Four Dimensional Imaging and Particle Tracking for Biological Applications and Beyond…

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Background

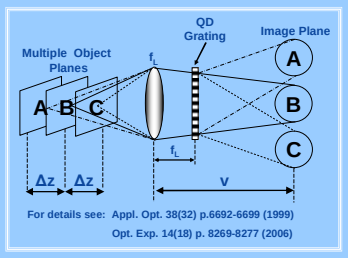

Figure 1: Multi-plane imaging by distorted diffraction grating

- **QD Grating** – Quadratically Distorted (QD) gratings (Figure 2) behave like a lens with a different focal length in each diffraction order.
- Combined with a lens, and fixed camera distance 'v' (Figure 1) different object planes are focused in each order.
- Separating the lens and grating by f_L ensures telecentricity (equal magnification in each image).
- Binary level phase gratings are used for their high photometric efficiency. Typically 84% of the incident light is diffracted into the first 3 orders (-1, 0, +1)

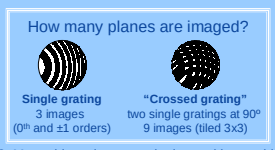

Figure 2: More object planes can be imaged by combining two (or more) QD gratings

Biological Image Gallery

Arabidopsis Mutant Plant Cells

Collaboration with David Logan, St. Andrews University

- **Sample:** GFP present in cell mitochondria, no cell walls present due to mutation.
- **Illumination:** Bright field with 520 nm LED (upper image), Epi-Fluorescence with 470 nm Laser (lower image).
- **3D Depth Imaging:** 1 µm between each image plane.

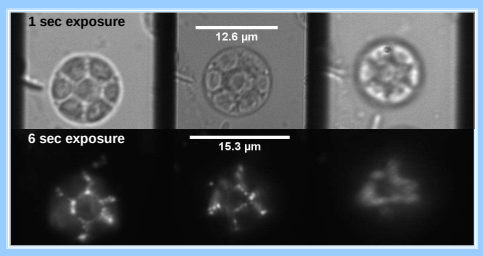

Figure 4: Bright field (upper image) and fluorescence (lower) images of mutant Arabidopsis cells on three depth planes.

Drosophila (Fruit Fly) Ovary

Collaboration with Ilan Davis, Oxford University

- **Sample:** GFP present in Drosophila Ovary.
- **Illumination:** Epi-Fluorescence (with GFP filter, 470nm).
- **3D Depth Imaging:** 7.3 µm between each image plane.

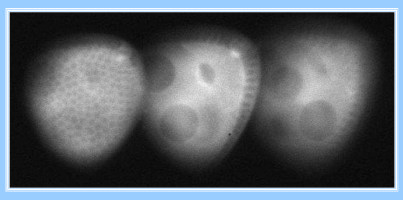

Figure 5: Partial view of a stage 8 Drosophila egg chamber. Left-most image: the small dark spheres in focus are the follicle cells, in the central image the oocyte is in the focus. The large dark spheres are two nurse cell nuclei.

Cell Division in HeLa (Cancer) Cells

Collaboration with Viki Allan, Manchester University

- **Sample:** Mitotic live HeLa (human cancer) cells.
- **Illumination:** DIC imaging with a 525nm LED.
- **3D Depth Imaging:** 1.63 µm between each image plane.

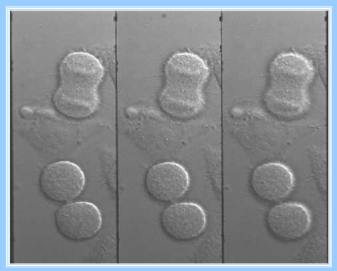

Figure 6: Mitotic HeLa cells. The upper cell is in the process of dividing, the lower cell pair has just finished. In the upper cell chromosomes (horizontal structures) are shown and in the lower cells vesicles (small bright spots) can be seen.

Programmed Cell Death in HeLa (Cancer) Cells

Collaboration with Viki Allan, Manchester University

- **Sample:** Apoptotic HeLa (human cancer) cells.
- **Illumination:** DIC imaging with a 525nm LED.
- **3D Depth Imaging:** 0.81 µm between each image plane.

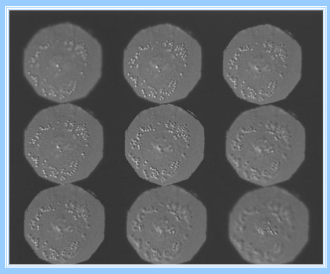

Figure 7: Vesicles within an apoptotic HeLa cell imaged on nine planes with crossed gratings. Reduced field of view has excluded the plasma membrane.

Particle Tracking

- **Experiment** – Nanohole 'fluorescent particles' (back-illuminated, 200nm dia.) mounted on precision piezo stages for true position measurement. Imaged at 100x, 4D system used to simultaneously image three depth planes.
- **Image Sharpness** – An image quality metric, maximized for an unaberrated (focused) image The three sharpness values obtained in each "snapshot" give unambiguous depth measurement (Figure 8).
- **MLE Algorithm** –The three sharpness measures in each image are combined into a maximum likelihood function that shows the most probable particle position.
- **Accuracy** – Comparing the MLE calculated depth position with the true position (from piezo stages). Over the focal volume of the grating (2.6µm) an average accuracy of 8.1nm is achieved (Figure 8).

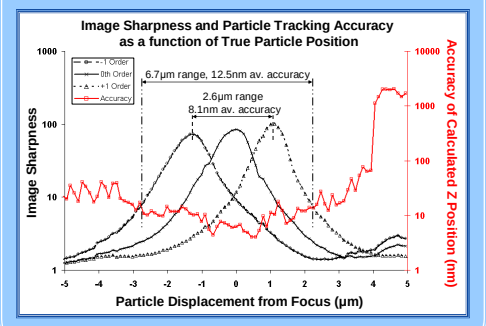

Figure 8: Image Sharpness for three simultaneously imaged depth planes (left axis). The MLE algorithm applied to this data provides unambiguous and accurate depth measurement (right axis).

Equipment Design

4D Imaging on a Commercial Microscope

Features –

- 1:1 relay lens setup does not alter image properties.
- Range of apertures used to limit Field Of View (FOV):
  - Rectangular apertures limit FOV in one axis for 3 plane imaging. Square apertures used for 9 planes.
  - Slider mount for interchangeability, or total removal.
- Grating:
  - Period matched to aperture size, maximizes image FOV.
  - Amount of QD combined with relay lens focal length and objective magnification determines Δz (see Figure 1).
  - Etch depth ensures balance of intensity in each image.

Benefits –

- Built from readily available, inexpensive components.
- QD gratings are easy to design, and customizable.
- System is compact and robust, suitable for inverted and upright microscopes.
- Suitable for any imaging mode - successfully tested with Bright and Dark field, Fluorescence, DIC & Phase Contrast.

Collaboration – Several 4D systems have been provided to Biology groups in the UK (see Image Gallery and our website for further details…).

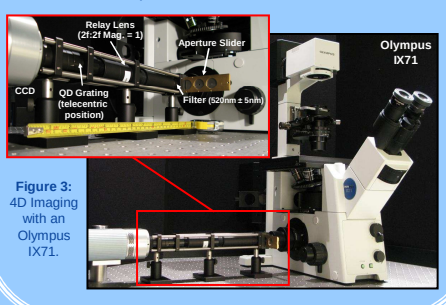

Figure 3: 4D Imaging with an Olympus IX71.

Future Work

Broadband Telecentric 4D Imaging

- **Problem** - Grating diffraction angle is wavelength dependent, causing lateral smearing of the non-zero order images for non-monochromatic input light.
- **Current Solution** – Use a narrow band filter (Figure 3). Photometrically inefficient!
- **Broadband Correction** – Broadband 3D imaging with white light illumination has been demonstrated previously (Opt.Comm **183**(1) p.29-36 (2000)).
- Blazed gratings disperse and re-collimate the input light across the QD grating whose period varies with width (Figure 2) thus preserving the diffraction angle for all wavelengths.
- **But…** this system was not telecentric.
- **The Next Step –** Development of a telecentric broadband 4D imaging system has begun (Figure 9).

And Beyond…

- Combining 4D imaging and particle tracking in applications from sperm motility studies to fluid flow in combustion engines. See our website for more!
- Incorporating spherical aberration correction into the QD grating design.

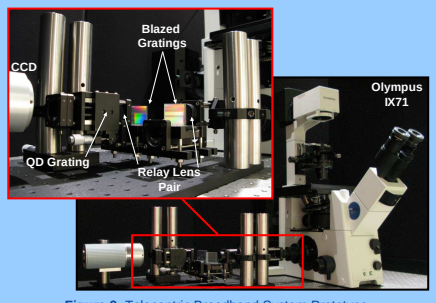

Figure 9: Telecentric Broadband System Prototype

Visit our group website for more details and our latest results: **http://waf.eps.hw.ac.uk** or email us: **waf@hw.ac.uk**