Institute for Integrated Systems

Tracking cells and particles in 3D using image sharpness

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Authorship

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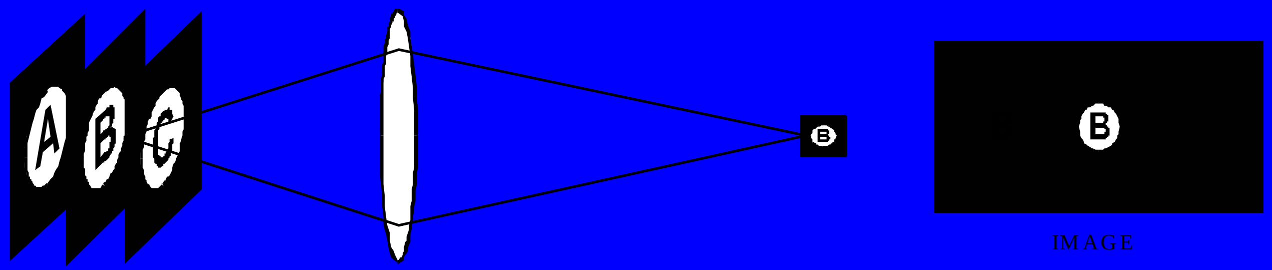
Thanks…

undergraduate project students en passent and making positive contributions…

- Aaron Weis
- Carola Diez
- Alan Baird, …

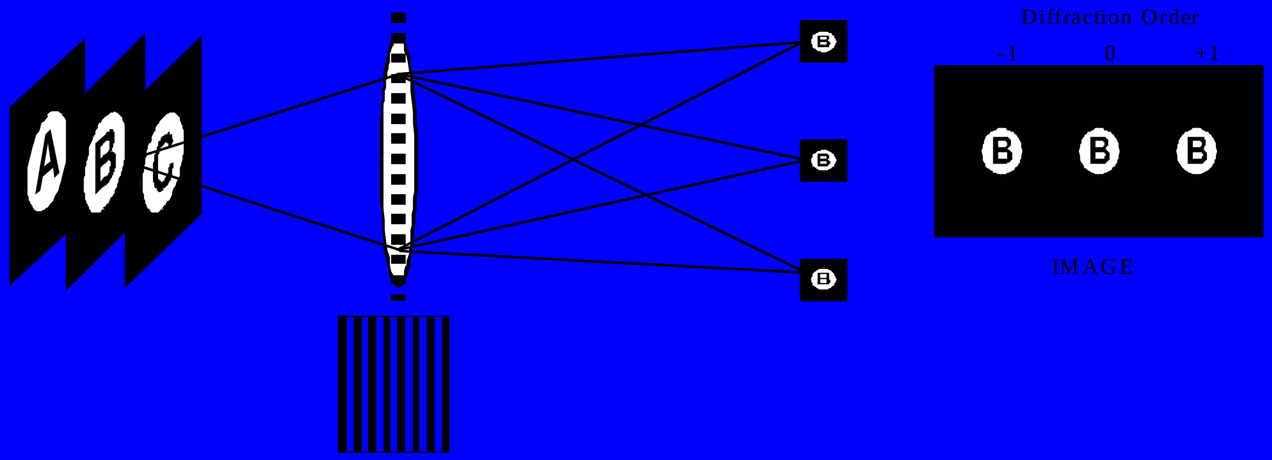
Conventional Imaging

- Conventional imaging system gives in-focus image of a single object plane



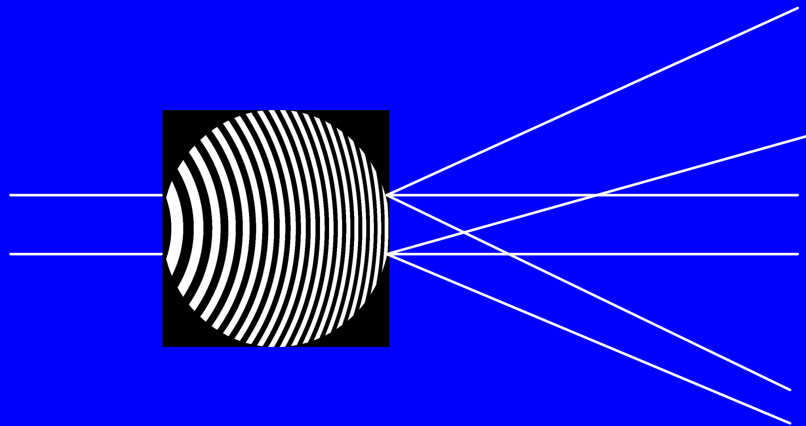
Conventional Imaging

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

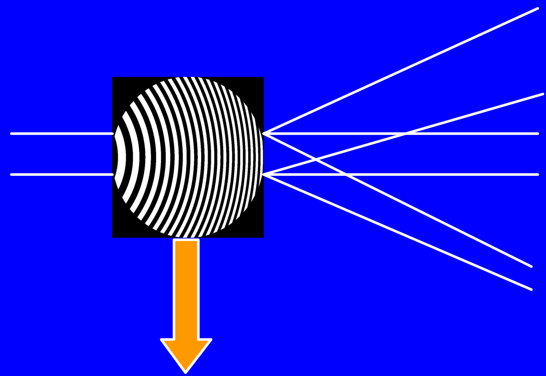


Diffractive Optics

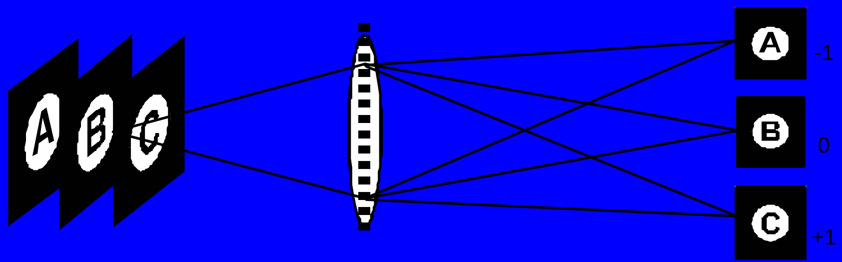
- Distorted grating gives different phase shift in each diffraction order
- Principle of detour phase ➔ holography
- Quadratic distortion ➔ wavefront curvature
- Acts like lens with different focal length in each diffraction order

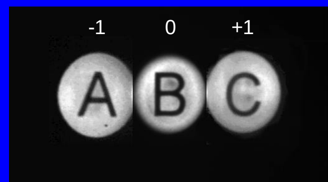


3-D Snapshot Imaging



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

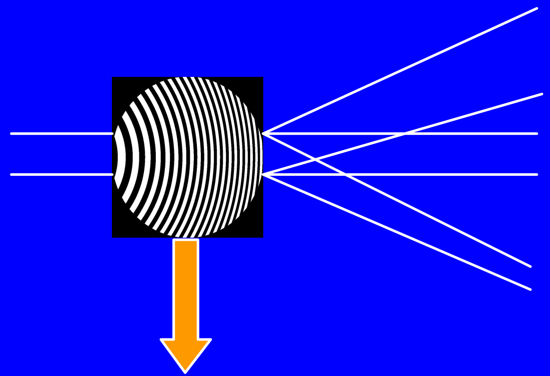




Blanchard & Greenaway

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

3-D Snapshot Imaging



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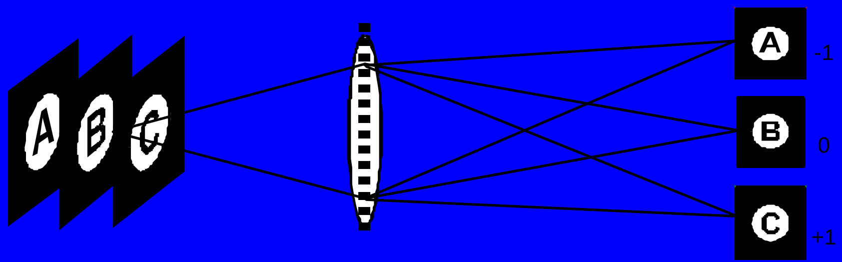
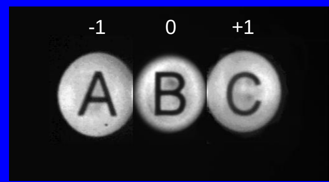


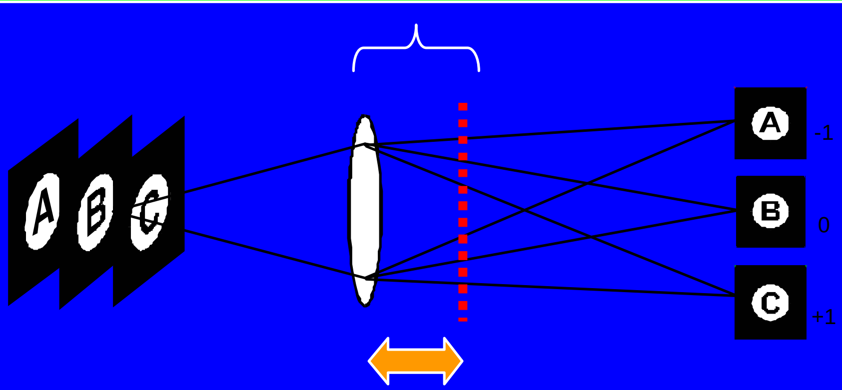


In-focus images of various z-planes are at different magnification

Telecentricity

Combination optical system

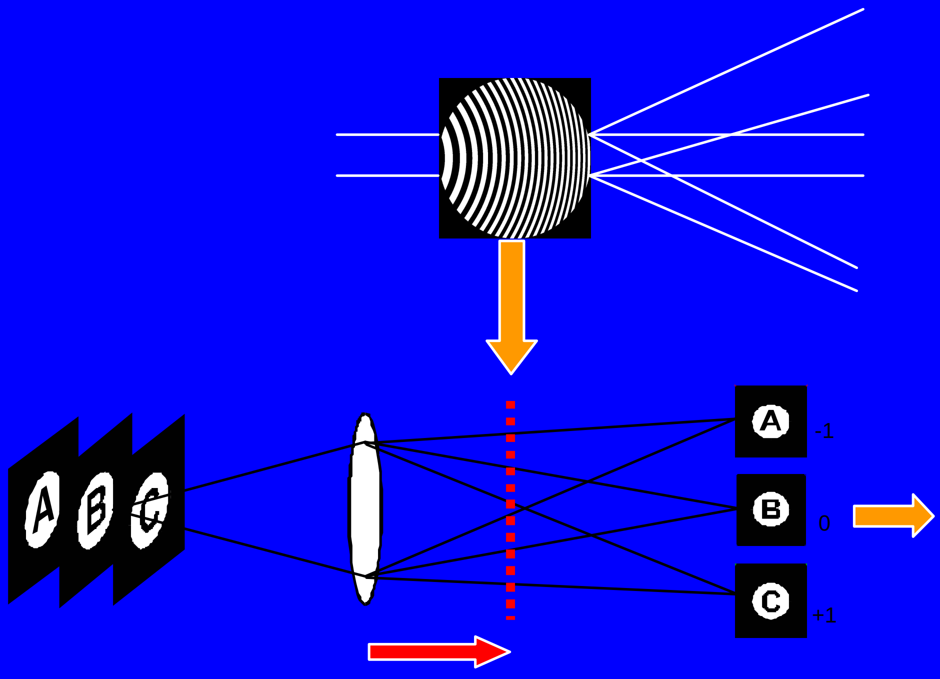
$$f_c = \frac{f_\ell \;{}_m f_g}{f_\ell + {}_m f_g - s} \text{ and } p_1 = \frac{s f_\ell}{f_\ell + {}_\mathrm{m} f_g - s} \text{ and } p_2 = \frac{s\left(f_\ell - s\right)}{f_\ell + {}_m f_g - s}$$



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

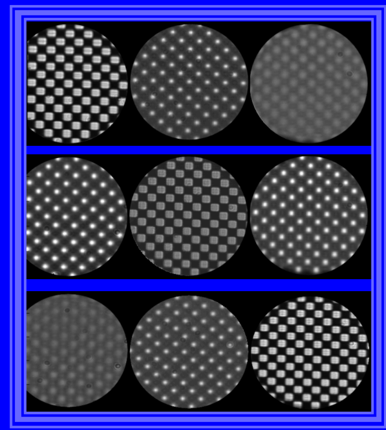
$$s = f_\ell \Rightarrow f_c = f_\ell \text{ and } p_1 = \frac{f_\ell^{\,2}}{{}_m f_g} \text{ and } p_2 = 0$$

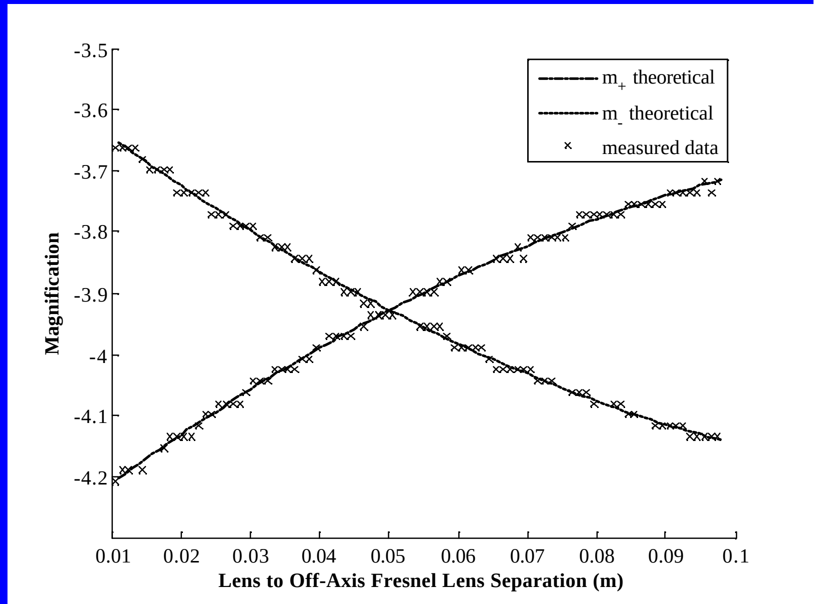
Telecentricity



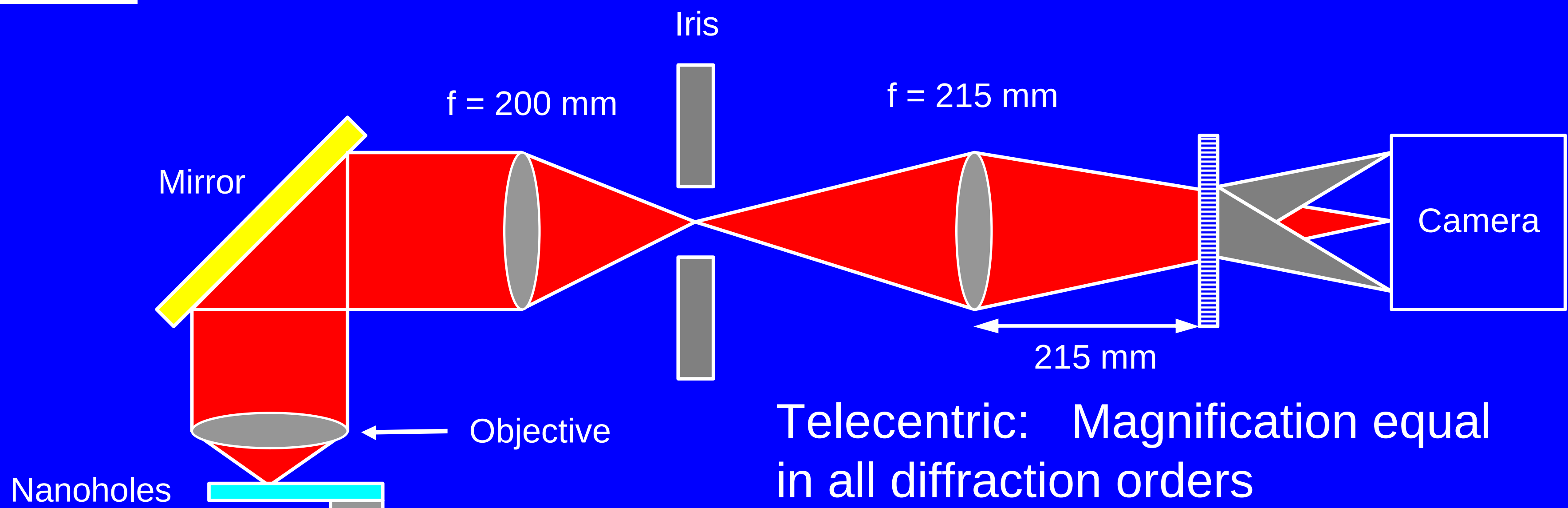
$$s = f_\ell$$

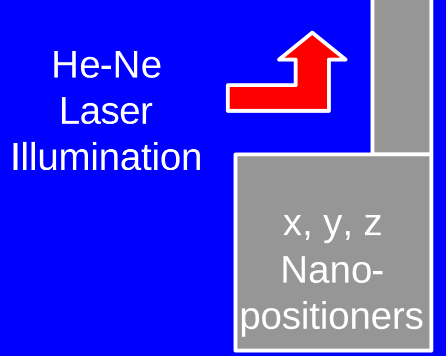
Telecentric performance



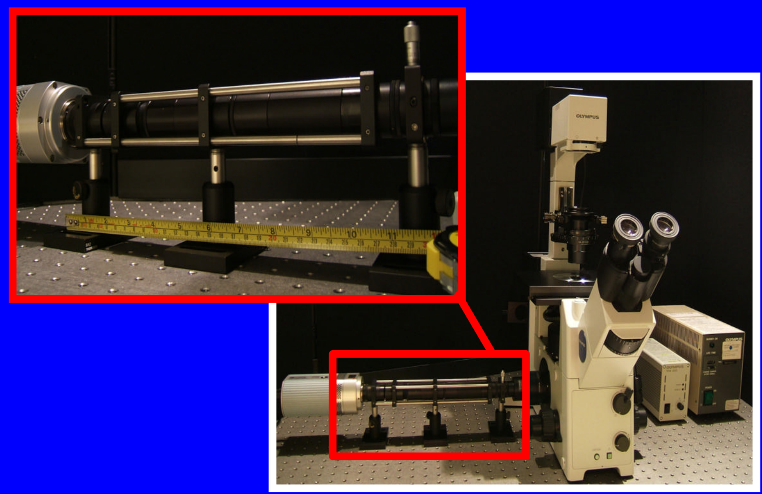


Experimental arrangement



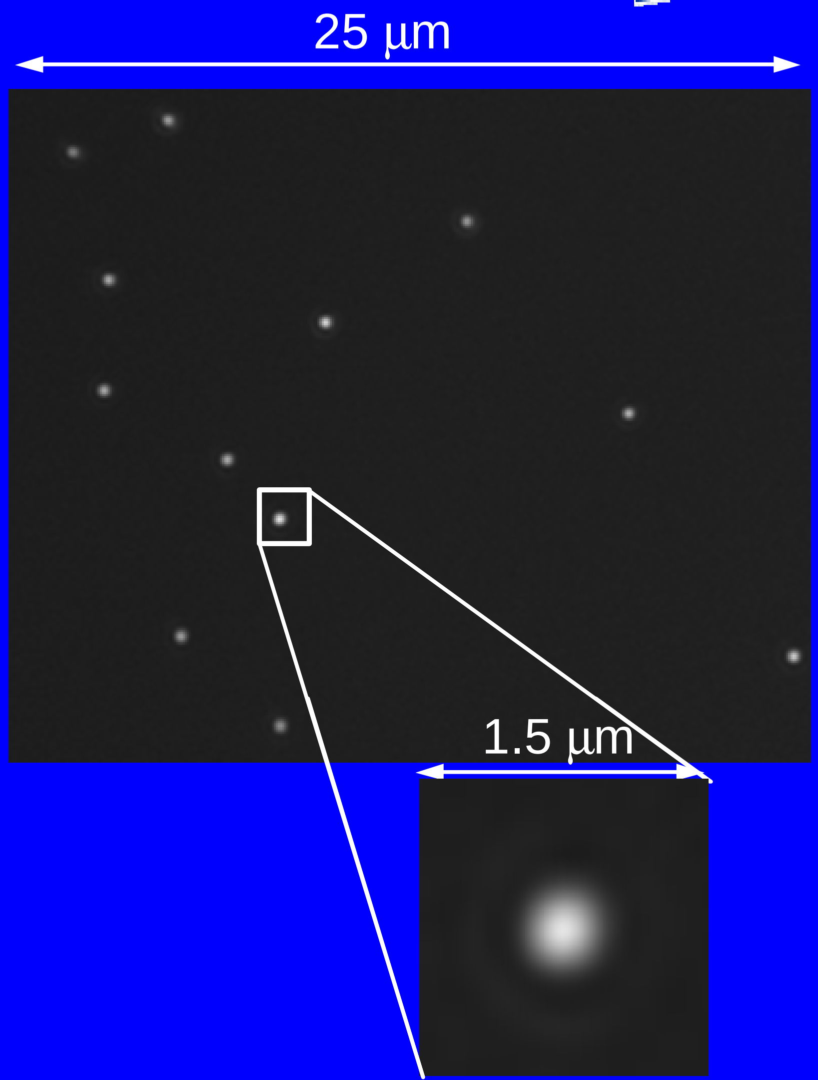


Telecentric: Magnification equal in all diffraction orders



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

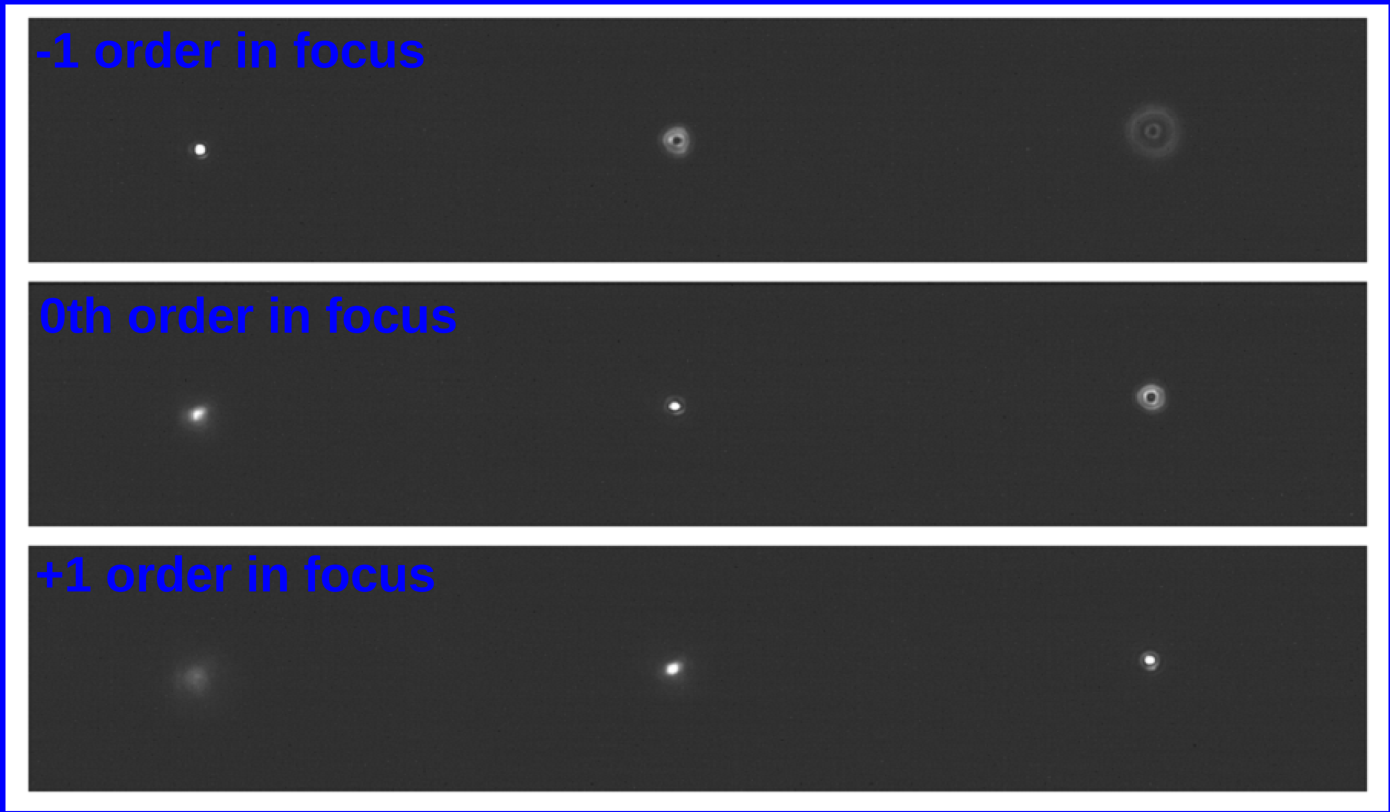
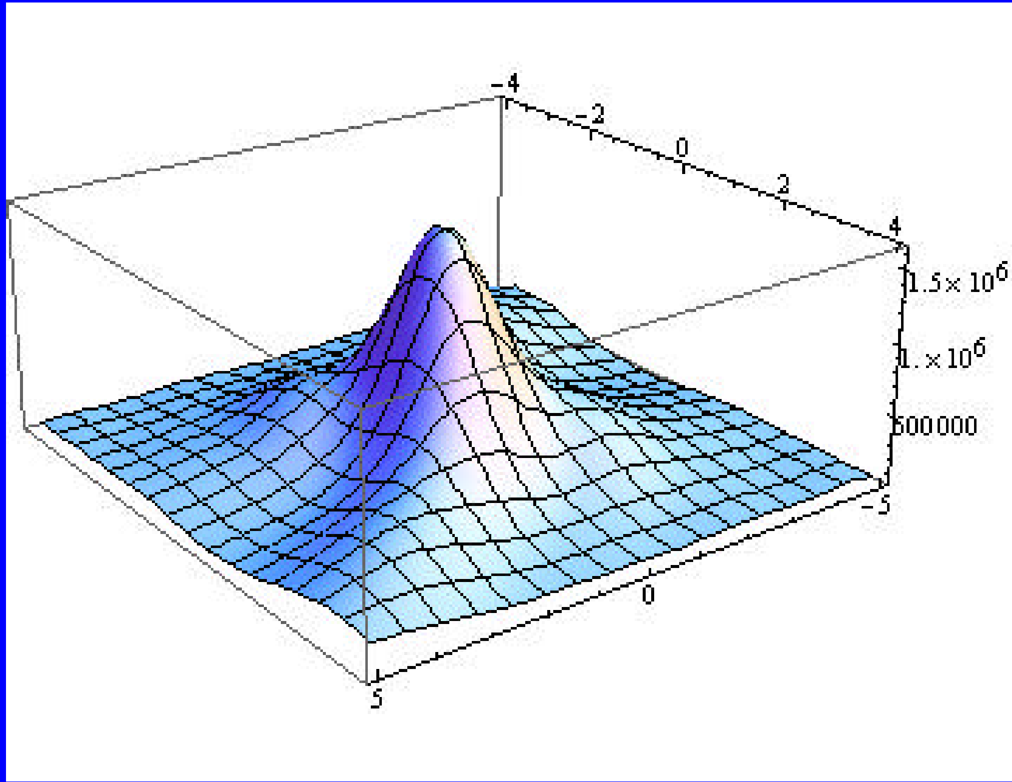


Image Resolution:
No grating = 233nm
With grating = 226nm and 231nm (for 0^{th} and ±1orders)

Image Sharpness

- Sharpness is the integral of the square of the image intensity
- Sharpness reaches a global maximum for an unaberrated image

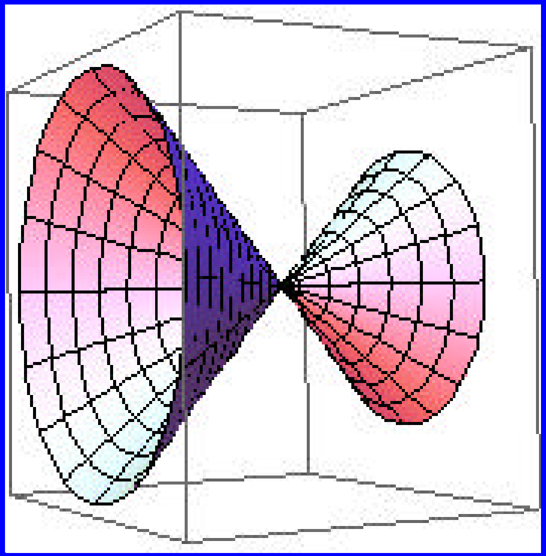


Tracking in 3-D

- Beam divergence from source depends on optical aperture

- Defocused image on non-source planes reduces intensity…

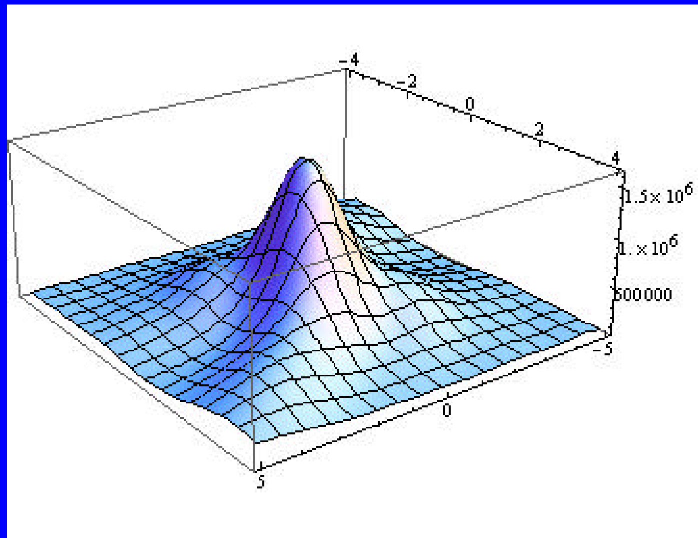
- …thus to a reduction in sharpness

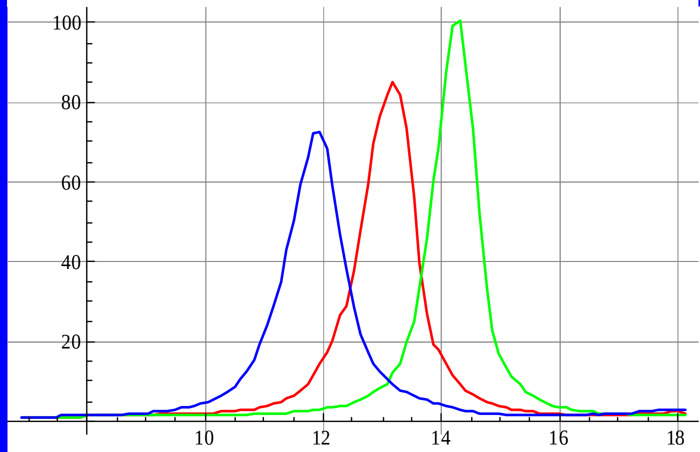


Suitable for real-time analysis and CMOS detector technologies

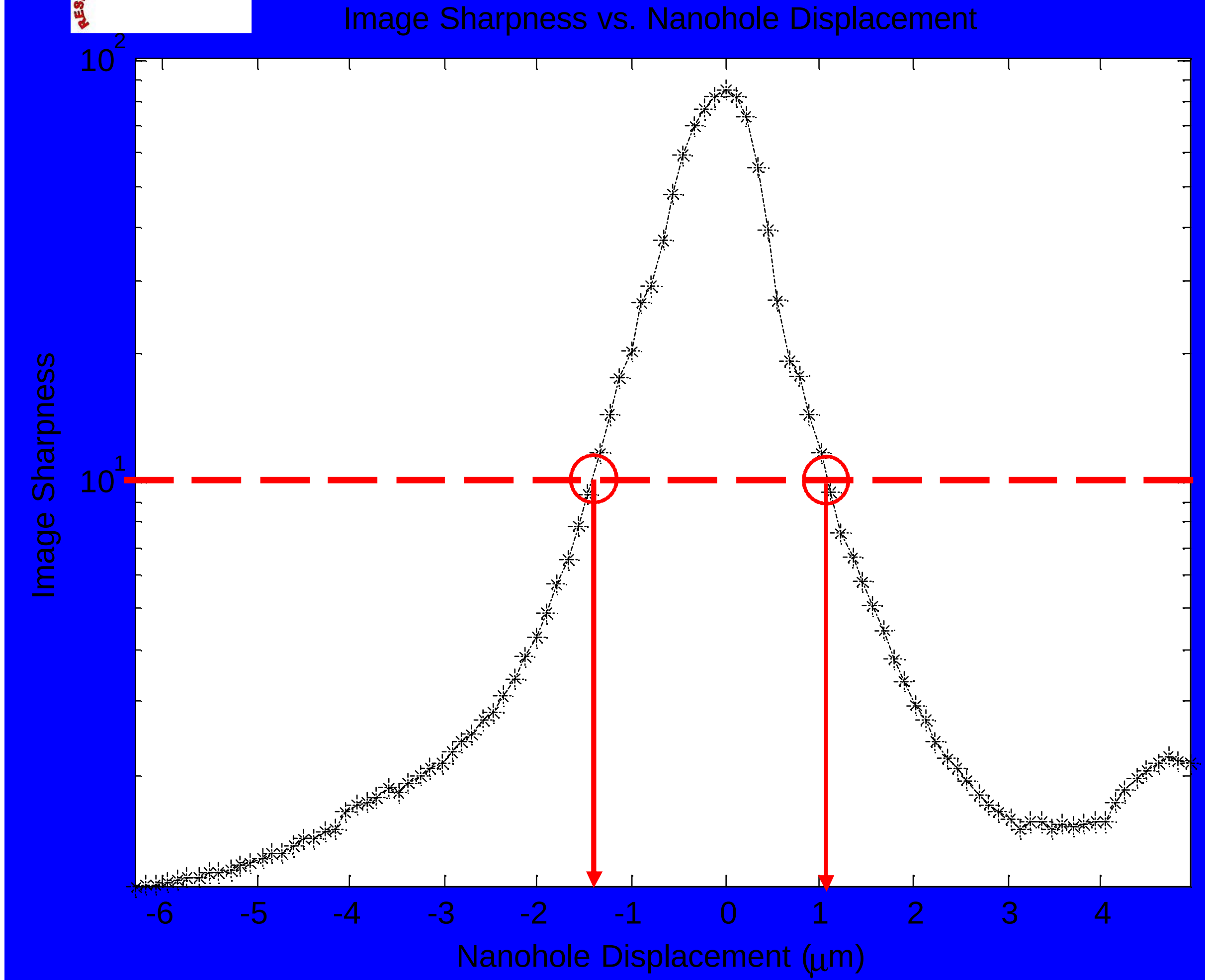
Ranging in Depth with Sharpness

- Qualitative agreement of simulation and experiment
- Calibrate from actual data
- Need accurate information on objective aberrations





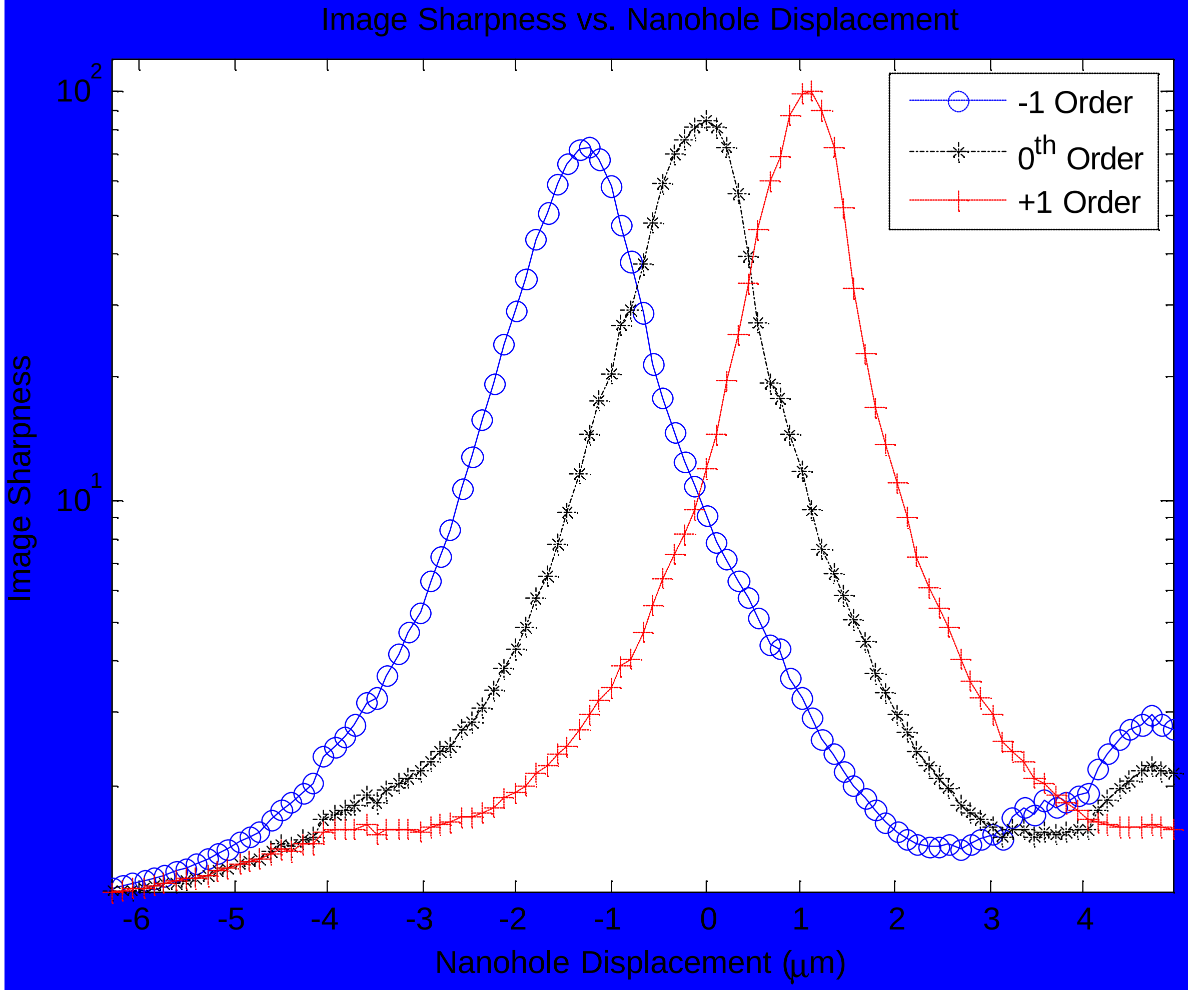
Position Measurement (Z)



Problem:

A single mage sharpness measurement gives ambiguous depth position

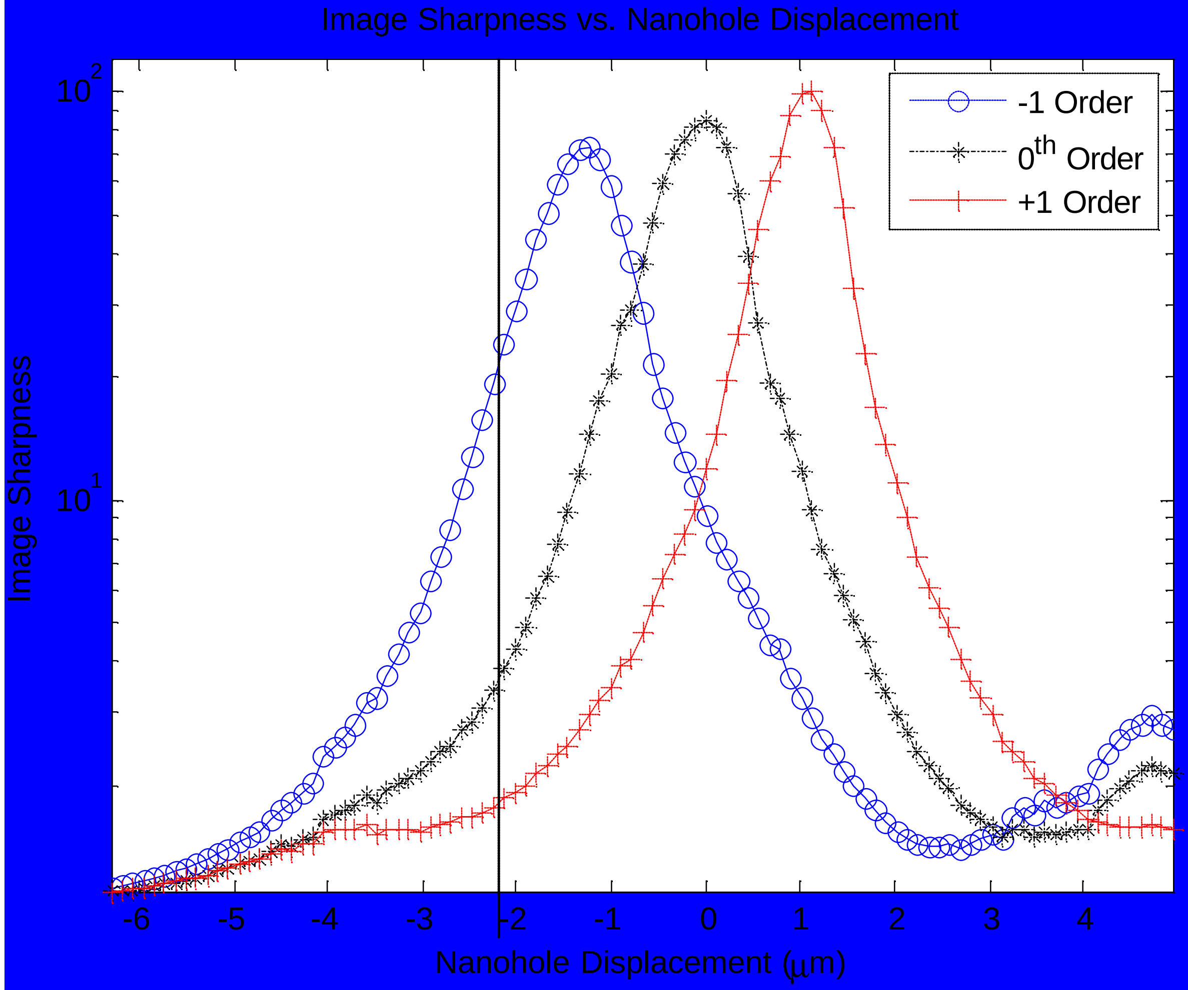
Unique depth indication…



Solution:

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

Unique depth indication…



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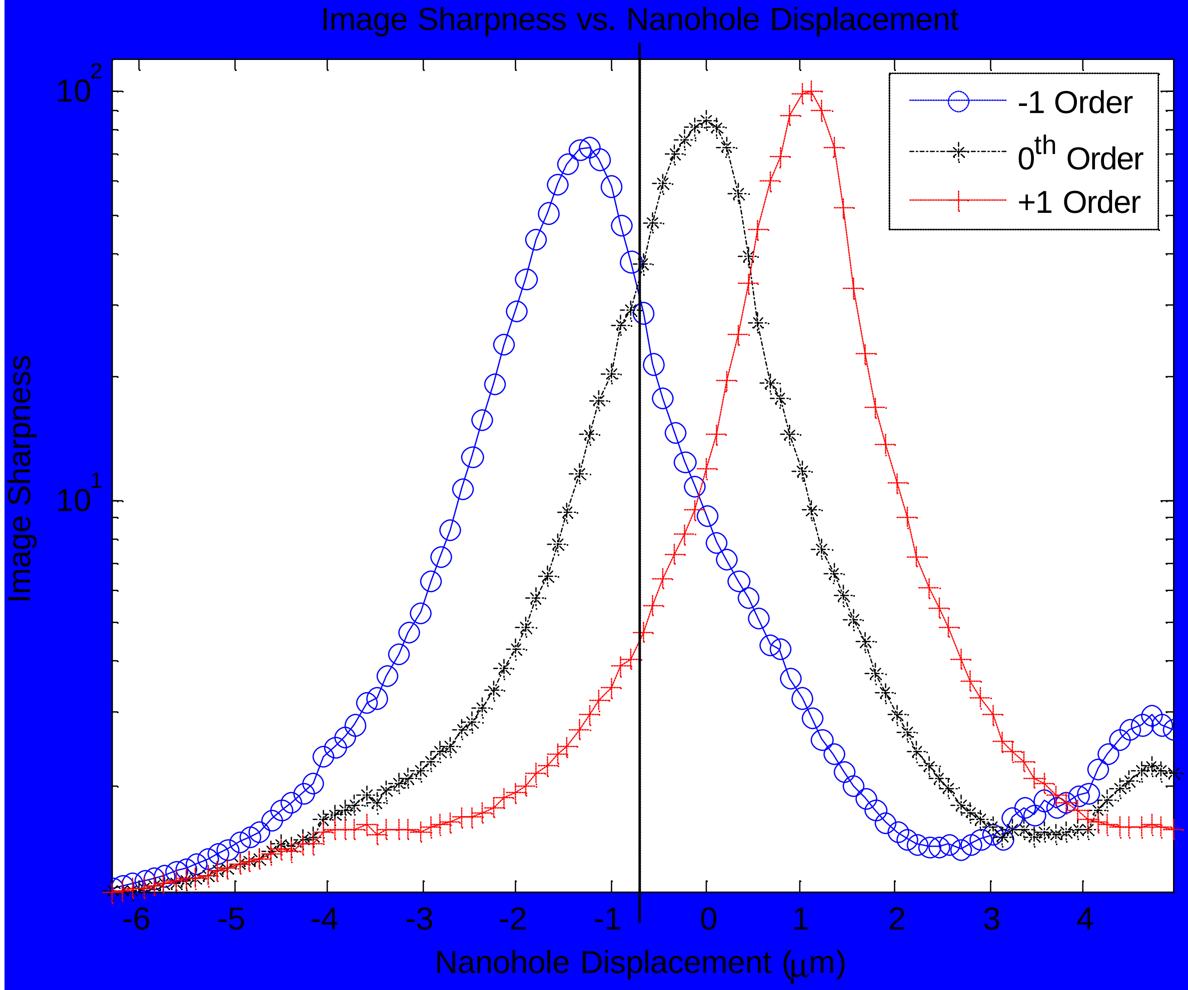

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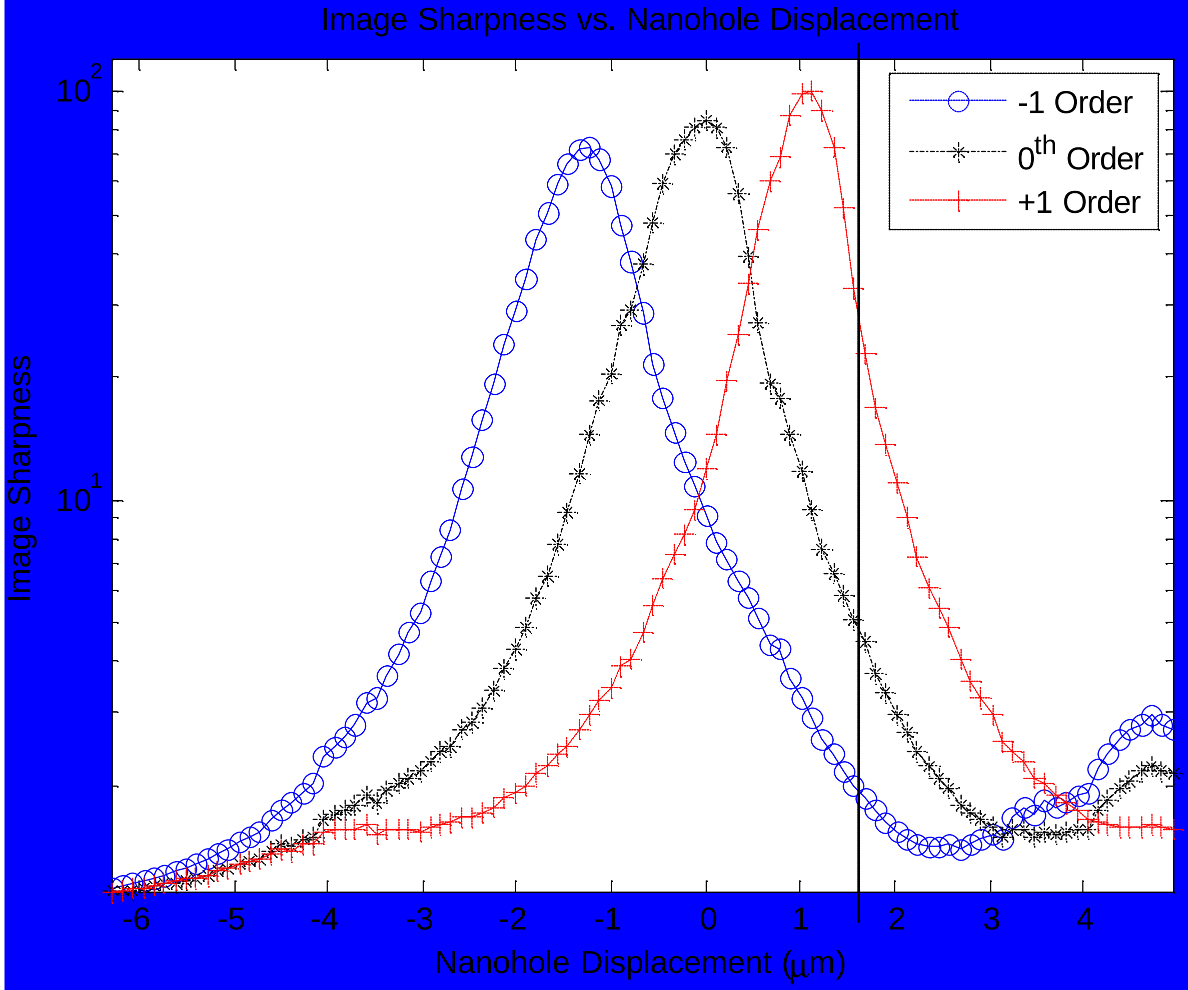

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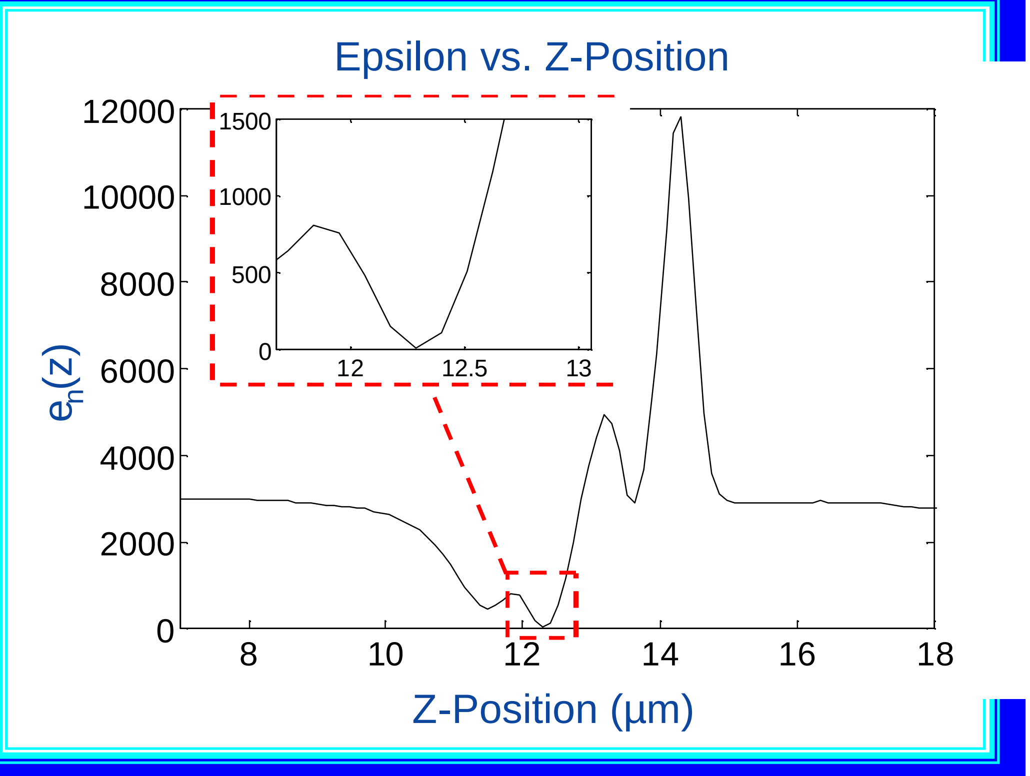
QD grating method gives 3 simultaneous image sharpness (one from each order) for each particle.

… from 3 sharpness measurements

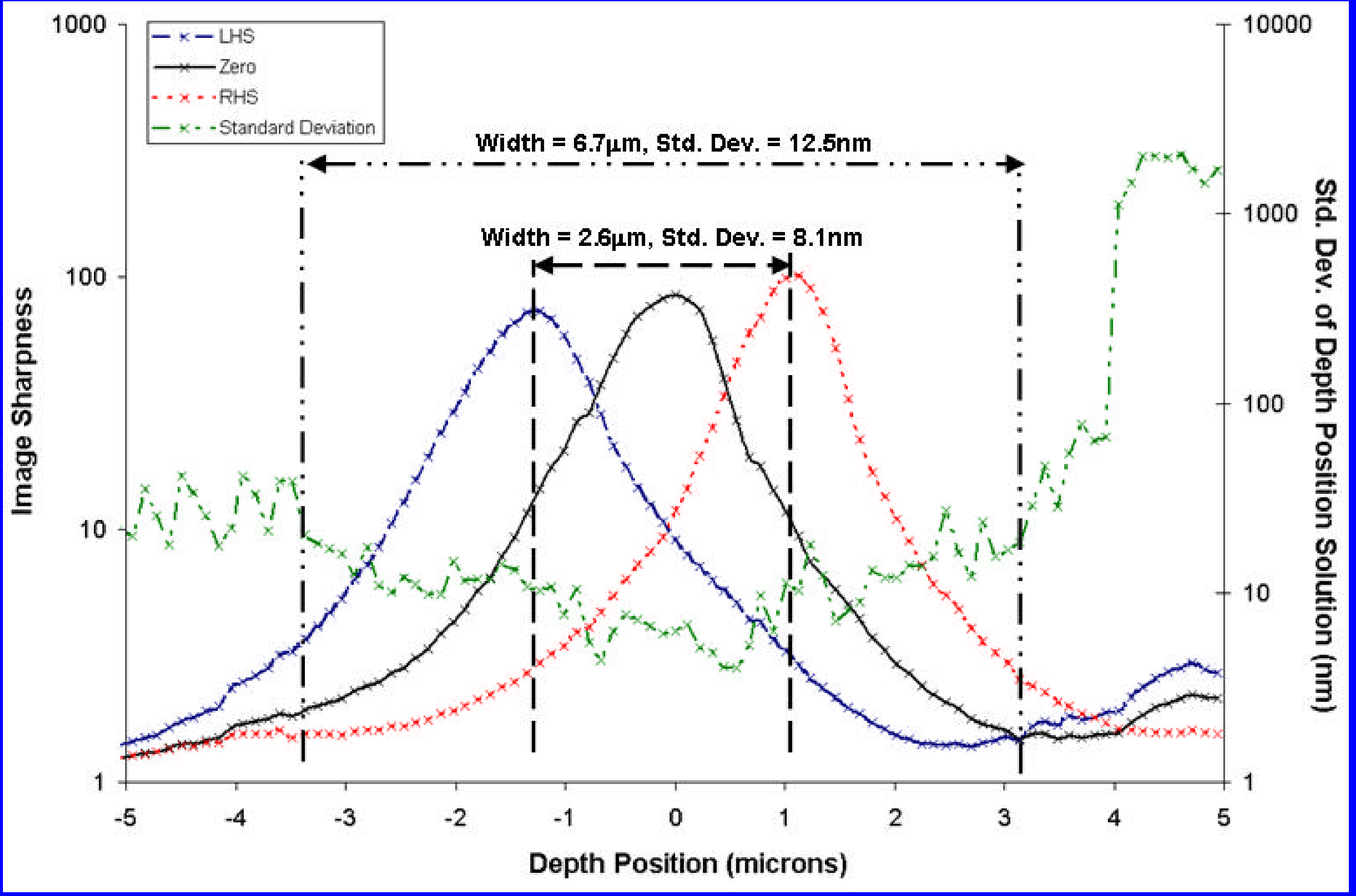
- Depth resolution (<50nm) using least squares

$$e(z) = \sum_{j=1}^{3} \left| S_j(z) - M_j \right|^2$$

- Likelihood-based analysis is better…



ML depth estimator



Future work

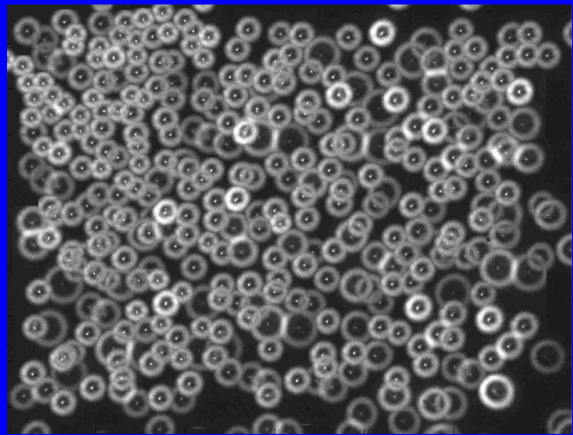
- Analysis of photon statistics
  - ➢ Sensitivity to photon noise contamination
- Assess real-time, but one-off, compensation of SA
  - ➢ Improve SNR
  - ➢ Optimised correction for central image
  - ➢ Correct differential SA in grating
- Polarisation sensitivity
  - ➢ Sub-? structures in grating

Summary

- 3D imaging using off-axis Fresnel lenses gives good-quality images of multiple in-focus planes
- Image sharpness measure in these planes gives a robust and high-accuracy depth-measurement tool
- Algorithm is simple and should be relatively straightforward to implement in real time
- Active compensation of SA should improve SNR

Ranging in Depth – Other Approaches

- Conical illumination allows greater particle density
  - ~450 particles in 410x310x120µm vol allows ~±180nm



Lin D et al., Optics Lett **33**(2008)907-907
(also Virtual Journal of Biomedical Optics)

- Anamorphic optics allows use of wavefront sensing or sharpness