Volumetric, time-sequenced imaging

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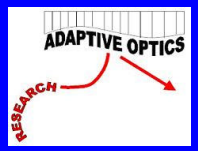
Thanks…

- Paul Dalgarno
- Richard Warburton
- Co-authors
- undergraduate project students en passent…
  - Aaron Weis, Carola Diez, Aurelie Putod, Alan Baird, Scott Aitken, …

Overview

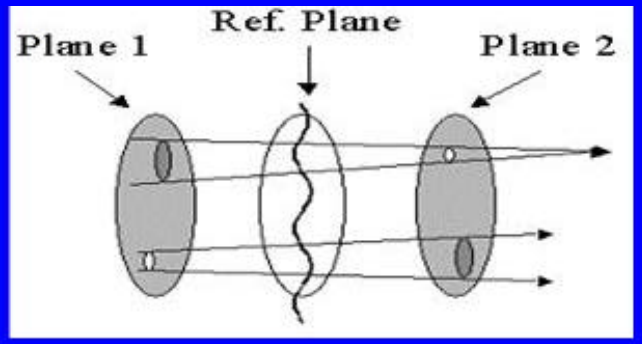
- Introduction and background
- 3D imaging of live cells
- Particle tracking
- Future work
- Summary



Introduction and background

Wavefront Sensing

- Zero order forms gives in-focus scene
- ± 1 diffraction orders give images of wavefront
- Phase-diverse wavefront sensing

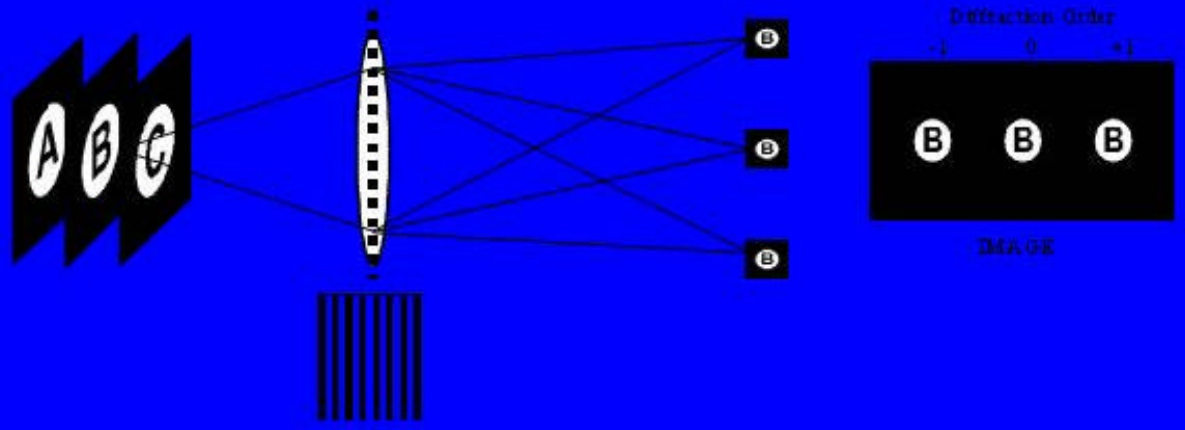


$$-\frac{k}{I}\frac{\partial I(r)}{\partial z} = \nabla^2 \phi(r)$$

$$\phi(r) = -k\int_R dr' G(r,r')\frac{\partial I(r')}{\partial z} = -k\int_R dr' G(r,r')\frac{I_1(r) - I_2(r)}{\Delta z}$$

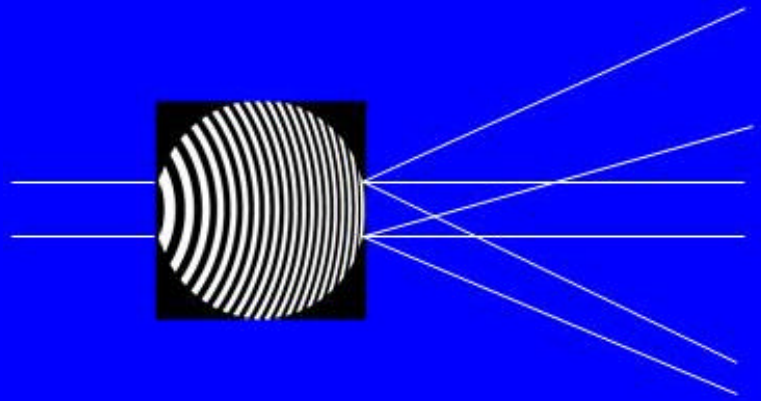
Conventional Imaging

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



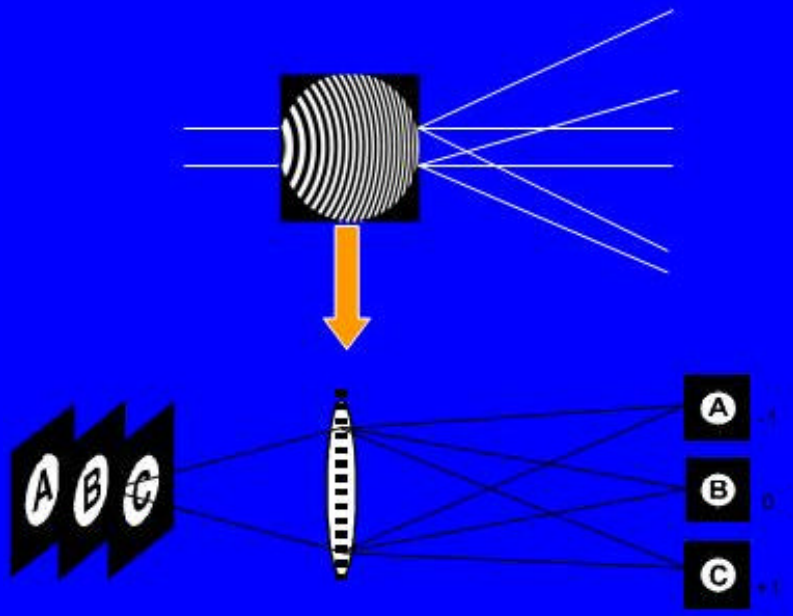
Diffractive Optics

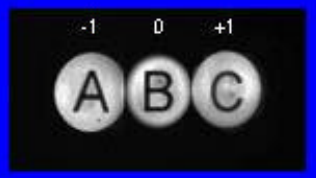
- Distorted grating gives different phase shift in each diffraction order
- Principle of detour phase ➔ holography
- Quadratic distortion ➔ wavefront curvature
- Acts like lens with different focal length in each diffraction order



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

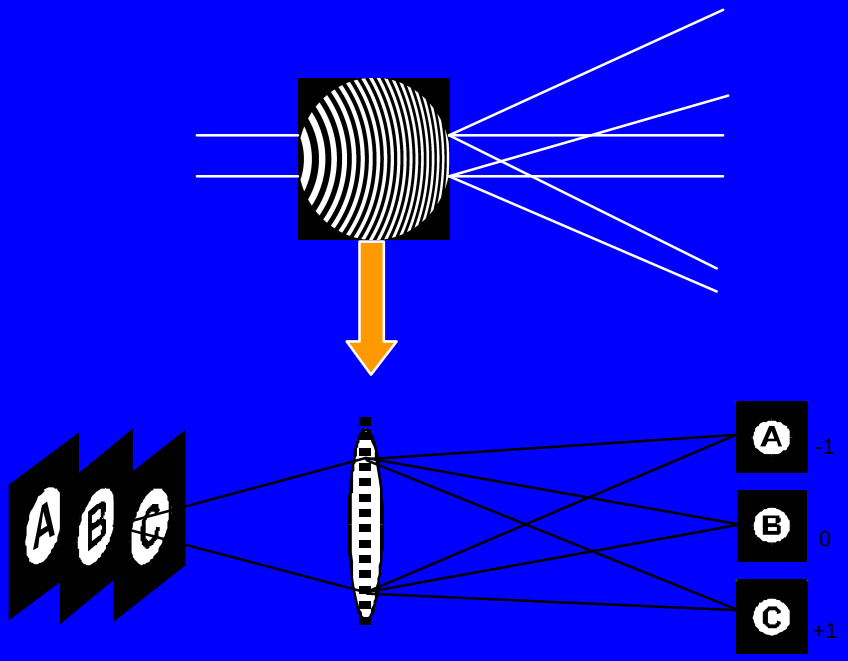


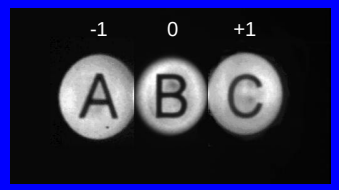


Blanchard & Greenaway

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

3-D Snapshot Imaging

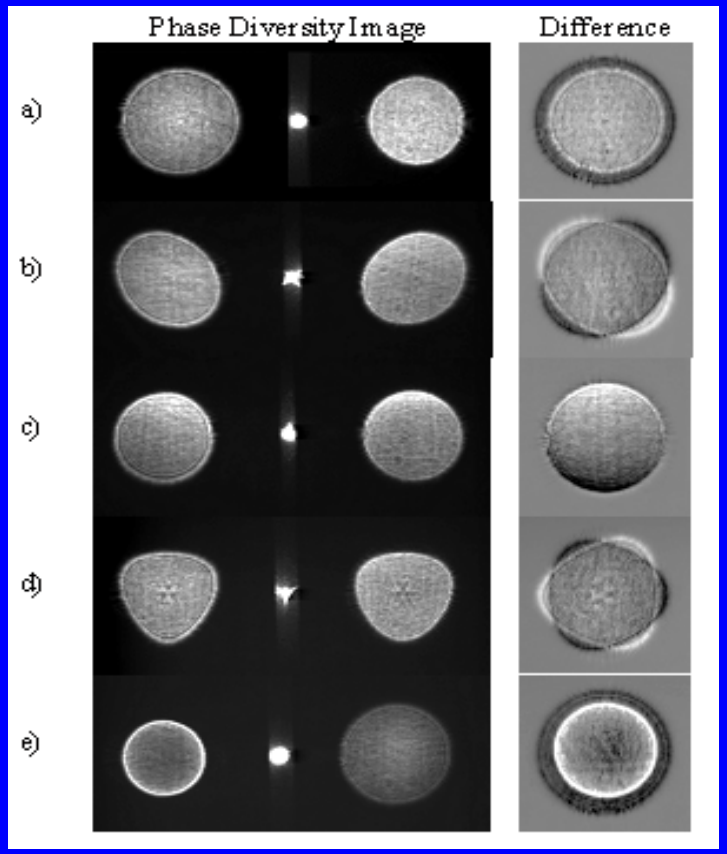




If the zero order delivers an image of the source, the ±1 orders image planes symmetric wrt the lens

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

Wavefront Sensing



Blanchard, Fisher, Woods & Greenaway

Applied Optics **39**(2000)6649-6655

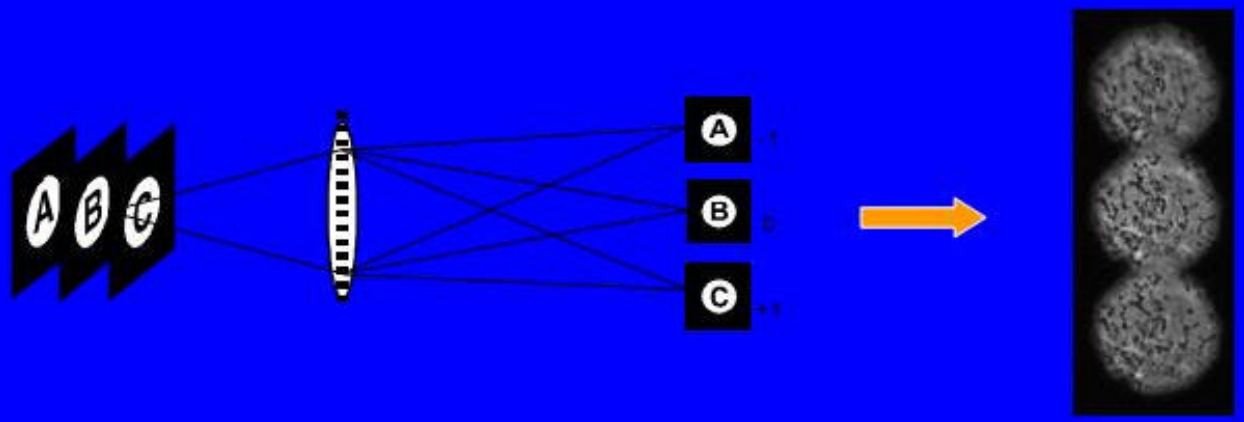
- Easy to see the wavefront aberrations by eye.

  a. Defocus
  b. Astigmatism
  c. Coma
  d. Trefoil
  e. Spherical Aberration

Defocus accuracy 0.7nm rms

➢ accurate tracking in 3D

3D imaging of live cells



3-D imaging in biological applications

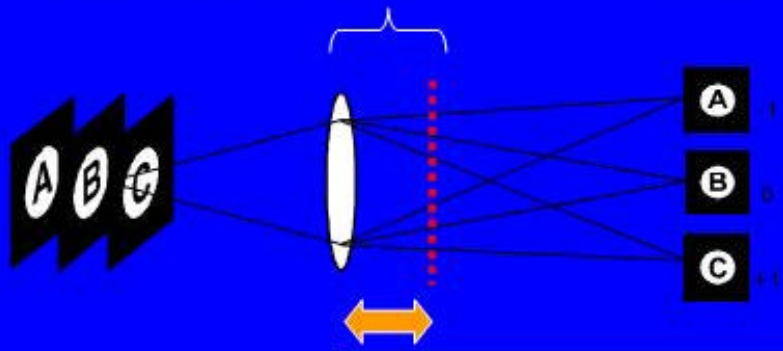
- Can magnification of images be equalized?
- Are gratings useable with microscopes?
- Is optical efficiency high enough?
- Can dispersion be corrected?

- ➢ Avoid re-scaling of large data volumes
- ➢ Minimize cost
- ➢ Minimize photo-damage
- ➢ Needed for fluorescent imaging

Telecentricity

Combination optical system

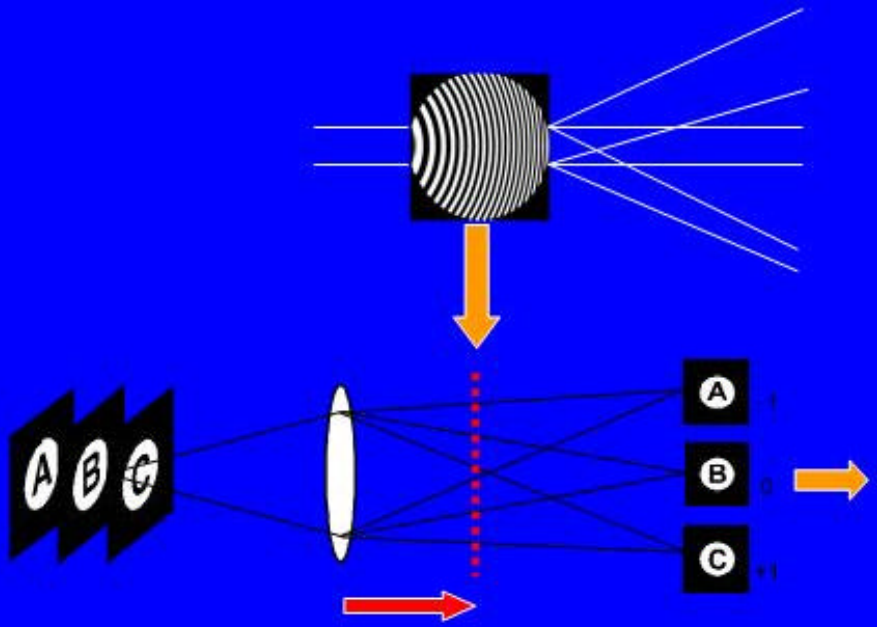
$$f_c = \frac{f_\ell \; {}_m f_g}{f_\ell + {}_m f_g - s} \text{ and } p_1 = \frac{s f_\ell}{f_\ell + {}_m f_g - s} \text{ and } p_2 = \frac{s\left(f_\ell - s\right)}{f_\ell + {}_m f_g - s}$$



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

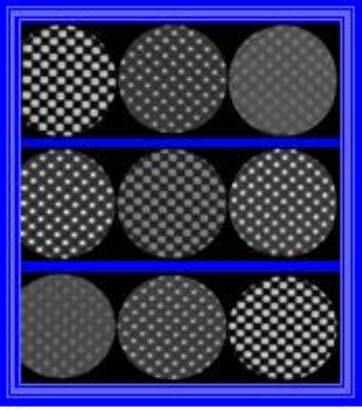
$$s = f_\ell \Rightarrow f_c = f_\ell \text{ and } p_1 = \frac{f_\ell^2}{{}_m f_g} \text{ and } p_2 = 0$$

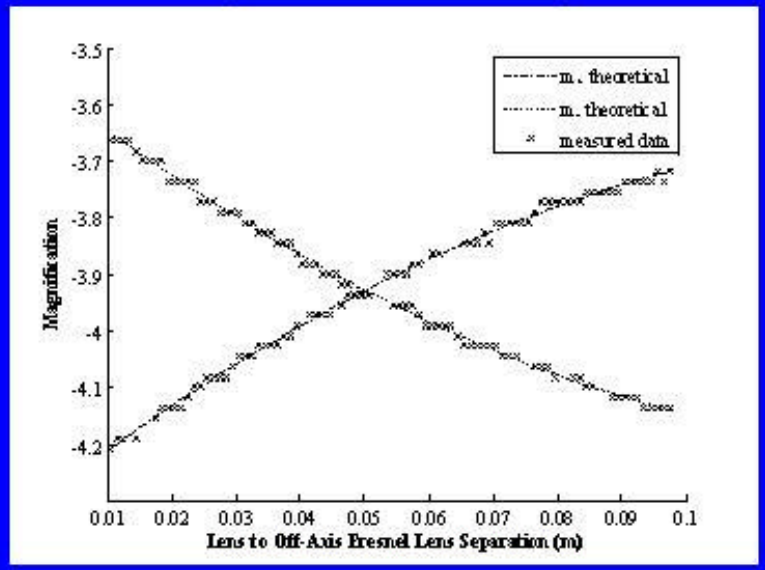
Telecentricity



$s = f_\ell$

Telecentric performance

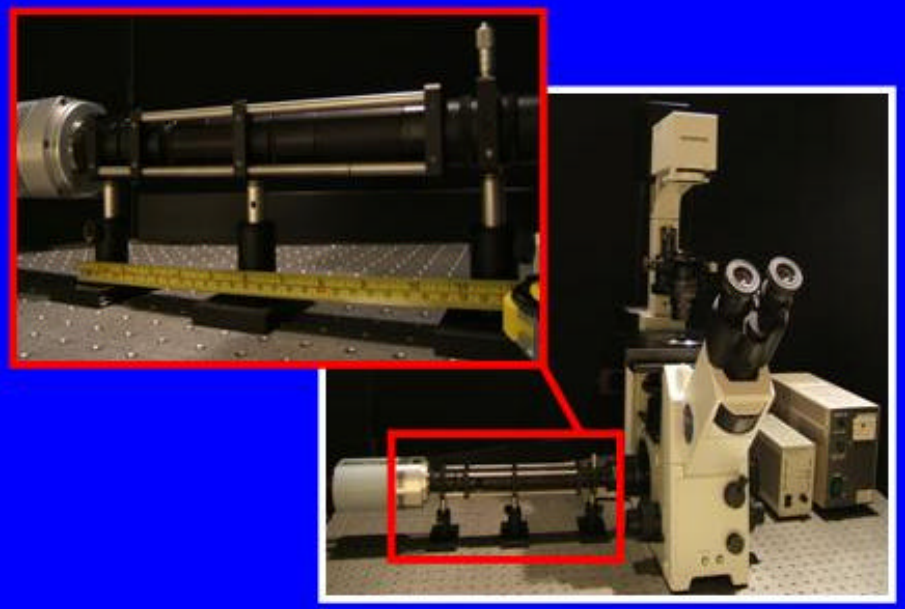




Practicality & Efficiency?

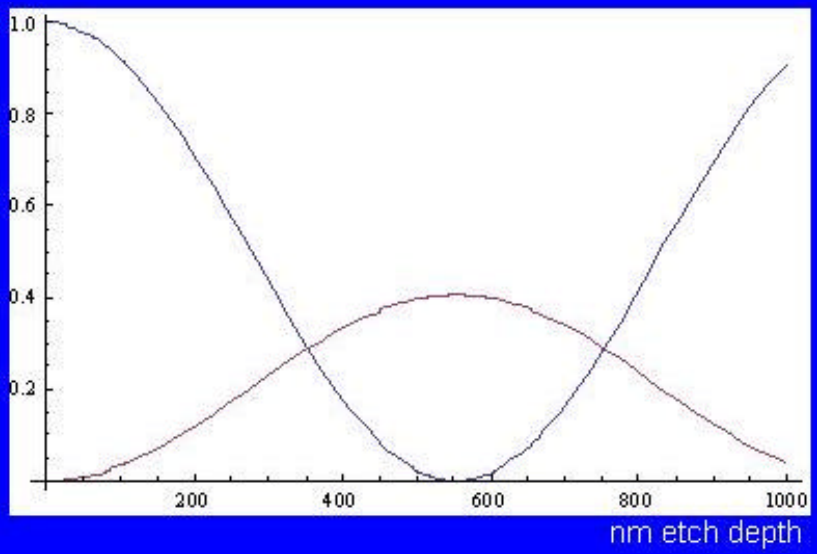
Practicality

- Used on inverted and upright systems



Efficiency

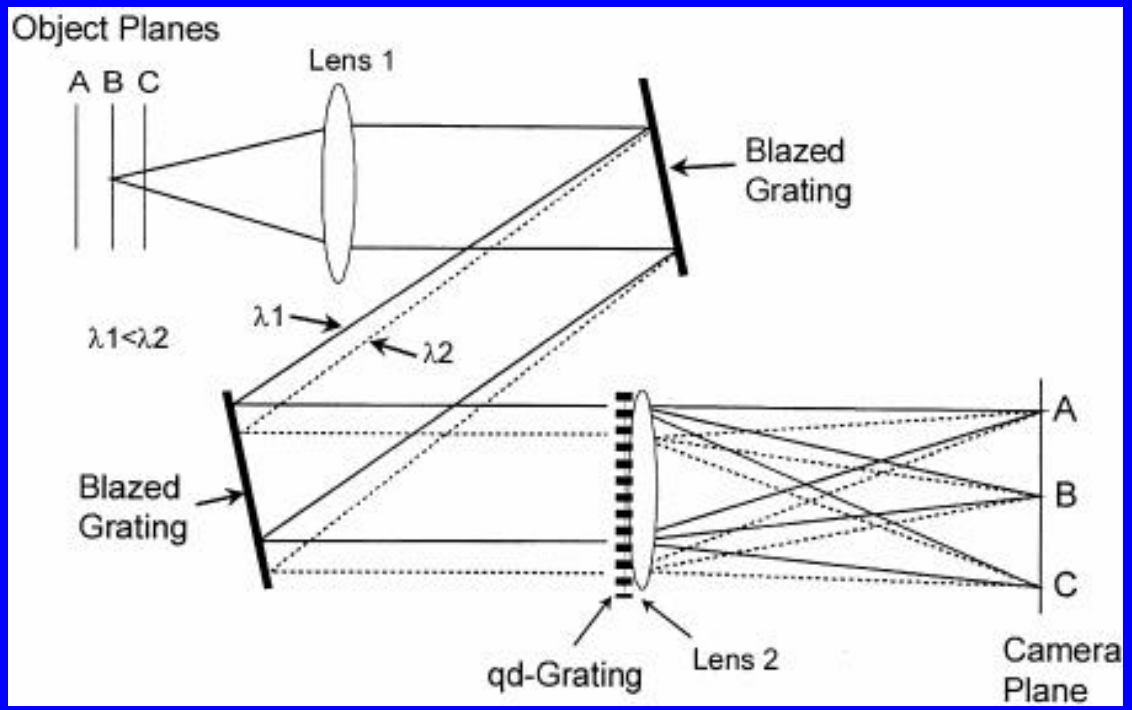
- Optical efficiency ~84% overall



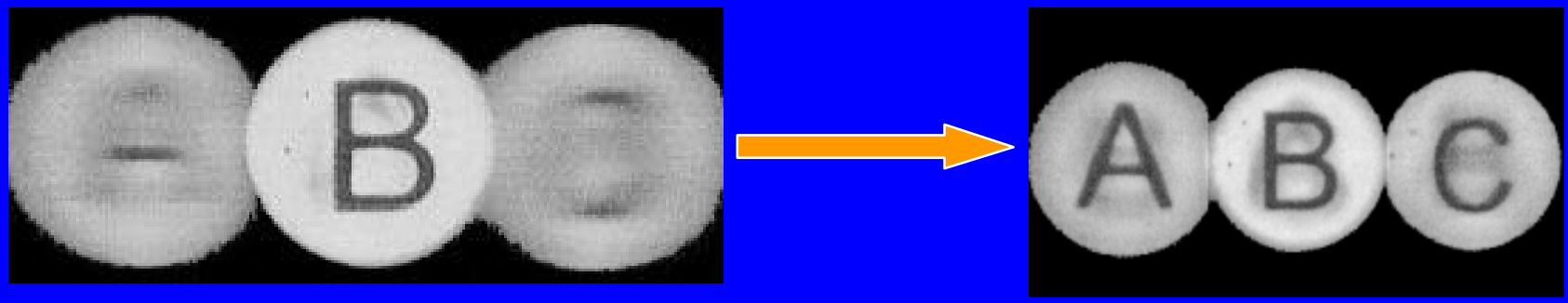
•(~28% each order)

Broadband Application

- Careful design achieve ‘white light’ imaging
- Unfiltered halogen light



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



Generalisation

- If original system is modelled as a compound system this analysis is easily generalised
- Spacings of layers in focus in different diffraction orders varies as

$$\frac{f_c^2}{m f_g}$$

with respect to system Primary Principal Plane

Control of depth

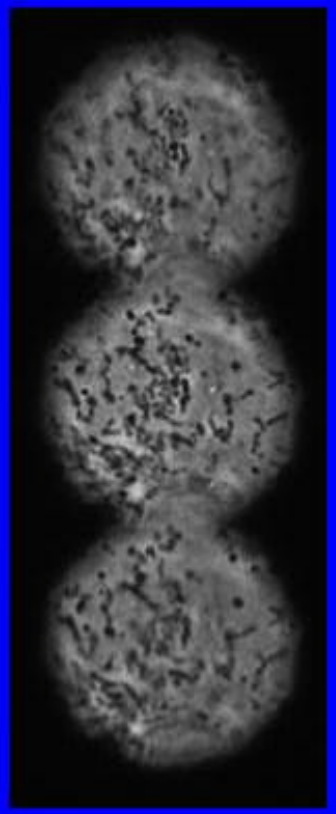
- As grating curvature increases
  - $\frac{f_c^2}{{}_m f_g}$ increases
  - in-focus planes move apart
- No point in using plane separation < depth of focus

Recall ${}_m f_g = \frac{R^2}{2mW_{20}}$

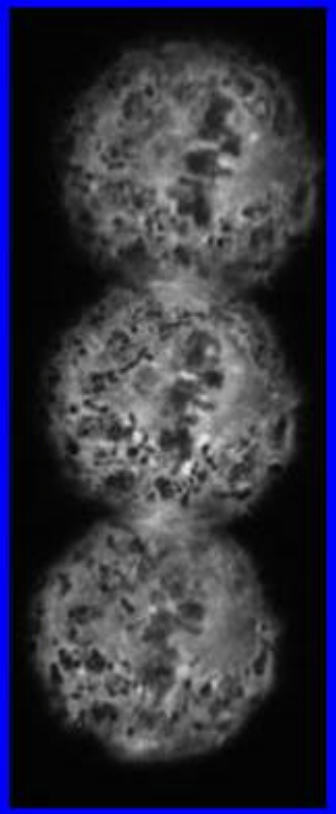
so…

in-focus plane separation increases with m and W_{20}

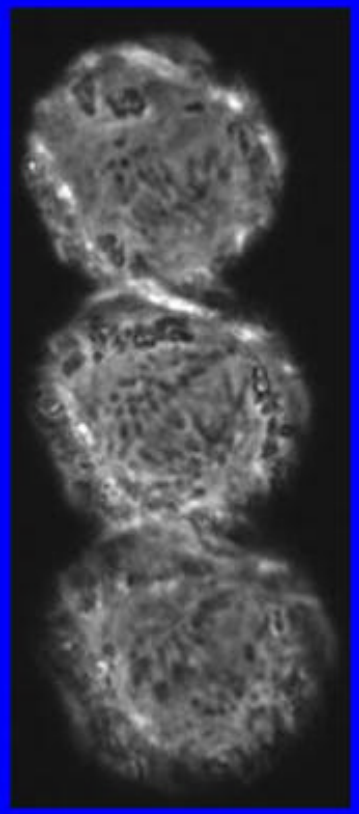
3-D Phase-contrast Movies



410nm



810 nm

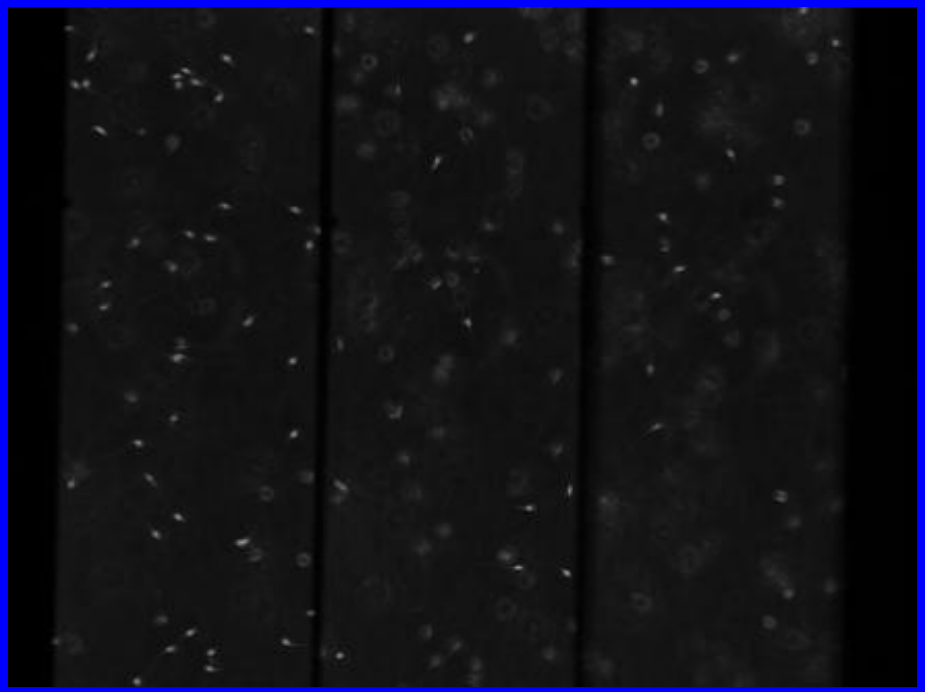


1.46µm

With Allan, Manchester

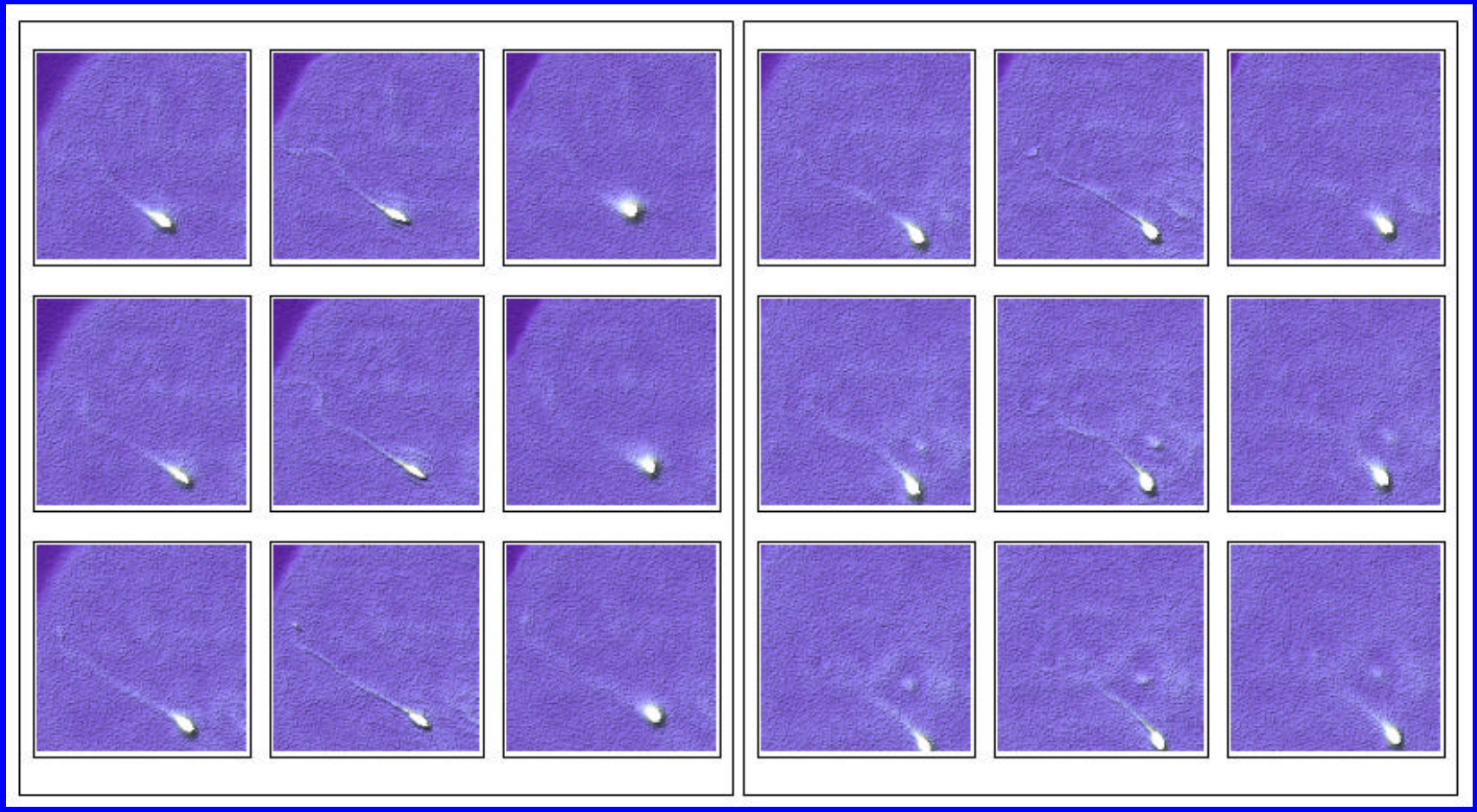
Motility Measurement

- 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



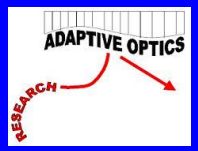
(with Kirkman-Brown, Birmingham)

Sperm Motility Data



Applied for 3-D imaging

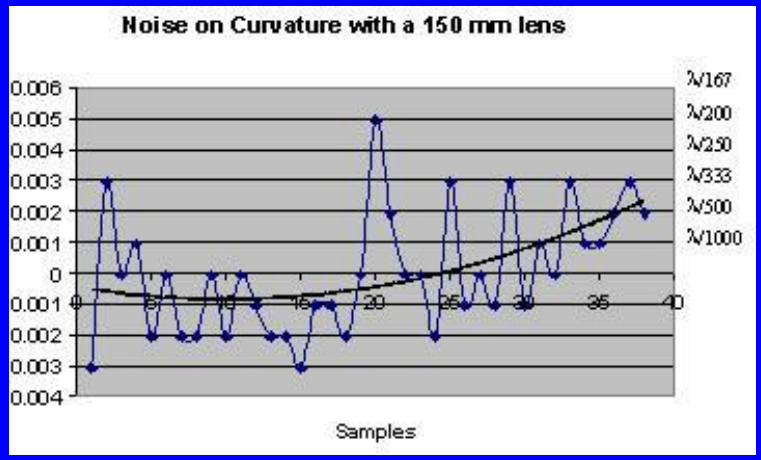
- 2 ways to use gratings for 3-D microscopy
  - Image in-focus layers with $\Delta d \geq$ depth of focus
    - Image 2 layers and range to tracer
      - (x,y) from centroid, *d* from wavefront shape
      - Density of tracers?
- Other possibility is to scan focus
  - Programmable or interchangeable lens



Particle tracking

Ranging in dynamic systems

- Current best wavefront measurements $\pm$0.7nm
- Equivalent to $\pm$22.4nm in particle position using f 2 optics
- Scales with $f_{\#}^2$
- Needs aperture to limit field of view
- Complexity of scene to be determined



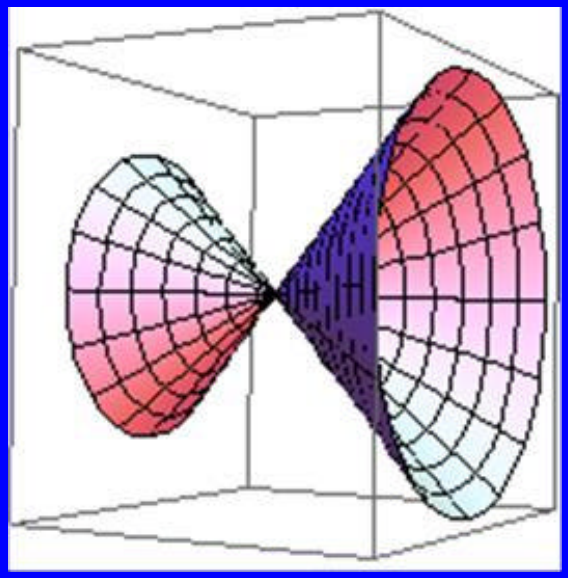
Ranging depth resolution

$$\Delta d = 8 f_{\#}^2 \Delta s$$

expt data shows focus control 250 x better than usual

Tracking in 3-D

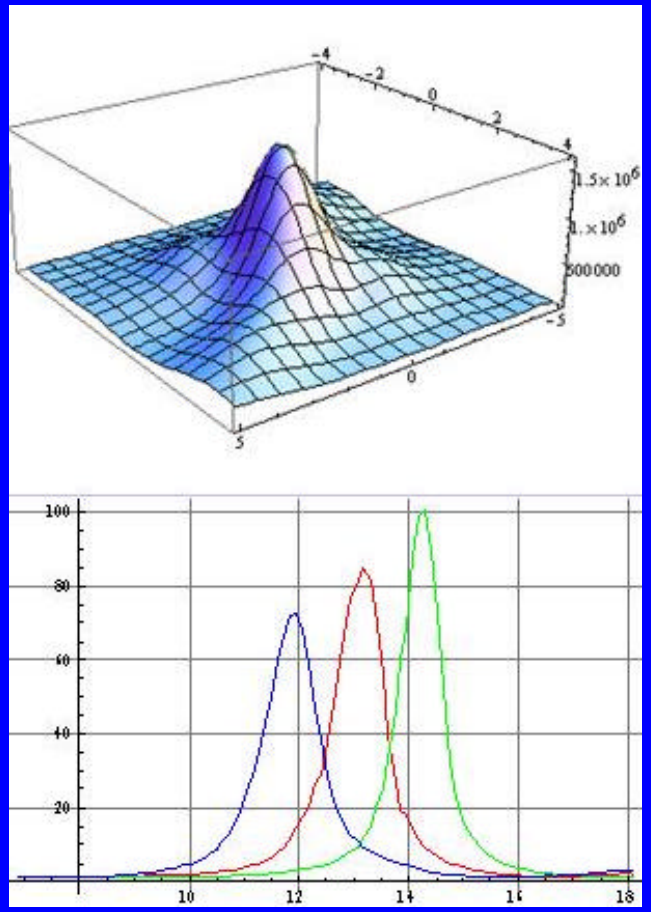
- Beam divergence from source depends on optical aperture
- Defocused image on non-source planes reduces intensity…
- …thus to a reduction in sharpness



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

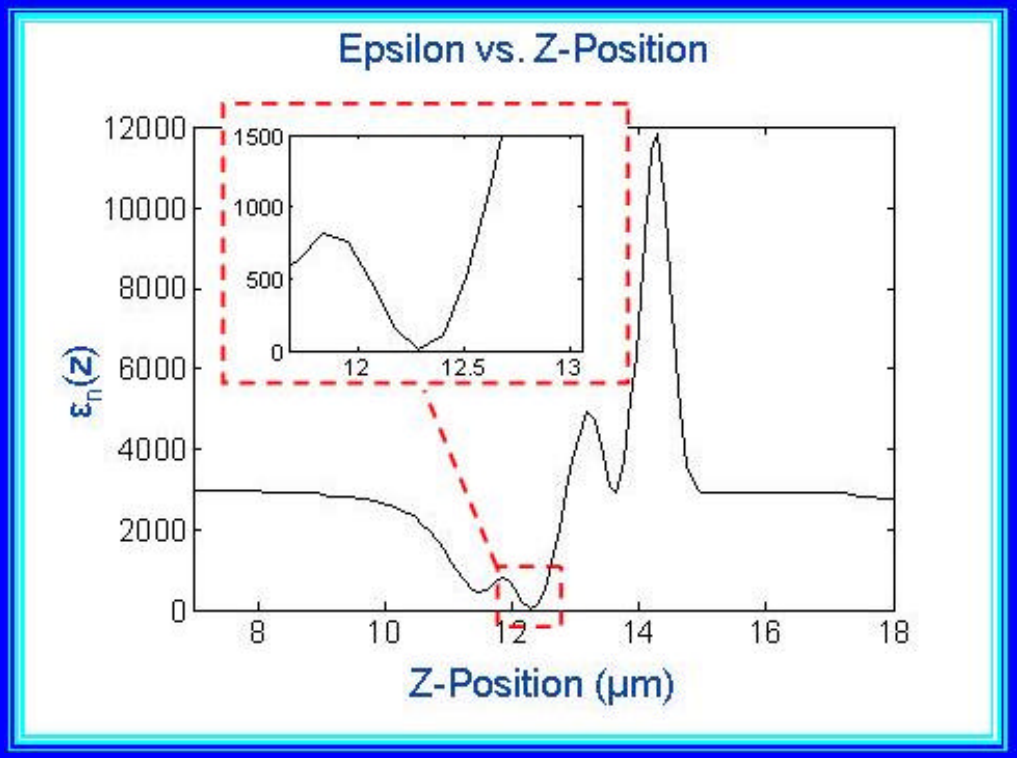


Ranging in Depth with Sharpness

- Depth resolution (<50nm) using least squares

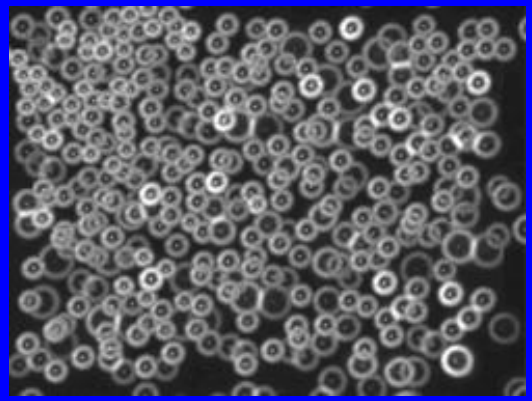
$$\varepsilon(z) = \sum_{j=1}^{3} \left| S_j(z) - M_j \right|^2$$

- Likelihood-based analysis looks very promising

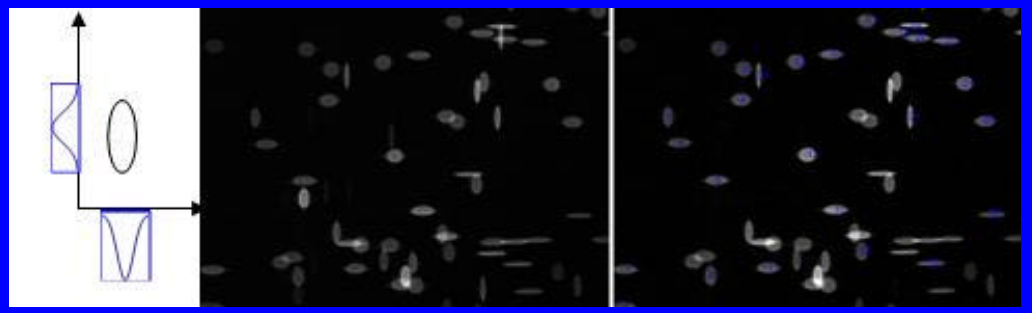


Ranging in Depth – Other Approaches

- Conical illumination allows greater particle density
  - ~450 particles in 410x310x120µm vol allows ~±180nm
- Anamorphic optics allows use of wavefront sensing or sharpness



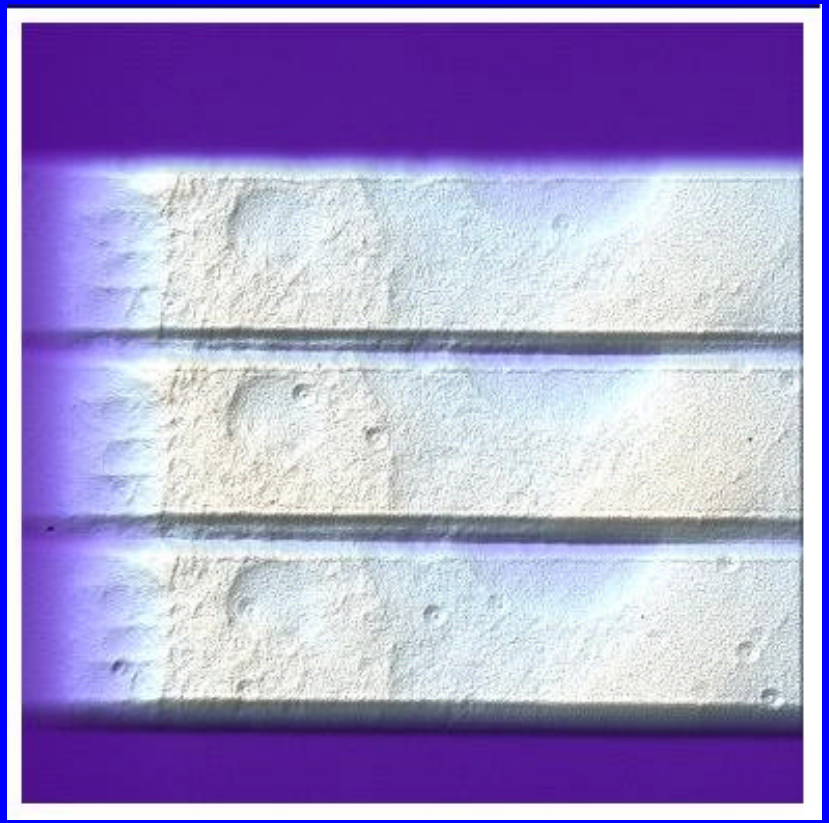
Lin D et al., Optics Lett **33**(2008)907-907
(also Virtual Journal of Biomedical Optics)



Future work

Sensitivity in fluorescence imaging

- EB1 maging micro-tubule dynamics is background limited
- Oocytes of drosophila using epifluorescence
- SNR very low
- Need to identify and track growth of microtubule ends



With Davis, Oxford – time-averaged image

Future Developments

- Reconstruct 3-D specimen using consistency in deconvolution
- Need to know OTF for the imaging situation (coherence/optics/sampling)
- CLEAN-type deconvolution looks promising

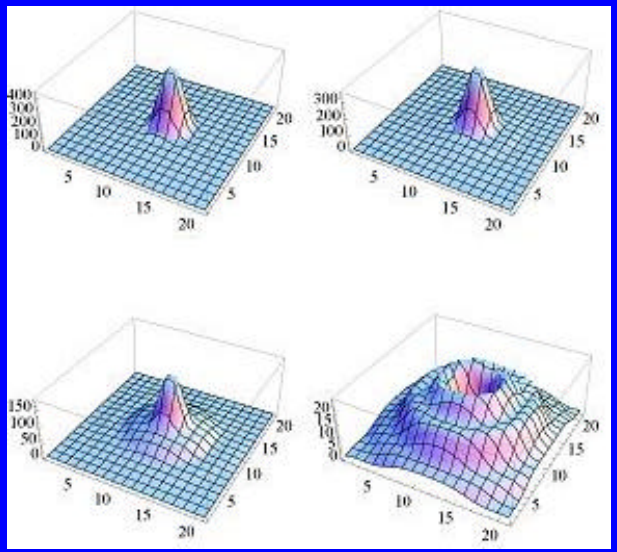
Fluorescence Imaging

- Expect spherical aberration from objectives
- Re-design gratings to correct all depth-dependent aberrations

- Consequent contrast loss
  - reduced SNR
- Improve Strehl ratio
  - Improves background-limited SNR
  - Relaxes focus requirements

Fluorescence Imaging

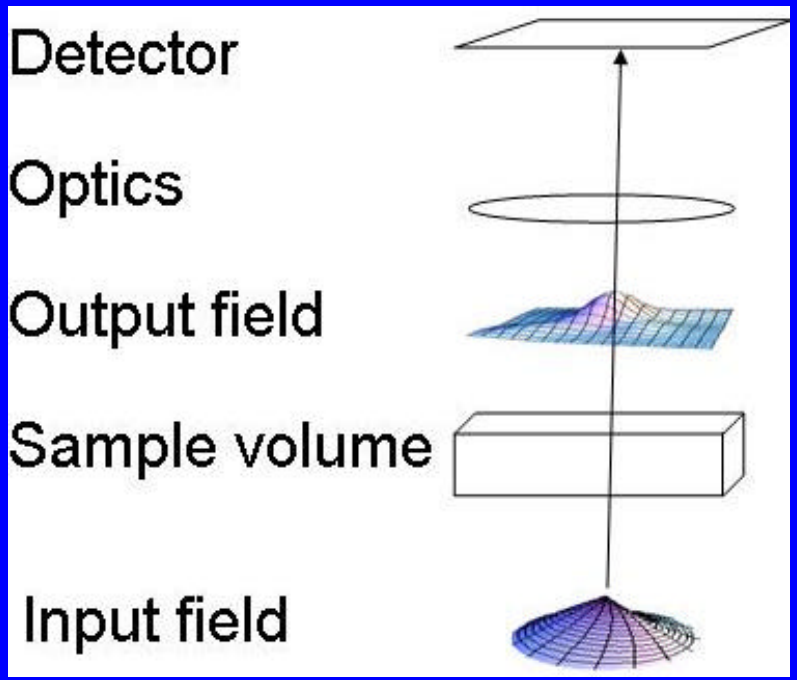
- Grating divides flux
  - 3 z-planes
- Signal strength is focus dependent



- SNR reduced by v3
- High Strehl gives good images in all z-planes
- Can easily recover more than v3 in multiple z-planes

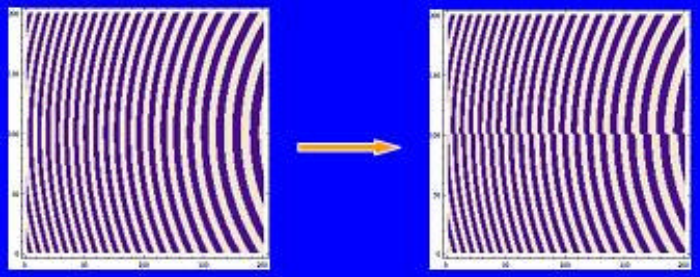
Future Microscopy Work

- Optics do analogue computations
- Use models to propagation within specimen volume
  - Model scattering, fluorescence etc
- How close can we get to this ideal?



Future Microscopy Work

- Start with phase reconstruction



3-D imaging with Hilbert transform

- Compensation of over-lying specimen

- Quantitative phase microscopy
  - Using differential phase diversity
  - Measure deposition/ thickness of materials
- Tracking vesicles close to the cell membrane
- Tomographic reconstruction

Summary

- Some issues (e.g. efficiency, field, flux…) to be resolved - application driven
- 3-dimensional imaging
  - using image slices tiled on single detector
- 3-dimensional imaging
  - tracking small number of particles in 3-D with high accuracy and high speed
- Collaboration with bio-medical groups for whom such techniques are beneficial

Other possibilities...

- Instead of grating use programmable lens
  - Wide range of object depths brought to focus electronically at same magnification
  - Thus fast scan with constant magnification is possible
  - Use LC lens
- Use wavefront sensing for sub depth of field positioning