Institute for Integrated Systems

3D data capture for studying dynamical processes

Alan Greenaway

Thanks…

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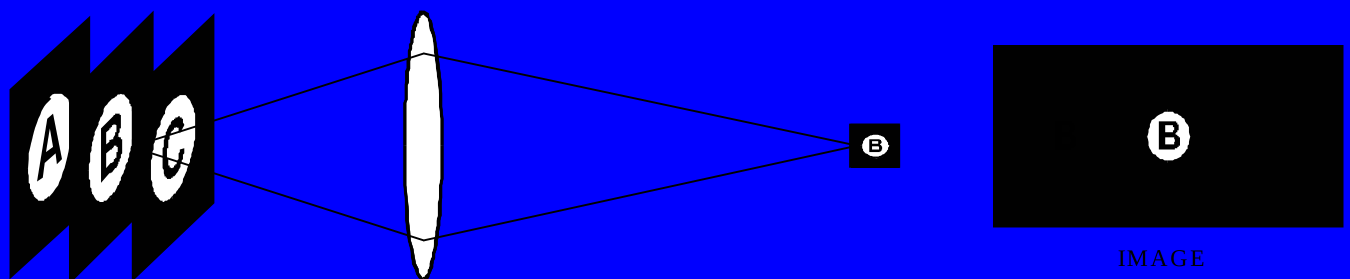
P.A. Dalgarno[1], H.I.C. Dalgarno[1], A. Putod[1], S. Aitken[1],
A Weis[1], C Diez[1], Alan Baird[1], R.J. Warburton[1]
D.P. Towers[2], C.E. Towers[2], N.C Angarita-Jaimes[2], S Chen[2]
D.J. Smith[3], J.C. Kirkman-Brown[3], J. Gaham[4],
V J Allan[5], R Southern[5], P Marsh[5],
I Davis[6], R Parton[6], K Lillie[6]

1 Dept Physics, SUPA/IIS, Heriot-Watt University, UK
2 School of Mechanical Engineering, University of Leeds, Leeds, UK
3 Centre for Human Reproductive Science, Birmingham Women's NHS Foundation Trust, Edgbaston Birmingham, UK
4 Cairn Research Ltd, UK
5 Faculty of Life Sciences, University of Manchester
6 Dept of Biochemistry, University of Oxford

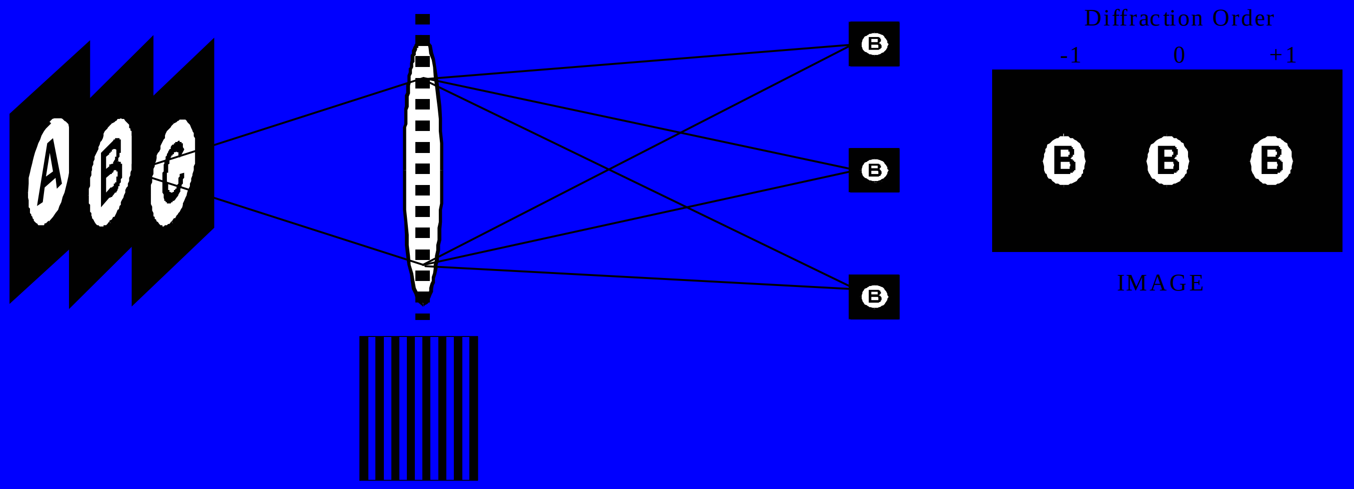
Basic principles

- Derived from wavefront sensing in astronomical adaptive optics
- Conventional imaging system gives in-focus image of a single object plane



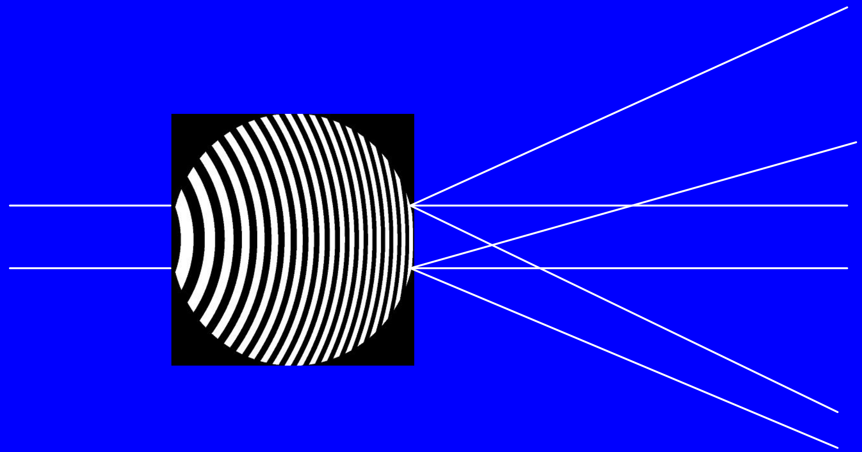
Basic principles

- Conventional imaging system gives in-focus image of a single object plane
- Combined with conventional grating gives multiple images of single object plane

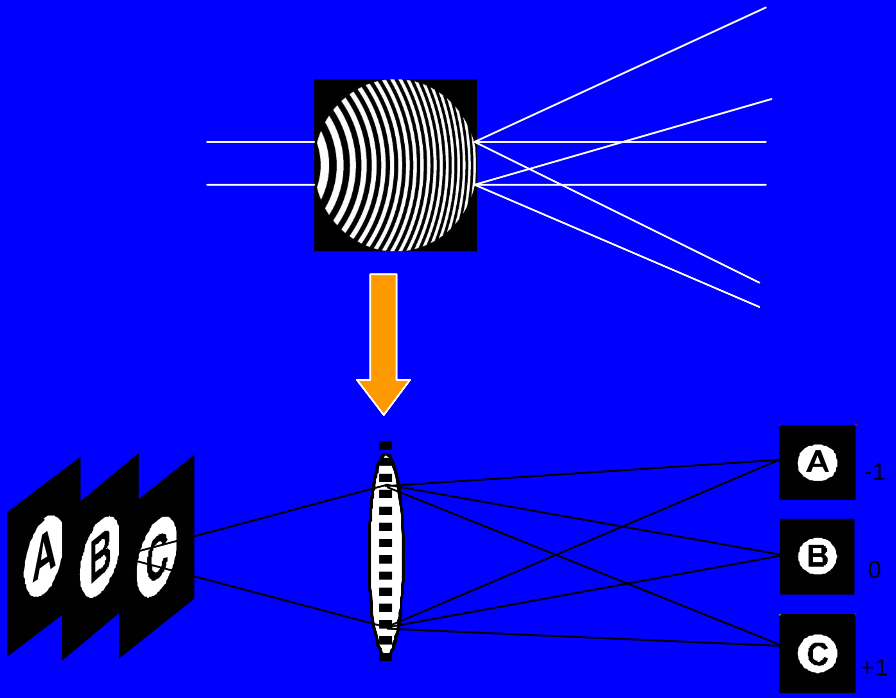


Diffractive optics

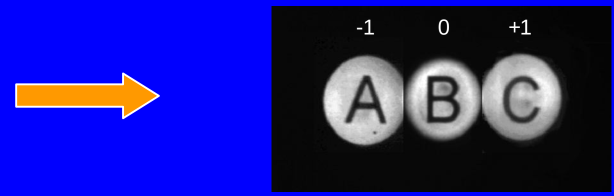
- Distorted grating gives different phase shift in each diffraction order
- Principle of detour phase ➔ holography
- Quadratic distortion ➔ wavefront curvature
- Acts like lens with different focal length in each diffraction order



3-D Snapshot Imaging



- Simple & cheap to manufacture
- Good control of divergence
- Phase grating etch gives energy- balance control
- High optical efficiency from binary grating



Blanchard & Greenaway

App.Opt. **38**(1999)6692

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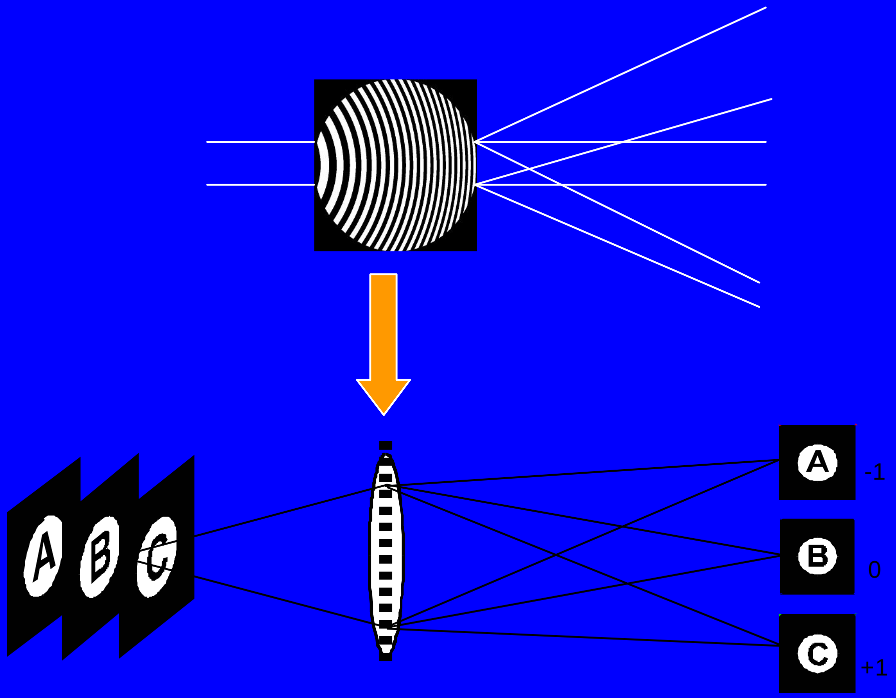

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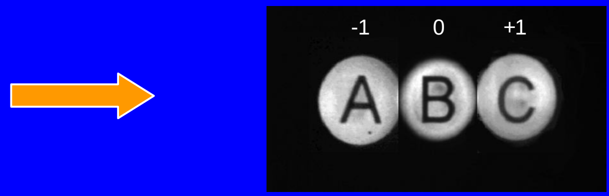
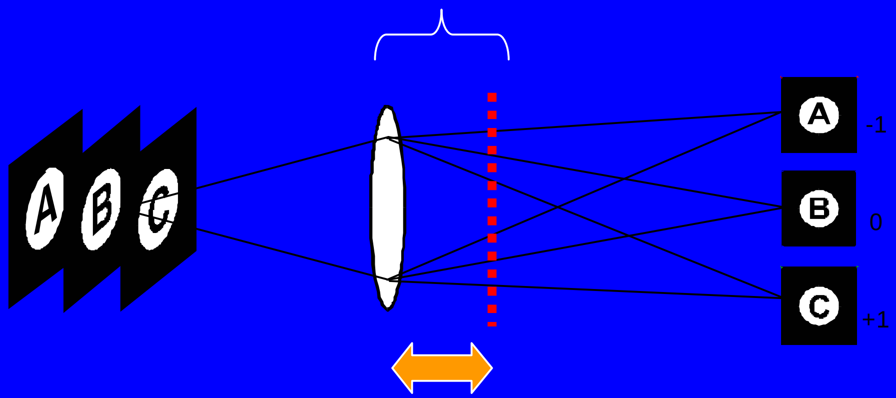


In-focus images of various z-planes are at different magnification

Telecentricity

Combination optical system

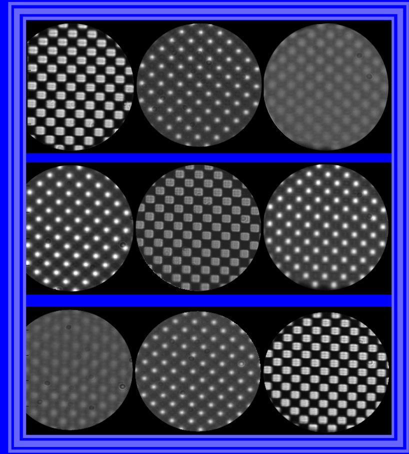
$$f_c = \frac{f_\ell \, {}_m f_g}{f_\ell + {}_m f_g - s} \text{ and } p_1 = \frac{s f_\ell}{f_\ell + {}_\mathrm{m} f_g - s} \text{ and } p_2 = \frac{s\left(f_\ell - s\right)}{f_\ell + {}_m f_g - s}$$

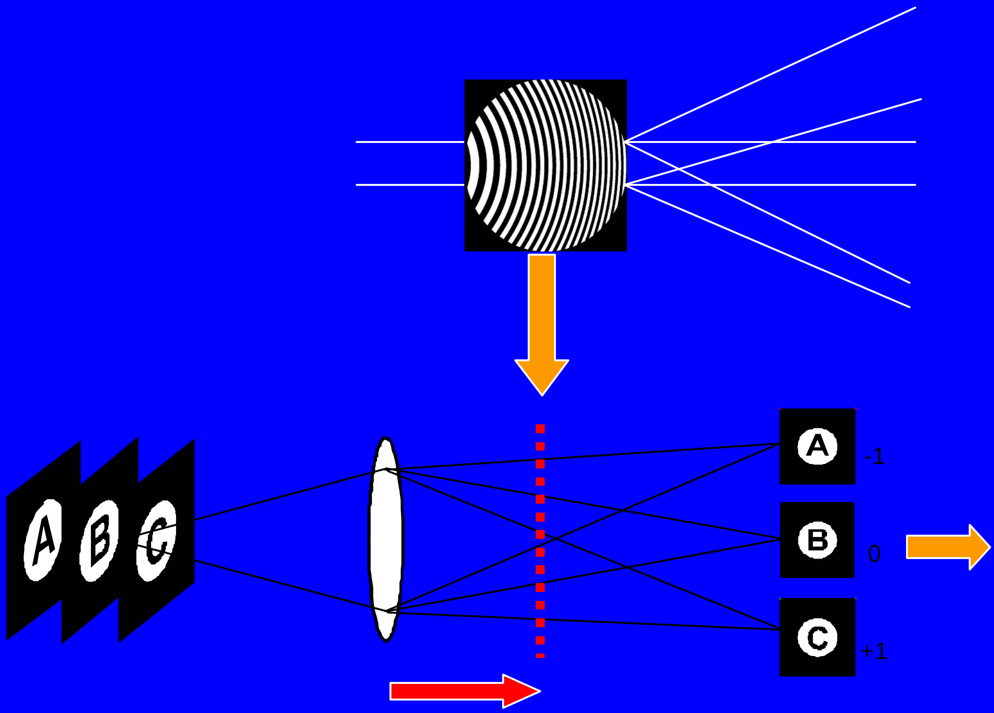


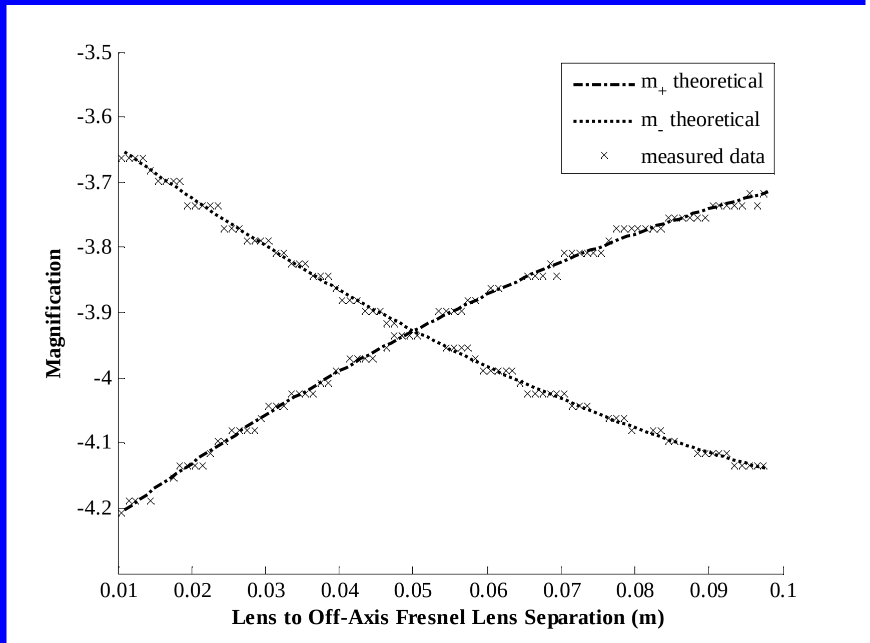
Djidel, Gansel, Campbell & Greenaway,
Opt Exp **14**(2006)8269-8277

$$s = f_\ell \Rightarrow f_c = f_\ell \text{ and } p_1 = \frac{f_\ell^2}{{}_m f_g} \text{ and } p_2 = 0$$

Telecentricity







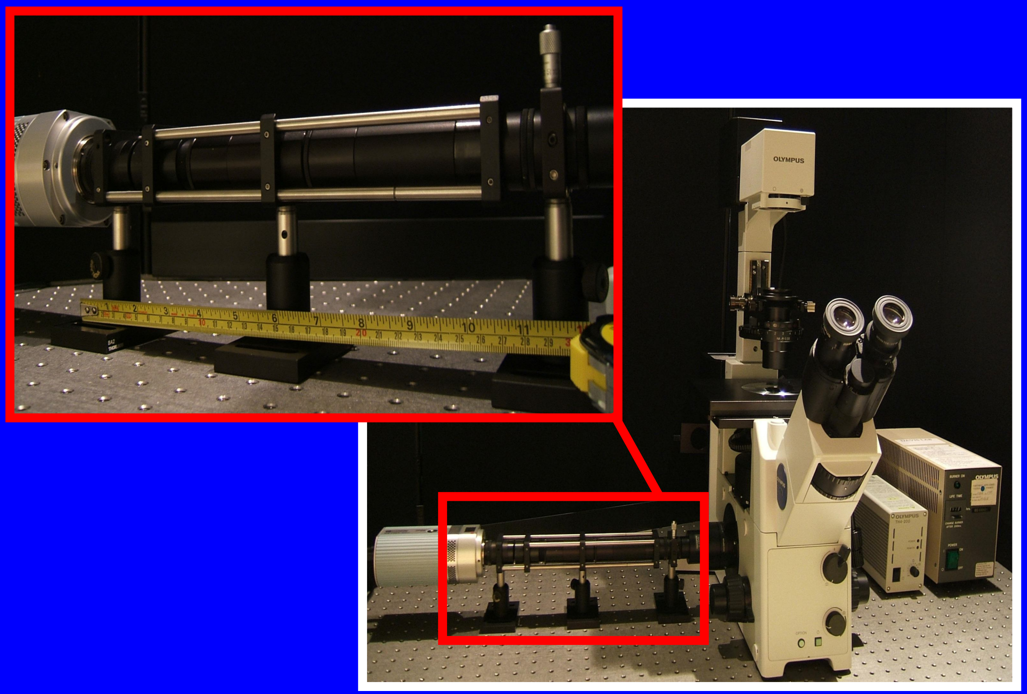
$$s = f_\ell$$

Telecentric performance

Practicality and Efficiency?

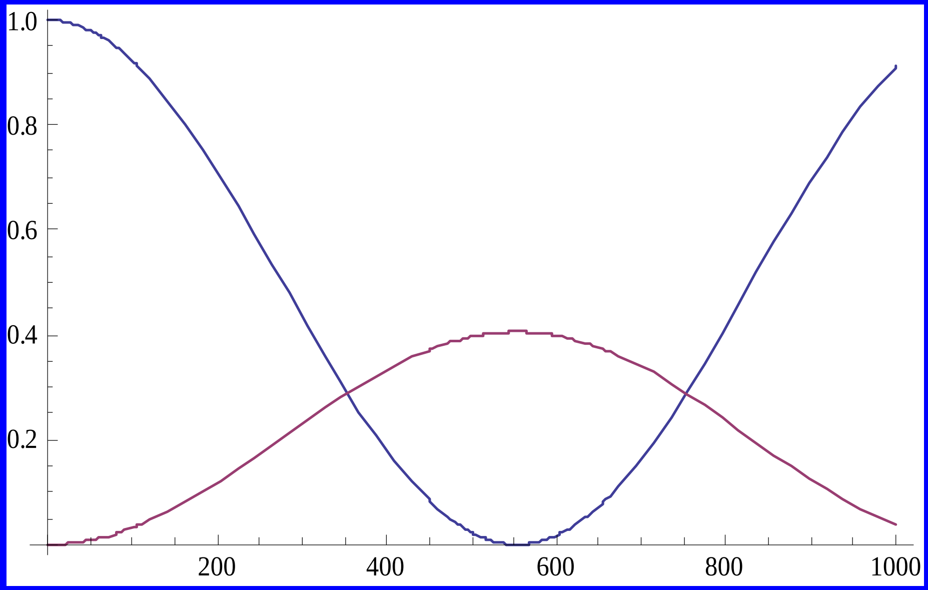
Practicality

- Used on inverted and upright systems



Efficiency

- Optical efficiency ~84% overall



- (~28% each order)

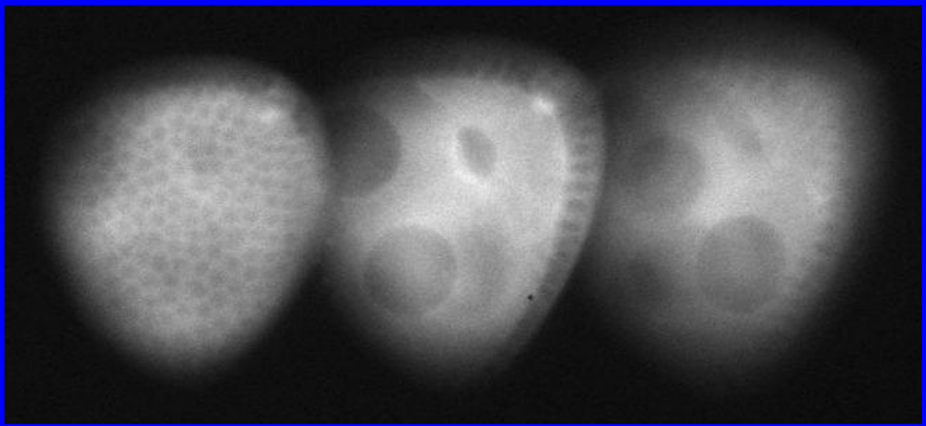
Initial results

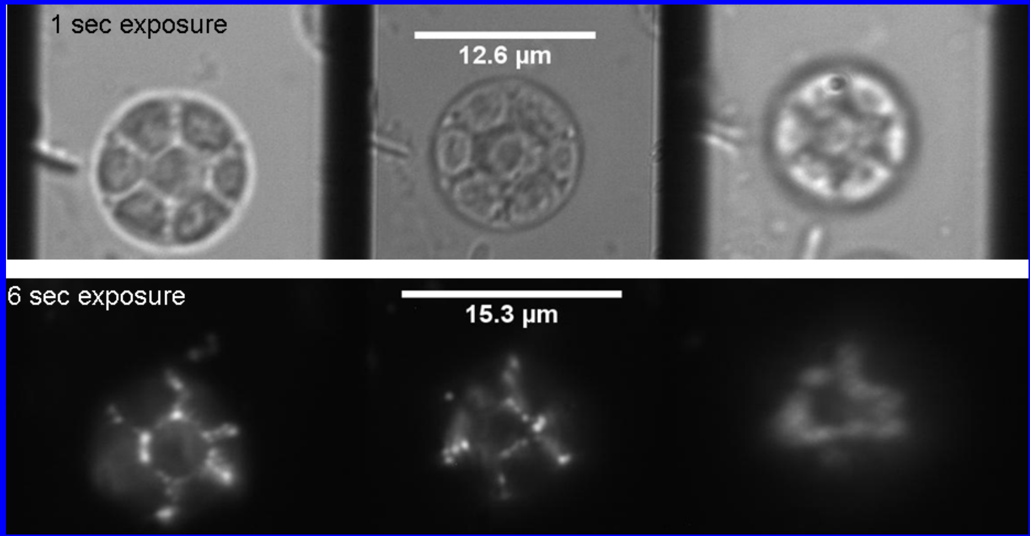
GFP in drosophila ovary

Epi-fluorescence images with 7.3µm between in-focus planes

Principal application microtubule dynamics through EB1 tracks

(with Davis, Oxford)





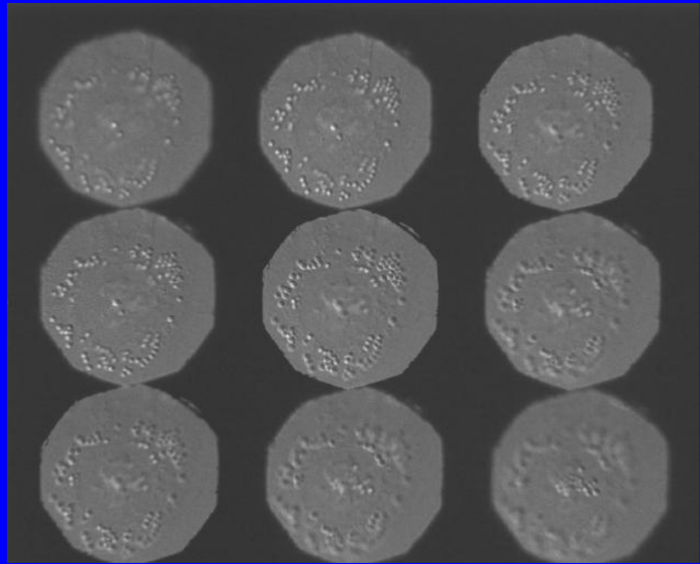
GFP in plant-cell mitochondria bright filed (upper row) and epi-fluorescence (bottom row)

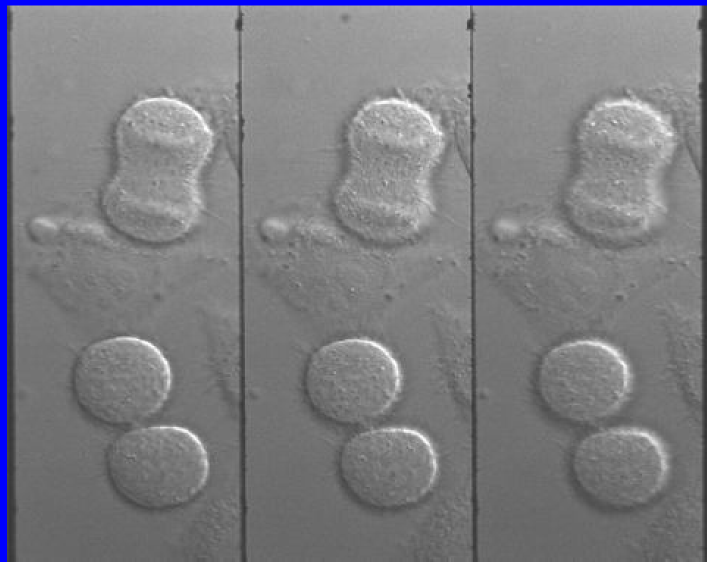
1µm separation between in-focus planes (with Logan, St Andrews)

3D movies

- 9 z-plane phase-contrast images of Hela cells
- Z-plane separation 810nm
- 3 z-plane DIC images from movie of Hela mitosis

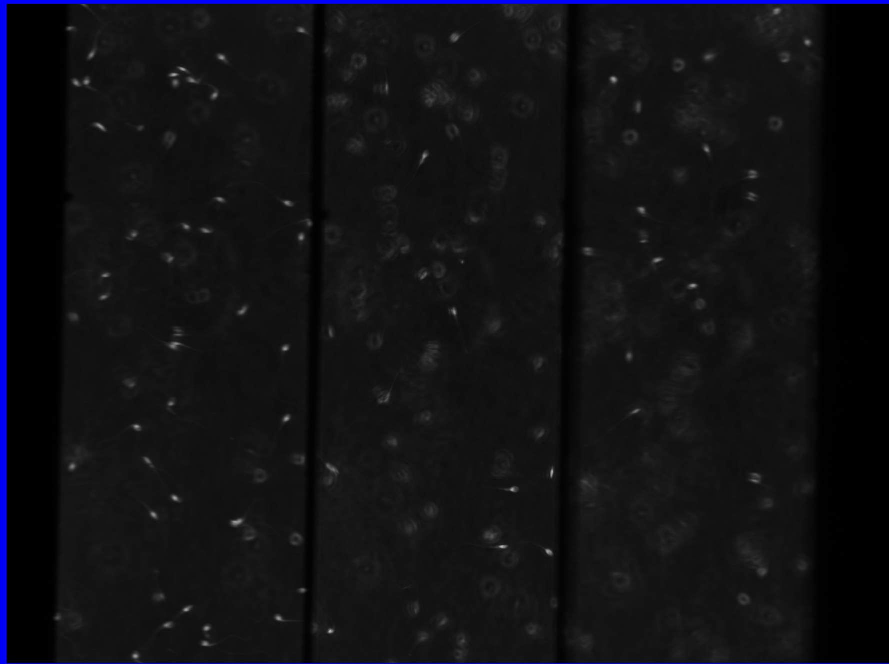
With Allan, Manchester





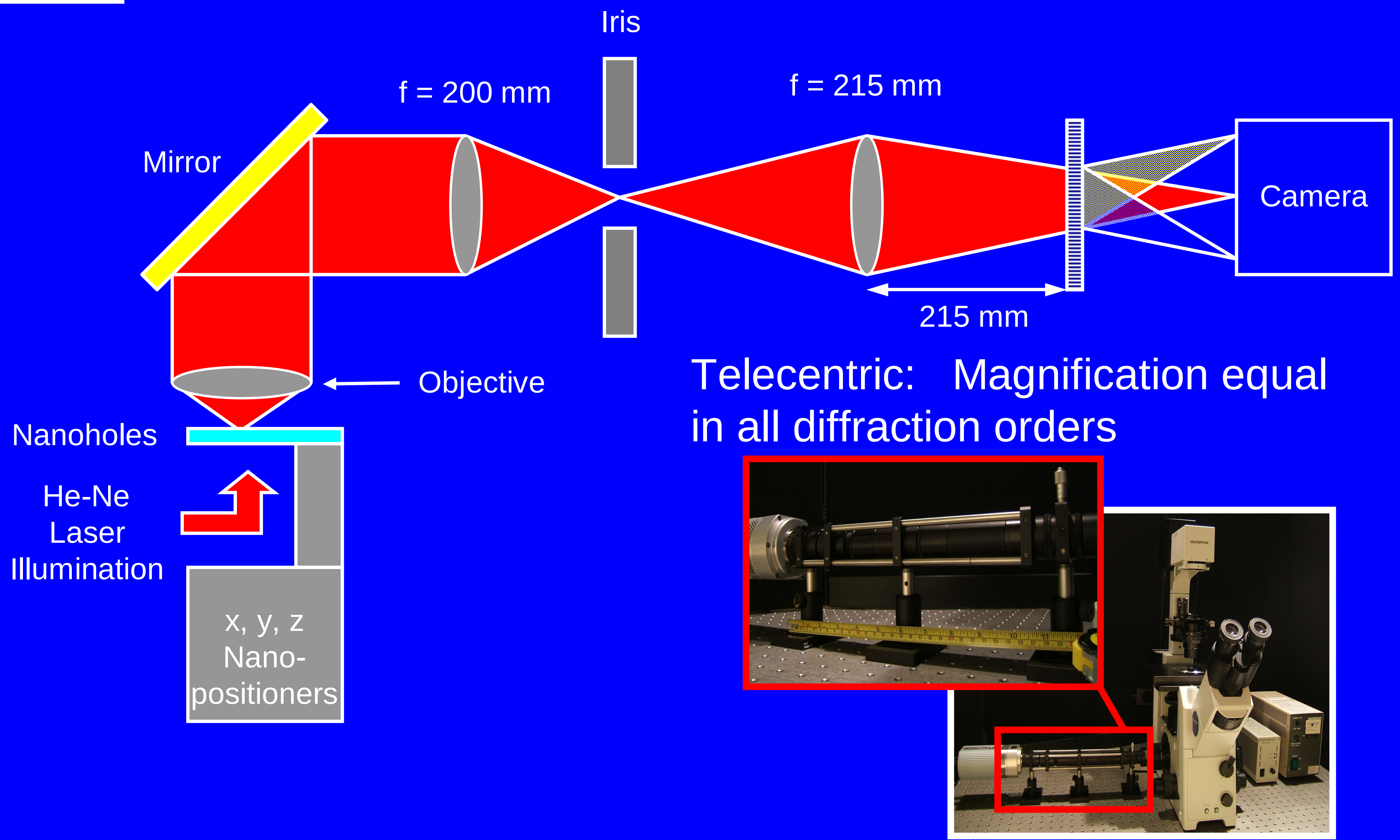
Motility Measurement

- Sperm motility from phase-contract and/or dark-field imaging
- Current approach uses apparent length as a proxy for out-of-plane deformation of tail
- Multi z-plane images provide extra information



(with Kirkman-Brown, Birmingham)

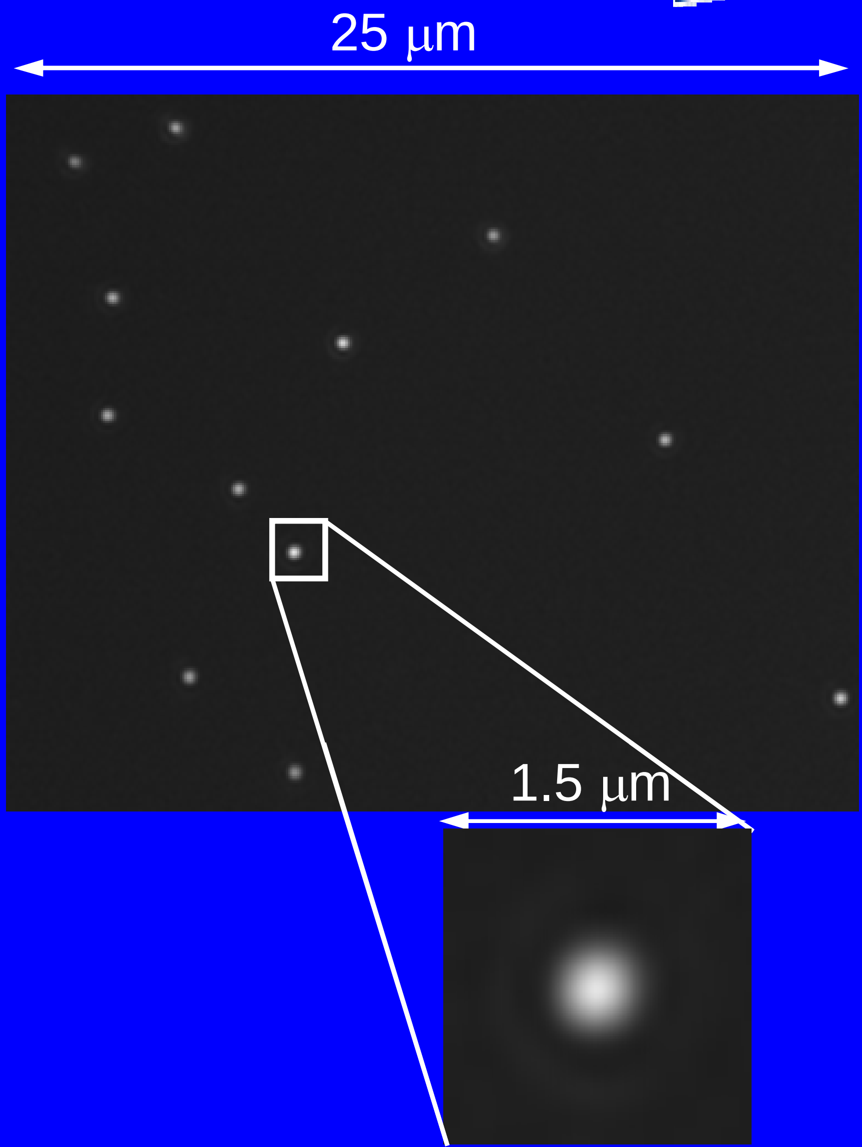
Experimental arrangement



Telecentric: Magnification equal in all diffraction orders

Nanohole test objects

- 210 nm diameter holes in Al foil
- Single point source
- Simulates fluorescent particle
- Mask / hole contrast $>10^4$
- Brightness limited only by illumination source



Resolution on 3 planes

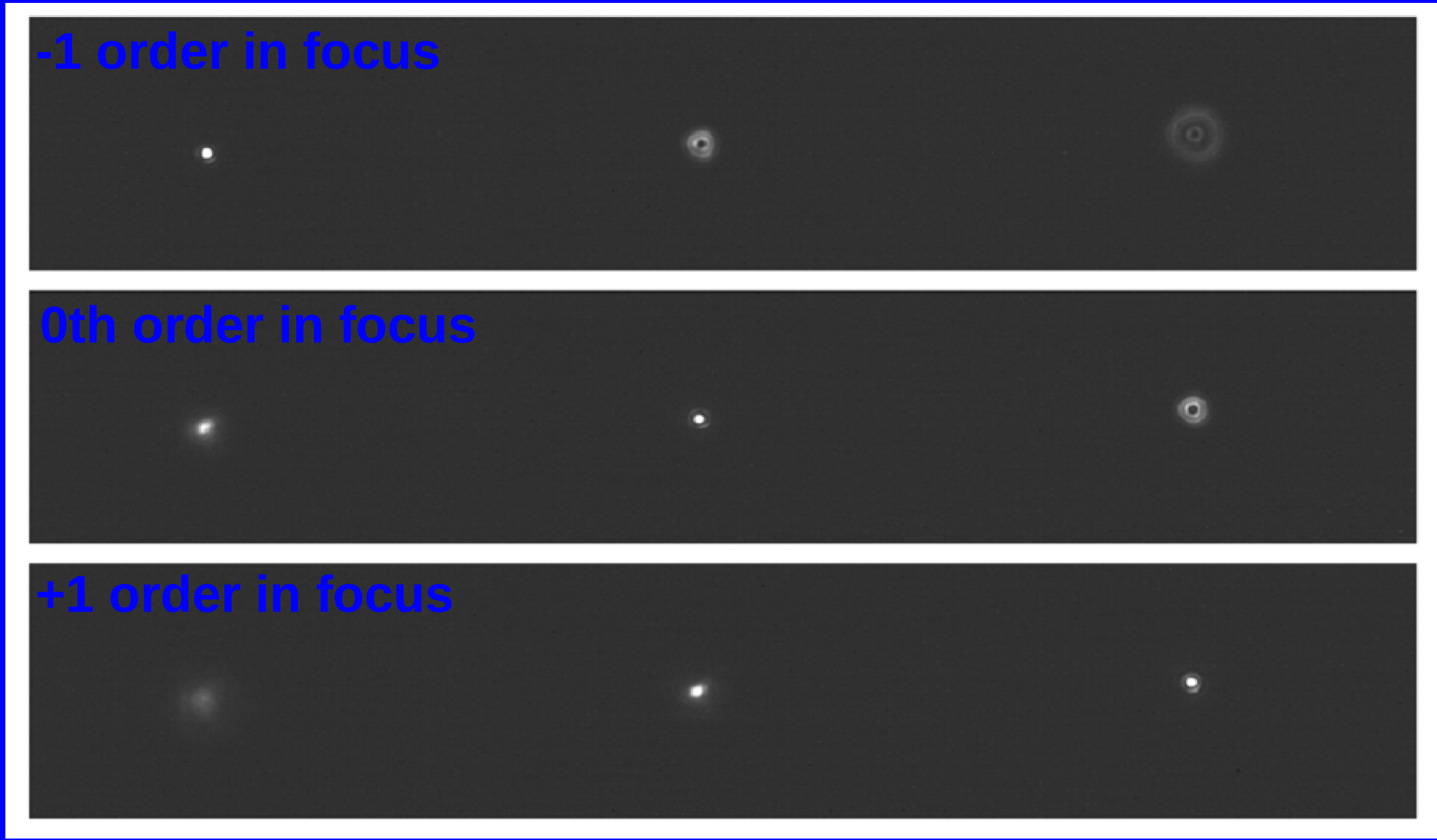
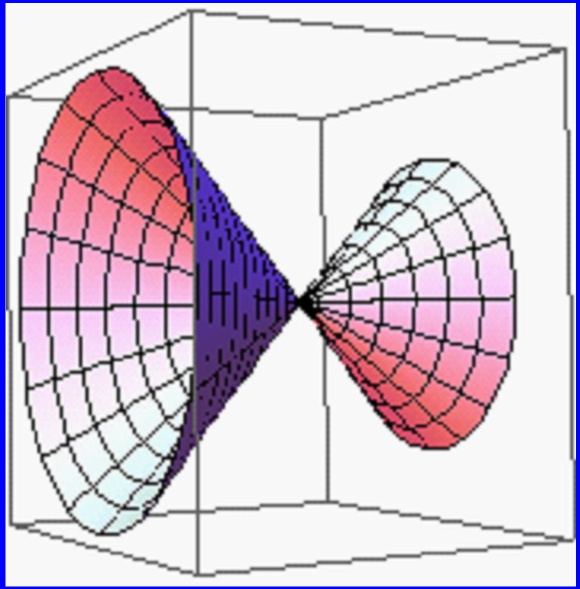
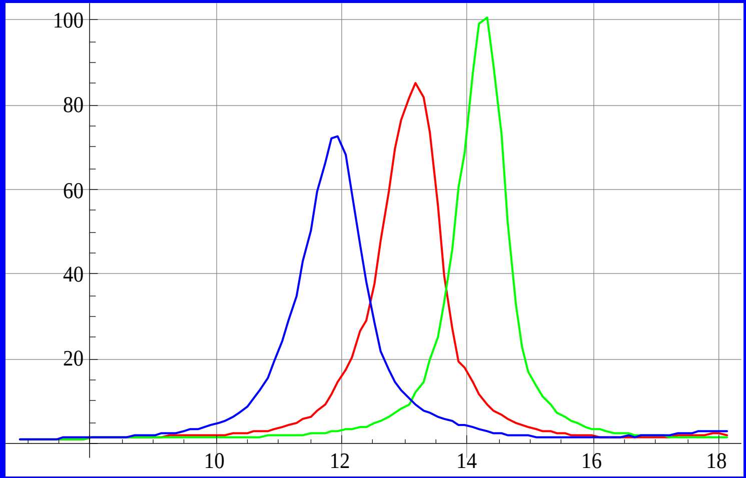


Image Resolution:
No grating = 233nm
With grating = 226nm and 231nm (for 0th and ±1orders)

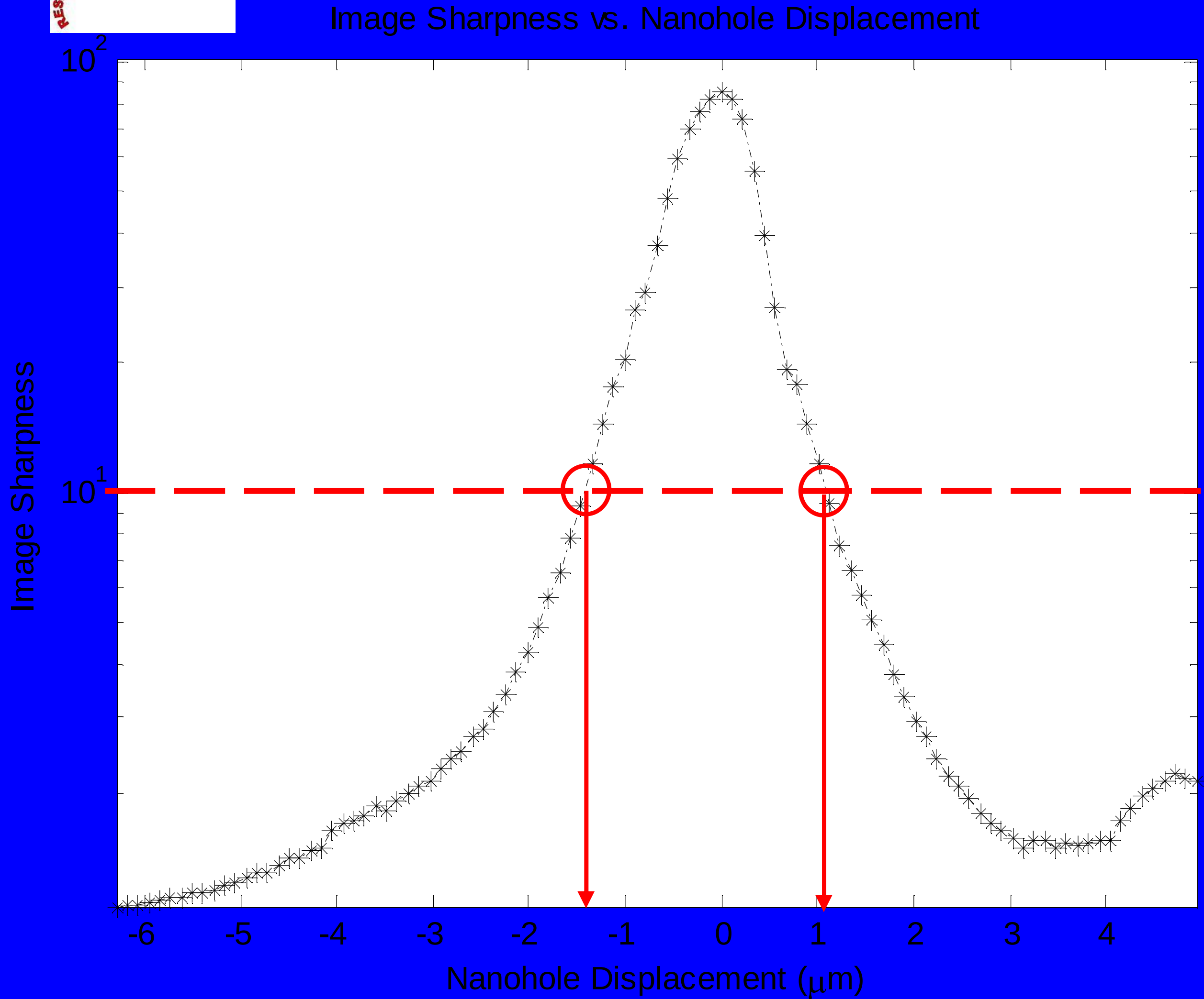
Ranging in Depth with Sharpness

- Beam divergence from source depends on optical aperture
- Defocused image on non-source planes reduces intensity…
- …thus to a reduction in sharpness (integral of intensity squared)
- Suitable for real-time analysis and CMOS detector technologies





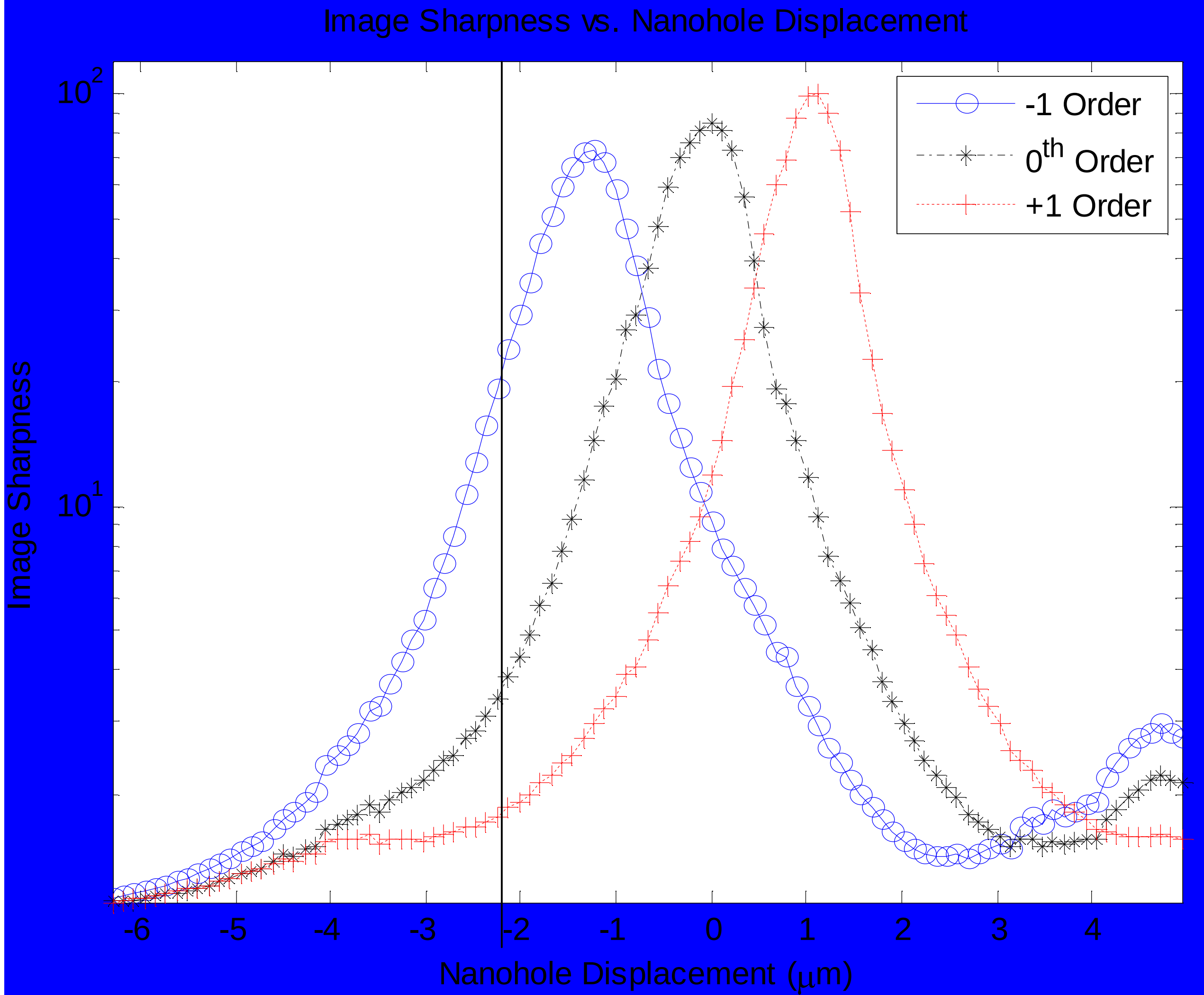
Position Measurement (Z)



Problem:

A single mage sharpness measurement gives ambiguous depth position

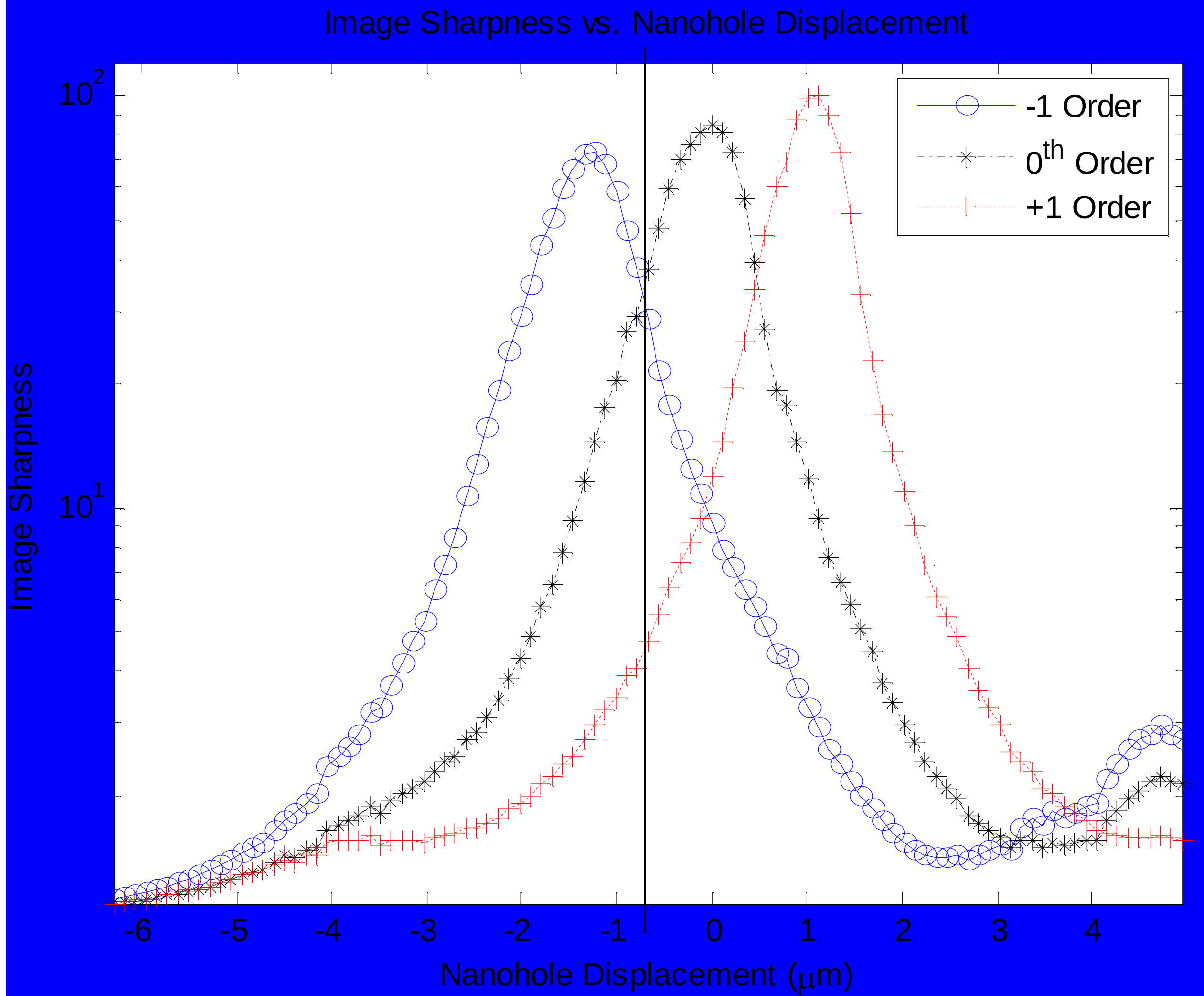
Unique depth indication…



Solution:

QD grating method gives 3 simultaneous image sharpness (one from each order) for each particle.

Unique depth indication…



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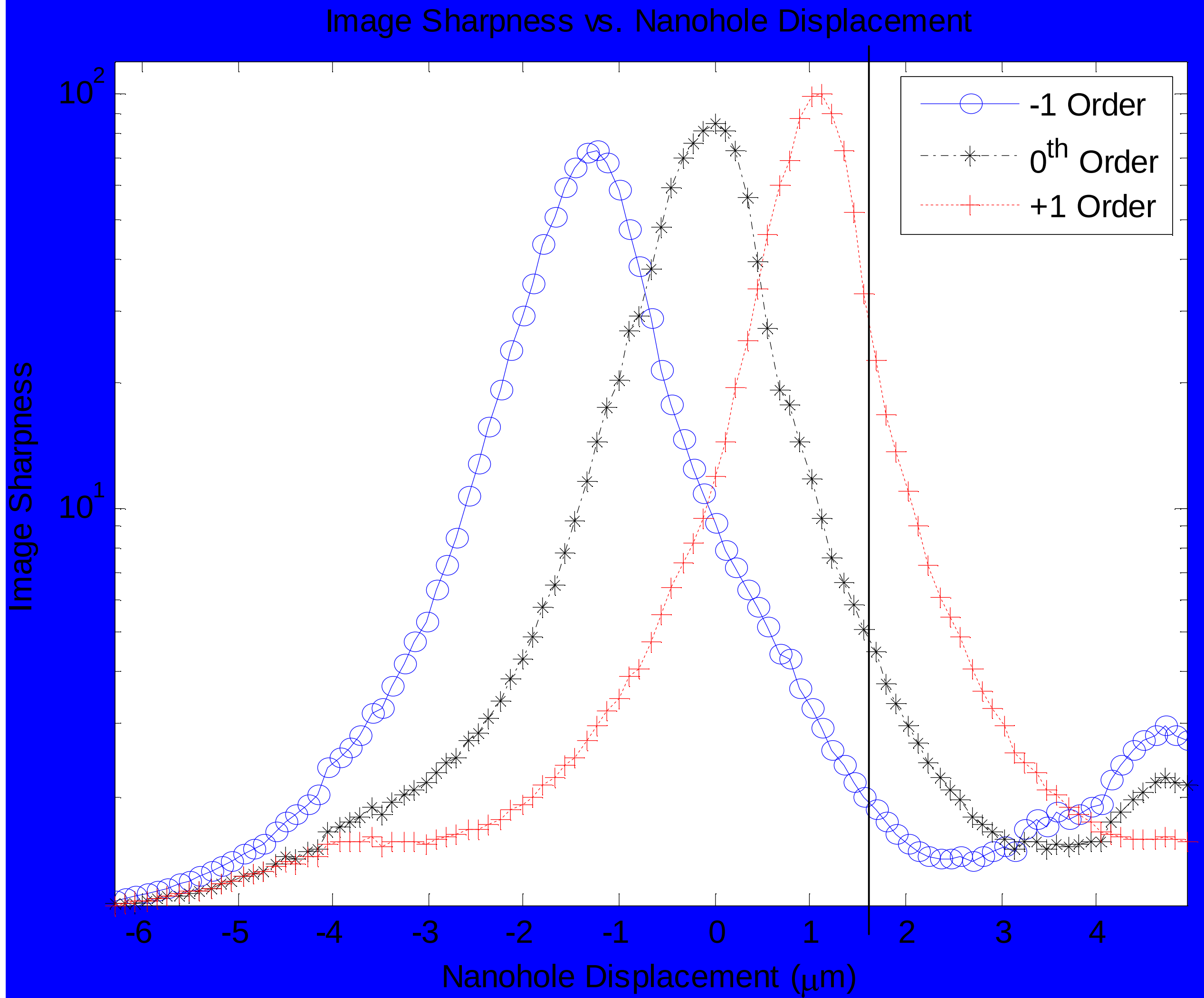

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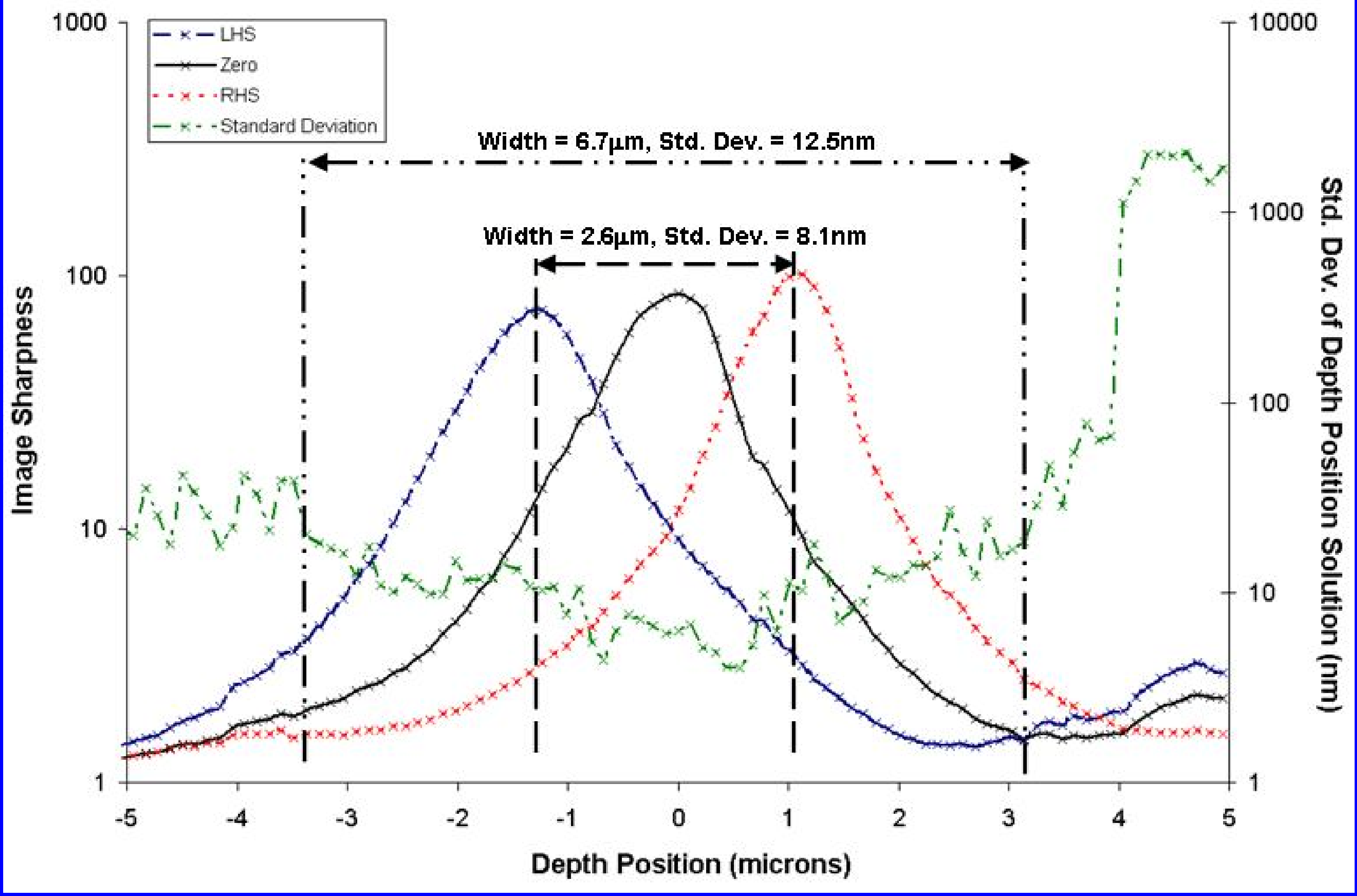
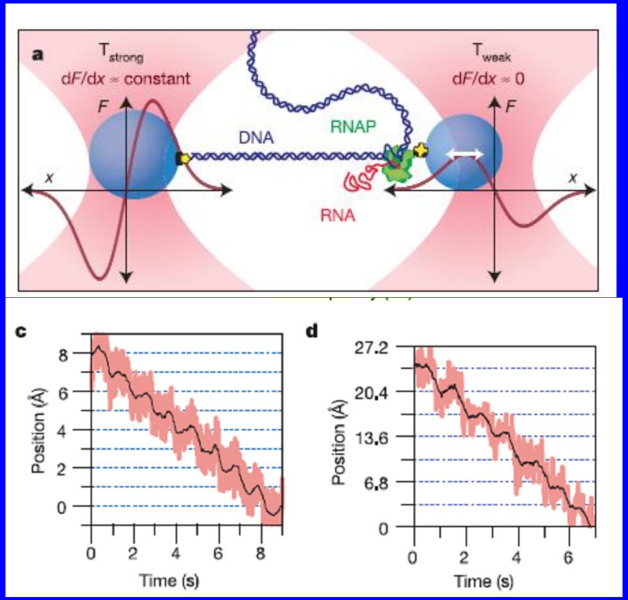
ML depth estimator



Molecular Biology

- Optical-trapping measurement of length at bp level
  - RNA polymerase measurements at Å-level accuracy



Abbondanzieri et al, Nature (2005)

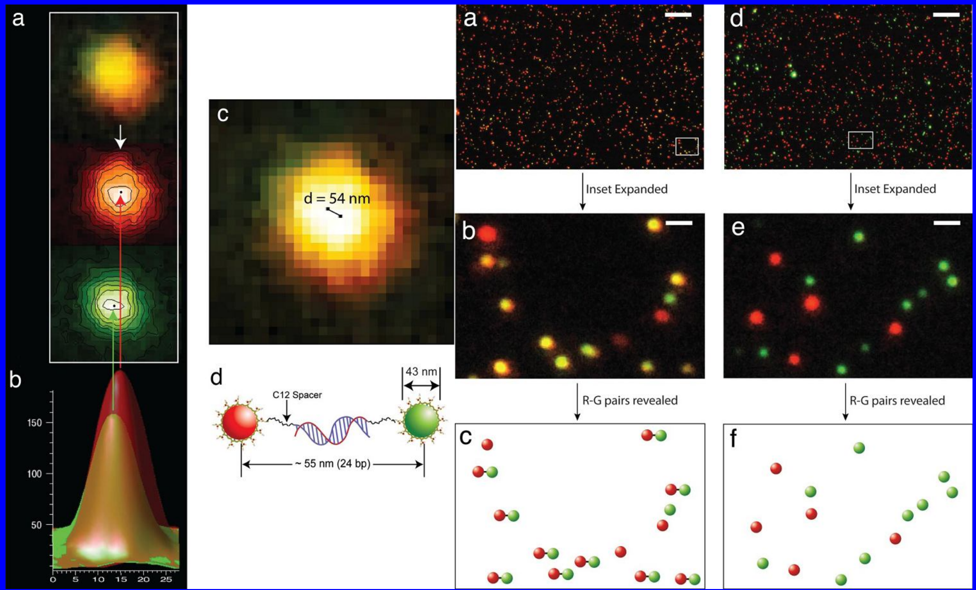
- Dual optical traps for force-displacement measurement

- Hierarchical folding in riboswitch aptameters
  - Greenleaf et al 2008

Molecular Biology

- Single-molecule detection with wide-field imaging in two-colour fluorescence

Agrawal et al, 2008

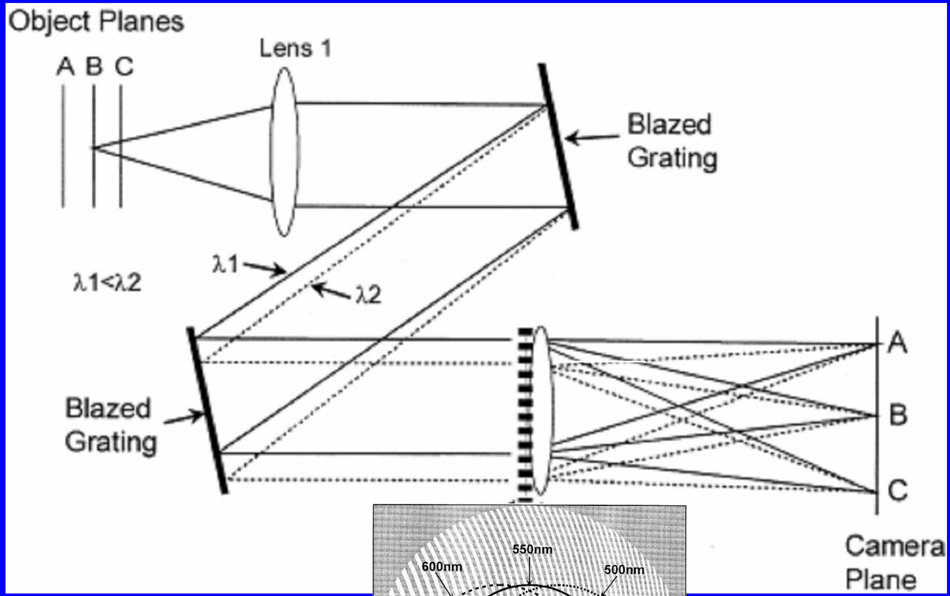


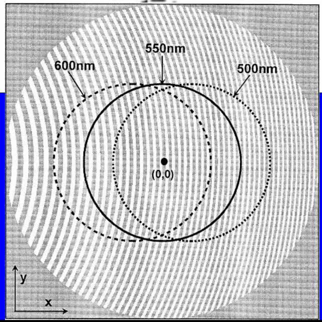
- ➢ Based on stellar spectroscopy techniques
- ➢ 2D colour images
- ➢ 10^6 photoelectrons per particle!
- ➢ Precise relative position required

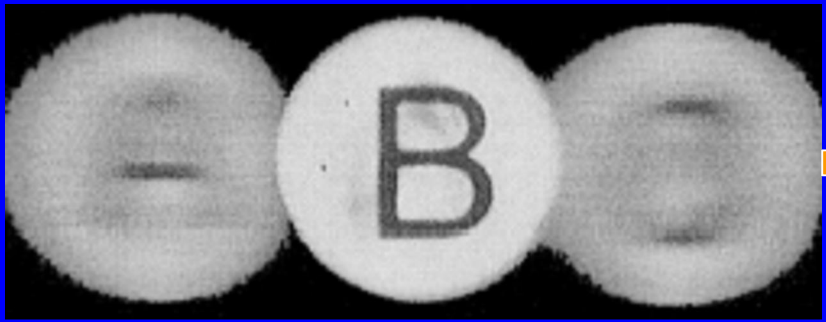
Broadband Application

- Careful design achieve 'white light' imaging
- Unfiltered halogen light

Blanchard & Greenaway, Opt. Commun. **183**(2000)29-36







Molecular tracking

- Sharpness-based tracking
  - nm capability demonstrated
  - Technique not optimised
  - Spatial accuracy limit - tbd
  - Camera-limited time resolution
- Track fluorescent proteins
  - FRET
- New fluorescent tags?
  - Bright, multi-coloured
  - Non-bleaching
  - 3-30 nm scale
  - Easily functionalised

Future

- Heriot-Watt has declared the Life-Sciences as one of its principal strategic themes for inter-disciplinary research and advertised 4 new posts:
  - Chair in Computational Biology
  - Chair in Bio-imaging Bio-marker technologies
  - Chair/Reader in Cell Biology
  - Chair/Reader in Chemical Biology
- See http://www.hw.ac.uk/hr/v_index.php
- If you are aware of anyone who may be interested please make sure that they know…