Tracking cells and particles in 3D using image sharpness

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Authorship

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– 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
3-D Snapshot Imaging

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

Combination optical system

\[ f_c = \frac{f_\ell m f_g}{f_\ell + m f_g - s} \quad \text{and} \quad p_1 = \frac{sf_\ell}{f_\ell + m f_g - s} \quad \text{and} \quad p_2 = \frac{s(f_\ell - s)}{f_\ell + m f_g - s} \]


\[ s = f_\ell \Rightarrow f_c = f_\ell \quad \text{and} \quad p_1 = \frac{f_\ell^2}{m f_g} \quad \text{and} \quad p_2 = 0 \]
Telecentricity

Telecentric performance

\[ s = f' \]

Lens to Off-Axis Fresnel Lens Separation (m)

Magnification

\[ m_{\text{theoretical}} \quad m_{\text{theoretical}} \quad m_{\text{measured data}} \]
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

-1 order in focus

0th order in focus

+1 order in focus

Image Resolution:

No grating = 233nm

With grating = 226nm and 231nm (for 0th and ±1 orders)
• 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...

Image Sharpness vs. Nanohole Displacement

Solution:
QD grating method gives 3 simultaneous image sharpness (one from each order) for each particle.
Unique depth indication…

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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

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

- Likelihood-based analysis is better...
ML depth estimator

Width = 6.7μm, Std. Dev. = 12.5nm

Width = 2.6μm, Std. Dev. = 8.1nm
Future work

• Analysis of photon statistics

• Assess real-time, but one-off, compensation of SA

• Polarisation sensitivity

➢ Sensitivity to photon noise contamination

➢ Improve SNR

➢ Optimised correction for central image

➢ Correct differential SA in grating

➢ 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

• Anamorphic optics allows use of wavefront sensing or sharpness

(also Virtual Journal of Biomedical Optics)