Towards four dimensional particle tracking for biological applications

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Background and Experimental Details

Distorted Diffraction Gratings give Imaging in 3D:

A weak, off-axis Fresnel lens combined with a conventional lens creates an imaging device that delivers simultaneous in-focus images of multiple object depths (see fig.1).

How does it work?

◆ Quadratic curvature of the grating rulings imposes a defocus detour phase on any incident wave-front → grating acts as a lens with a different focal length in each order.

◆ For constant image distance (v) the in-focus image in each order corresponds to a different object depth. Object plane separation (Δz) is determined by the curvature of the grating.

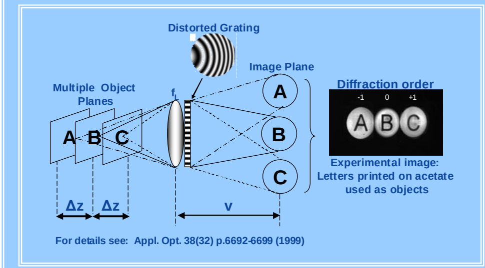

Figure 1: Multi-plane imaging with a distorted diffraction grating

◆ Phase gratings: optically more efficient. Etch depth sets energy balance of the diffraction orders.

◆ For telecentric imaging (equal magnification in ±1 orders) the lens – distorted grating separation should be 0 or f_L (Opt. Express **14**(18) pp.8269-8277 (2006)).

3D Imaging in Microscopy:

◆ A high magnification microscope delivers an image of the sample to an optically accessible plane (iris plane in fig.2). The grating-relay lens combination images multiple object planes either side of the magnified image plane.

◆ Sample: 215 nm diameter holes ("nanoholes") in an metal mask are illuminated from below, creating perfect point sources, simulating fluorescently tagged bio-particles.

◆ High precision piezo positioners are used to move the nanoholes in X,Y and Z. This position information allows experimental verification of our particle tracking algorithm.

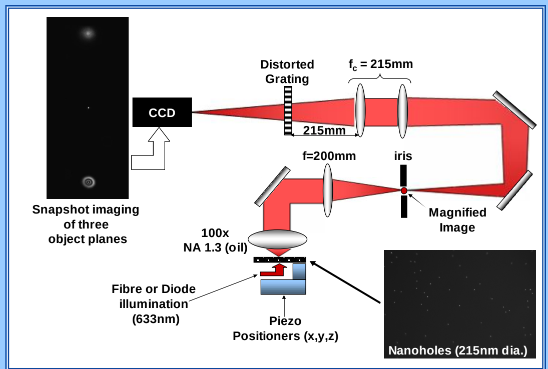

Figure 2: Schematic of the 4D Particle Tracking System

Particle Tracking at the Nano-Scale

Depth Imaging – The Z-Series:

A Z-series is a sequence of optical sections collected at different levels perpendicular to the optical axis (the Z-axis) within a specimen (see fig.3).

◆ Usually captured as a sequential series of images → inherent time delay.

◆ **Advantage of the grating method :** Simultaneous capture of images on 3 (or more) planes facilitates rapid Z-series acquisition to study fast dynamic processes. Also less likely to "lose" tracked particles during imaging (if they move away from one measurement plane they will appear in another).

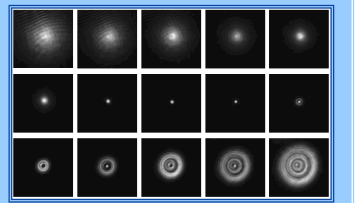

Figure 3: A through focal Z-series of a single nanohole

3D Position Measurement using the Z-Series:

◆ The centroid of each image is used to find the position in X and Y.

◆ The particle's position in Z is calculated using an Image Sharpness measurement.

What is Image Sharpness and how is it calculated?

◆ Image Sharpness - measure of image quality, dependent on the degree of aberration (e.g. defocus) in the image, maximised for a focussed image.

◆ The Image Sharpness is the integrated area under the Modulation Transfer Function squared (MTF²) of a given image.

◆ Fig. 4 shows the area under the MTF decreasing as the level of defocus aberration on the wave-front is increased (→ Image Sharpness decreases too).

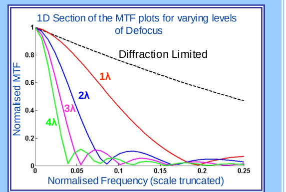

Figure 4: Area under the MTF decreases as wave-front aberration increases

◆ The Image Sharpness can also be calculated by integrating the square of the original intensity image.

Image Sharpness as a function of particle position – Experimental Results:

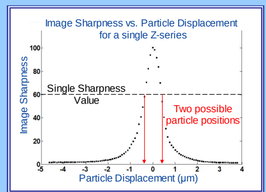

Figure 5: Image Sharpness vs. Particle Displacement for a single Z-series (no grating) - gives ambiguous result.

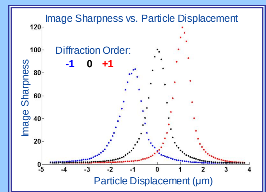

Figure 6: Image Sharpness vs. Particle Displacement - tracking a single nanohole on 3 planes with the grating

◆ The width, height and shape of the Image Sharpness curve produced by a Z-series depends on the aberrations present in the system (see figs 5 & 6).

◆ **Problem:** Fig. 5 shows that for a single Z-series the Image Sharpness gives an ambiguous measure of the particle's Z-position.

◆ **Solution:** The grating provides 3 images at each position. Comparing the calculated Image Sharpness for the image in each diffraction order gives an unambiguous Z-position measurement (fig. 6).

Accuracy : We have demonstrated single particle tracking (of a nanohole) through a 50x50x6 μm volume with a repeatability accuracy of better than 50nm in each direction.

Live Cell Imaging and Beyond!

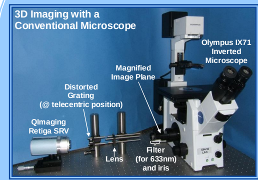

Figure 7: 3D imaging with a standard commercial microscope (microscope courtesy of Prof. Ilan Davis, Oxford University).

◆ **Application** - Multi-plane imaging allows fast capture of Z-series to study dynamic processes *in vivo.*

◆ **Benefit –** Our 3D imaging system is easy to incorporate into existing systems. It is unobtrusive, inexpensive and uses well established imaging techniques.

◆ **Demonstration** - Our system is added onto a commercial microscope (see fig. 7).

◆ **Application** - Multi-plane imaging facilitates multiple target particle tracking for Particle Imaging Velocimetry.

◆ **Benefit** - Potential 30% saving in fuel consumption in combustion engines through improved fuel mix.

◆ **Demonstration** - Initial experiments mapping the trajectory of a fluorescent bead provide 3D resolution from a single viewpoint that matches state-of-the-art accuracy (fig. 8).

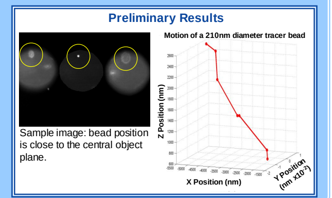

Figure 8: Mapping the trajectory of a fluorescent bead.

Future Work

Particle Tracking:

◆ Fluorescent particle tracking at Brownian motion speeds (potential application to virus tracking and cell dynamics).

Phase Contrast Imaging – Benefits:

◆ Transmitted light technique - useful for imaging whole cells, no addition of potentially damaging contrast agents/dyes.

◆ Multi-plane imaging can be used to observe rapid changes in cell shape (e.g. during mitosis).

◆ **Demonstration** – Fig. 9 shows 3D phase contrast images captured using the system shown in fig. 7, with a 100x phase contrast objective (equipment courtesy of Dr Viki Allan, University of Manchester).

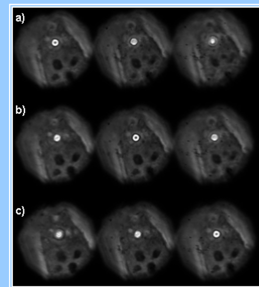

Figure 9: 3D Phase contrast images of a 2μm diameter polymer bead phagocytosed into a Chinese Hamster Ovarian fibroblast cell. **a-c** bead is brought to focus in the -1, 0 and +1 orders sequentially. (sample courtesy of Dr. Lynn Paterson, Heriot-Watt University).

Extension to Wave-front Sensing – Benefits:

◆ Only requires further analysis of the data, not additional equipment.

◆ Would provide information about system and specimen-induced aberrations.

◆ Characterisation and subsequent rejection of non-defocus aberrations will increase the accuracy of the Z-position measurement.

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