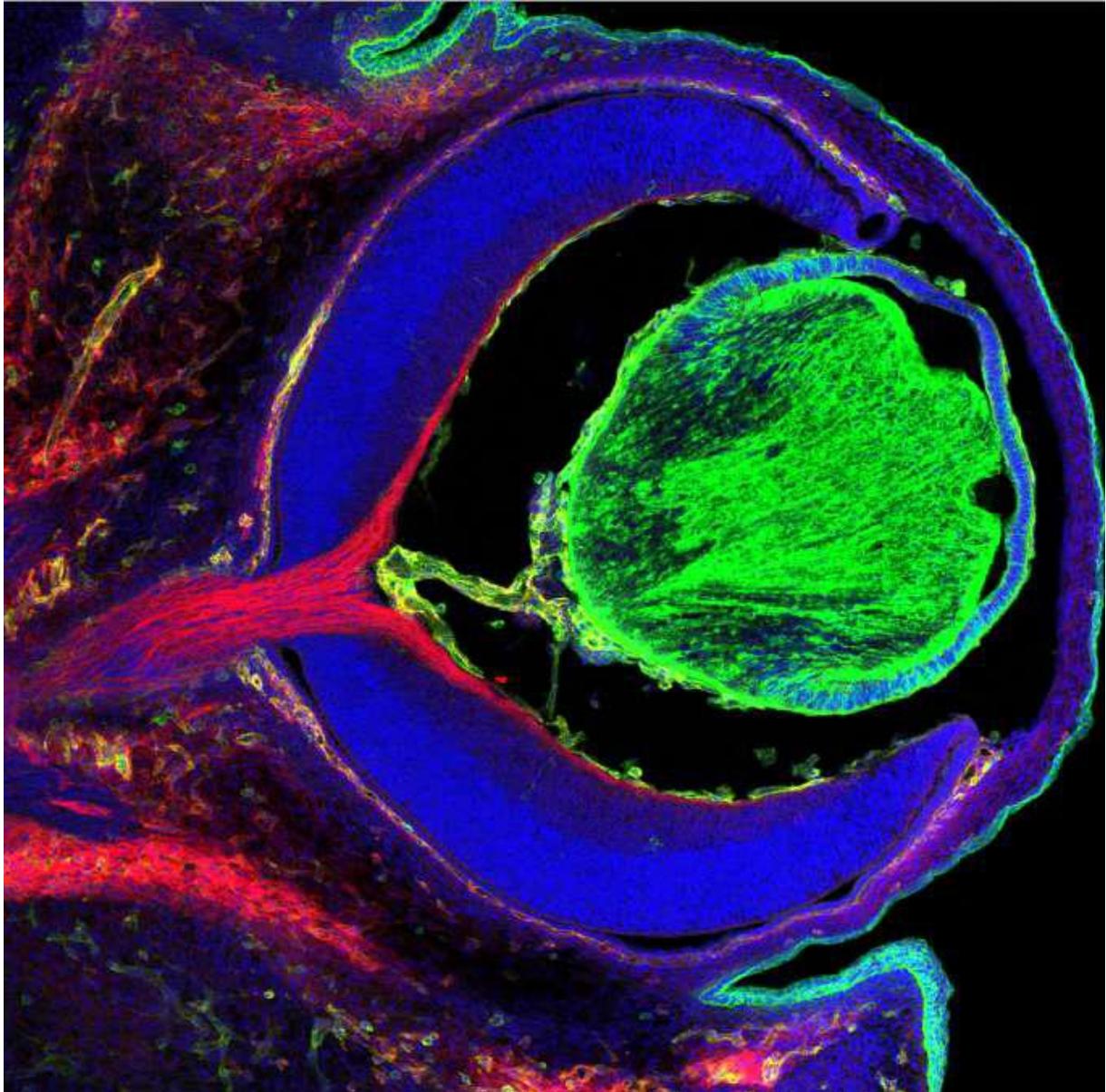
One can use specimens on slides, 35mm glass-bottomed plates, multi-well plates etc... It's best to check with us to ensure we have the correct specimen holder before you start.

We can train you how to use this live-cell system ([£250 for the 2-hour session](#))

Use is [£40ph during the day](#) and [£20ph overnight, after 6pm](#).

Confocal Microscopy II: Zeiss LSM700

Our workhorse confocal microscope



The Zeiss LSM700 is a confocal microscope allowing the acquisition of high-resolution images of fluorescently-stained specimens. Cells and tissue sections can be examined. The sample must be on a slide, however, and covered with a thin, sealed, glass coverslip.

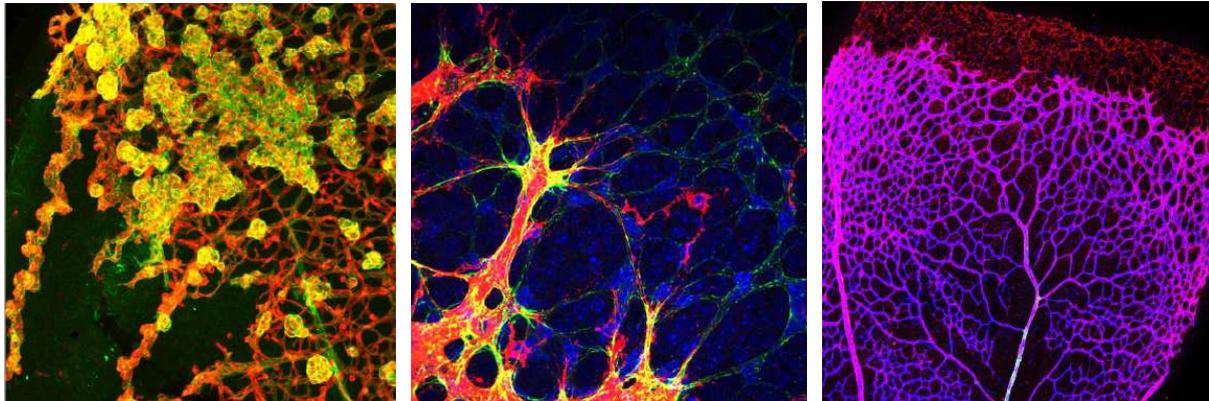
Plastic slides can be used, (such as those for growing cells directly onto) but these are difficult to generate z-stacks from, as they flex under the high-power lenses, fluoresce in a range of colours and sometimes physically distort at high laser power.

With the exception of the 10x air and 15x objectives the 'working distance' of the lenses is short. This limits the depth of specimen that can be imaged.

We charge [£28 per hour](#) to use this microscope.

Confocal Microscopy III: Zeiss LSM710

Workhorse microscope with a programmable stage

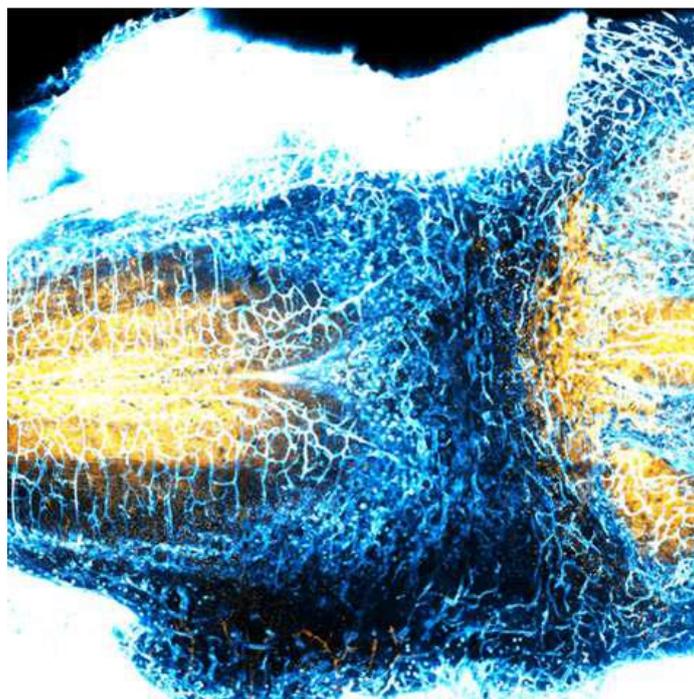


(Thanks to A. Fantin and C. Ruhrberg for the images above).

The Zeiss LSM710 is a confocal microscope allowing the acquisition of high-resolution images of fluorescently-stained specimens. Cells and tissue sections can be examined, however, the sample must be on a slide and covered with a thin, sealed glass coverslip.

The microscope has a programmable stage allowing one to automatically collect multiple single images or multiple stacks of images, which can then be montaged into large, high-resolution composites.

With the exception of the 10x air and 10x water objectives the 'working distance' of the lenses is short. This limits the depth of specimen that can be imaged. The machine has spectral unmixing built-in and can image fluorescent dyes and separate fluorescence from dyes even if they have overlapping emission spectra. (Thanks to M. Tata and C. Ruhrberg for the image below.)



Confocal Microscopy IIIB: Zeiss LSM710 Airyscan

Pushing the resolution limit

We have augmented the Zeiss 710 with an Airyscan detector. This is a 32-channel gallium arsenide phosphide photomultiplier tube (GaAsP-PMT). This system does not use a pin-hole, instead collecting an array of 0.2 Airy unit images as scanning progresses. From this data the scanner 'pixel-reassigns' the signal, back-calculating the 'true' source of the light: a sort of hardware deconvolution.

The detector is very sensitive, and this, combined with the efficient light-path, a high NA 63x oil-immersion lens and the array system, results in improved resolution and signal-to-noise ratio.

We can achieve an **increase in resolution of 1.4 fold**, to around **140 nm** in the xy domain and **450 nm** in the axial (z).

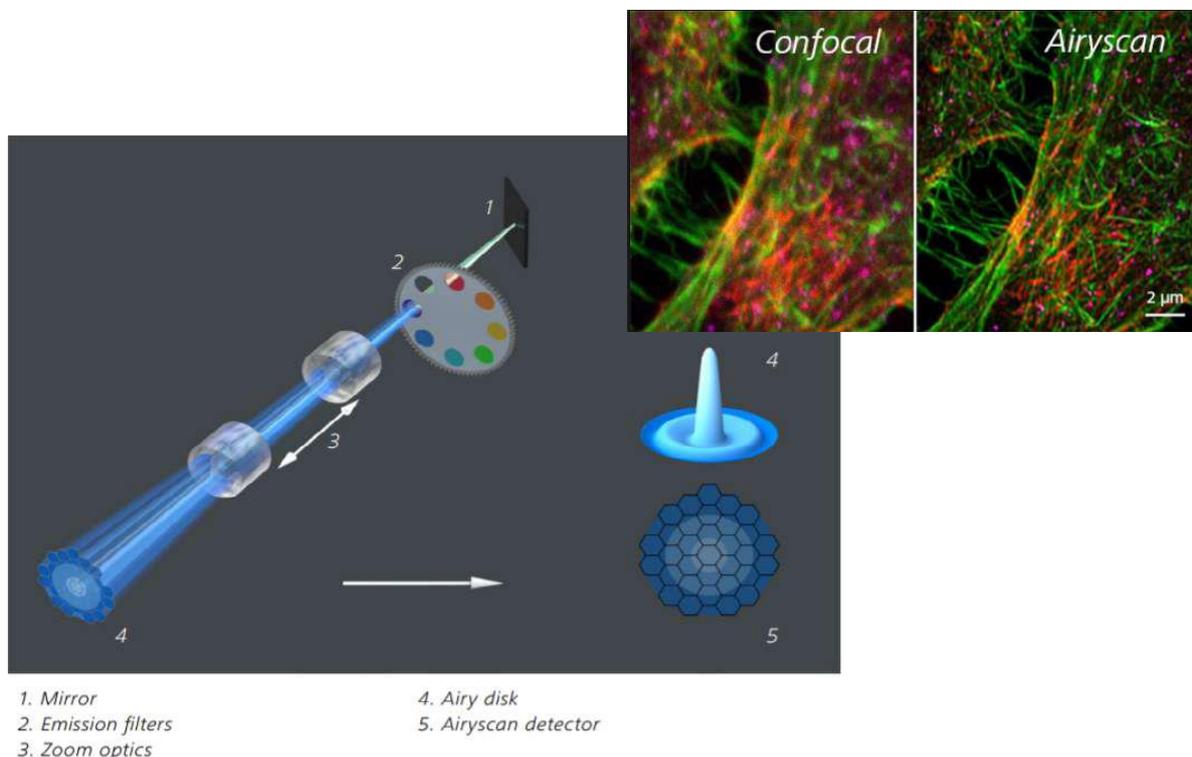
This compares to approximately 250 nm and 650 nm for conventional confocal microscopy.

This is surprisingly useful as lots of things we are interested in the department fall within this range: synaptic clefts, ribbon synapses, cilia, basal bodies, mitochondria, polysomes etc.

Multiple channels can be sequentially assigned to it so you can get three colour images from it, all 'super-resolved'.

The increase in resolution is not as high as can be obtained from SIM, PALM, STORM or STED types of super-resolution microscopy, but it is not associated with many artefacts and requires no special sample preparation.

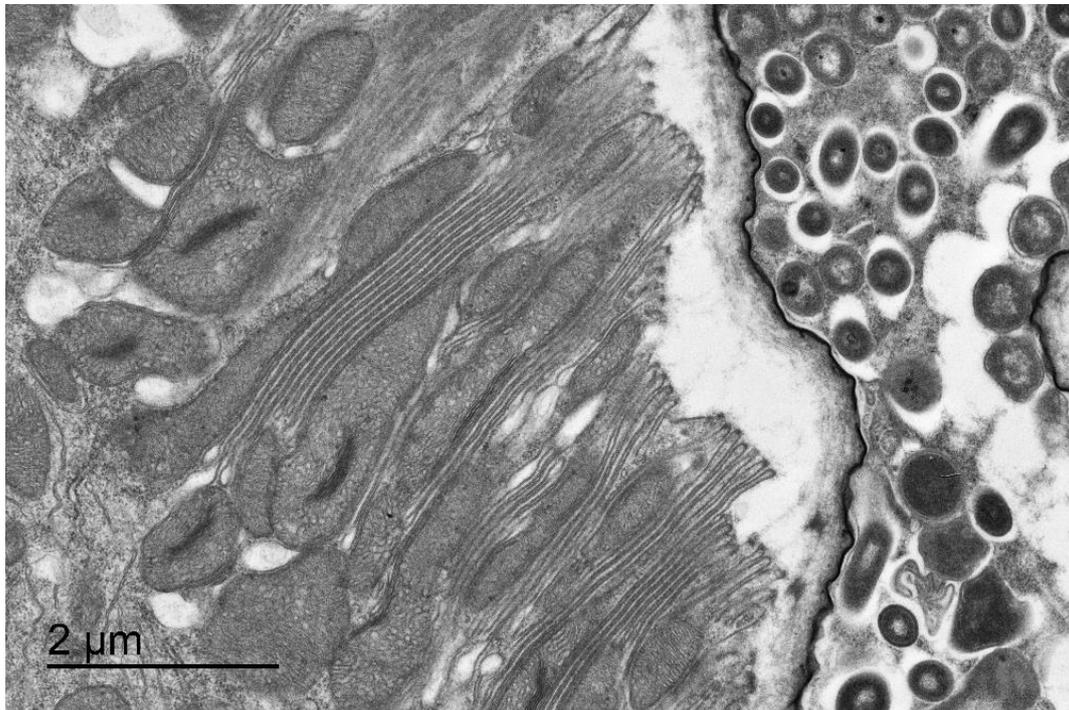
We provide additional training on how to use it at no extra cost.



Images from Zeiss Ltd

Transmission Electron Microscopy (TEM)

Exploring subcellular structure in ultra-thin sections



TEM is usually performed on fixed, osmicated (treated with osmium tetroxide), completely dehydrated specimens. They are infiltrated with epoxy resins, cooked into hard blocks and cut into 50nm-thick sections with a diamond knife on an 'ultra-microtome'. We can look at cells or tissues.

Sections are collected onto copper grids (3mm across) and stained with lead or uranium salts. They are imaged using a 'flooding' beam of electrons (up to 120kV). The electrons produce light on a scintillator and the resultant photons are collected using a CCD camera. The image produced is actually the 'electron shadow' produced by the heavy metals in the sample and from the stain. We can obtain very high resolution from this technique, down to around 1nm.

The secret of good TEM is to fix and infiltrate samples very well. Unfortunately the use of glutaraldehyde in the fix means antigenicity is lost and antibodies rarely recognise the sections.

We offer a full sample preparation service ([£300 for up to 10 specimens](#)).

Sectioning is quite time-consuming and so costs an extra [£500 for 10 specimens](#).

We can train you how to use the microscope ([£100 for training and then £30 an hour](#)).

We can also put semi-thin sections (650nm) on glass slides and stain them with toluidine blue; this produces a nicely preserved overview of the tissue which can be imaged on a light microscope.

In the very near future we hope to augment our new Jeol 1400 plus TEM with tomography so we will be able to make high-resolution, 3D reconstructions from slightly thicker (250 nm) sections.

TEM II: Cryo-immuno electron microscopy.

Labelling of antigens using immuno-gold.



Cryo-immuno EM is a way of labelling antigens such that we can localise them in the TEM.

The specimens are 'gently' fixed to try to preserve antigenicity, embedded in gelatin and infiltrated with a cryoprotectant. They are then mounted on a pin and frozen in liquid nitrogen. The frozen block can then be sectioned on a cryo-ultramicrotome and semi-thin sections (250 nm) can be collected and put onto slides, or ultra-thin sections (50 nm) put onto specially-prepared copper grids.

The 'semis' (which, in spite of the name are pretty thin sections) can be stained with antibodies and looked at by fluorescence. This is a useful technique for looking at heavily pigmented tissues (eg the RPE) in which it is hard to localise antigens using conventional sectioning.

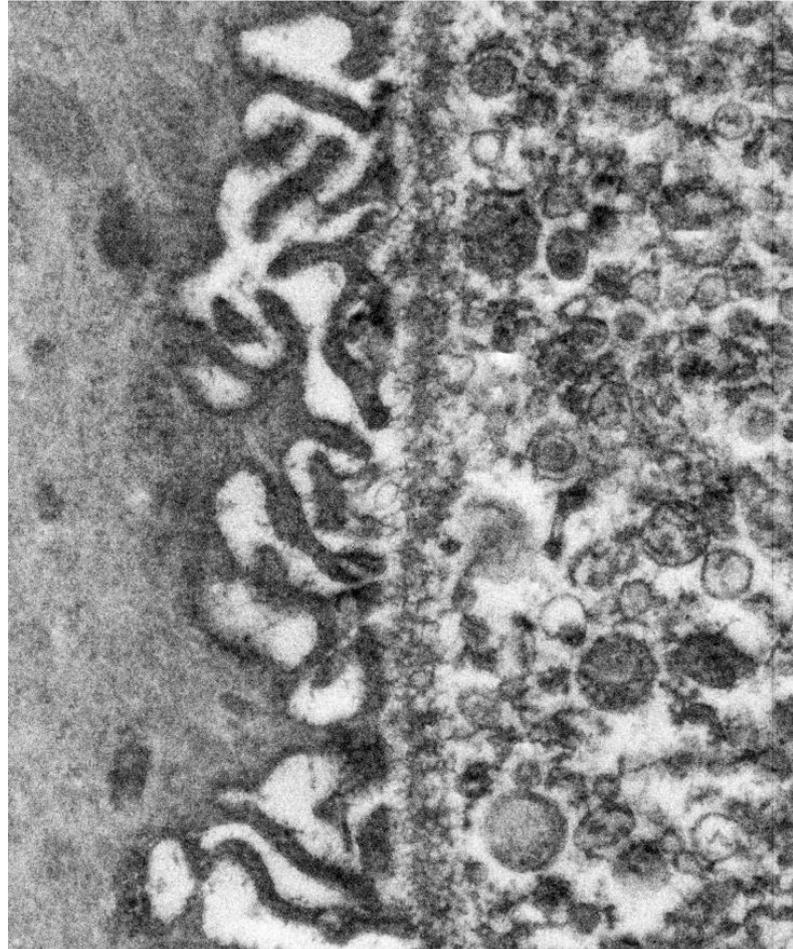
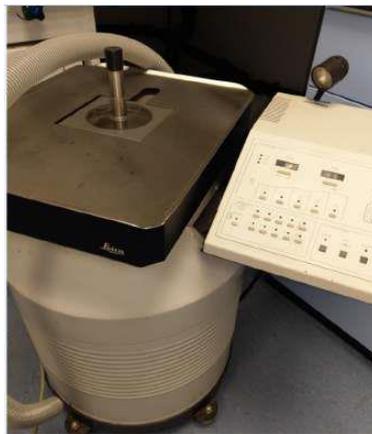
The 'ultras' on grids can be stained with primary antibodies and gold-conjugated secondaries or antibody-binding proteins and then imaged in the TEM. It is also possible to increase the signal using gold or silver enhancement.

The technique is quite specialised and requires a certain degree of skill and experience. There is a continual balance between preserving ultra-structure and antigenicity. So you need VERY good antibodies and it helps if the antigen is abundant.

We can perform all aspects of this protocol but given its complexity [price is on demand](#).

TEM III High-Pressure Freezing/Freeze substitution

Preservation of subcellular structure in ultra-thin sections



HPF-FS is a means of attaining the best possible preservation of cellular ultra-structure. Conventional TEM preparation has a tendency to introduce distortions of membranes at the ultra-structural level.

When good preservation of membranes is the over-riding concern in an experiment we can take fresh or 'gently' fixed material and freeze it incredibly quickly (20 000 degrees per second). This ultra-fast freezing does not allow ice crystals to form in the specimen which otherwise would damage internal membranes.

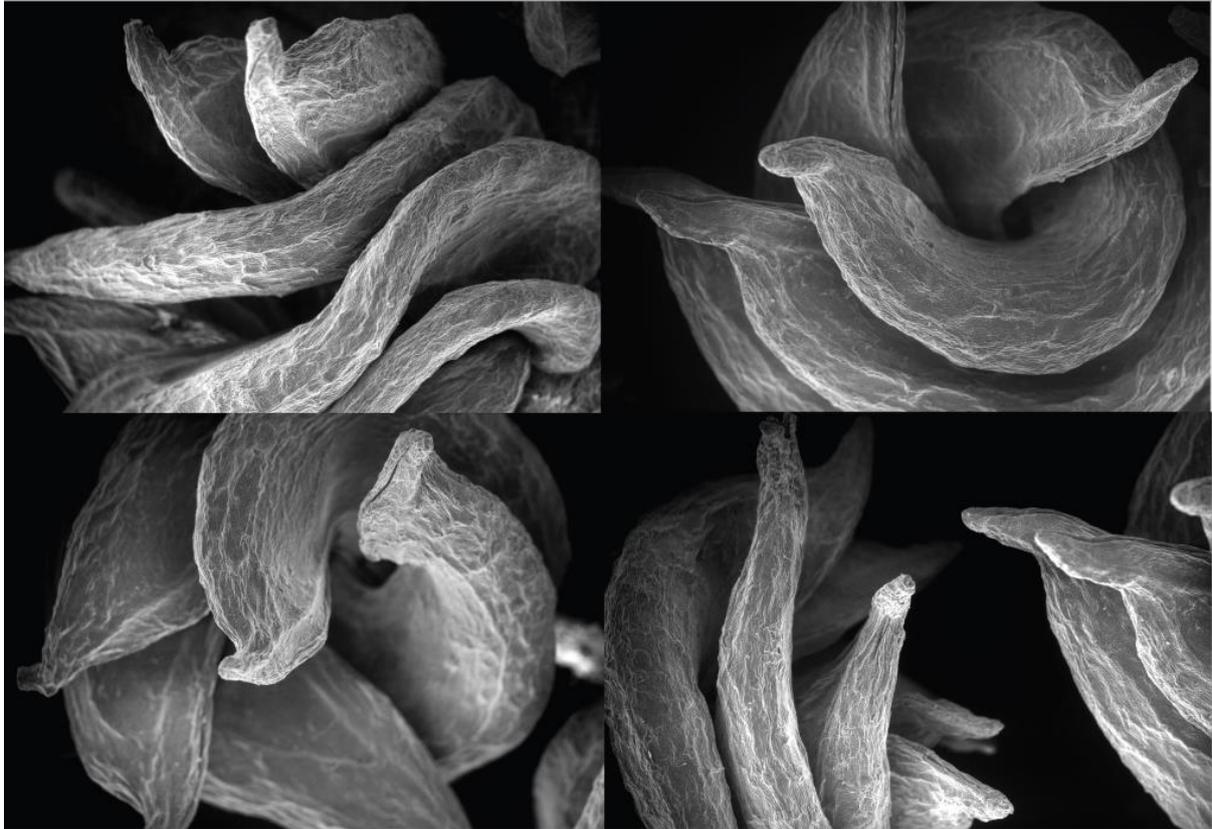
The frozen specimen is then infiltrated with heavy metals over several days at very low temperatures. The resultant specimen can then be embedded in resin and prepared and sectioned for conventional TEM.

The resultant images have superior preservation of membranes.

We can perform all aspects of this protocol which has many variants ([Prices on demand](#))

Scanning Electron Microscopy (SEM)....

Secondary electron detection to explore surfaces



SEM is usually performed on fixed, osmicated (treated with osmium tetroxide), completely dehydrated specimens. We can look at cells or tissues.

Specimens are mounted onto aluminium stubs in the preferred orientation. These are then coated with a layer of platinum or gold to make them conductive.

A scanned, low-voltage electron beam passes across the surface and electrons are emitted by the heavy metals in the sample. These 'secondary-electrons' are collected by a detector to give images of the metal-coated surface.

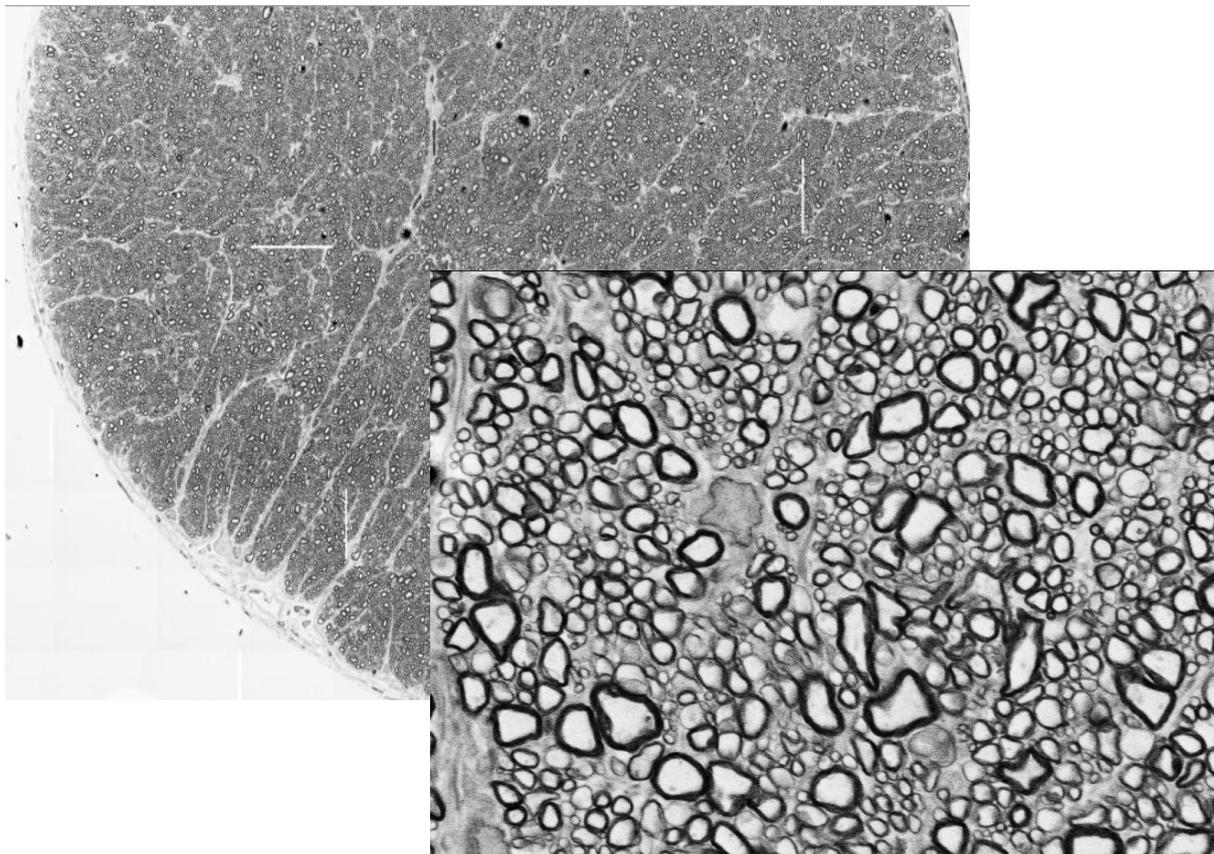
The secret of good SEM is to preserve delicate structures during dehydration which usually involves using liquified carbon dioxide at high-pressure or exotic solvents.

We offer a full sample preparation service ([£200 for up to 5 specimens](#))

We can train you how to use the microscope ([£100 for training and then £30 an hour](#)).

Scanning Electron Microscopy (SEM) II: Backscatter

Exploring subcellular structure in semi-thin sections



SEM can be performed on semi-thin (around 2000 nm) resin sections put onto glass slides. The samples are then heavily stained with metals (uranium or lead salts). The sections are coated in a thick layer of carbon which makes them conductive but is quite transparent to the electron beam.

In 'backscatter mode' we use high energy (20kV) electrons which bounce off the nuclei of the heavy metals in the specimen. These are collected by special detectors and generate an image which looks like a slightly lower-resolution TEM image.

The benefit of using this technique is that the SEM can take relatively large specimens: several centimetres not 3mm like copper grids in the TEM. We have stitching software which allows the machine to take many high magnification images (say 5000x times) and stitch them together to generate a large montage.

The example above is an optic nerve. (Thanks to [K. Eastlake](#) and [A. Limb](#) for the images)
This technique is fairly time-consuming and the resolution is not as high as TEM.

We offer a full sample preparation service ([£700 for up to 10 specimens](#))

We can train you how to use the microscope ([£100 for training and then £30 an hour](#)).

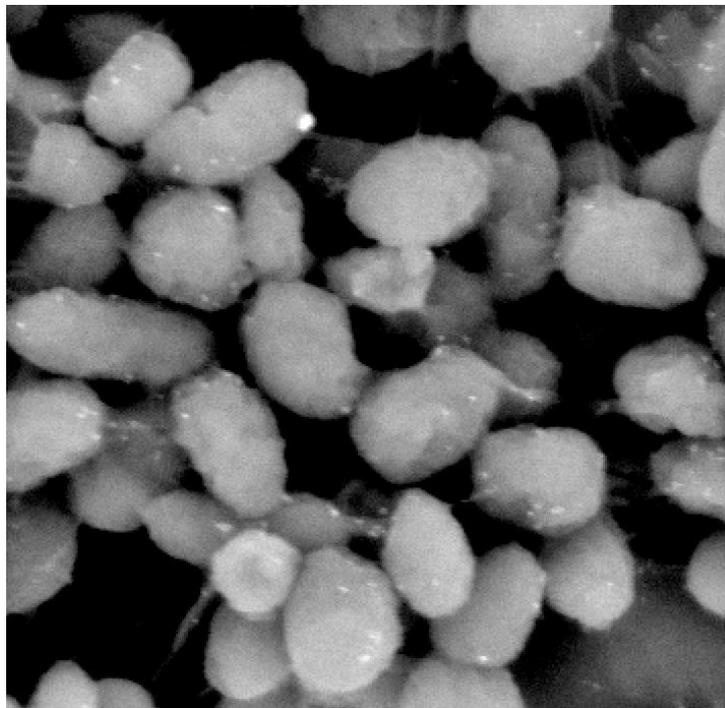
Scanning Electron Microscopy (SEM): IIB Backscatter with gold-particle labelling.

Labelling subcellular structures.

SEM is usually used in 'secondary electron mode (SE) with a thin layer of platinum on the surface. This allows collection of low-energy electrons which give very high resolution images of surface structures (by interacting with the surface electron shells of the sample).

If you can manage to label any antigens in your sample with nano-particles of heavy metals you can combine the high resolution of the SE mode with the backscatter signal (which bounces off the heavy atomic nuclei).

The hard part of this technique is in sufficiently fixing the sample whilst maintaining any degree of antigenicity.



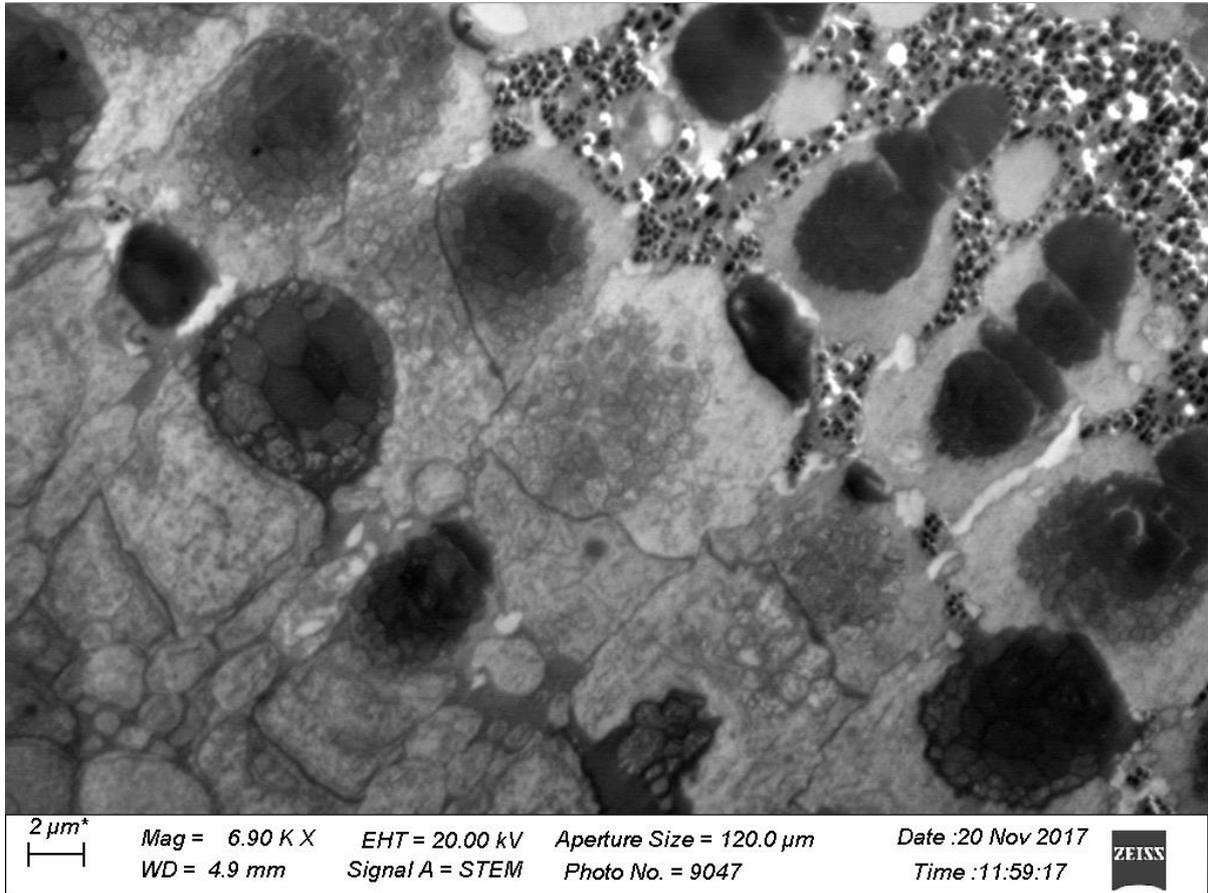
Sometimes cells are partially stripped of their membranes to allow the antibodies in. Usually primary antibodies are added to partially-fixed samples. Then secondary antibodies or binding proteins are added *en bloc* to the sample which are conjugated to gold. These find the primary antibodies and can potentially be imaged directly or 'enhanced' by using silver or gold salts which precipitate on the nano-particle producing a nice big electron target.

The sample is then more strongly fixed, usually coated with a thin platinum layer or a thick carbon one, and imaged in the SEM in both secondary and backscatter modes. (as seen above [thanks to M. Baily](#)).

This is a bit of a specialised technique but we help you set it up and can put you in contact with people who have done it.

Scanning Transmission Electron Microscopy (STEM) III

Exploring subcellular structure in ultra-thin sections



STEM (Just to be confusing!) is a way of getting a transmission image out of a scanning microscope.

Ultrathin samples on grids are made the same way as for TEM. Cells and tissues can be observed.

The sample is put into a special carousel holder and a STEM detector is positioned on the far side of the sample so electrons pass through the sample as the beam scans. In theory, this could produce an image as good as TEM but with a *scanning* beam. In practice, because the highest voltage electrons we can generate are around 20kV rather than the 80kV of the TEM, the resolution is reduced.

It has particular use when you want to look at whole grids. (The image above is a zebrafish retina)

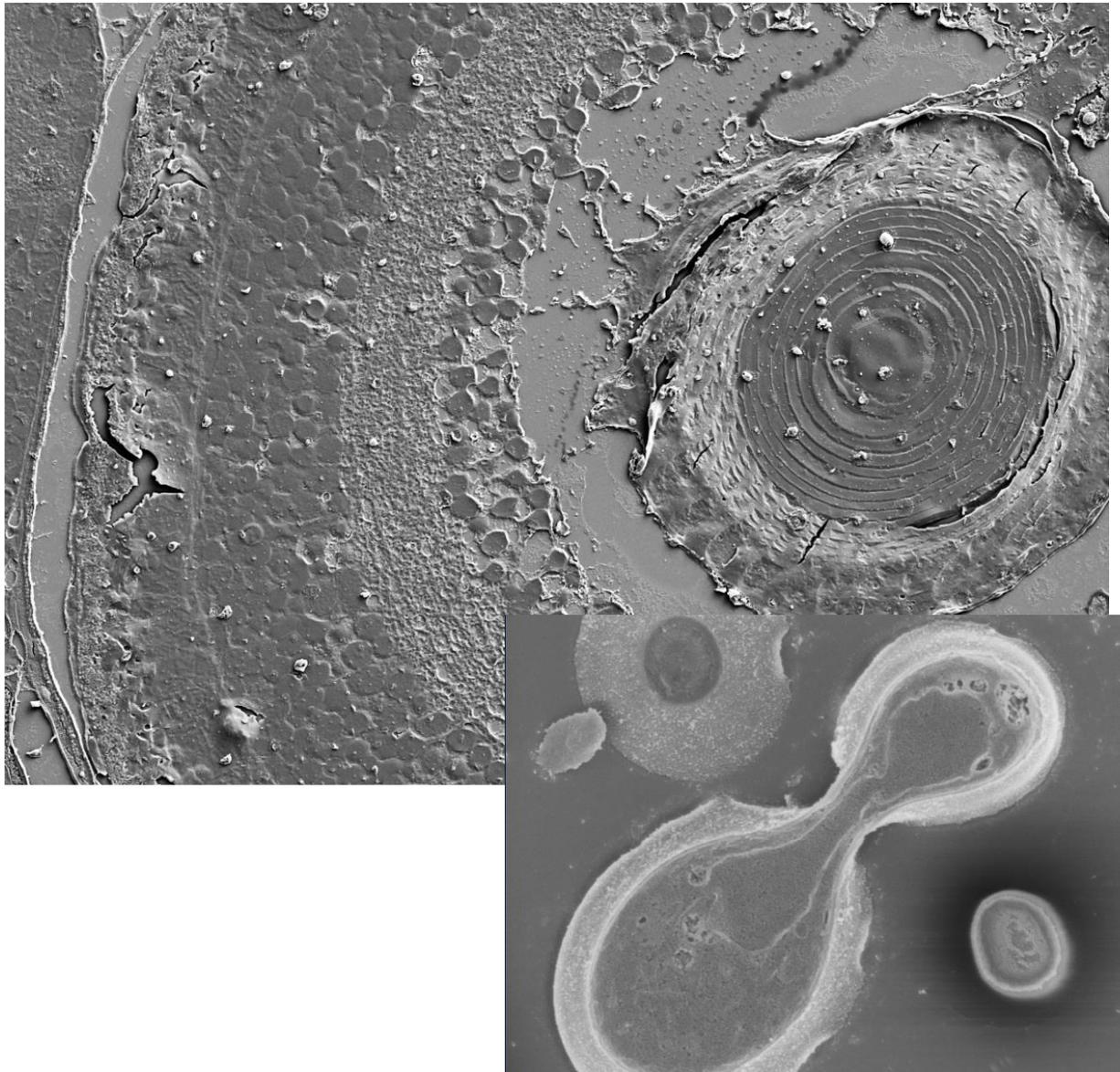
It is also useful when you want to look at crystalline inclusions in samples.

We offer a full sample preparation service (£700 for up to 10 specimens)

We can train you how to use the microscope (£100 for training and then £30 an hour).

Scanning Electron Microscopy (SEM): IV Etching.

Examination of interior surfaces of tissues and cells.



Resin Section etching

This is done using various solvents to expose the ultrathin sections within. Etching can be complete, to remove all the resin, or partial, leaving the metal-labelled structures proud of the surface.

The resultant sections can be coated with platinum or carbon and then imaged in the SEM. The technique is useful for showing the gross shape of internal structures on various scales.

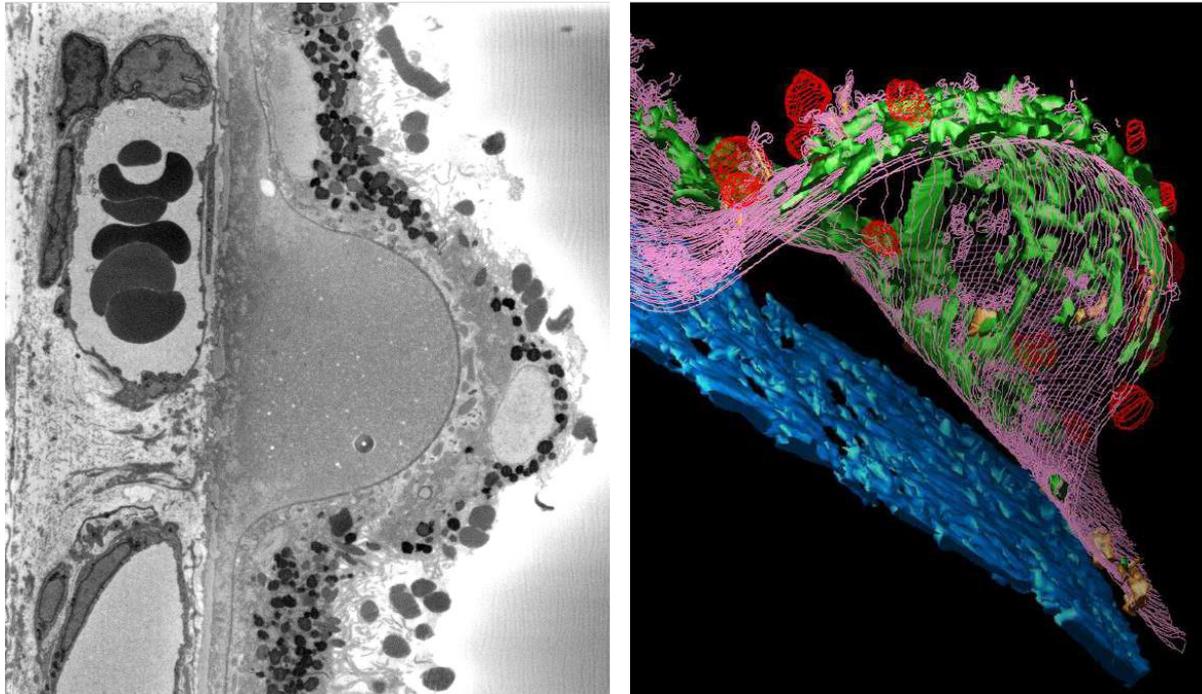
The large example above is a zebrafish, the small one a single budding yeast.

We offer a full sample preparation service ([£700 for up to 10 specimens](#))

We can train you how to use the microscope ([£100 for training and then £30 an hour](#)).

Serial Block-Face Scanning Electron Microscopy (SBF-SEM) also known as 3-View

Using backscatter on serially-exposed surfaces to generate a 3D image.



As described in “SEM II backscatter” it is possible to image a semi-thin section in backscatter mode.

If you stain the sample with loads of heavy metals (lead salts, uranium salts and osmium twice!) it is possible to image the polished surface of a resin block with tissue in it even if you don't make the whole sample conductive with a carbon coat.

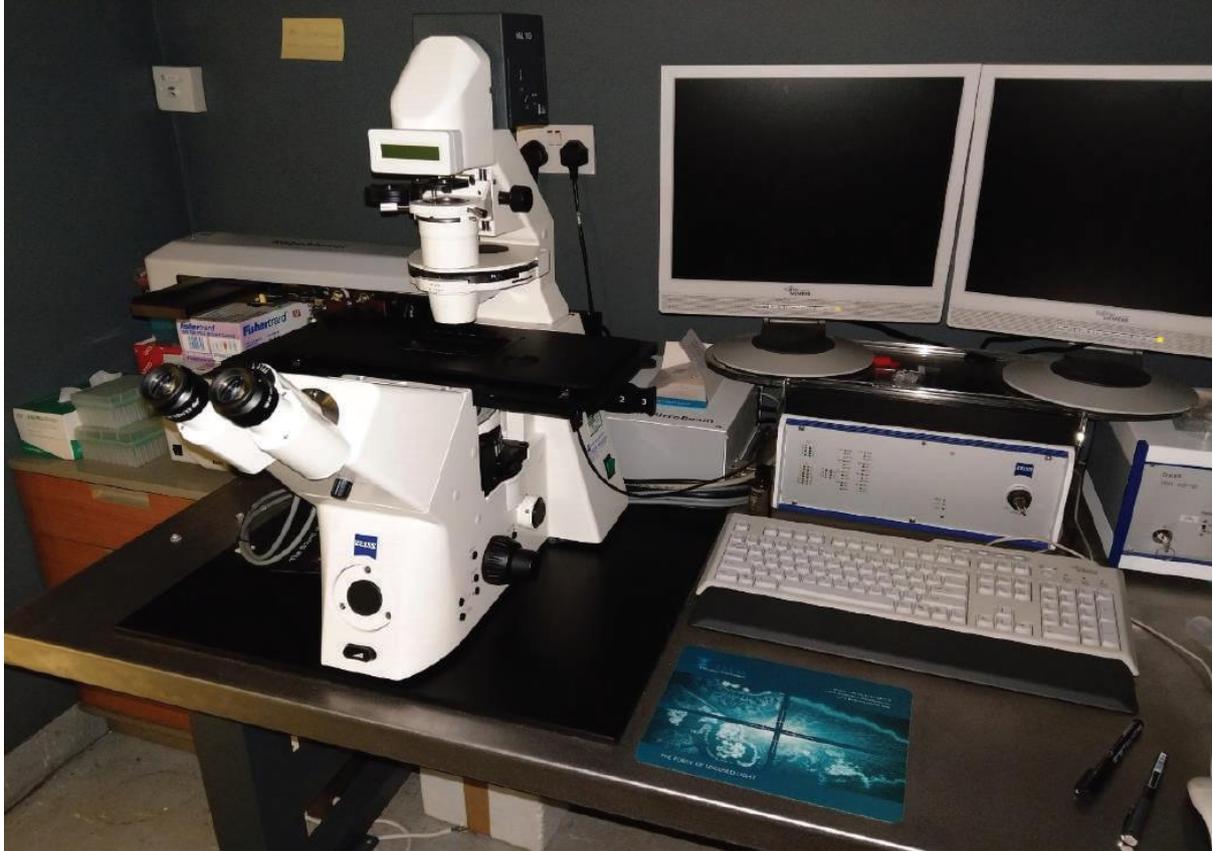
If you have an ultramicrotome built into your SEM machine you can cut a layer of sample away and image again... and again ... and again. Luckily we do! (Gatan 3View). This allows you to generate a big Z-stack of images you can then analyse and 'segment' (i.e. pick out 3D structures.)

The limitations are: You can only look at very small blocks (0.65 x 0.6 x 0.5 mm). You have to use a special detector for very low energy backscatter electrons (lowers resolution). The samples are difficult to prepare and have a high failure rate. When it works though it is amazing!

We charge £600 to make up to 10 specimens and then another £600 each to mount them on pins and run them overnight in the machine.

Zeiss P.A.L.M. Laser Microdissection Microscope

Laser microdissection and capture of tissue.



PALM MicroBeam allows capture of laser microdissected specimens - cryosections, paraffin sections, OCT sections, native tissue, live cells etc.

In our department it is usually used to isolate small pieces of tissue on which to subsequently perform transcriptomics or genotyping.

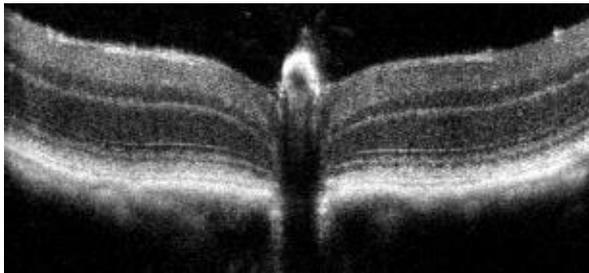
Use of the equipment is set at [£30 an hour](#).

We do not provide technical advice on how to use this piece of equipment, but know who can!

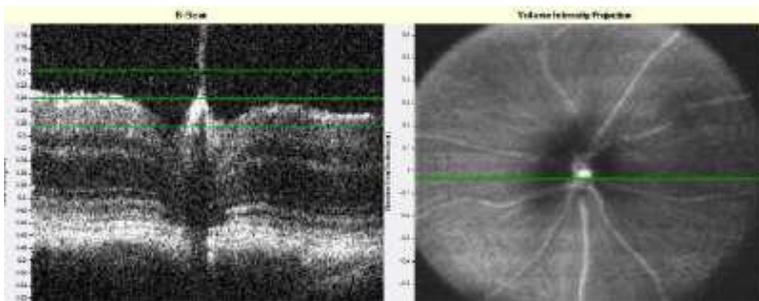
SD-OCT (Spectral Domain Optical Coherence Tomography)

In Vivo Imaging for rodents and zebrafish

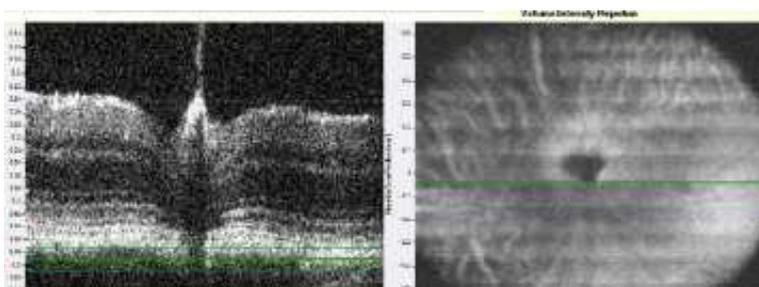
SD-OCT measures the reflectivity of tissue microstructures as a function of depth. A 2D tomographic slice is called a B-scan. Multiple adjacent OCT B-scans create a 3D volume. SD-OCT provides *in vivo* observation of ocular tissues, including all retinal layers. Studies to quantify retinal thickening and thinning are easily done with either manual caliper tools or automated thickness measurement over the whole volume.



Volume intensity projections precisely select the layers to review “*en face*”.

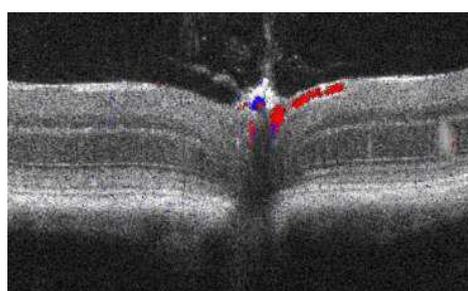
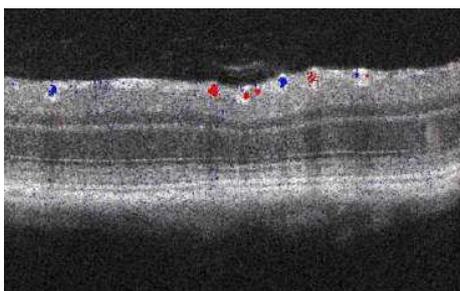


Segmented projections of inner retinal vessels



Segmented projections of choroidal vessels

It is also easy to analyse blood flow in the retina using doppler mode.



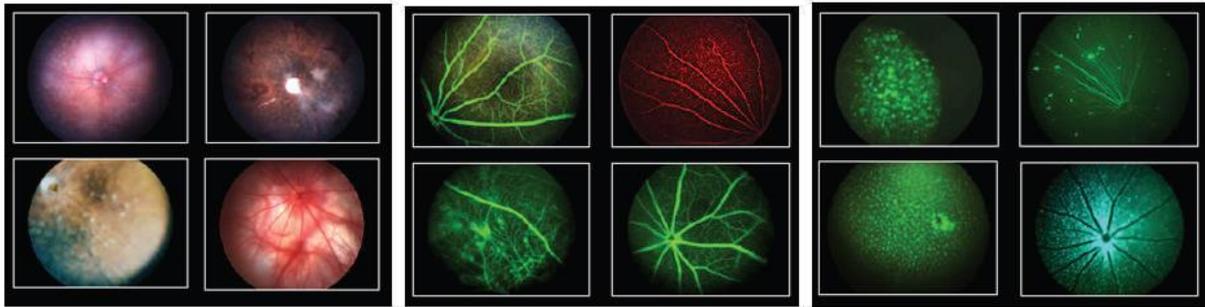
MICRON III – Retinal Imaging Microscope for rodents

Micron III is an *in-vivo* retinal imaging system for mice and rats which can take brightfield, fluorescent and angiogram images at less than 4 micron resolution. It is very simple to use and align using both the camera and animal stands.

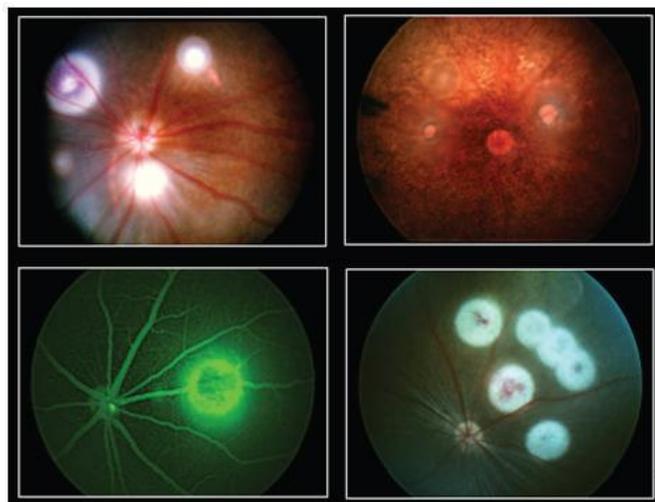
Brightfield

Angiograms

Fluorescence



With a separate Image Guided Laser Injection attachment you can deliver quick and precise laser energy in CNV studies.



3D Printing

We can bring your beautiful 3D virtual renders into the physical world by 3D printing and can provide consultation for creating printable models of your files.

We are equipped with a fused deposition modelling machine (XYZ printing Da Vinci 1.0) which uses ABS for quick overnight prints.

The cost is £2 per hour.

Reptile skin 'osteoderm' printed by Vasilis Theofylaktopoulos for Sergio Bertazzo, UCL Department of Medical Physics and Biomedical Engineering



For more advanced pieces (higher resolution or more exotic/permanent materials) the printers at the Bartlett school of architecture can be used.

Hi-res osteoderm printed by Vasilis Theofylaktopoulos



Public Engagement

If you wish to highlight your research we can help you create and share videos of processes and 3D models with the wider public audience. We can provide help with presentation of your data through our accounts on YouTube (video) and Sketchfab (3d models).

Equipment details

Zeiss 700 upright

Objectives: 10x Air, 16x Water/glycerol, 20x Air, 40x Oil, 60x Oil

Zeiss 710 upright

Objectives: 10x Air, 10x Water, 20x Water, 40x Water, 63x Water, 63x Oil (optimised for Airyscan)

Leica SP8 inverted

Objectives: 10x Air, 20x Oil, 40x Oil, 63x Oil

PALM (laser capture) inverted

Objectives: 5x Mixed, 10x Air, 20x Water, 40x Oil, 63x Oil

TEM Jeol 1400

80-120kV (200x – 150000x magnification)

SEM Zeiss Sigma

Field Emission / variable pressure

3-View / SBF-SEM

Gatan 3-View door fitted to our Zeiss Sigma

Nikon Ti-E

Objectives (long working distance): 4x Air, 10x Air, 20x Air, 40x Air, 60x Air, plus 60x Oil

Fluorescence filters: DAPI, FITC, TRITC, Cy5, CFP, YFP, MCherry

Camera: Photometrics CoolSnap HQ2 (14-bit)

Micron III

Lenses: mouse and rat optimised

Filters: GFP, RFP, YFP (available optionally)