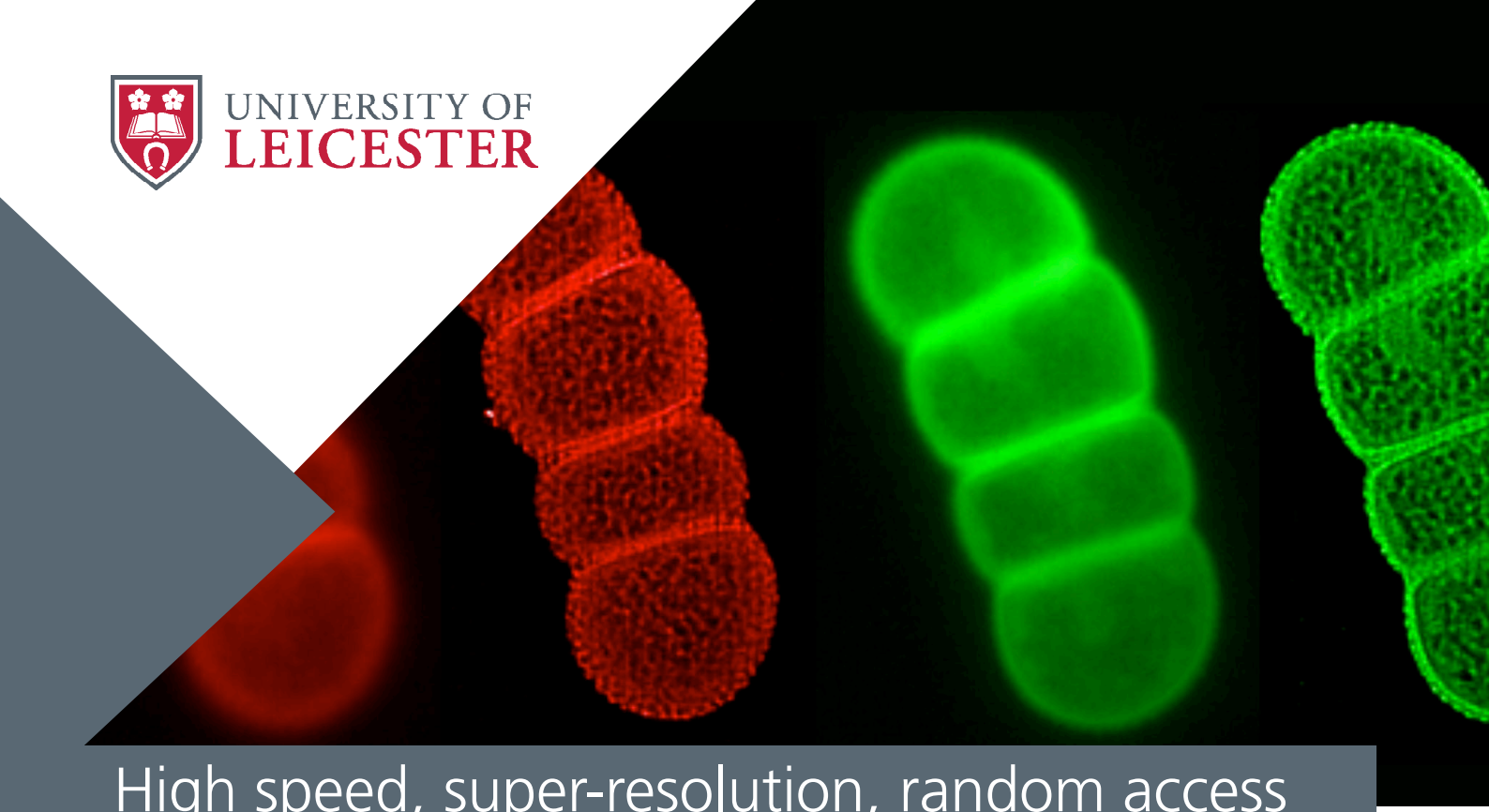
UNIVERSITY OF LEICESTER



High speed, super-resolution, random access multiphoton microscopy (SuperRAMP)

- Super-resolution imaging
- Class leading 0.5 mm imaging depth
- Highly versatile

Challenge

There is currently a huge demand for optical technologies that can exceed the diffraction limit to provide super-resolution images. Most existing super-resolution systems are highly complex and limited in functionality. Those that provide the highest resolution are generally slow and work best in fixed tissue using fluorophores with specialist characteristics. Most super-resolution methods can only work on thin specimens or are limited to depths of a few tens of microns at most.

Solution

A new multiphoton, super-resolution microscope has been developed at the University of Leicester that is capable of multicolour, super-resolution imaging at high speeds to depths of up to 0.5 mm. It provides up to two-fold improvement in lateral (xy) and axial (z) resolution compared to existing multiphoton microscopes. It is highly versatile allowing users to choose between optimum speed for functional imaging of living tissue or optimum resolution at lower speeds. It can scan in standard multiphoton mode with a large field of view at speeds of up to 80 frames per second or defined points of interest identified by super-resolution imaging at speeds of up to 30 kHz. It can also be used for programmable photoactivation for the release of caged compounds or optogenetic control of excitable tissues. A cost-effective single photon version is also available for use with thinner specimens.

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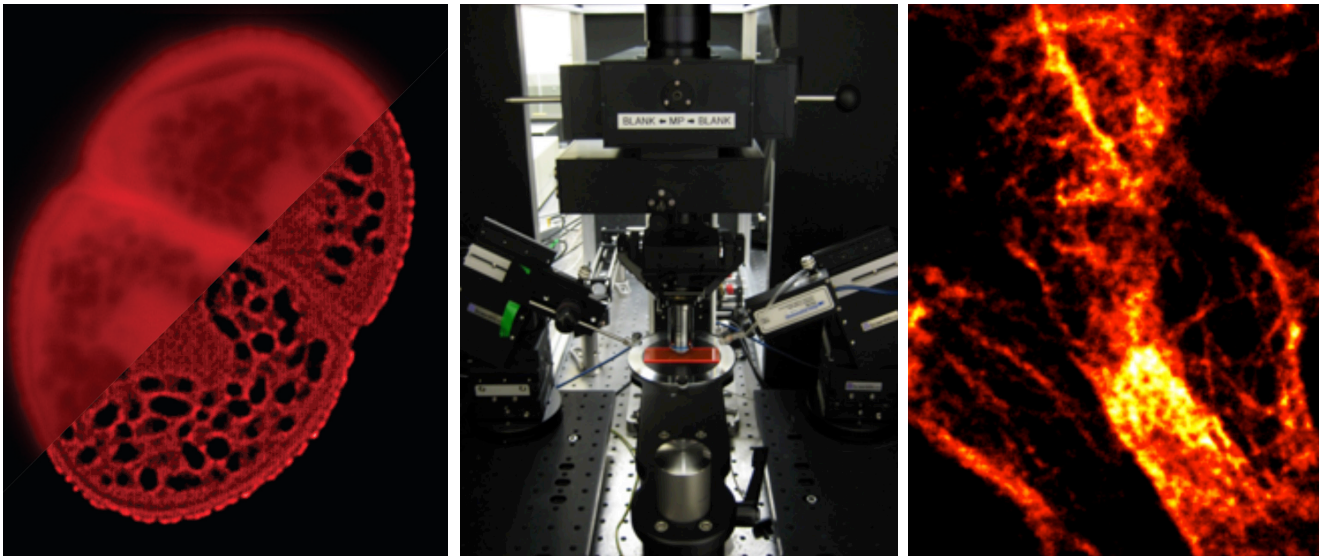
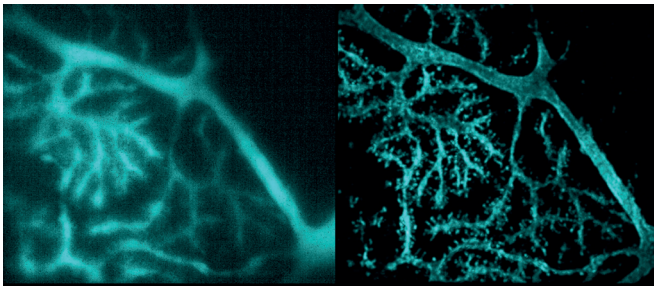


Images from left to right: 1) Comparison of multiphoton (top) and SuperRAMP (bottom) images of pollen autofluorescence. 2) The SuperRAMP microscope. 3) SuperRAMP image of fluorescently-labelled tubulin in neuroblastoma cells.

Benefits

- Improves lateral (xy) and axial (z) resolution up to two-fold compared to a standard multiphoton microscope.
- Produces super-resolved images to a depth of up to 0.5 mm in living tissue.
- Super-resolution imaging at speeds of up to 1 Hz.
- High speed random access mode: Scans super-resolved points of interest at up to 30 kHz.
- Video mode: Up to 50 frames per second (512 x 512 pixels).
- Multiple fluorophore imaging of fluorescent proteins, standard fluorophores and secondary antibodies (Alexa, CY2-5), calcium indicators (Fluo-4, Oregon Green, Calcium Green) and nuclear markers (DAPI, Hoechst).
- Dual emission imaging and dual excitation imaging with a second laser.
- User programmable photo-activation including release of caged compounds or optogenetic activation.
- Large fields of view – 200 x 200 µm at 20x magnification.
- Single photon version for super-resolved imaging of thin specimens.



Standard multiphoton (left) and SuperRAMP (right) images of a fluorescent neuron >100 µm below the surface of a mouse brain slice.

Market

Multiphoton microscopes provide optical sectioning deep within tissue. They reduce phototoxicity by selectively illuminating fluorophores in the focal plane allowing longer imaging of living specimens. Most current systems scan relatively slowly. Our new SuperRAMP system doubles the resolution compared to standard multiphoton systems whilst operating at comparable depths. It provides a flexible and versatile scanning system that can be switched rapidly to allow full frame scanning at video rates or random access scanning at kHz speeds to capture the fastest biological signals. It can also deliver super-resolution imaging at depth for non-biological specimens, for example in the superconductor industry.

IP status

The technology underpinning this microscope is protected by two patent applications:

Improvements in or relating to super-resolution microscopy. PCT/GB2014/053137.

Improvements in or relating to structured illumination microscopy. PCT/GB2015/050810.

Are you an imaging company looking to develop super-resolution products?

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