CCI

CENTRE FOR CELL IMAGING

UNIVERSITY OF LIVERPOOL

LIFE CHANGING | World Shaping

A Technology Directorate
Shared Research Facility

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CCI CENTRE FOR CELL IMAGING

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LSM 880 BioAFM

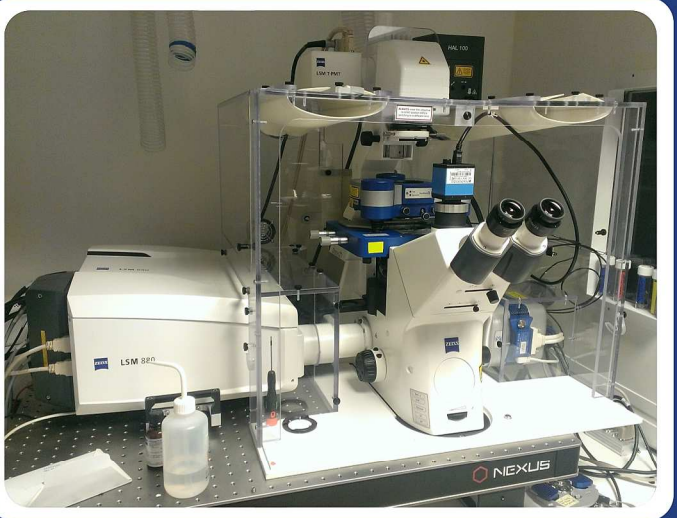
Specifications

Zeiss Axio Observer Z1

10 20 40 63 100

405 488 561
458 514 633

TEMP CO_2 O_2 SPEC AFM TIRF



Technology Focus

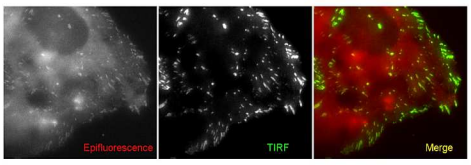
The BioAFM combines three key technologies:

CONFOCAL MICROSCOPY

The Zeiss 880 generation of Confocal Laser Scanning microscopes (LSMs) combine high sensitivity, speed and optical resolution. They excel at **optical sectioning**, flexible spatial sampling and custom emission profiles.

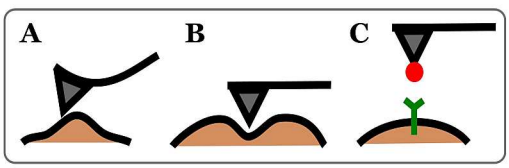
TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF)

TIRF microscopy allows imaging of fluorescent structures within ~100nm of the glass surface. The technique is **fast** with low background signal, making it ideal for the study of surface interactions or vesicle delivery.



ATOMIC FORCE MICROSCOPY (AFM)

AFM has many applications in the realm of cell biology. Advanced cantilever and tip design allow the application of AFM to studies of (A) membrane topology, (B) physical perturbation, (C) protein-protein interaction, and many more.



This piece of equipment was funded by BBRSC grant number BB/M012441/1

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LSM 880 MULTIPHOTON

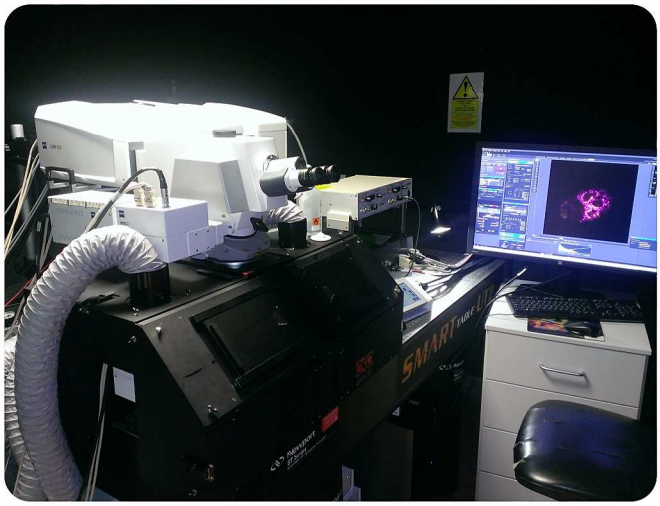
Specifications

Zeiss Axio Examiner Z1 (upright)

2.5 10 20 40 63

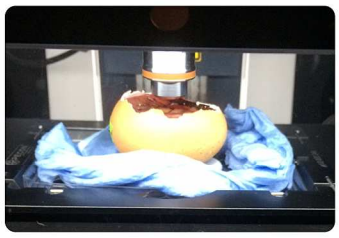
405 458 488 514 561 633 1080

TEMP CO_2 O_2 SPECTRAL UNMIXING MP AIRY



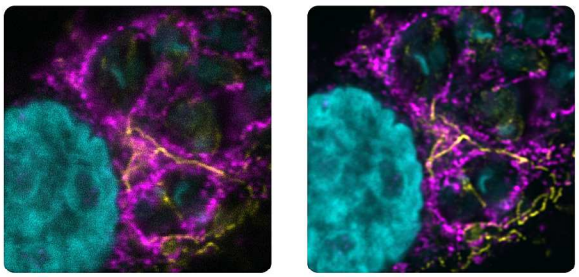
Technology Focus

The 880 Multiphoton is unique in the facilty as being the only upright microscope. The presence of the objectives above the sample, allows imaging of samples that are not amenable to inverted microscopes such as the in ovo chick embryo model (below).



The microscope also has a 1080nm **multiphoton laser** which allows for deep tissue penetration, photodamaging experiments and **Second Harmonic Imaging** with minimal scattering.

The system also has installed a first generation Zeiss **Airy Scan** module. This allows for super-resolution imaging of data without the need for special probes or optics.



Staining shows DNA (cyan), microtubules (yellow) and lysosomes (magenta). Sample courtesy of Sarah Berry (Helen Price Group, Keele)

Above, the same macrophage sample is seen imaged with confocal (left) and Airyscan (right).

This piece of equipment was funded by MRC grant number MR/M009114/1

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LIGHTSHEET

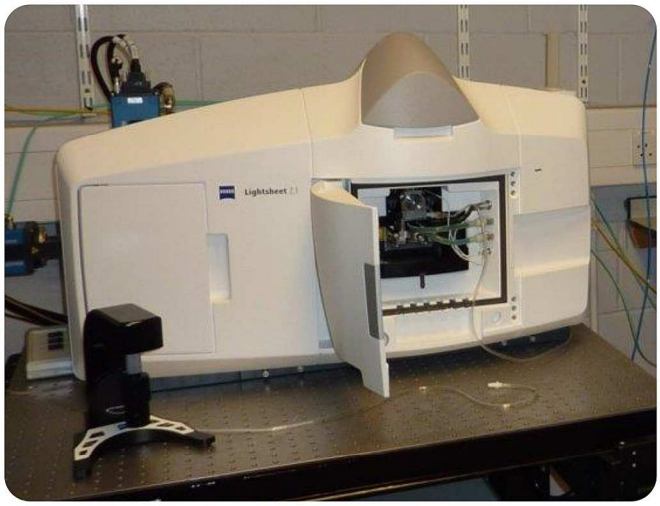
Specifications

Zeiss Lightsheet Z1

5 20 40

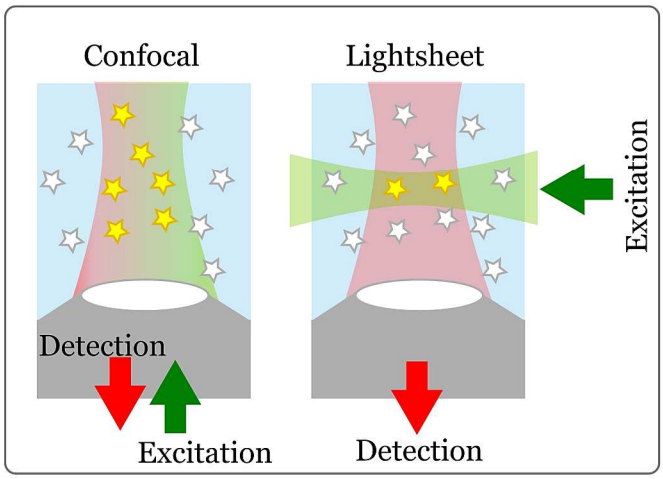
405 488 561 638

TEMP CO_2

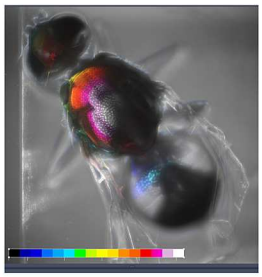


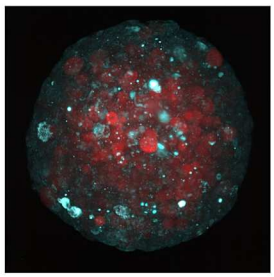
Technology Focus

Confocal Microscopes focus laser light into a point which is then scanned across the sample. Lightsheet (LS) imaging is **gentler** on the sample as only the fluorophores in the same plane as the focus are excited. As a further advantage, the z-resolution is improved due to a reduction in out-of-focus light.



Lightsheet microscopy allows imaging of relatively **large samples**, such as adult *Drosophila melanogaster* (about 4mm in length - below left).





Spheroids (above right), a model of tumor formation, are also highly amenable to this technique and allow visualisation of, for example, cell migration or metabolic gradients within tumours.

This piece of equipment was funded by BBRSC grant number BB/L014947/1

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LSM 780 FLIm/FRET

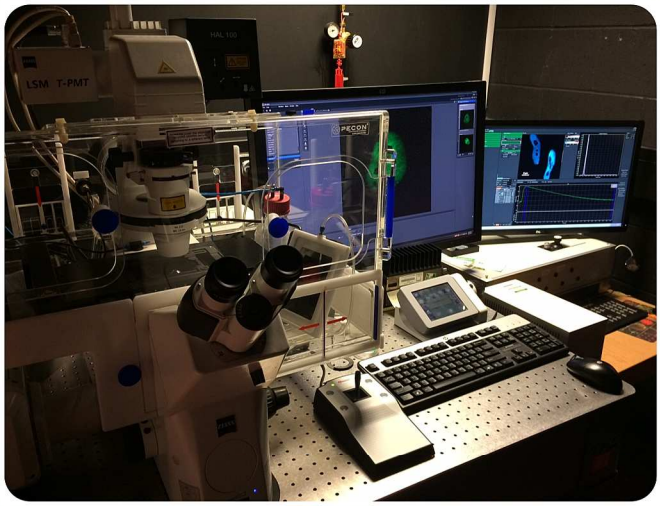
Specifications

Zeiss Axio Observer Z1

10 20 40 63 100

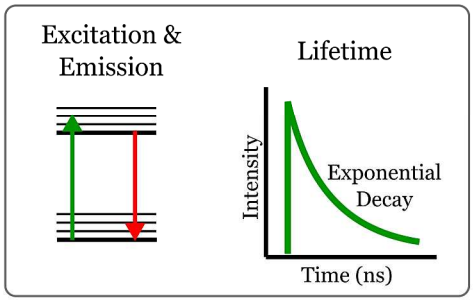
440 458 488 514 561 633

TEMP CO_2 O_2 SPECTRAL UNMIXING FLIm



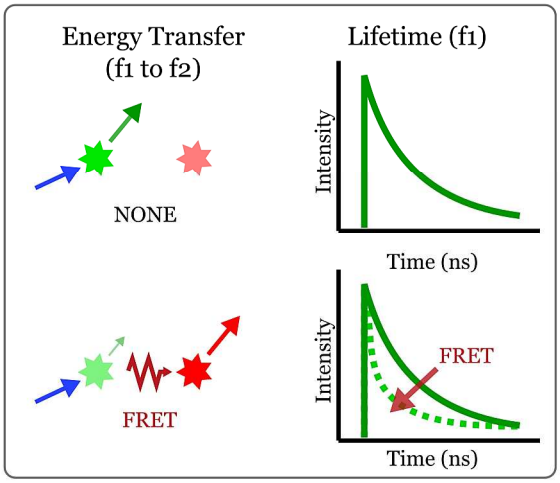
Technology Focus

Fluorescence is the mechanism by which energy (in the form of photons) is absorbed by a fluorophore then emitted at a longer wavelength. The time that a fluorphore exists in an excited state, before releasing a photon and returning to ground state, is characteristic of the fluorophore and local environment. We measure this using **Fluorescence Lifetime Imaging** (FLIm).



The lifetime is also affected by non-radiative energy transfer and the presence of other flurophores.

As the lifetime depends both on radiative and non-radiative energy transfer, a measurable decrease in lifetime is seen during **Förster Resonance Energy Transfer** (FRET). Using this method allows for a rigorous and quantitative measurements of protein-protein interactions.



This piece of equipment was funded by MRC grant number MR/MK015931/1

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CCI CENTRE FOR CELL IMAGING

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EPIFLUORESCENT

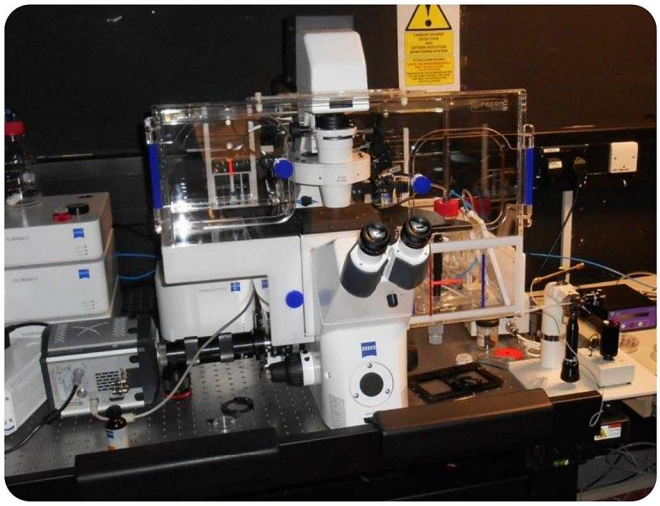
Specifications

Zeiss Axio Observer Z1

10 20 40 63 100

Mercury Arc Lamp

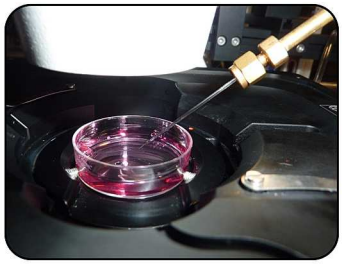
TEMP CO_2 O_2 DIC MICRO-INJECTION

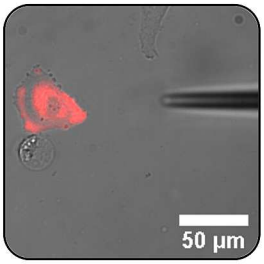


Technology Focus

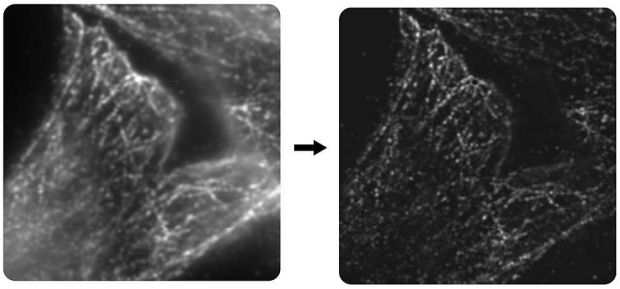
Unlike the confocal microscopes, the epifluorescent uses an arc lamp to illuminate samples. This polychromatic light source is tuned by filters and dichroic mirrors providing good **flexibility** for different imaging setups.

This microscope has environmental and oxygen control as well as a **micromanipulator**. This allows cells to be microinjected with peptides, DNA, dyes or antibodies, and followed real-time using brightfield or fluorescence imaging.





The combined speed and sensitivity of the Andor iXon Ultra 897 camera, makes this system ideal for High Speed, High Sensitivity applications. One such application is fluctuation-based **super-resolution** imaging (SOFI).



By taking fast consecutive images, the pixels can be correlated in space and time, providing an improved resolution, below the Abbe diffraction limit.

This piece of equipment was funded by MRC grant number MR/K015931/1

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LSM 710

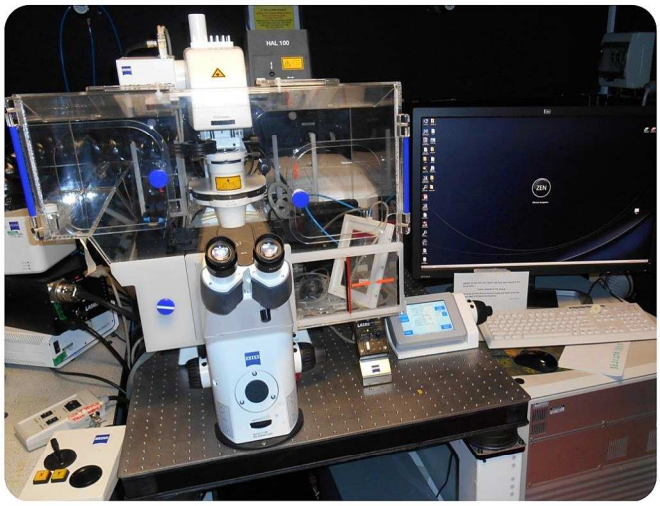
Specifications

Zeiss Axiovert 200M

10 20 40 63 100

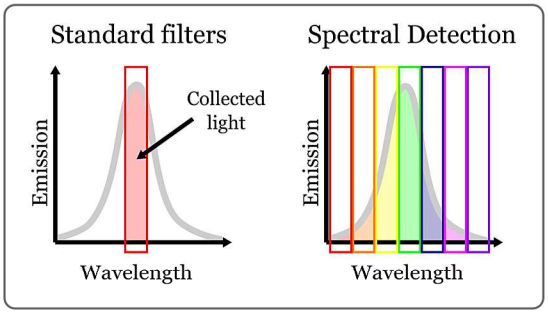
458 488 561
477 514 633

TEMP CO_2 SPECTRAL UNMIXING



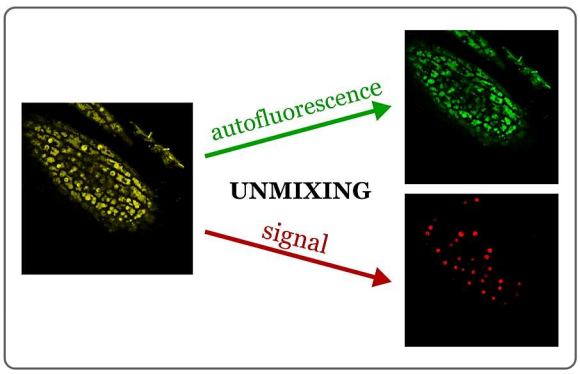
Technology Focus

Traditional fluorescent microscopy relies on a single emission filter to select an output range. **Spectral detection** allows for the acquisition of data across a range of wavelengths, accumulating more of the emitted light.



Spectral detection also allows multiple spectra to be '**unmixed**' if you know the shape of the individual spectra. This is particularly useful to spatially separate closely related fluorophores (eg. Alexa488 and eGFP).

Some samples, particularly those from plant tissues, produce a lot of **autofluorescence**, which makes it impossible to identify signals from other fluorescent proteins.



The example above shows a leaf of *Arabidopsis* expressing a red fluorescent protein and imaged in spectral mode. Only after unmixing can the autofluorescence (green) be optically separated from the protein of interest (red).

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PHOTOTHERMAL

Specifications

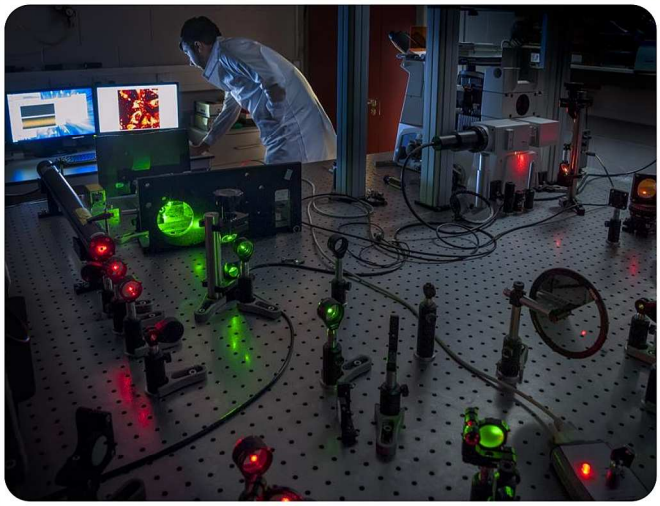
Nikon Eclipse Ti-E

40 63

523
633

Mercury Arc Lamp

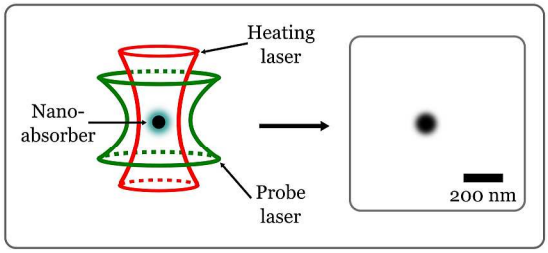
TEMP



Technology Focus

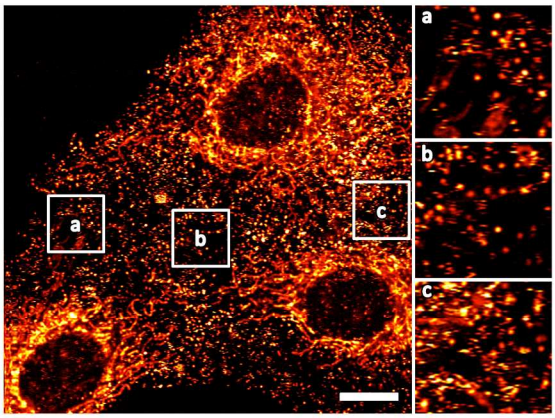
Metallic nanoparticles have myriad uses as labels and carriers. Unfortunately they are not inherently fluorescent and so have traditionally relied on fixed techniques such as EM to visualise.

Photothermal imaging provides a solution to this problem by probing the location of nanoparticles through their laser scattering properties.



A heating beam (shown in red) is absorbed by the nanoparticle, while a probe beam (green) measures the scattering caused by a local change in refractive index (shown in blue).

This technique is highly suited to a range of applications especially those, such as single-molecule tracking, that benefit from high signal to noise.



Here, fibroblast growth factor 2 (FGF2) was labelled with gold nanoparticles. The data help to explain how FGF2 interacts with ligands on the cell surface.

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LSM 510 MULTIPHOTON

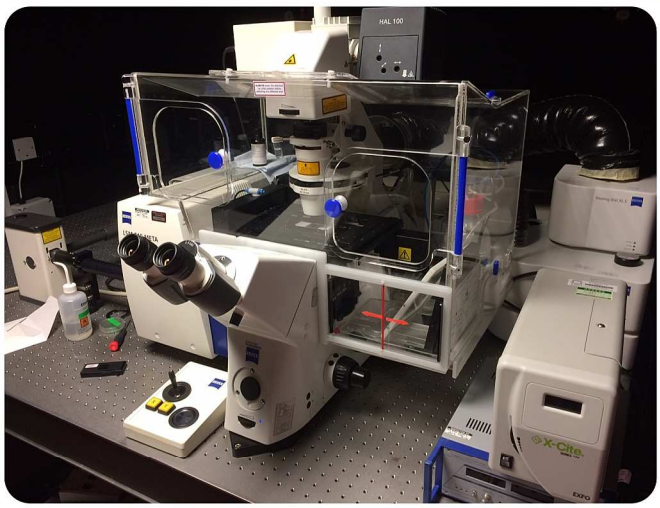
Specifications

Zeiss Axiovert 200M

10 20 40 63 100

458 488 561 800
477 514 633

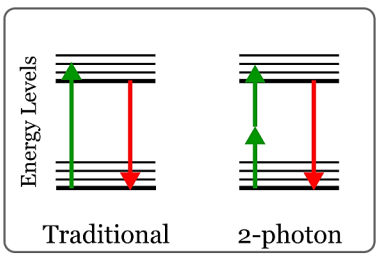
TEMP CO_2 SPECTRAL UNMIXING MP



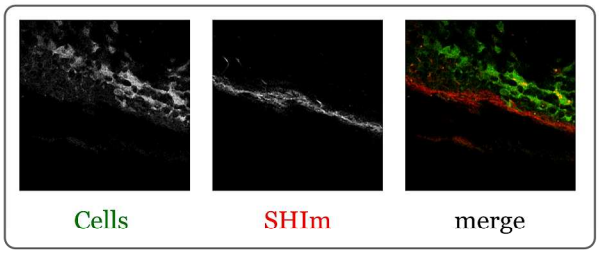
Technology Focus

Unlike traditional fluorescence microscopy, **multiphoton** (MP) microscopy excites samples with a lower-energy laser for reduced scattering, less bleaching and greater sample penetration.

Two co-incident photons, each with lower energy, excite the fluorophore. This happens only where the beam is focussed, reducing the confocal volume and as a result, the optical section.



The greater depth of penetration combined with cutting edge detector technology allows for unprecedented sensitivity in **thick samples** such as spheroids, tissue sections or (below) deep inside lymph node tissue.



Above, labelled cells were imaged inside the lymph tissue with multiphoton imaging, while **Second Harmonic Imaging** (SHIm) was used to visualise collagen in the sample.

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