Advanced Optical Imaging for the Biomedical Sciences

J. Nylk*[1,2], Z. Yang[1], M. Mazilu[1], T. Vettenburg[3], M.V.G. Kristensen[1], C.A. Mitchell[1,2], E.C. Campbell[2], J.M. Pullman[4], M.B. Prystowsky[4], F.J. Gunn-Moore[2], K. Dholakia[1]

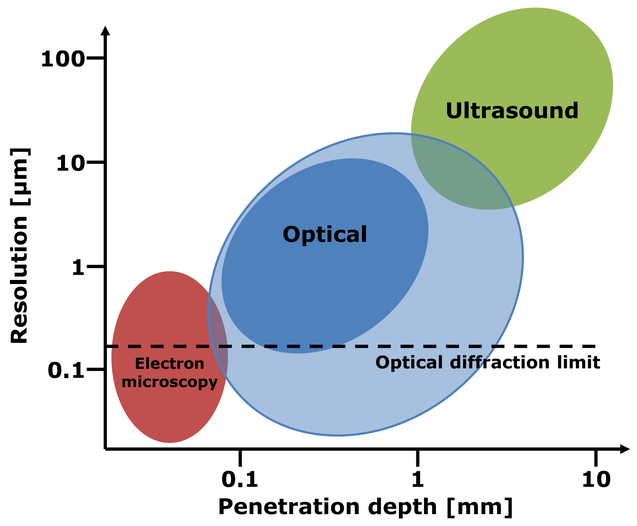
[1]SUPA, School of Physics & Astronomy, University of St Andrews, North Haugh, St Andrews, Fife, KY16 9SS, UK
[2]School of Biology, University of St Andrews, North Haugh, St Andrews, Fife, KY16 9TF, UK
[3]Present address: Dept. of Bioengineering & Aerospace Engineering, Universidad Carlos III de Madrid, Madrid, Spain
[4]Department of Pathology, Albert Einstein College of Medicine, NY, USA
**contact: jn78@st-andrews.ac.uk*

Introduction

Advanced beam shaping is pushing optical microscopy beyond traditional limitations.

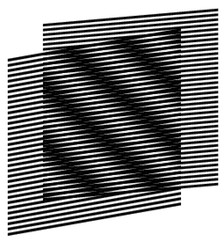
Non-Gaussian illumination can **increase imaging volumes** and **lower photo-damage** in high resolution light-sheet microscopy.

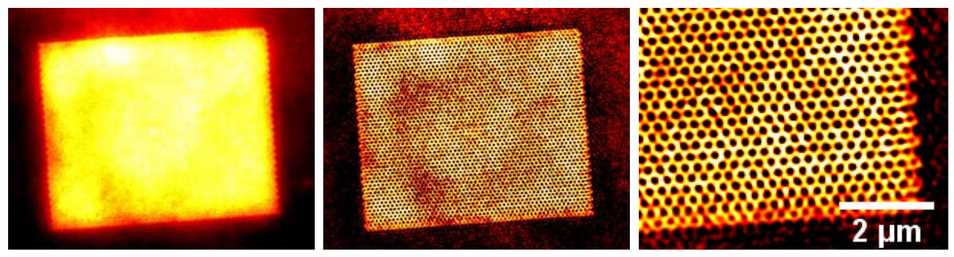
Compact optical trapping solutions can be incorporated into super-resolution microscopy platforms to **add functionality** without impacting on image quality.



Structured Illumination

Structured illumination microscopy (SIM) is a super-resolution technique that uses Moiré fringes to access higher spatial frequencies that the microscope can resolve. SIM gives **resolution 2 times smaller than the diffraction limit** and is one of very few super-resolution techniques **fast enough for live imaging**.

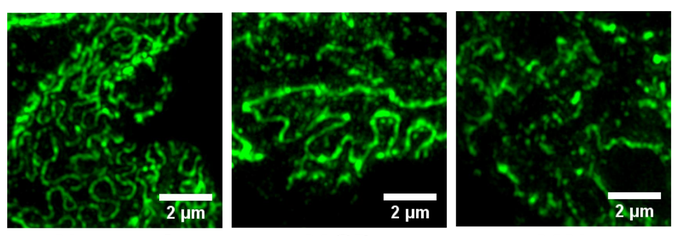


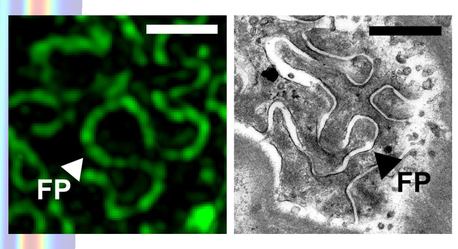


Right: Epi-fluorescence (left) and 3D-SIM (middle, right) images of photonic crystal structures coated in fluorescent layer.

Even doubling the resolution facilitates a number of previously inaccessible studies.

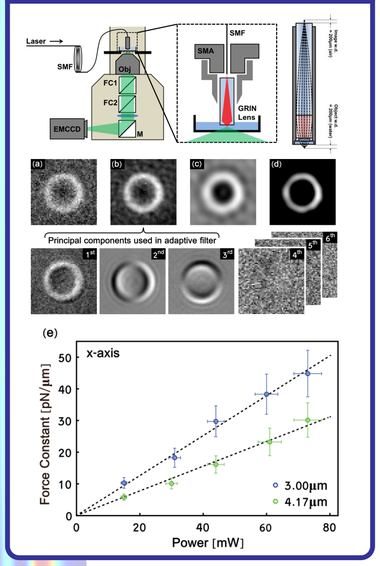
SIM could replace electron microscopy (EM) for a number of diagnostic procedures[1].





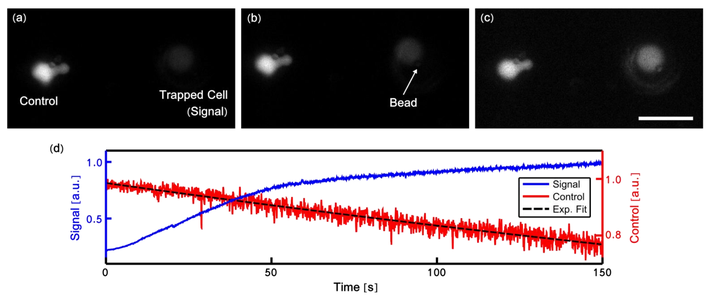
Above: 3D-SIM images of renal biopsy tissue slices stained with α-podicin (green). Morphological changes between normal (left), minimal change disease (MCD; middle), and membranous nephropathy (MN; right) are visible.
Left: Correlation of 3D-SIM and EM images of podocyte foot processes (FPs) in normal tissue. Scale bar: 1µm.

GRIN Lens Optical Trap



Optical trapping enables spatiotemporal control of events and interactions under the microscope. Gradient refractive index (GRIN) lenses can make very compact optical trapping probes for use on any inverted microscope[2].

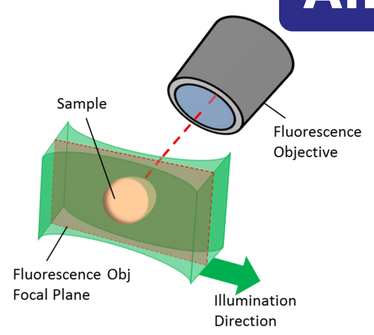
The trap quality was characterised using principal component and power spectrum analyses, and used to induce immune synapse formation.



Publications

1. Pullman, J. M. *et al*, *Visualization of podocyte substructure with structured illumination microscopy (SIM): A new approach to nephrotic disease*, in preparation.
2. Nylk, J. *et al*, *Development of a graded index microlens based fiber optical trap and its characterization using principal component analysis*, Biomedical Optics Express **6**(4) (2015), p. 1512-1519.
3. Vettenburg, T. *et al*, *Light-sheet microscopy using an Airy beam*, Nature Methods **11** (2014), p. 541-544.
4. Yang, Z. *et al*, *A compact Airy beam light sheet microscope with a tilted cylindrical lens*, Biomedical Optics Express **5**(10) (2014), p. 3434-3442.
5. Nylk, J. *et al*, *Depth enhanced light-sheet microscopy using a compensated propagation-invariant Airy beam*, in preparation.

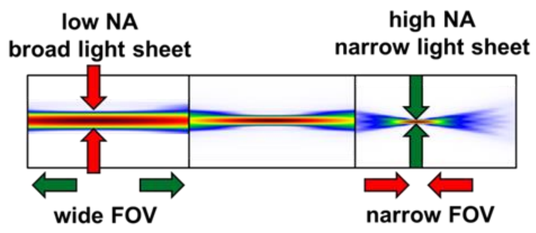
Airy Beams for LSM

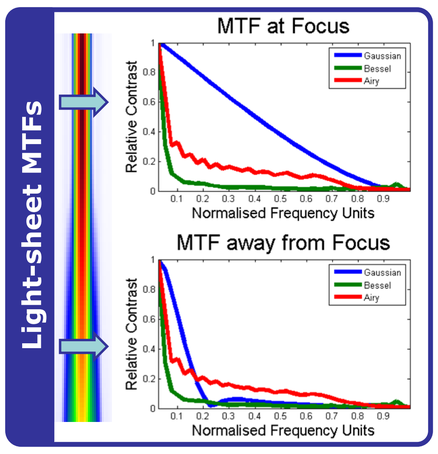


Light-sheet microscopy (LSM) is an emerging microscopy method with perpendicular illumination and fluorescence detection optics. LSM is particularly favoured for its **selective illumination** and **wide-field detection**. LSM facilitates rapid, high-contrast images of large volumes with minimal photo-damage.

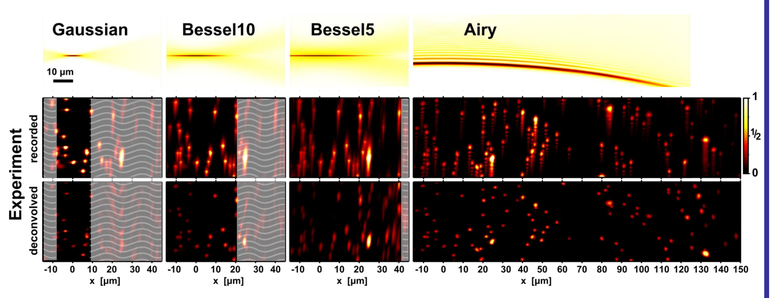
A standard Gaussian light sheet becomes more divergent as the sheet width is reduced, limiting the field-of-view (FOV) of very high resolution LSM.

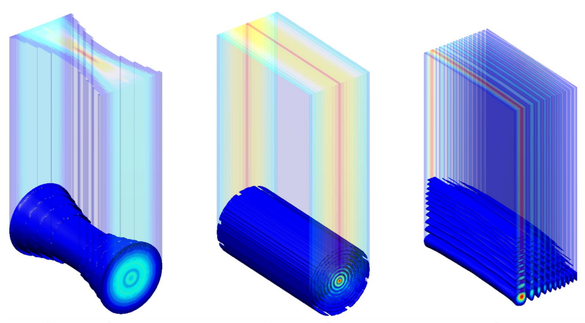
- **Use propagation-invariant beams**

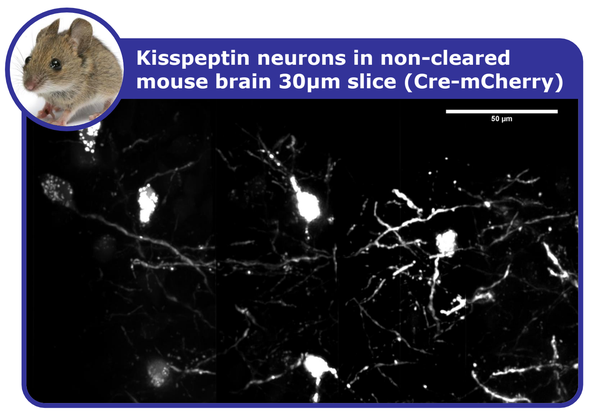




Fourier analysis of the light-sheet cross-section (MTF) reveals that the **Airy light-sheet intrinsically supports high spatial frequencies (resolution)**[3]**.**







Specialty beam shaping optics are expensive which limits use. Low cost is essential for wide usage. **Aberrations in simple optical components can be exploited**[4]**.** An Airy light-sheet can be created with the cylindrical pupil function, where α controls the length and width of the Airy light-sheet:

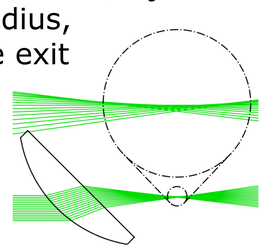
$$P(u,0) = exp\left(2\pi i \alpha u^3\right)$$

The Seidel wave aberrations for a lens are:

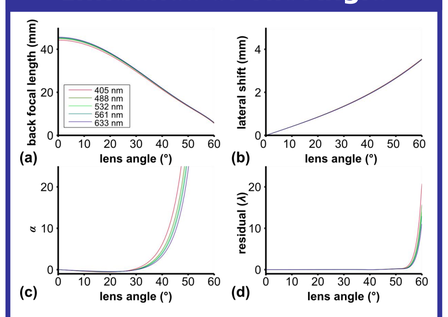
$$\phi(\rho,\theta,h_0) = Bh_0{}^3\rho\cos\theta - \frac{1}{2}(2C\cos^2\theta + D)h_0{}^2\rho^2 + Eh_0\rho^3\cos\theta - \frac{1}{4}F\rho^4$$

E is the coma aberration coefficient, h_0 is the object height, ρ is the pupil radius, And θ is the polar angle at the exit pupil of the lens.

The coma term will yield a cubic phase and an Airy beam



Influence of Tilt Angle



Simulations of focal length (a), optic axis displacement (b), cubic (c), and higher order phase modulation (d) as a function of lens angle.

Attenuation of the illuminating light-sheet limits imaging at depth and causes degradation of image quality with propagation.

For propagation invariant beams there exists a relationship between the transverse coordinates in the pupil plane and the axial coordinates in the focal volume. An amplitude mask in the pupil plane of such beams can pre-compensate for attenuation[5].

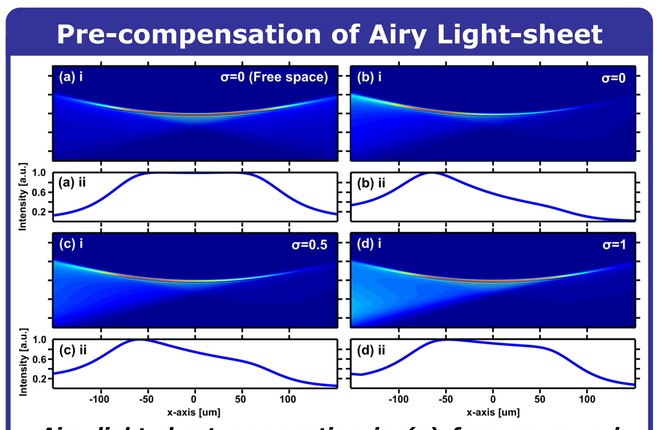
The pupil function for a compensated Airy light-sheet:

$$P(u,0) = exp(\sigma u)\cdot exp\left(2\pi i \alpha u^3\right)$$

where the σ parameter controls the amount of compensation.

This allows **delivery of more power to greater depth** in a specimen **without increasing the illumination peak power**.

Pre-compensation of Airy Light-sheet



Airy light-sheet propagating in (a) free-space and (b) absorbing media with no compensation. (c,d) compensation restores light-sheet intensity.

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SUPA
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RS Macdonald