Three dimensional imaging on the nanoscale: applications in single cell biological imaging and nano-particle tracking

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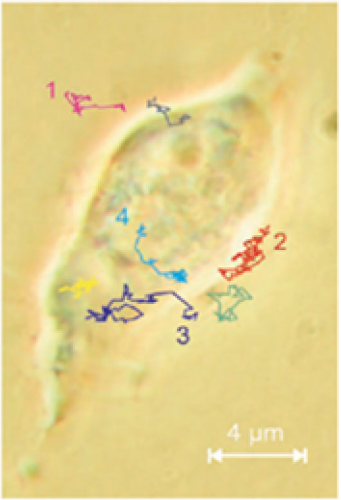
Motivation

What is Needed?

- Biological processes are dynamic
  - Requires 4D Imaging (3D and time)
  - Requires real time particle tracking
- Single live cell investigation
  - Nanometre precision required
  - Limit cell exposure to harsh environments



AAV-Cy5 particles entering a single living HeLa cell

Seisenberger et al, Science 294 (2001)

What Can we Provide?

Application of Wavefront Sensing, Adaptive Optics and Astronomy technology to Biophotonics….

3D Imaging by Diffraction Grating

The Idea: By ‘bending’ the straight rulings the grating we can change its imaging properties.

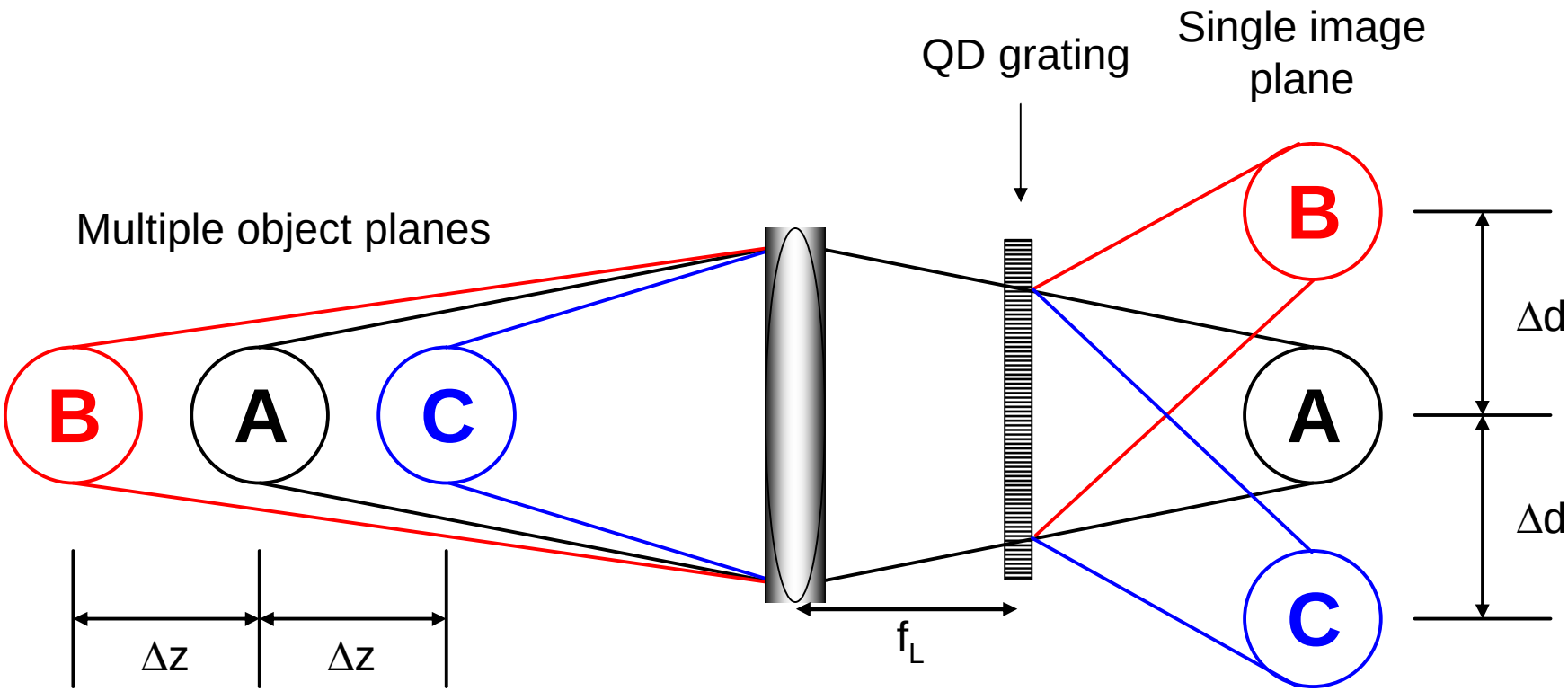
How it works: Detour phase is the phase shift added to each diffraction order by the distortion.

Opt.Lett 29(23) p2707 (2004)
Appl.Opt. 39(35) p6649 (2000)

3D Imaging: Distorting the diffraction grating to produce a quadratic (defocus) detour phase results in an important property:

<u>The focal length in each diffraction order is different</u>

Simultaneous Imaging of Multiple Planes

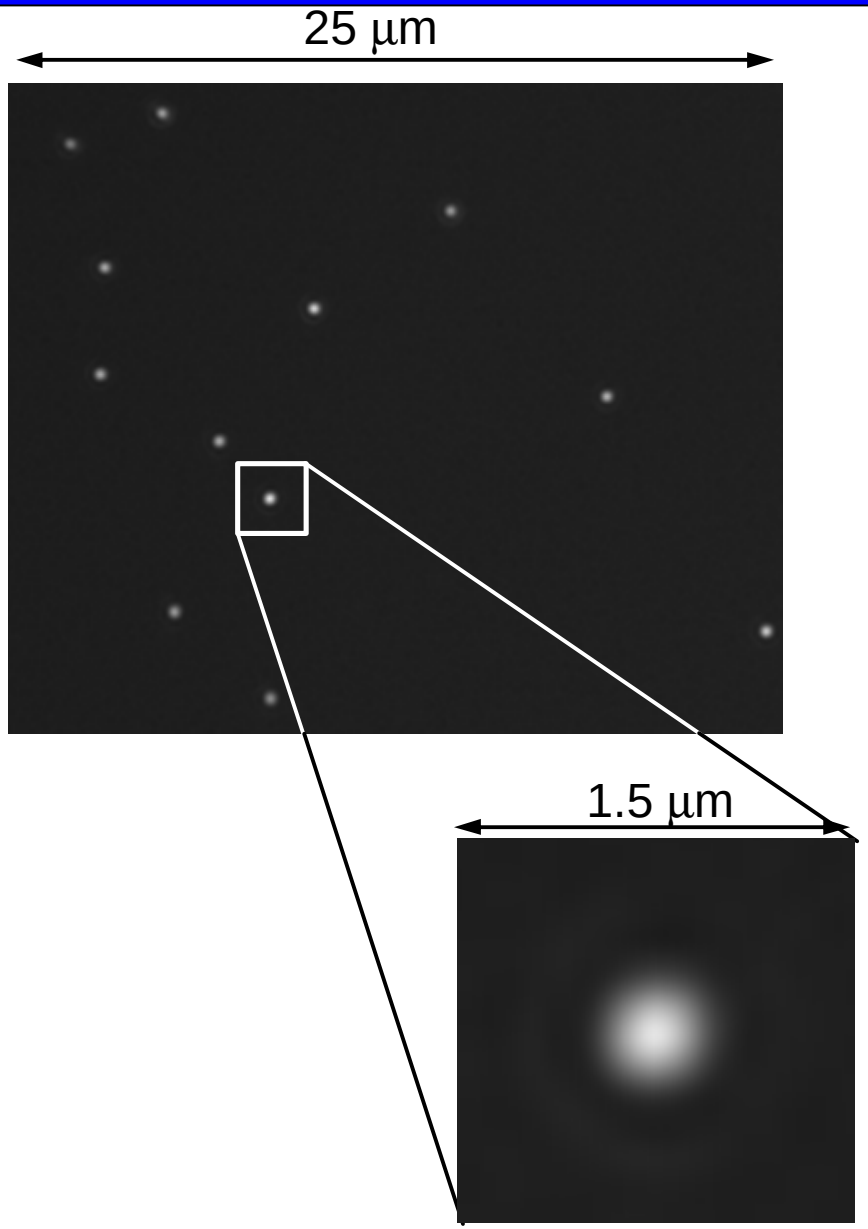


Separating the lens and grating by f_L provides telecentric imaging

ΔZ and Δd can be tuned through grating design parameters

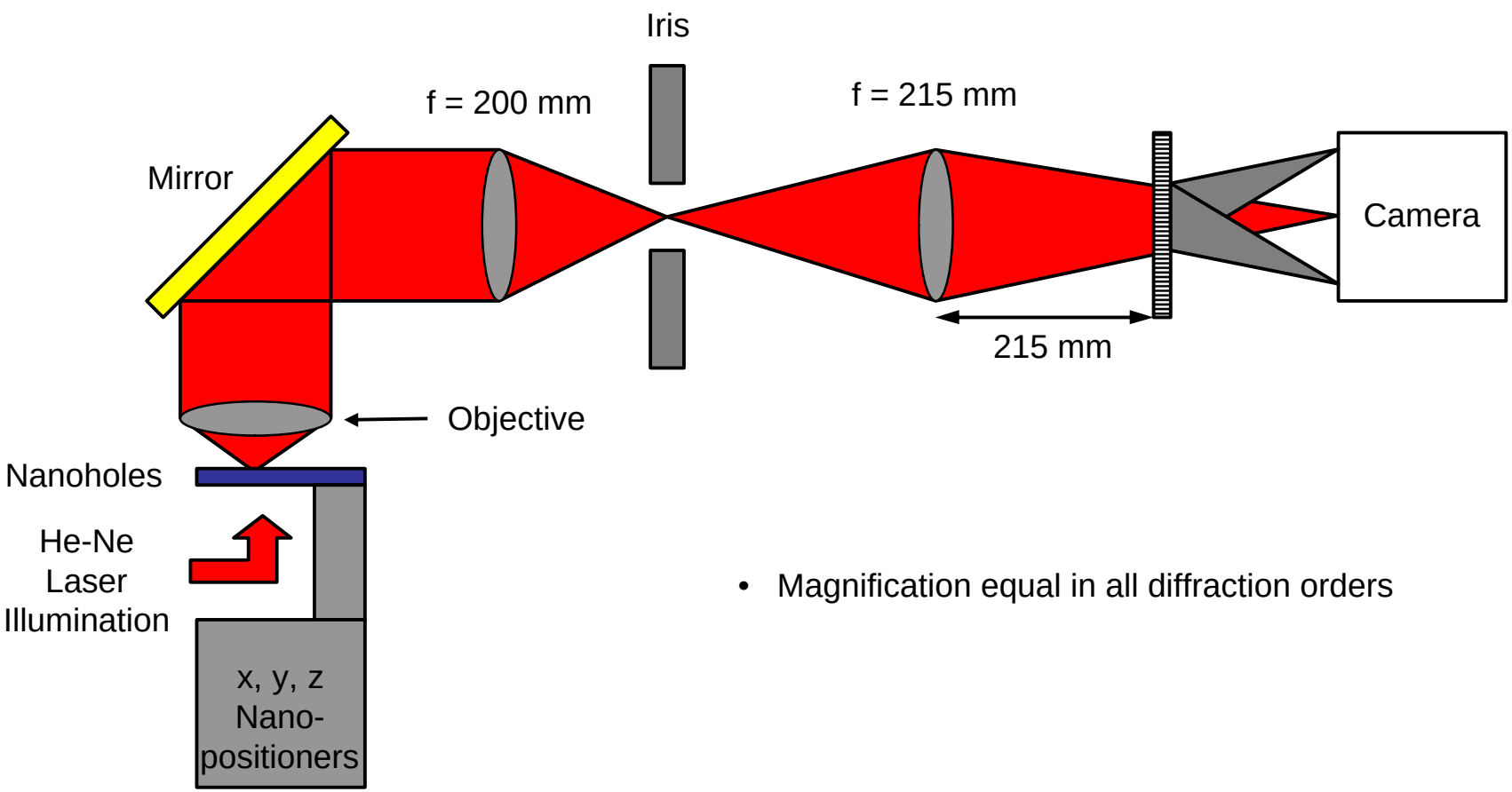
Nanoholes

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



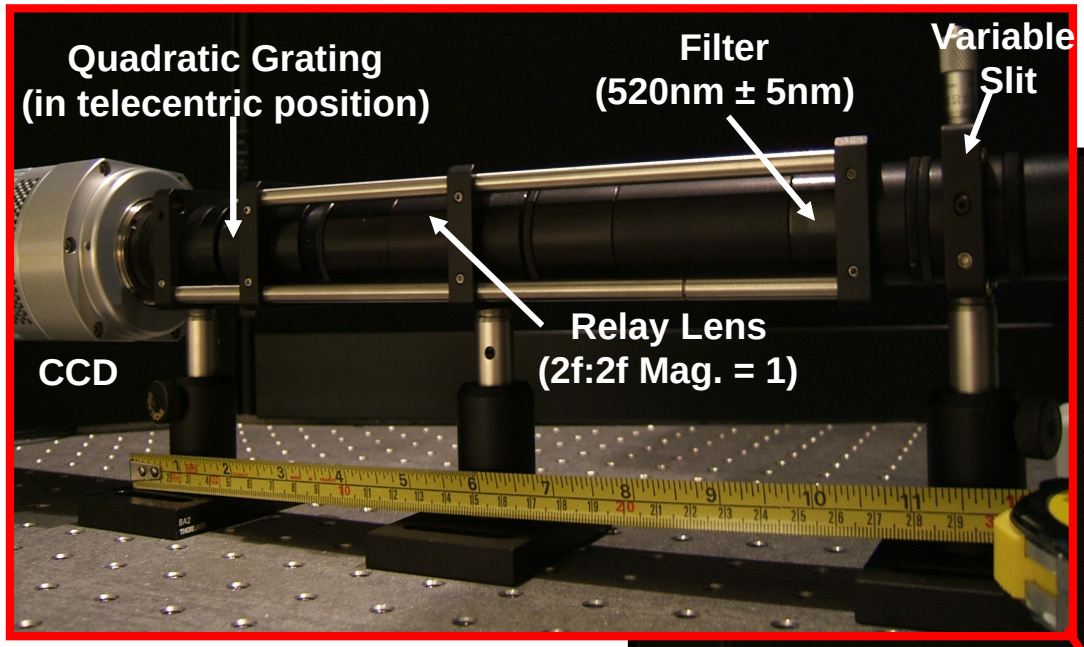
http://waf.eps.hw.ac.uk/

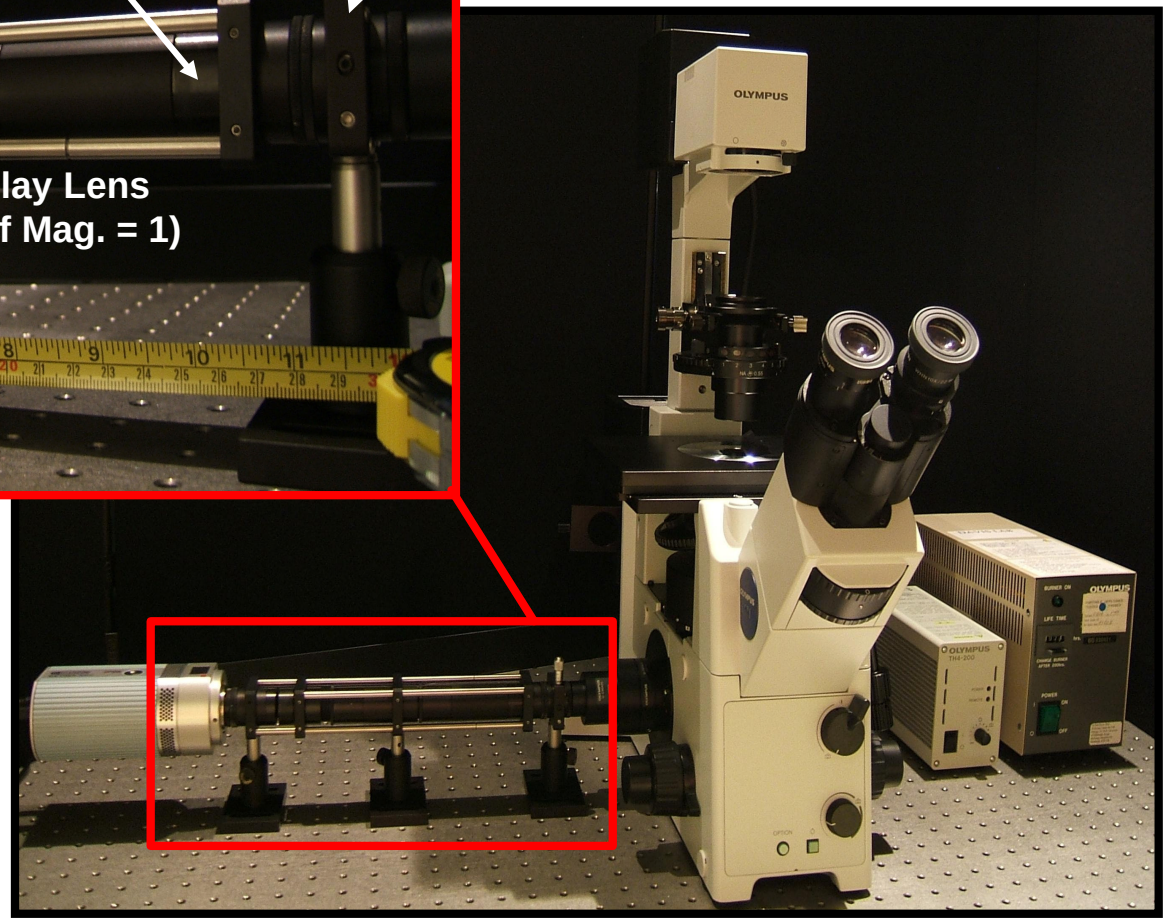
Experimental Setup



- Magnification equal in all diffraction orders

Integration with Commercial Systems

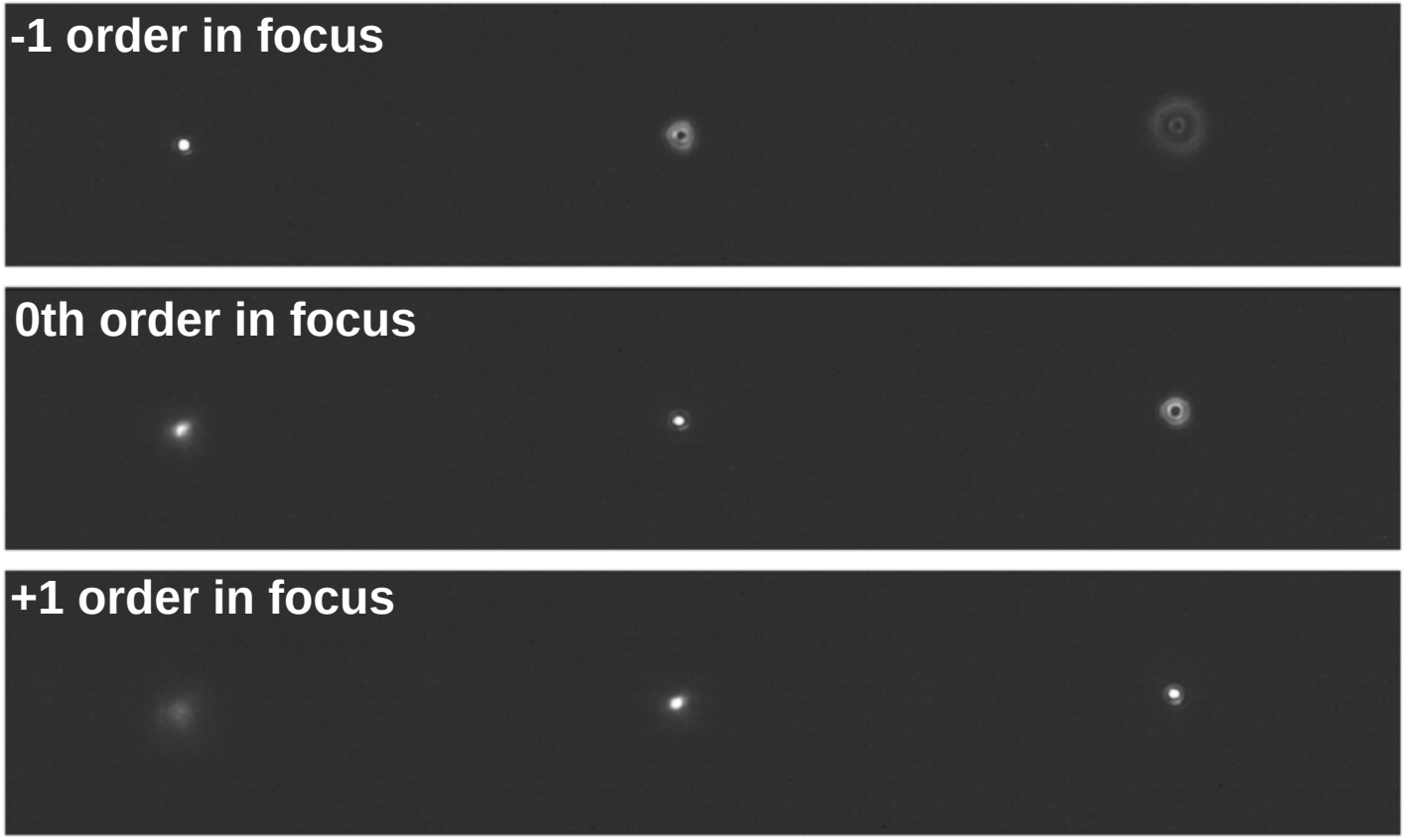




The 4D Imaging System mounted on an Olympus IX71.

The close-up illustrates the compact size of this bolt-on.

Resolution on 3 planes



Measured Resolution:

Without grating = 233nm
With grating = 226nm and 231nm (for 0th and ±1orders respectively)

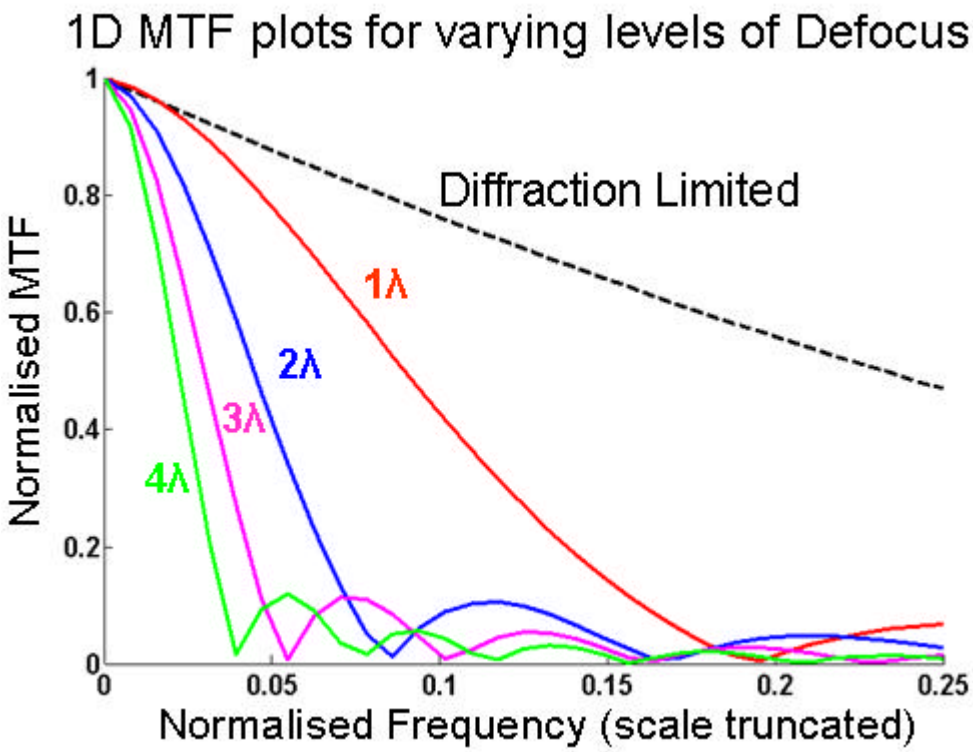
Image Sharpness

What is Image Sharpness?

- It is a measure of image quality (related to the area under the MTF)
- It varies according to the level of aberration present in the image.

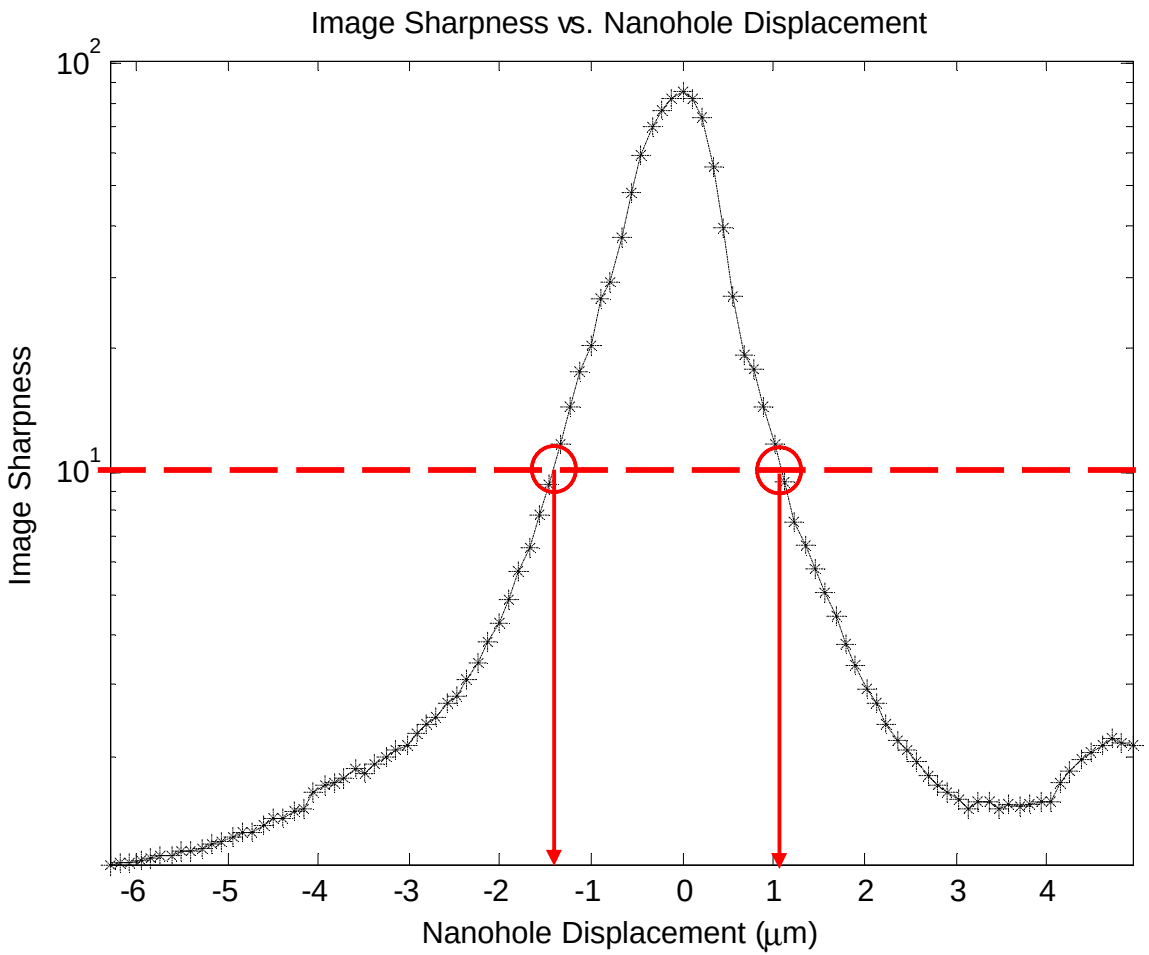
Application:

- It can be used as an accurate metric for determination of range from a set of defocused images.
- It can be calculated easily and quickly, and could be done “on-chip” with a CMOS detector for high speed particle tracking.



The area under the MTF decreases as defocus aberration increases (Image Sharpness also decreases)

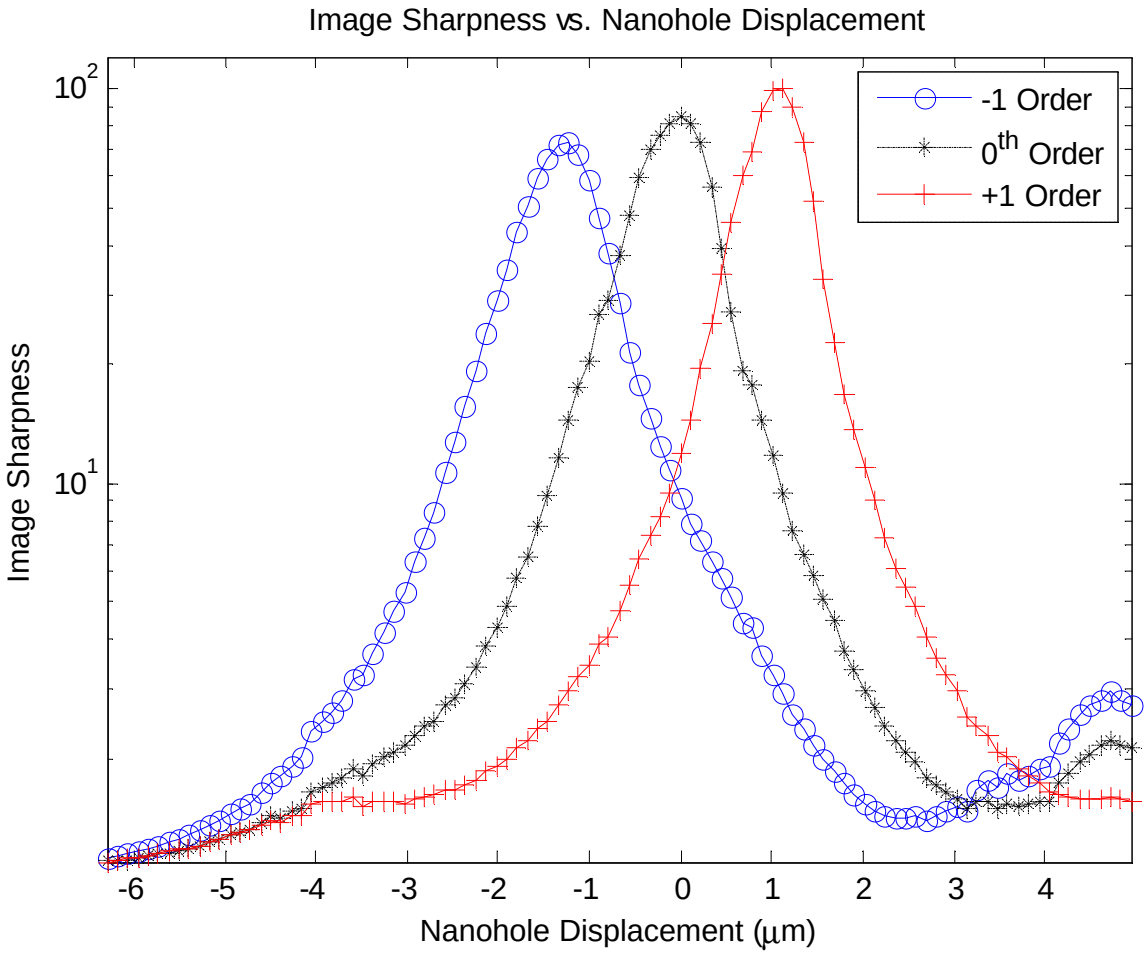
Position Measurement (Z)



Problem:

Image Sharpness measurements from a single through-focal series produces ambiguous depth position

Position Measurement (Z)

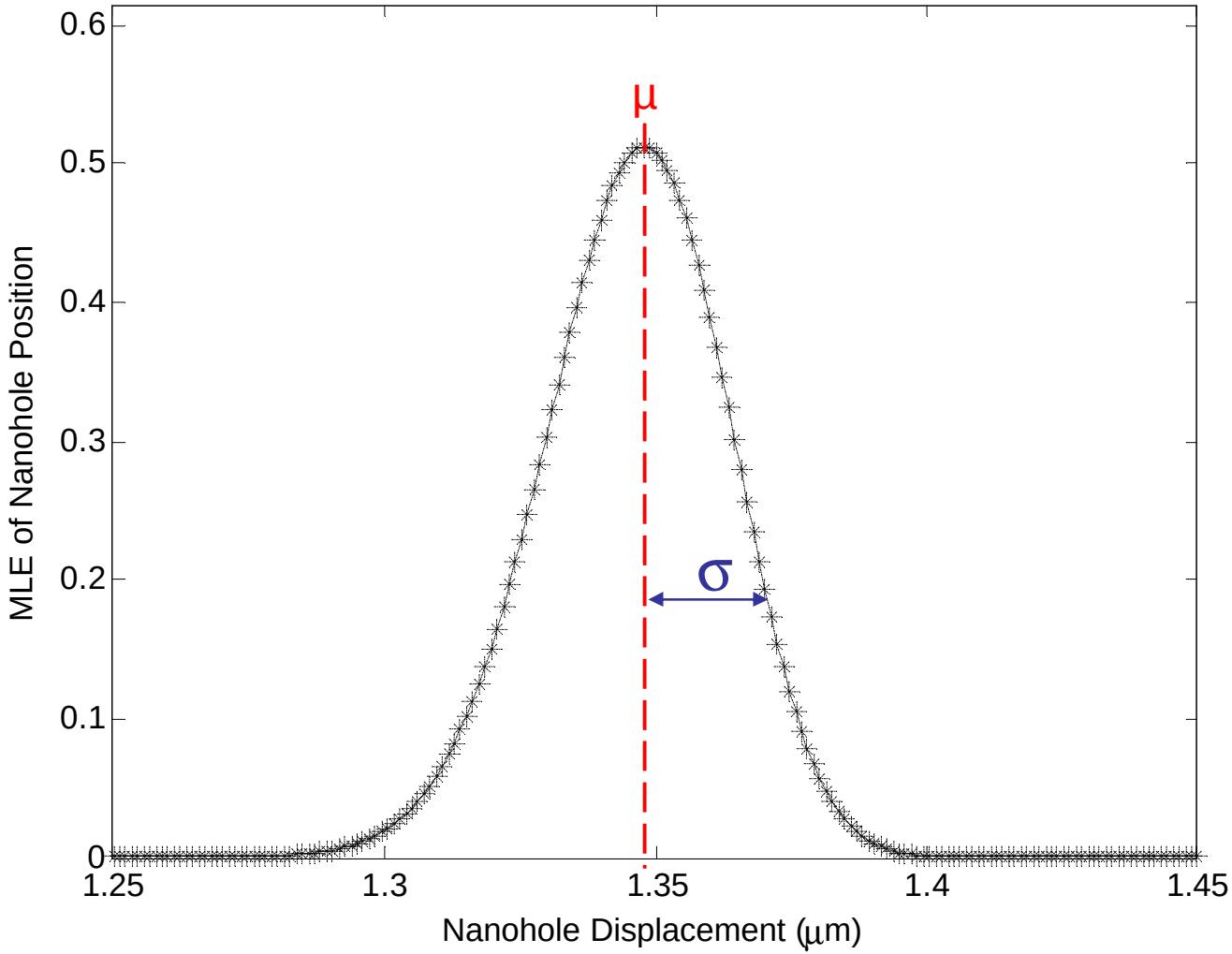


Solution:

QD grating method provides three measurements of Image Sharpness (one from each order) at each depth position.

How can we use this information to provide an unambiguous depth position – and how accurate will it be?

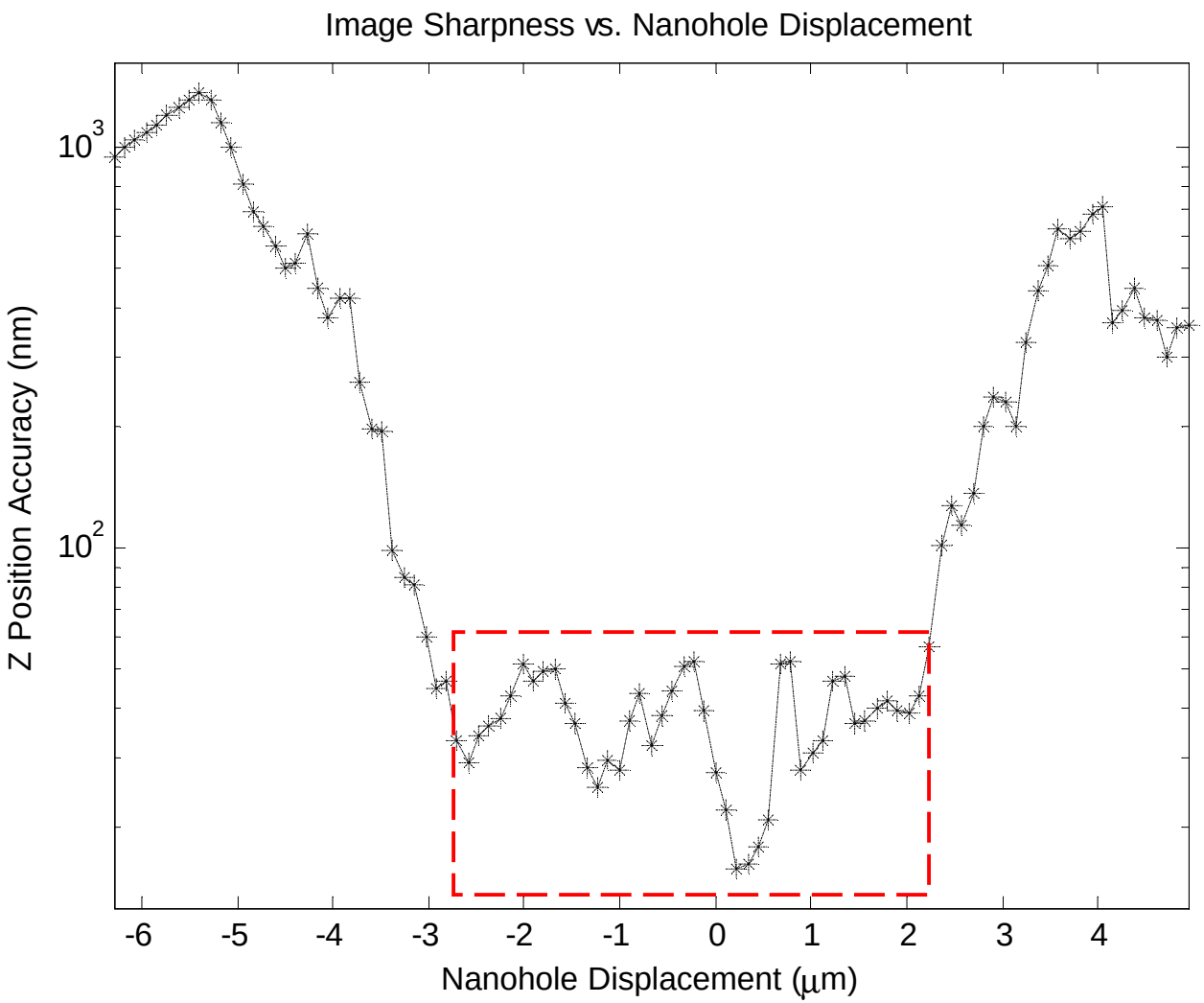
MLE Approach



Our Maximum Likelihood Estimator based algorithm, given a single snapshot image, produces a Likelihood Function as shown.

Most *probable* depth (Z) position for the Nanohole = $\mu \pm \sigma$.

MLE Approach



Focal volume was 3.3 μm

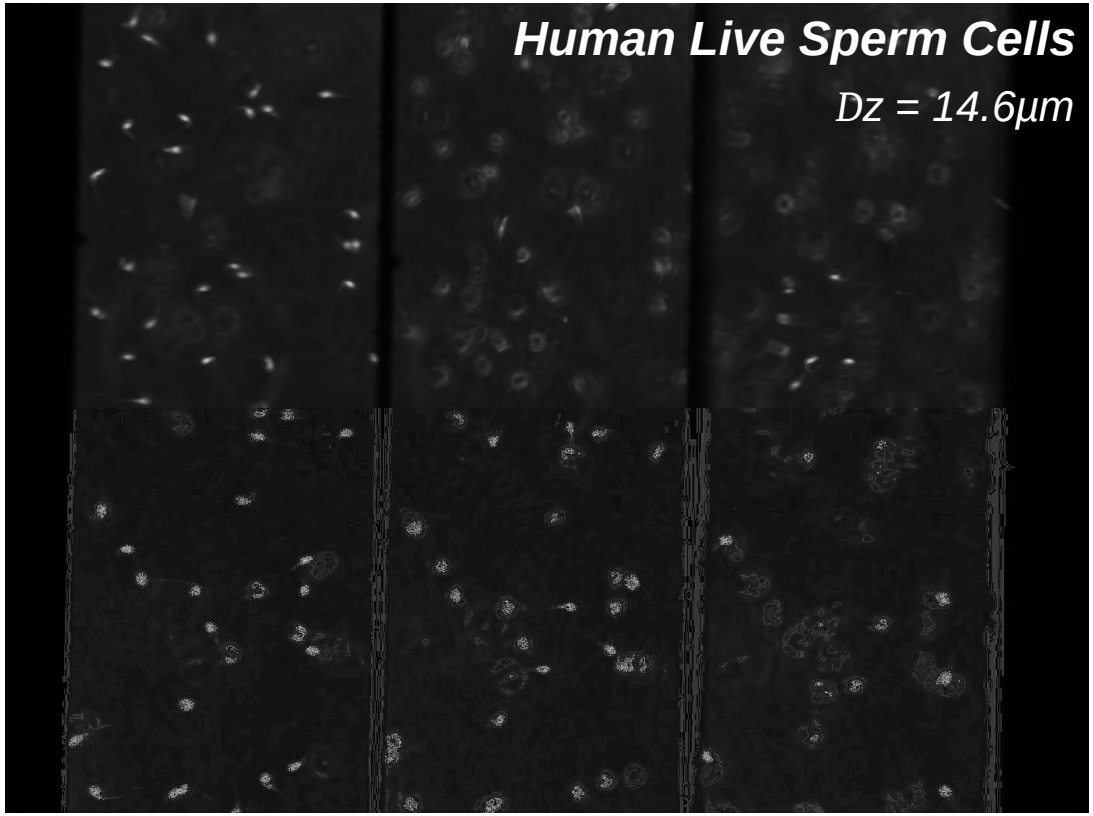
Best results given over 4.6 μm range.

Over this region the accuracy is approx. ±20nm.

In the centre of the focal volume the accuracy is ±8nm

Motility Application Highlight

Aim – To study of the complex tail movement of sperm cells.



Collaboration with Medical School, University of Birmingham and Cairn Research

- **Current Technique –** 2D tail length used to estimate the 3D tail shape.
- **Our Approach** – To combine our 4D imaging technique and particle tracking algorithm.

Conclusions

- 4D Imaging can be achieved using a simple diffraction grating approach.
- This same system, coupled with an image sharpness MLE algorithm can provide fast, accurate, particle tracking.
- The system is cheap, robust, and easily adaptable to commercial biological microscopes.

Website

A copy of the presentation is available at:

http://waf.eps.hw.ac.uk

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