

Two-Photon Excitation Microscopy (TPEM) for Chemical Visualisation and autofluorescence techniques

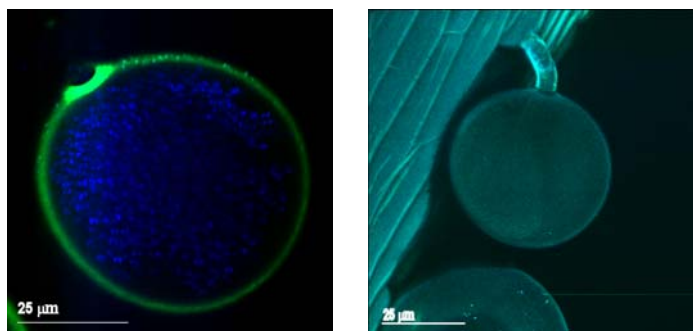
A new approach to nanoparticles—TPEM

The use of TPEM

TPEM allows the visualisation of specimens in conditions as close as possible to their natural state. The technique uses a scanning laser to excite the sample; the emitted fluorescence is detected to produce a single image, or a 3D reconstruction of the sample. TPEM Imaging using sample autofluorescence has been pioneered at Lancaster Environment Centre (LEC).

(<http://www.lec.lancs.ac.uk/ccm/research/visualisation/index.htm>)

Using TPEM a sample can only be excited through the simultaneous absorption of two low energy photons; Simultaneous absorption can only occur within a sub- μm volume at the focal point, generating a single thin ($< 0.2\mu\text{m}$) 'optical section'. By successively adjusting the position of the focal point, multiple optical sections can be generated throughout a sample. By combining these a 3D reconstruction of the sample can be generated.

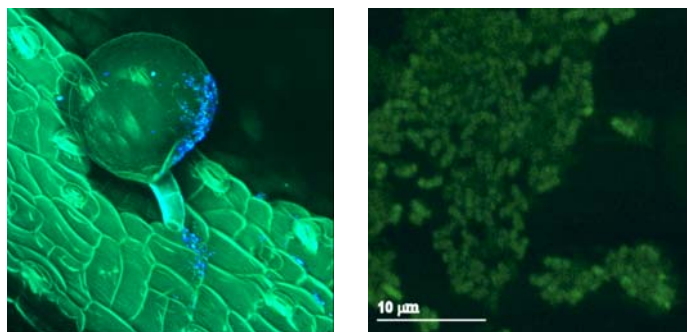


A single optical section of a living pollen grain and 3D reconstruction of a living fungal spore

Case Studies:

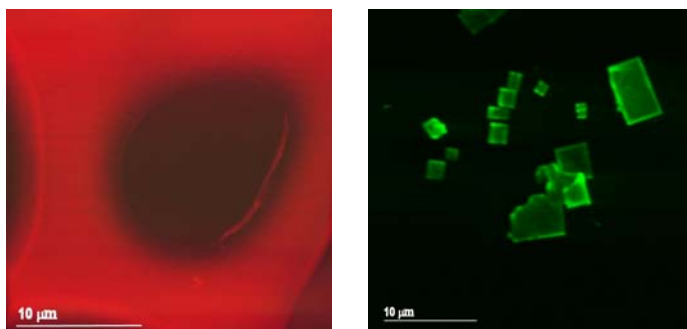
- **Atmospheric Pollutants:** Visualising the uptake of atmospheric organic pollutants to living vegetation - New insights for pollutant transport, toxicity, in-plant degradation and chemical fate modelling.
- **Soil Bound Chemical Residues:** Visualising chemical movement from soils to living plant roots - new insights into plant uptake, transport, storage in situ degradation, and contaminated land remediation strategies.
- **Agrochemicals:** Visualising agrochemical behaviour following application to living vegetation, quantification of diffusion rates, modes of activity, storage and degradation

- **Bacteria:** Visualising bacterial interactions within cellular complexes and mixed media; including pollutant degrading *Pseudomonas*.



Crop protection products (blue) on diseased leaves. *Pseudomonas* bacteria

- **Synthetics:** Visualising the behaviour of chemicals within a range of synthetics, including plastics foams, waxes, polymers and a range of specific particulates



A 3D reconstruction of a foam complex and select crystal complexes

TPEM has a wide range of applications, many of which are currently being developed or already in operation here at Lancaster. The application of TPEM in the field of Nanotechnology is one particularly exciting development at LEC.

Nanotechnology

Using our expertise of TPEM and environmental chemical fate, a research programme is currently being developed, at Lancaster, focussed around the behaviour of nanoparticles in the environment. This research initiative will be initially centred on nanoparticle aggregates (0.5-5 μm).

It is envisaged that the above research will have strong industrial applications, exploring the environmental fate and the chemical interactions of nanoparticles as well their behaviour in synthetics, pharmaceuticals and personal care products.

