

Image analysis and open problems in biophotonics

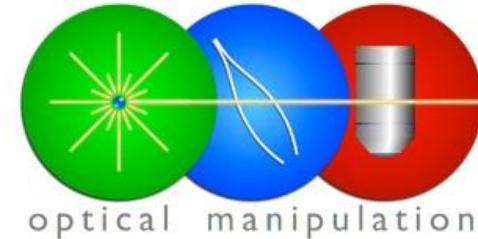
Jonathan Nylk*
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* jn78@st-andrews.ac.uk

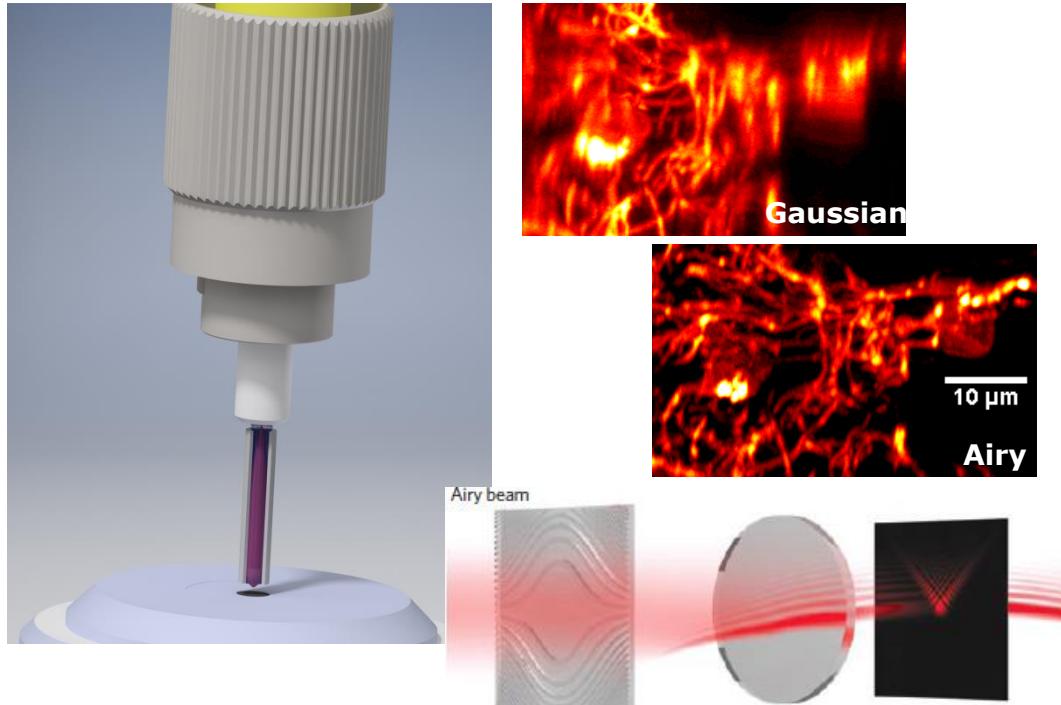
 [@jonathan_nylk](https://twitter.com/jonathan_nylk), [@OpticManip](https://twitter.com/OpticManip)



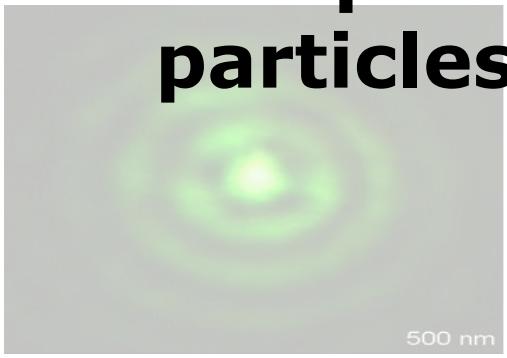
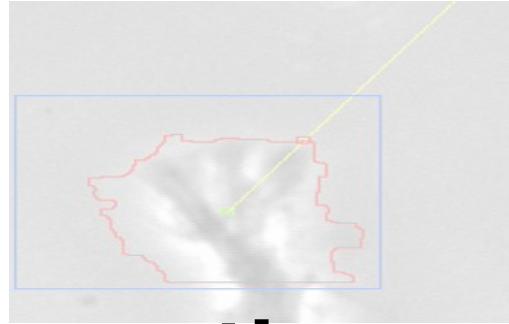
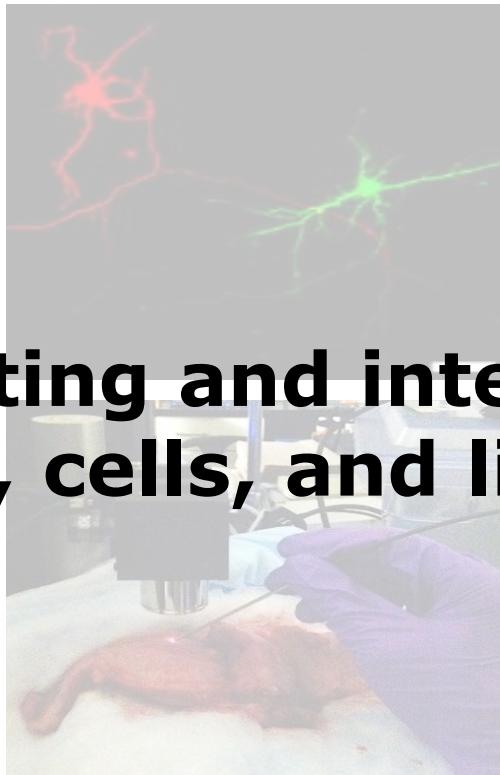
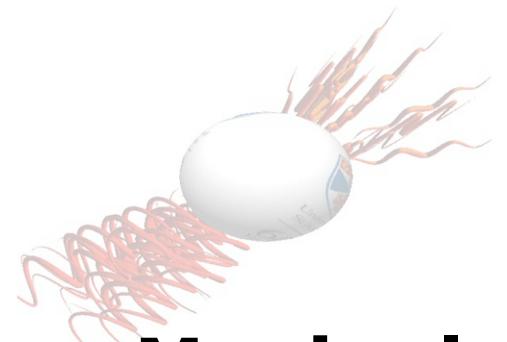
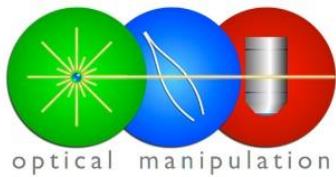
University
of
St Andrews



optical manipulation

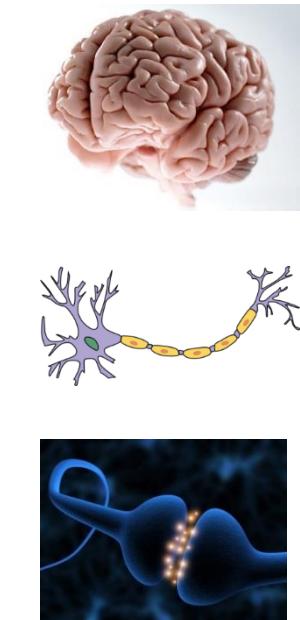
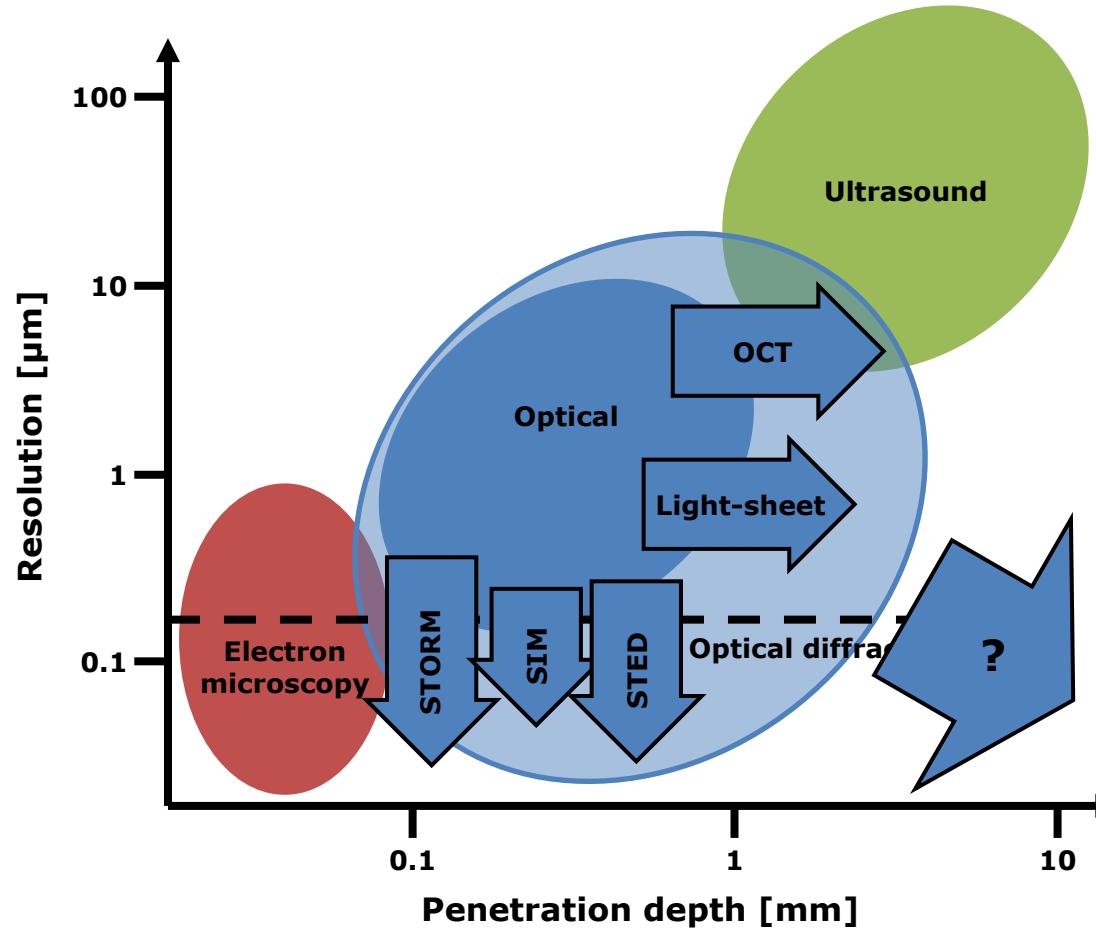


Optical Manipulation

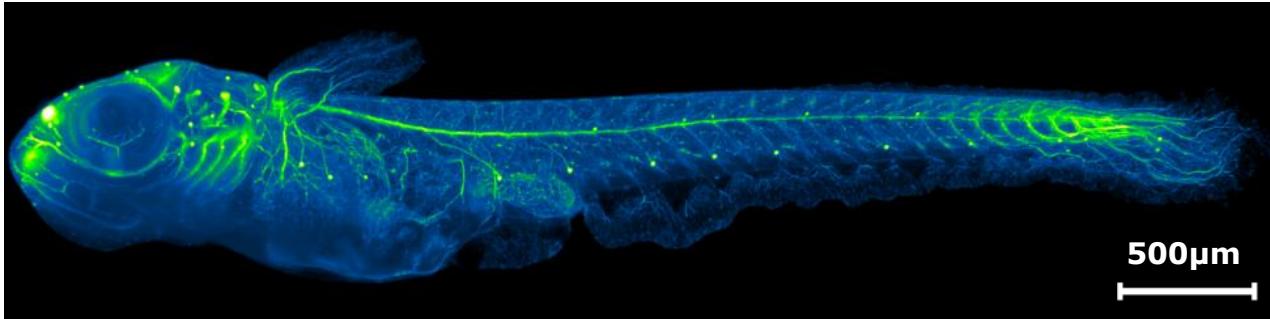


**Manipulating and interrogating
particles, cells, and light itself**

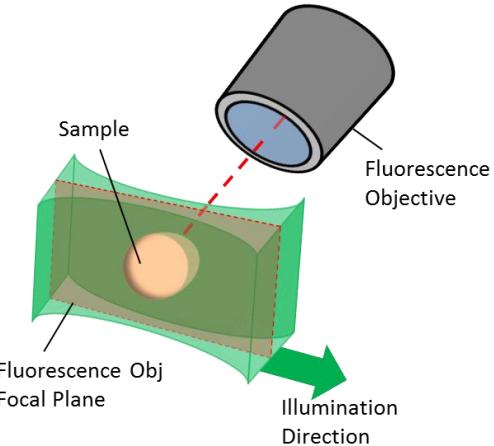
The optical imaging domain?



Light-sheet microscopy



Keller, P. J. & Stelzer, E. H. K., Science (2008)

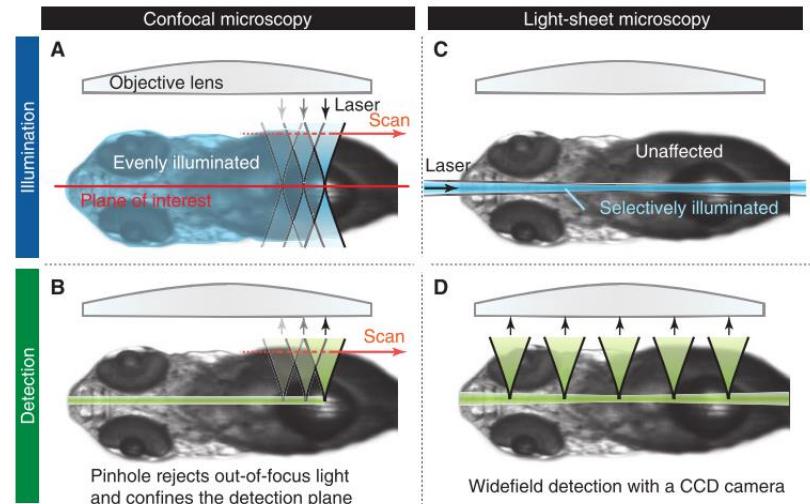


Selective illumination

- High contrast
- Low photo-bleaching/-toxicity

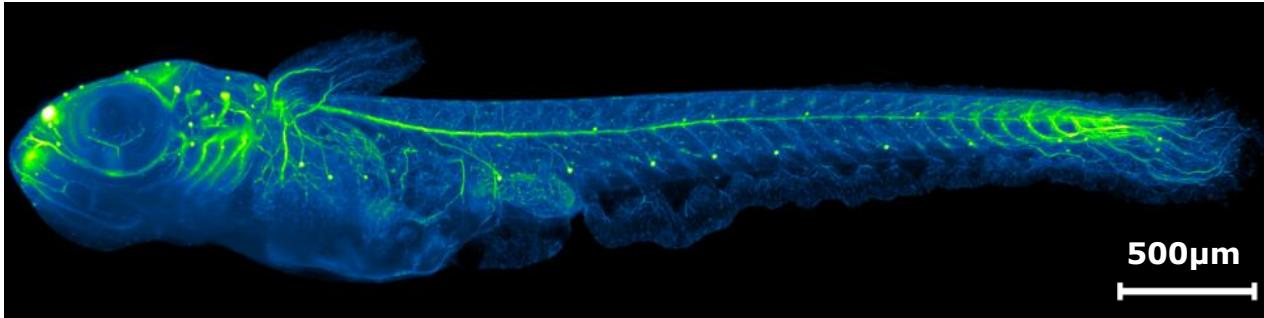
Wide-field

- Large volumes
- Fast acquisition

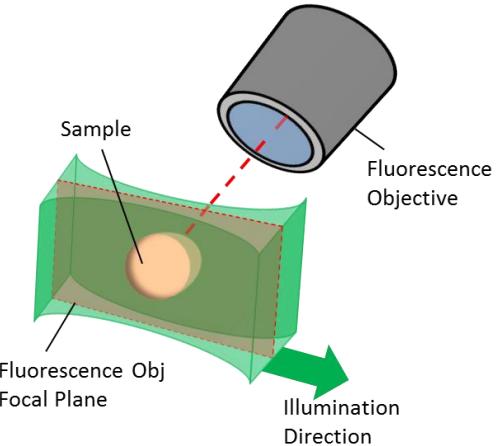


Huisken, J. & Stainier, D. Y. R., Development (2009)

Light-sheet microscopy



Keller, P. J. & Stelzer, E. H. K., Science (2008)

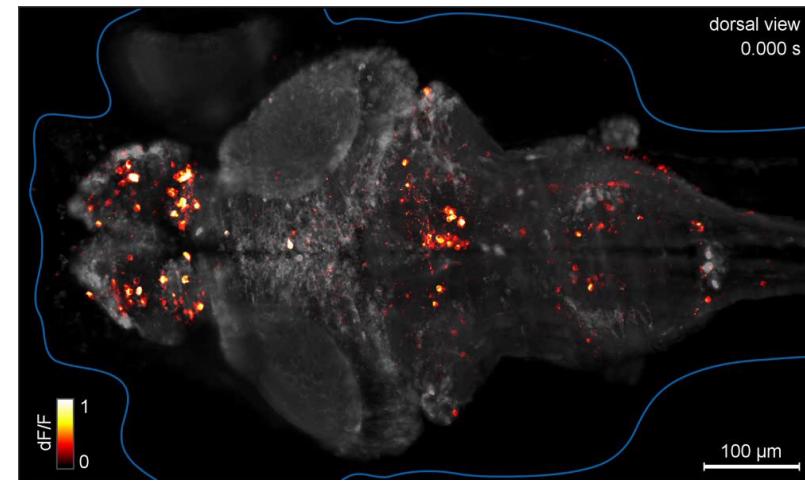


Selective illumination

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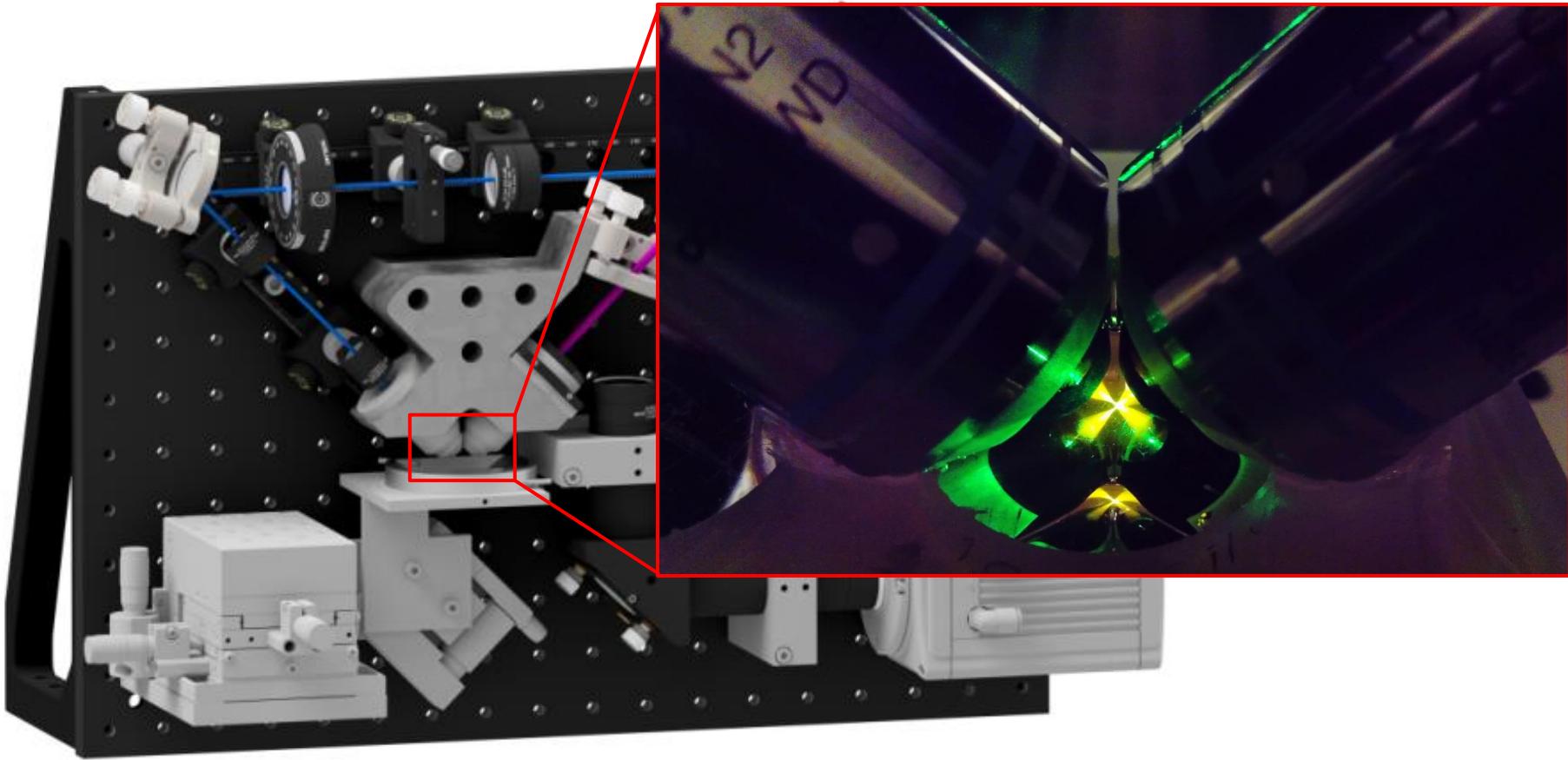
Wide-field

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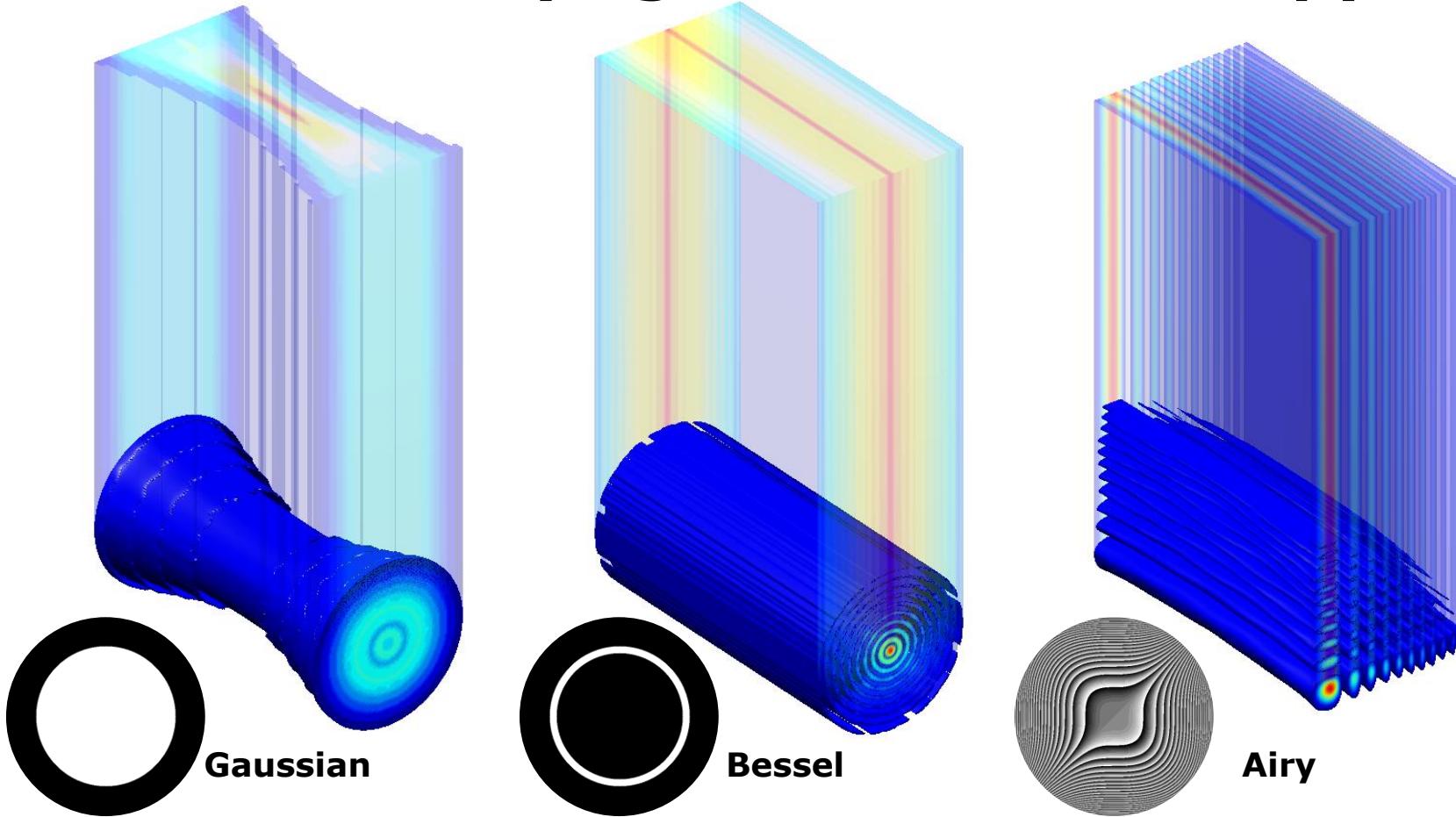


Ahrens, M.B. et al, Nature Methods (2013)

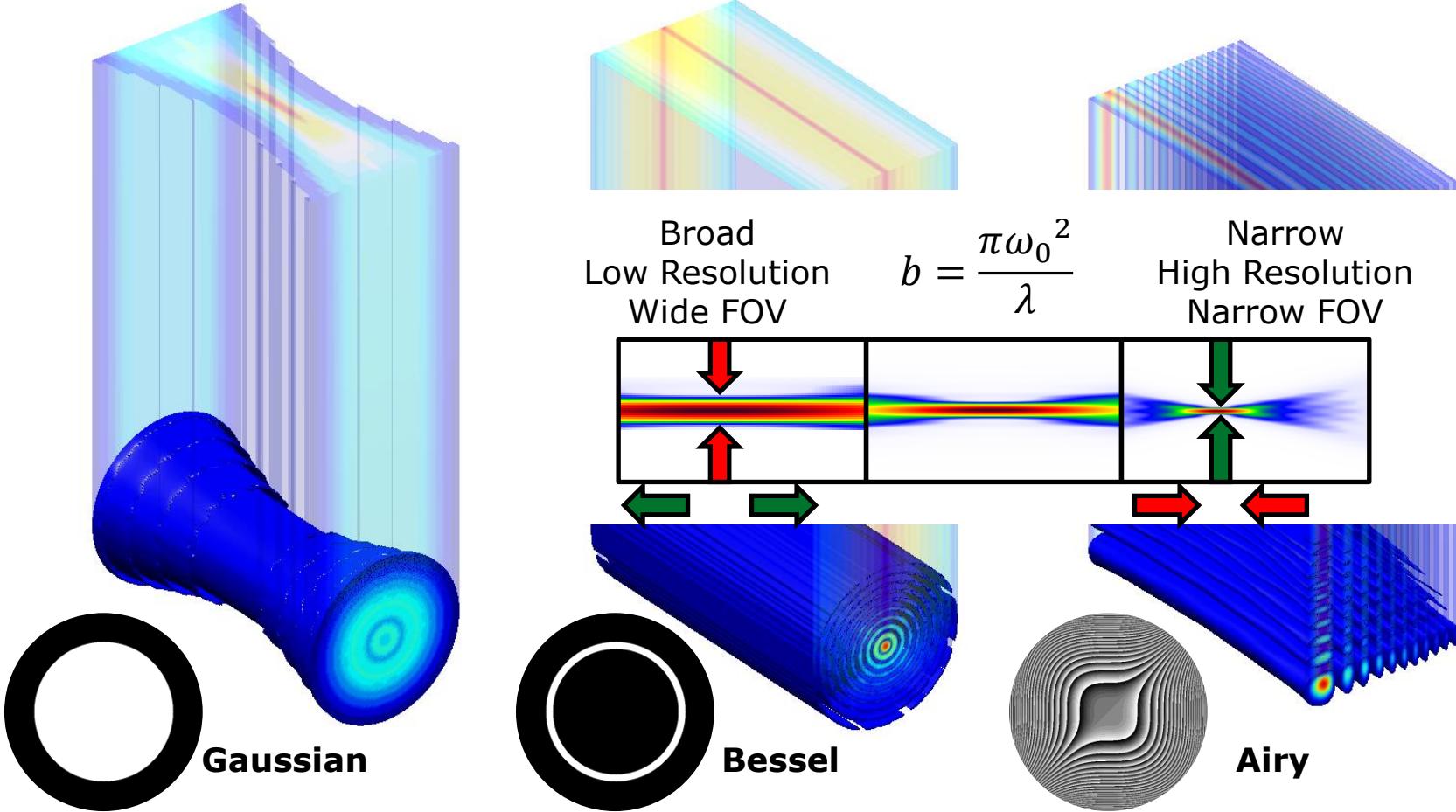
What does a light-sheet microscope look like?



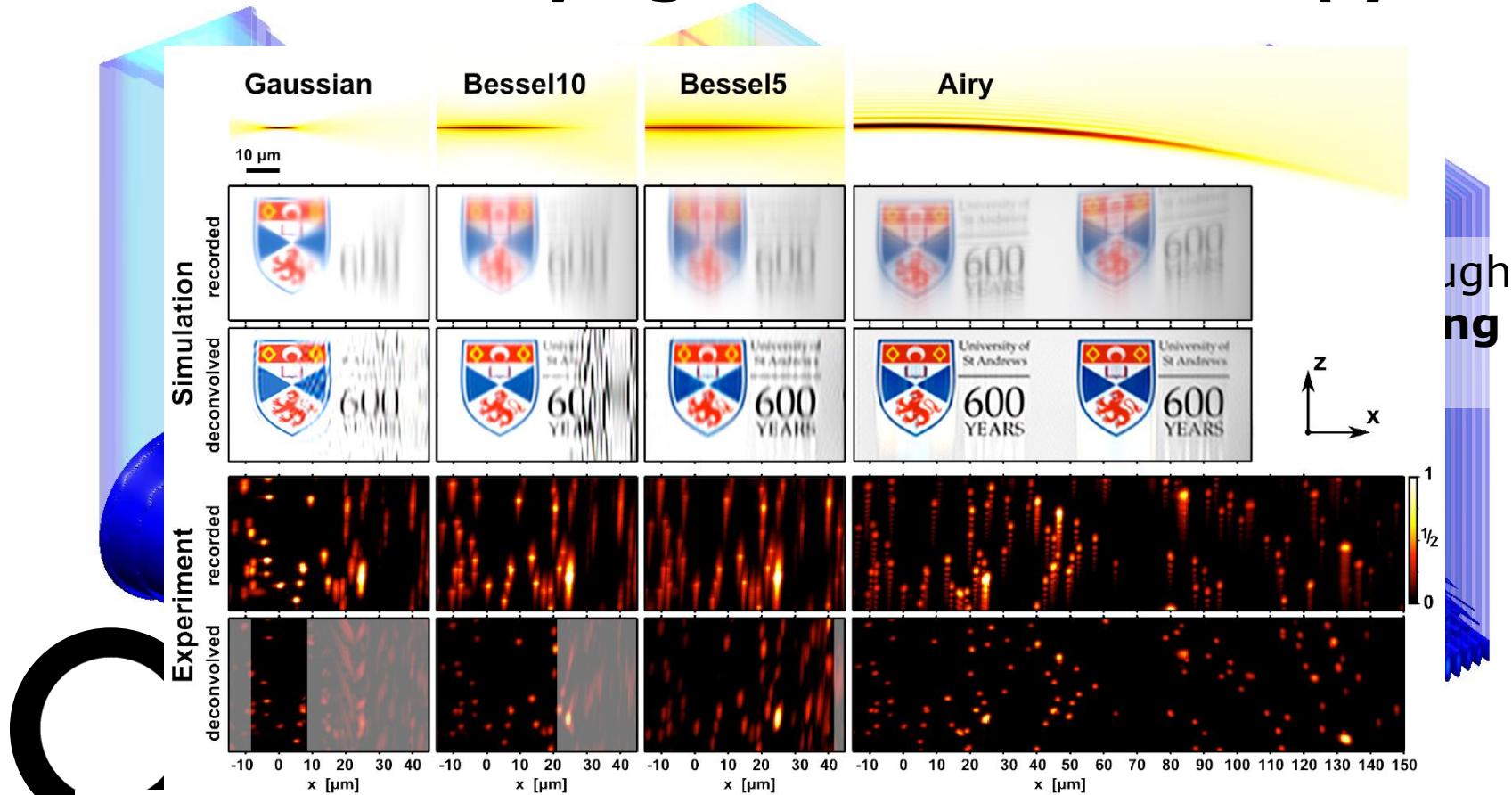
Evolution to Airy light-sheet microscopy



Evolution to Airy light-sheet microscopy

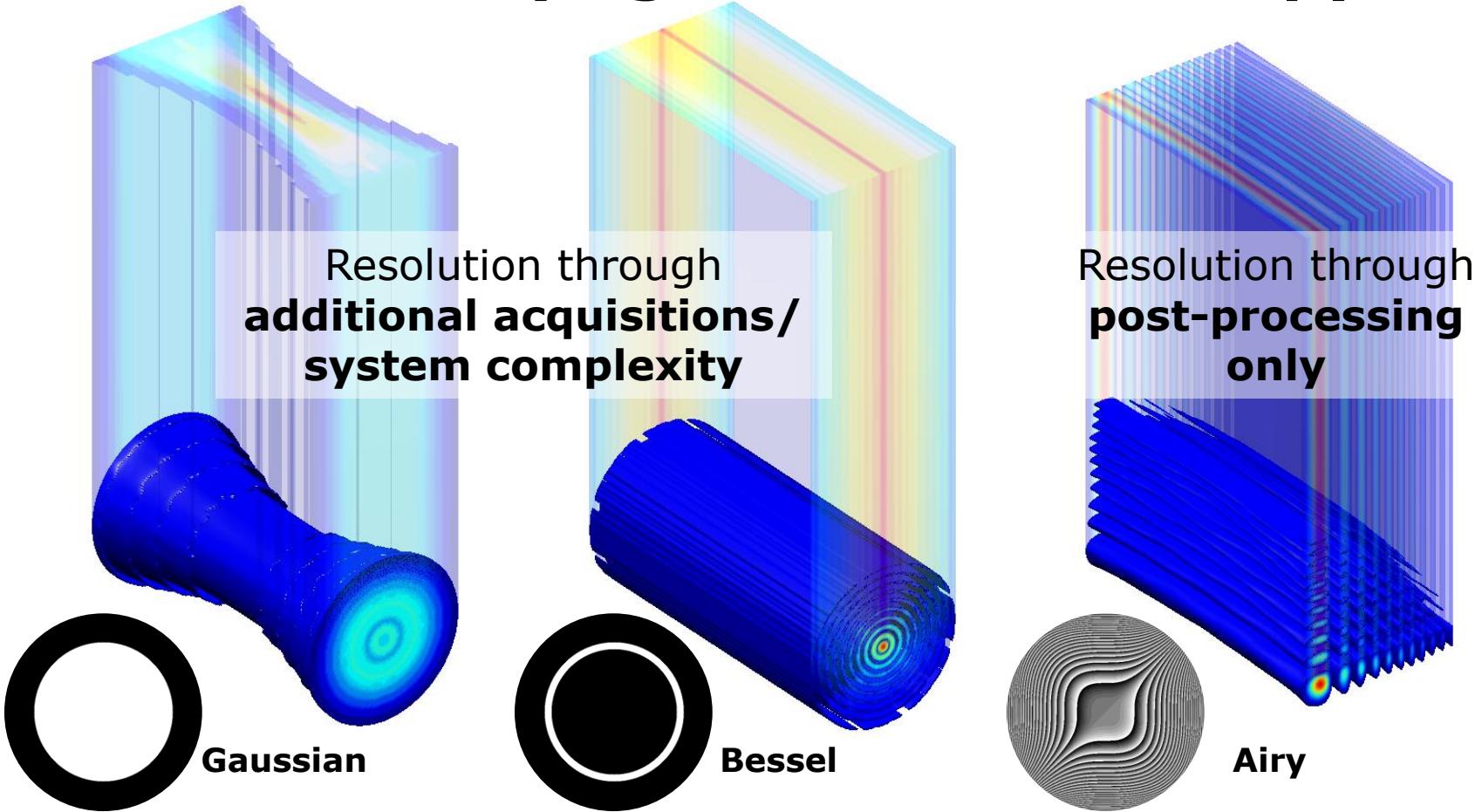


Evolution to Airy light-sheet microscopy



Vettenburg, T. et al, "Light-sheet microscopy using an Airy beam", Nature Methods **11**(5), 551-544 (2014).

Evolution to Airy light-sheet microscopy



Deconvolution – Wiener filter

Imaging is a convolution:

$$D(r) = (H \otimes S)(r)$$

OR multiplication in Fourier space:

$$\tilde{D}(k) = \tilde{H}(k) \times \tilde{S}(k)$$

So deconvolution is a division:

$$\tilde{S}(k) = \frac{\tilde{D}(k)}{\tilde{H}(k)}$$



Divides by zero!
Artefacts!

$S(r)$: Object

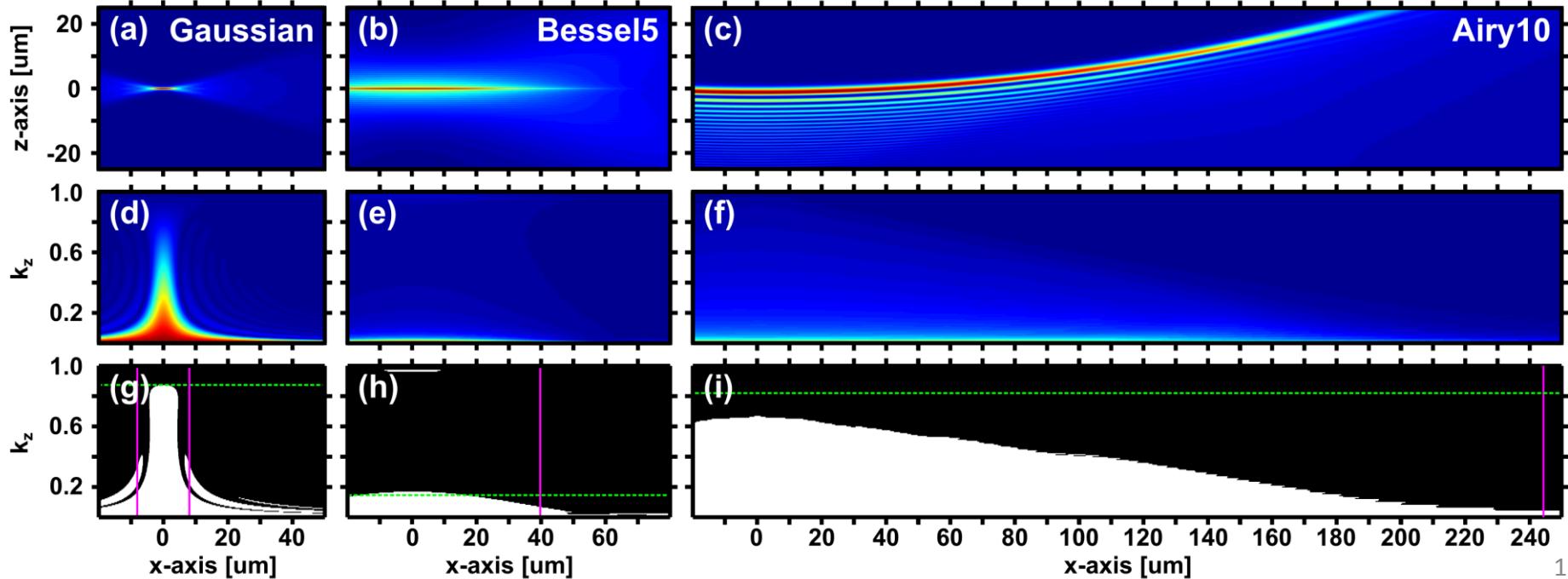
$D(r)$: Image

$H(r)$: Point-spread function

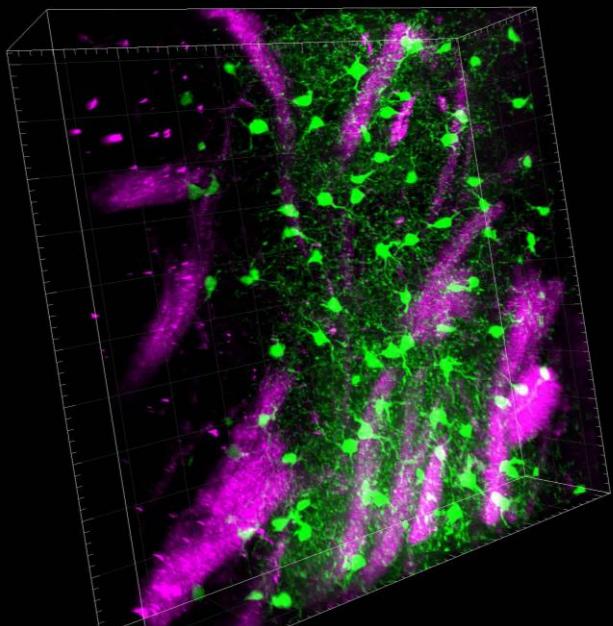
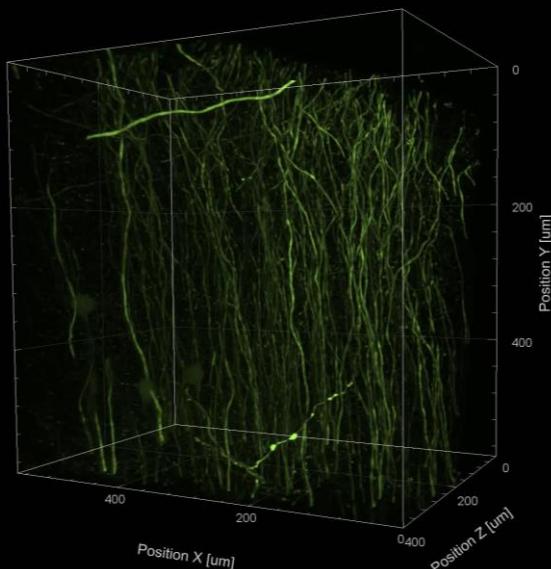
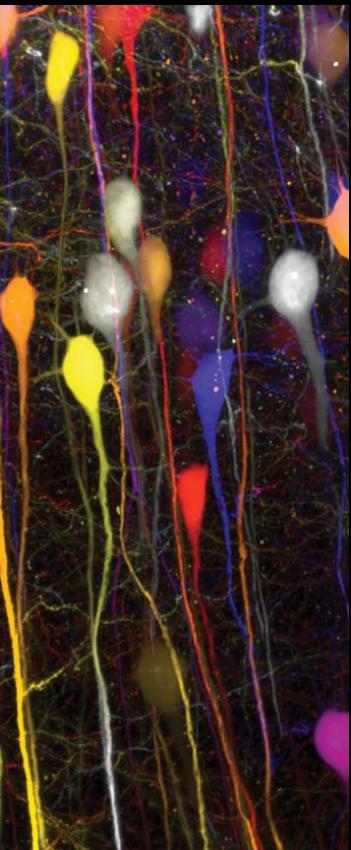
**A better way:
Minimum mean square error filter**

$$\tilde{S}(k) = \tilde{D}(k) \frac{\tilde{H}(k)^*}{|\tilde{H}(k)|^2 + SNR(k)^{-2}}$$

Modulation transfer function (MTF)

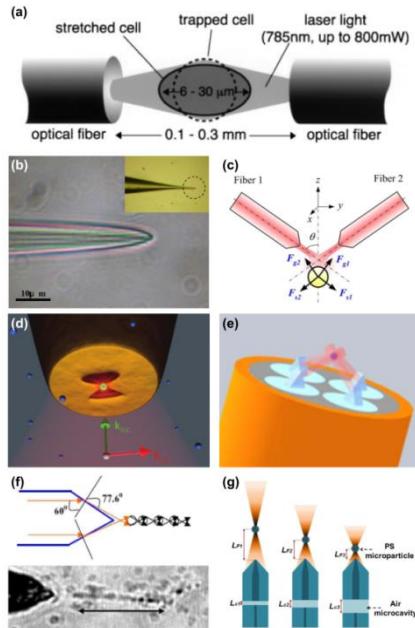


Airy LSM commercialised with M Squared Lasers



Images courtesy of M Squared Lasers
and King's College London

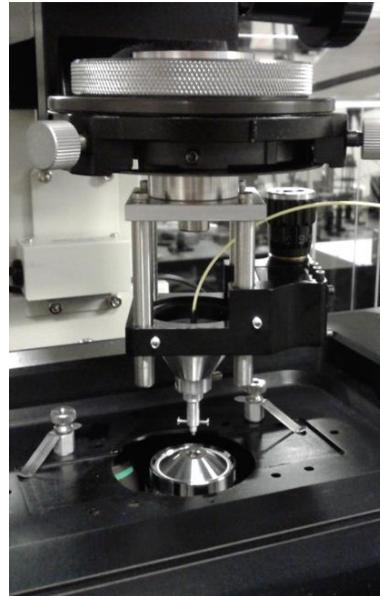
Mesoscale trapping



Compact
Low NA
Short range
Low cost



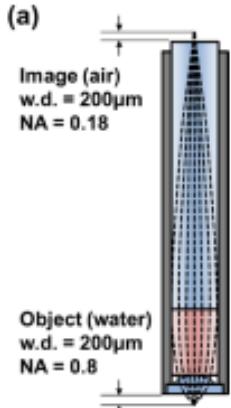
Compact
High NA
Long range
Low cost



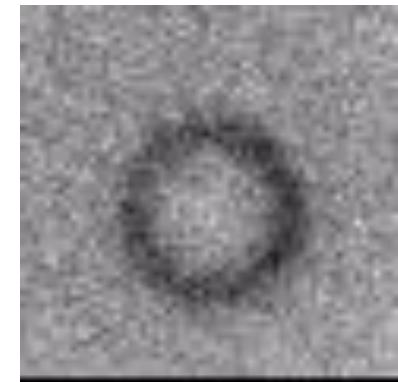
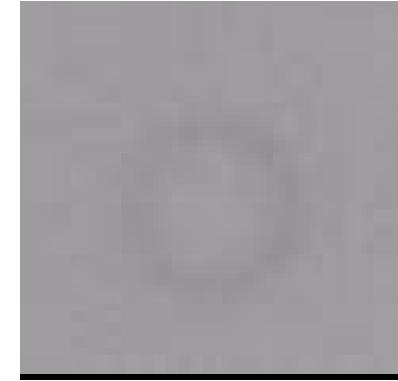
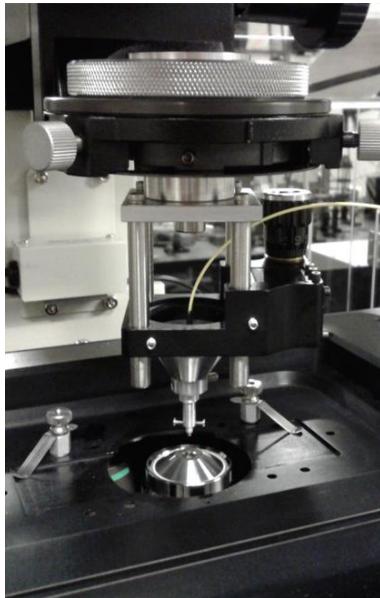
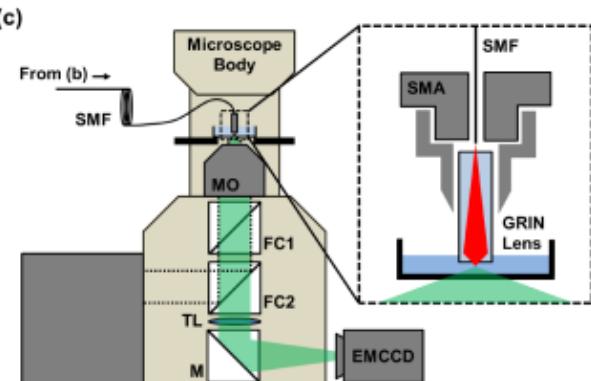
Bulky
High NA
Long range
High cost

Mesoscale trapping

GRINTech

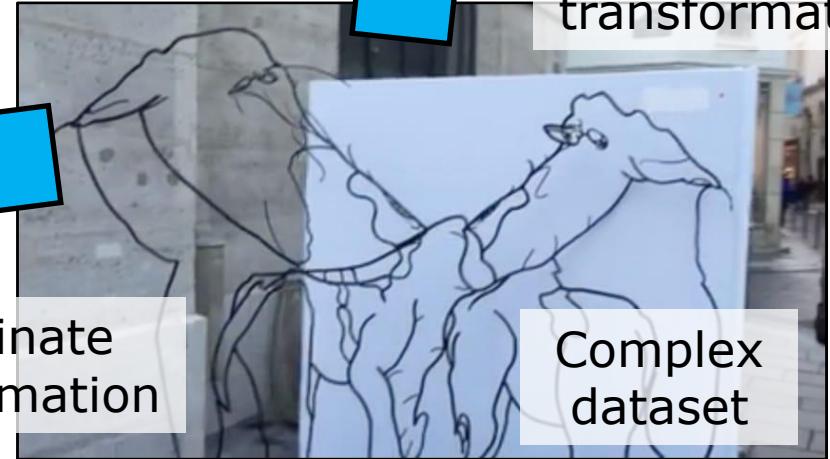
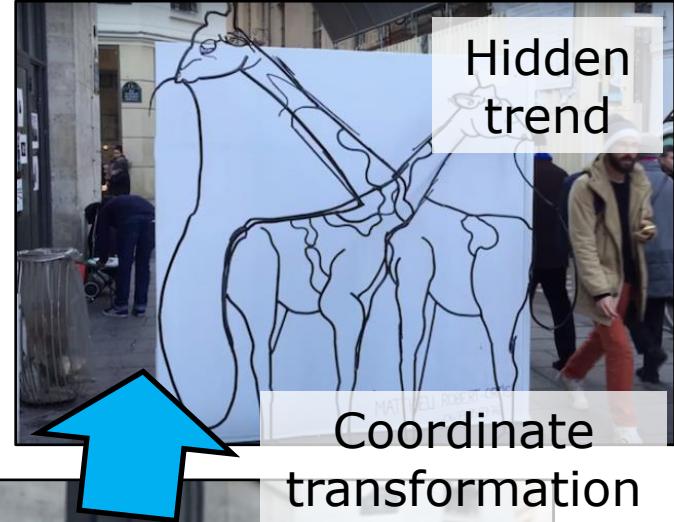


Commercial
GRIN micro-
objective lens
positioned on
single-mode
fibre



Characterize *in situ* on any
microscopy platform

Principal component analysis



Principal component analysis

$$C_{ij} = \sum_n \sum_m M_{nm}^i M_{nm}^j$$

$$\lambda^{(k)} V_i^{(k)} = \sum_j C_{ij} V_j^{(k)}$$

$$M_{ij}^{(1)} = \frac{1}{\sqrt{\lambda^{(k)}}} \sum_m M_{ij}^m V_m^{(k)}$$

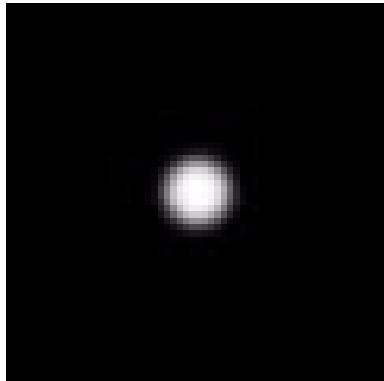
$$c_k^j = \sum_n \sum_m M_{nm}^j M_{nm}^{(k)}$$

$$\tilde{M}_{nm}^j = \sum_{k=1}^{k_{max}} c_k^j M_{nm}^{(k)}$$



Analyse inter-frame spatial correlations to extract fundamental particle motions

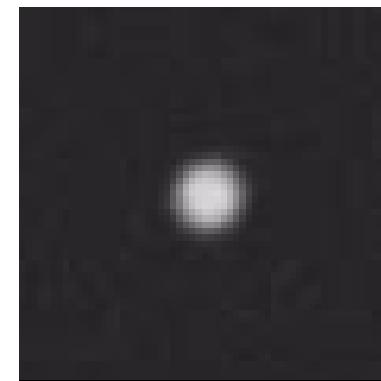
Simulations – Gaussian particle



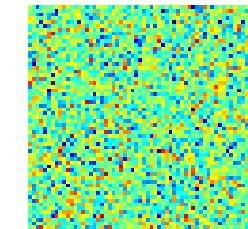
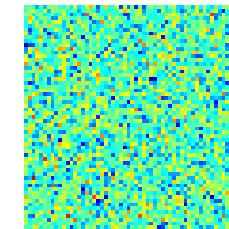
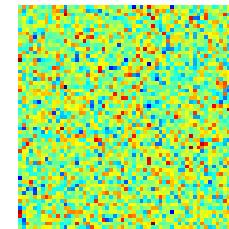
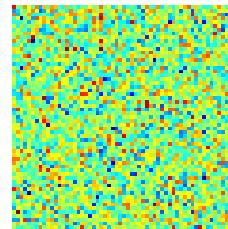
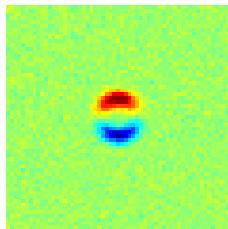
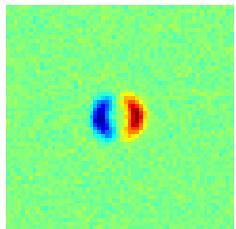
Actual



Image

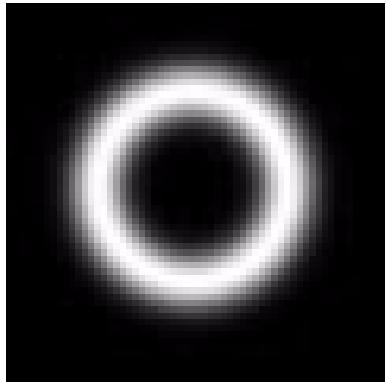


Reconstruction

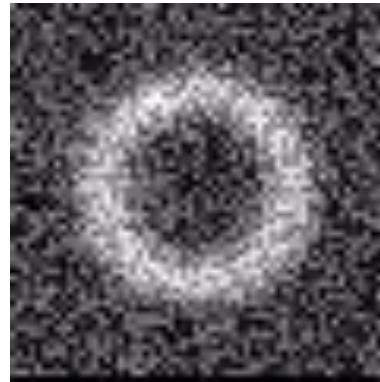


Principal components

Simulations – Ring particle



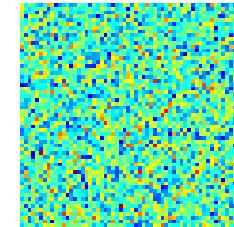
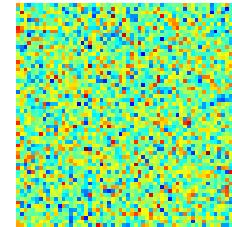
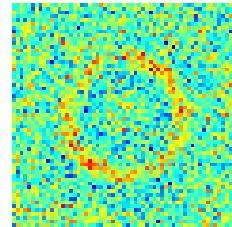
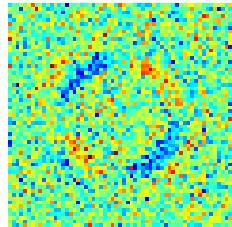
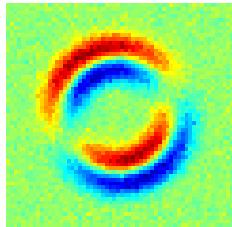
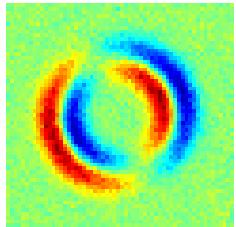
Actual



Image



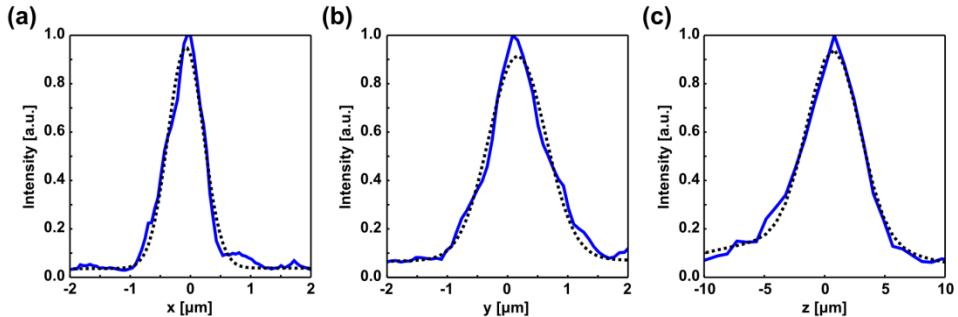
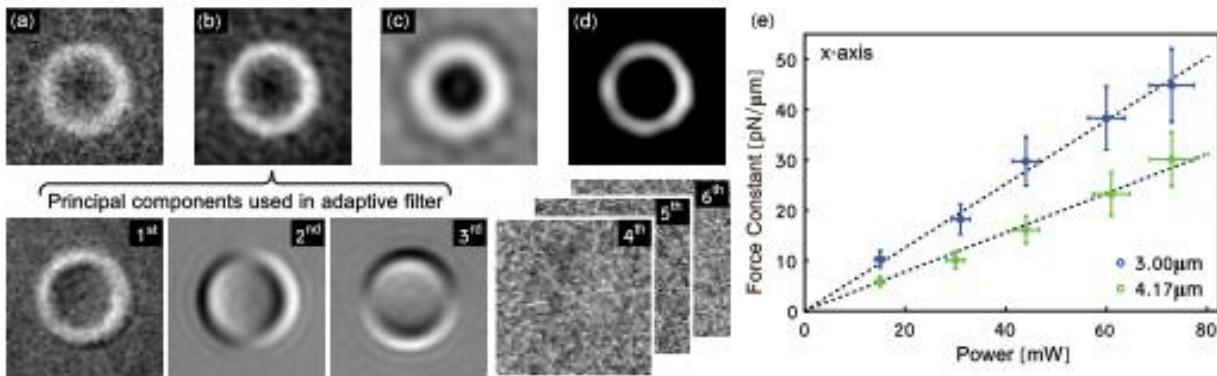
Reconstruction



Principal components

Trap characterisation

- Acquire
- PCA filter
- Low-pass
- Threshold
- k_{trap}



$$K_{\text{trap}, x} = (0.63 \pm 0.01) \text{pN } \mu\text{m}^{-1} \text{ mW}^{-1}$$
$$K_{\text{trap}, y} = (0.522 \pm 0.006) \text{pN } \mu\text{m}^{-1} \text{ mW}^{-1}$$

$$\text{FWHM}_x = 0.8 \mu\text{m}$$
$$\text{FWHM}_y = 1.3 \mu\text{m}$$
$$\text{FWHM}_z = 6.2 \mu\text{m}$$