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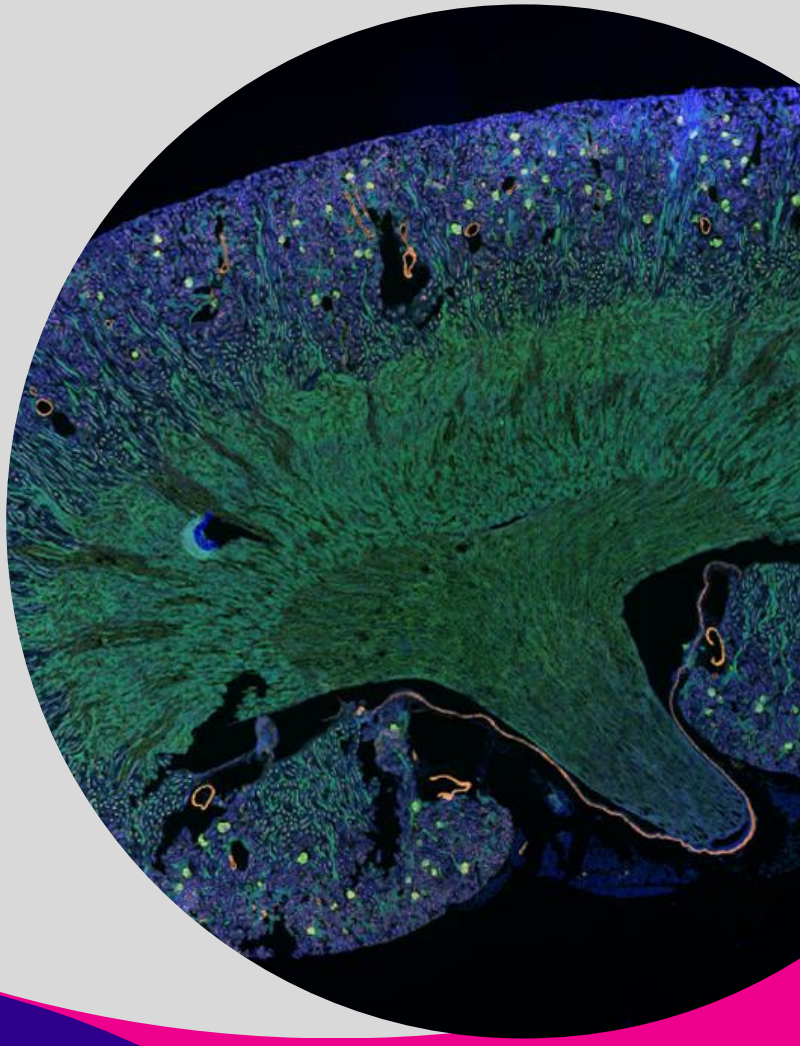
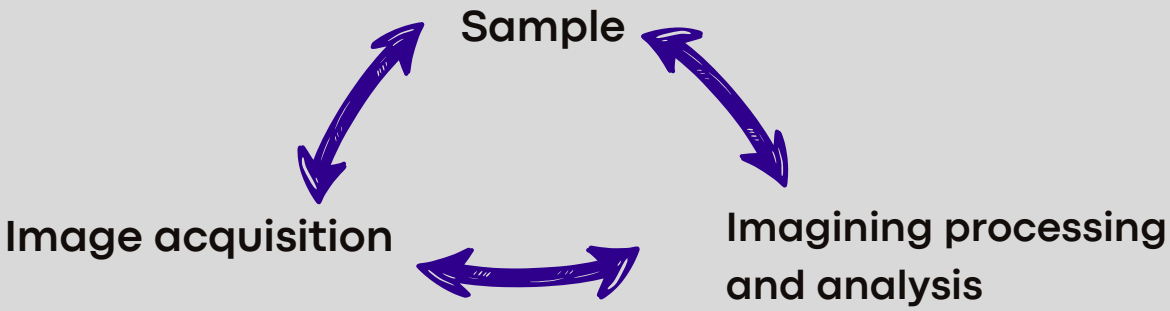
Microscopy and Imagining TTP



Instruments, training and research support for microscope-based experiments

Software and software support for bioimage data analysis.

Imaging experiments: Key to successful bioimaging is an appreciation of the interplay between sample preparation, image acquisition, and digital image analysis.



Sample preparation

We provide pre-imaging advice on sample preparation, suitable for the instruments in our facility. We also have a small library of fluorescent labels for different cellular compartments, suitable for live or fixed cells.

Instruments

Which microscope? Help to select the right instrument for your experiment. Get the data you need from live or fixed samples – from single molecules to tissues.

A) Widefield Microscopes
a. Phase Contrast
b. Fluorescence

B) Confocal Microscopes
a. Fixed Cells and Tissues
b. Live cells and explants

C) Plate scanning
a. Incubated live cell experiments.
b. Evos

D) Slide Scanning
a. Multiplex

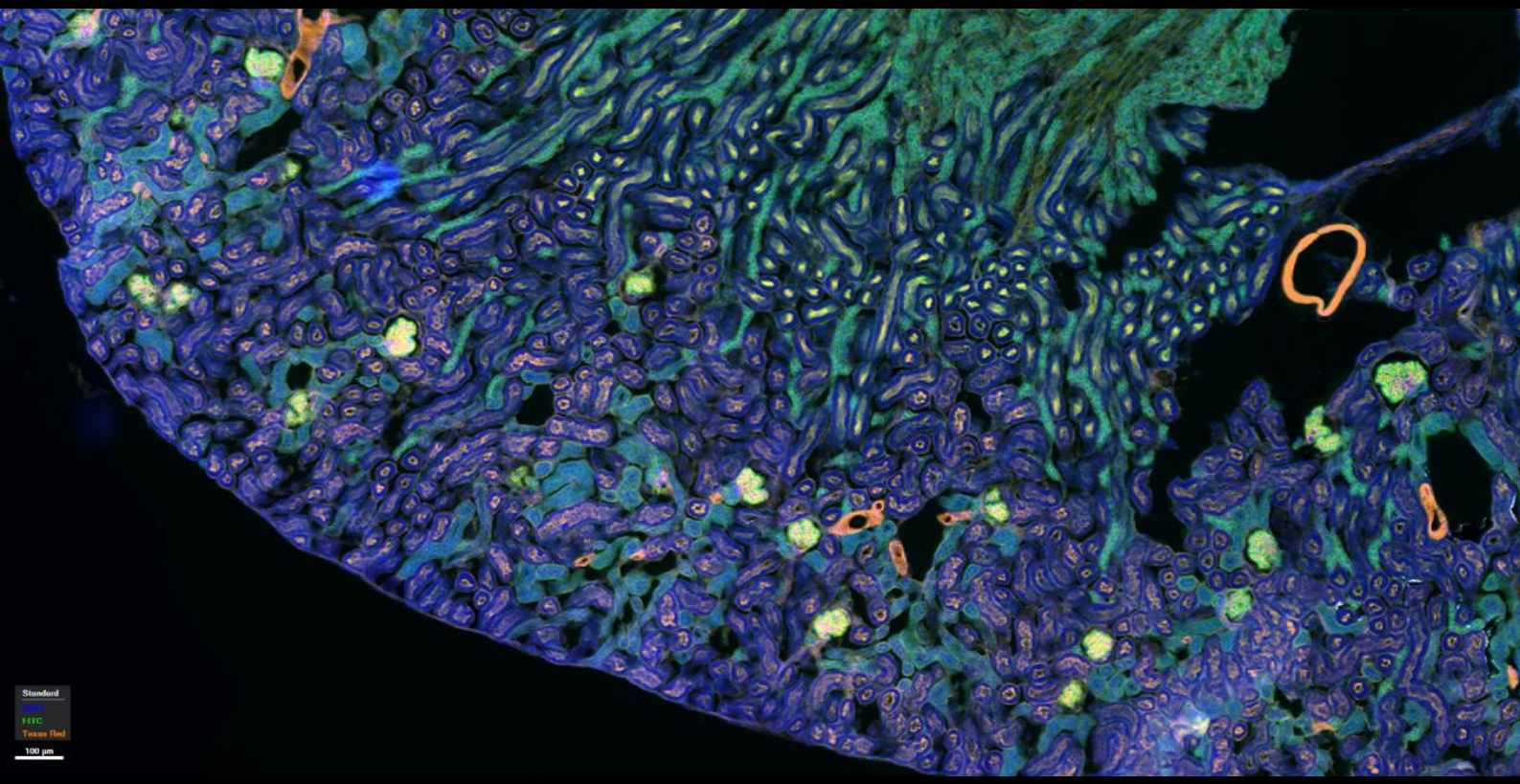
E) Super-resolution
a. Airy Scan
b. TIRF

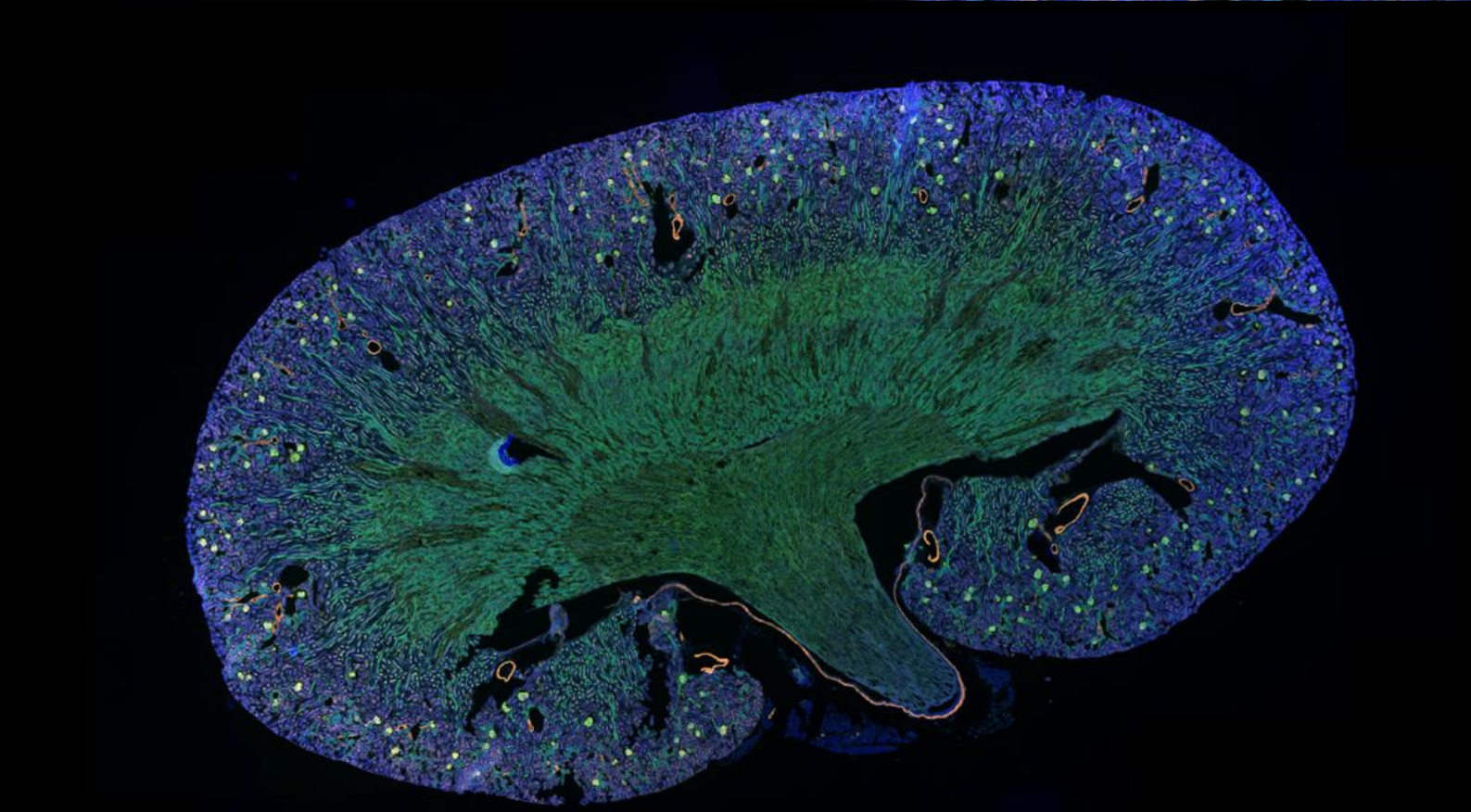
BioImage Analysis

Software:

• Imaris • Huygens • ImageJ • MATLAB • Python • Cellprofiler

We can design and implement bespoke image analysis workflows to automate data processing, analysis and visualisation



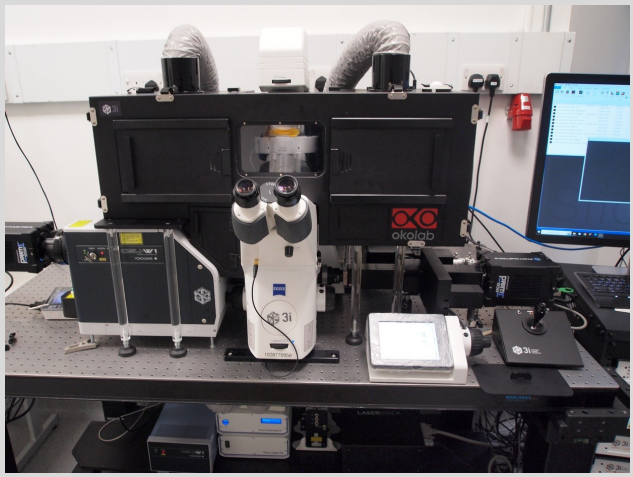


Mouse Kidney section

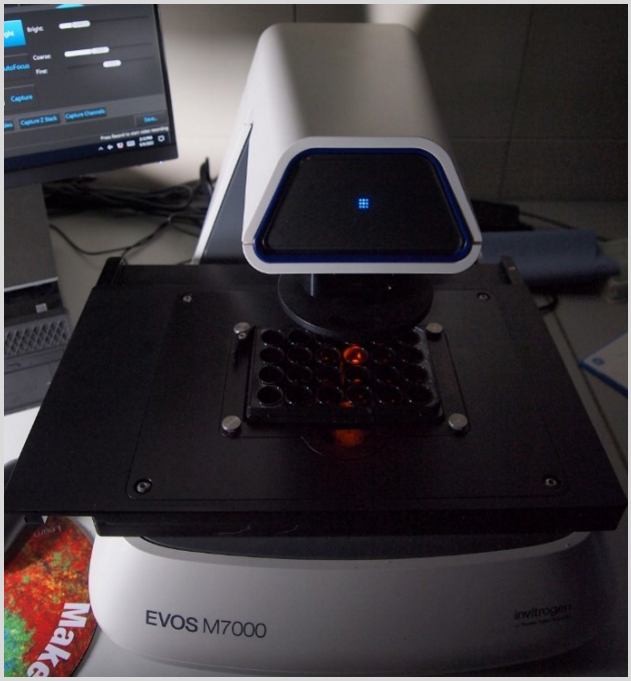
Instruments

3i Marianas Spinning Disk with TIRF and Laser Ablation

This instrument has 4 laser lines, 2 x sCMOS sensors (for Spinning Disk) and 1 x sCMOS sensor (for TIRF) and is capable of imaging up to 40fps. It is fully equipped for time-lapse imaging.
Ideally suited to multi-parametric experiments where resolution and fast imaging is required. The ablation laser can be used to activate photoactivatable fluorescent proteins during a time-lapse acquisition and all components, Spinning disk, TIRF and ablation can be used interchangeably during one experiment.



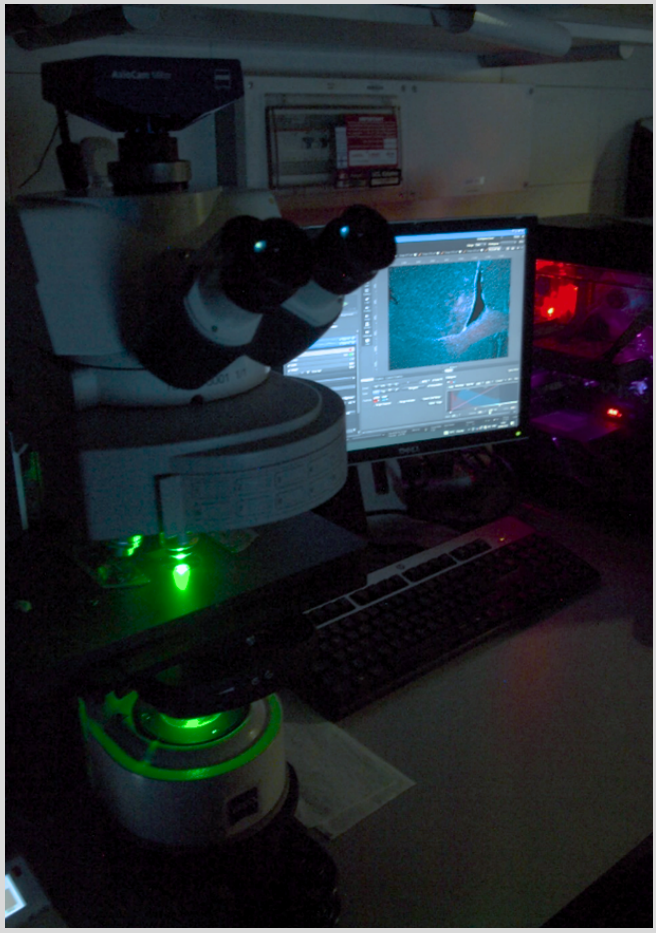
Evos M7000



The Evos M7000 is a multipurpose widefield based imaging system. It is equipped with a colour camera to image coloured stains as well as a monochrome camera for fluorescence imaging.
The instrument is capable of imaging up to 5 channels (4 fluorescent and 1 transmitted light) in a sequence and along with its scanning stage, can be set to automatically image wells in multi-format plates as well as other cell culture vessels.
This instrument is the go-to ‘all-rounder’ providing acceptable research grade images.

Zeiss Z1 inverted, Zeiss Z1 upright, Zeiss Cell Observer

These widefield-based imaging systems are all optimised for fluorescence imaging. They all have an LED excitation source for targeted excitation of fluorophores as well as CCD-based camera systems.



The Zeiss Cell observer is optimised to image live cells over time. There are atmosphere and temperature controls available as well as optimised filter sets to capture widely available fluorescent proteins.

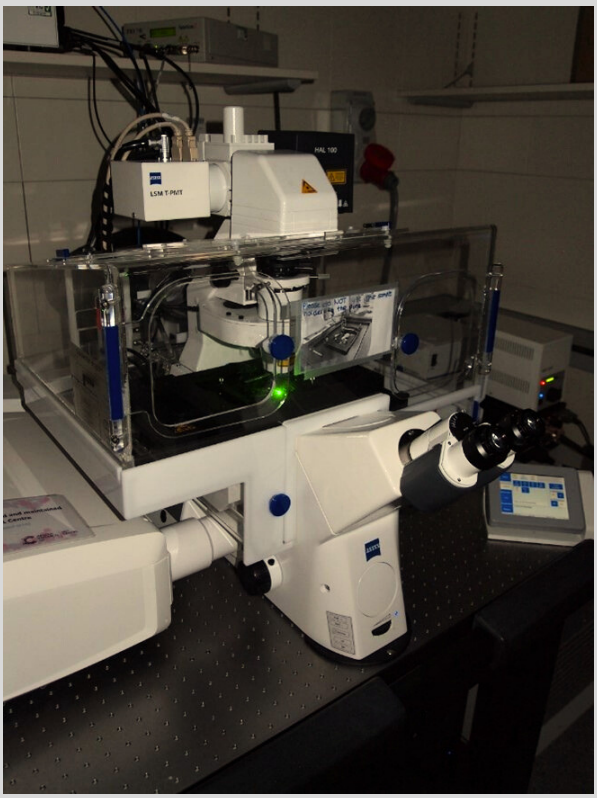
The Zeiss Z1 inverted and upright microscopes are ideal to image fixed, coverslipped samples. The high-end objectives enable highly resolved images to be acquired.

These instruments are ideal for quantitative microscopy analysis and provide a relatively high sample/imaging throughput.

Incucyte Zoom and Incucyte SX5

These purpose-built time lapse imagers have been designed to permanently image from within a cell culture incubator. As a result, researchers are able to image live cells over extended periods of time. The instruments can image Phase contrast for cell growth, death and morphology analysis as well as multiple selected fluorescence channels.
Universal plate holders enable regular culture plates, dishes and flasks to be placed in the instrument for imaging. LED excitation and sensitive CMOS cameras enable for gentle imaging that reduces overall phototoxicity.
The sensitive camera enables detection of low concentrations of fluorescent dyes or proteins.

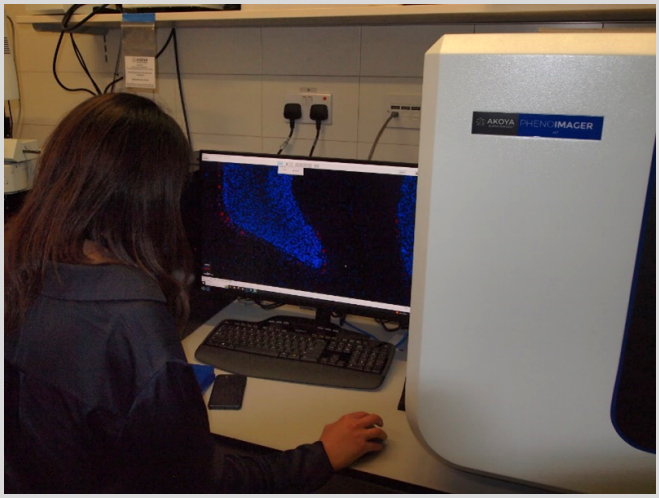
Zeiss LSM880 and LSM900 confocal microscopes

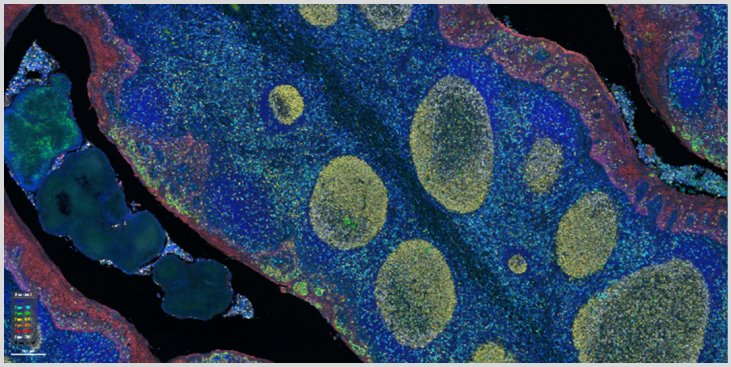


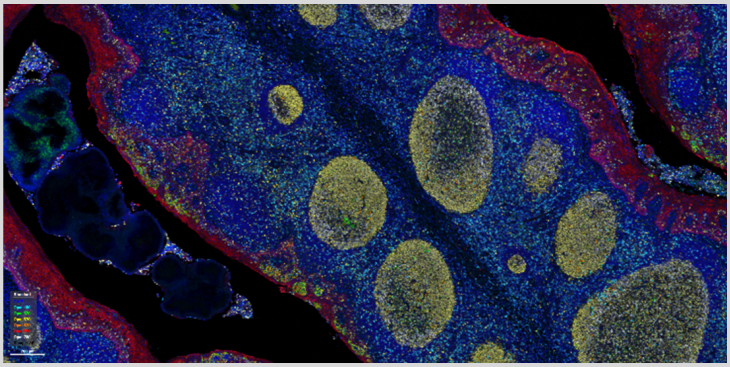
Our confocal microscopes can generate high resolution 2D, 3D and 4D datasets. Utilising powerful lasers for excitation, chromatically corrected objectives and sensitive PMT detectors able to capture single photon events. Multiple detectors can be used simultaneously for applications such as FRET.
These confocals provide a high level of customisation to enable the researcher to capture the perfect dataset. Z stacking can be used to reconstruct 3D images enabling complex analysis methods such as colocalization studies.
The Airyscan detector available on the LSM880 combines Airyscan deconvolution and sensitive detectors which enables researcher to acquired high-quality data that can be resolved sub 200nm.

Vectra Polaris and Phenoimager HT

These imaging cytometers are designed to image tissues stained either with coloured or fluorescent stains. The instruments are tuned with special unmixing algorithms to allow for imaging of up to 8 fluorescent channels on a single tissue section. The data generated can then be analysed using InForm commercial software with built in machine learning. After training the software to correctly segment and identify cells, the hardware can then be programmed to automatically image only areas of interest. The instruments are semi-automated and only require minimal user input. They can hold up to 200 slides (Vectra) or 80 slides (Phenoimager HT) and can be left scanning in an automated fashion







Human tonsil section stained with 8 fluorescent colours and DAPI and acquired on the PhenoImager HT.
LEFT: Raw data captured no unmixing. RIGHT: Raw data after spectral unmixing algorithm has been applied.

Image Analysis

We have expertise in image analysis using both open-source software as well as commercially available software.

Our most popular 3D analysis software is Imaris, Oxford Instruments. This purpose-built analysis package enables researchers to accurately analyse their 2D, 3D and 4D datasets. The software accepts file formats produced by all the instruments in the TTP enabling researchers to transition from acquisition to analysis seamlessly. We also have Huygens deconvolution software available which helps researchers in cleaning up their data prior to analysis.

If you require a custom analysis modality, TTP staff can work with you directly using a range of open-source, commercial and programming analysis to fit your specific requirements.

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