REFRACTOMETRY USING DIGITAL FOURIER MICROSCOPY

Katrina Seet, Paul Blazkiewicz, Robert Vogel, Simon Corrie, Timo Nieminen and Andrei Zvyagin

School of Physical Sciences
School of Information Technology and Electrical Engineering
University of Queensland, 4072, Australia
Email: seet@physics.uq.edu.au

Digital Fourier Microscopy (DFM) is an adaptation of the digital holography [1,2]. Comprehensive sample information is recorded with a single CCD frame at the output of the Fourier holography optical circuit. Individual angular spectra of sample constituents provide a basis for, firstly, efficient image processing to pick up small scatterers on the crowded background of the sample [1], and secondly, determination of refractive index of scatterers individually and in ensemble [2]. Finally, rapid 3D reconstruction in software allows true perception of the sample morphology.

In this paper, we report on the determination of refractive index and size of silica and polystyrene microspheres. An image of the sample containing microspheres was obtained by performing discrete Fourier transforms of the recorded hologram in software. The optical angular spectrum pattern and its profile, shown in Fig 1(a) and (b), were obtained by cropping a single sphere in the image (Fig (b) inset) and performing an inverse Fourier transform. Fitting the optical angular spectrum with that of calculated using Mie theory yielded sphere diameters provided their refractive index was specified by the manufacturers. To test the reliability of our method, we determined the sphere diameters and results are plotted as a size histogram, in Fig 1(c). The mean diameter of $2.00 \pm 0.10 \mu m$ agrees with the manufacturer’s value of $2.01 \pm 0.05 \mu m$.

The determination of microsphere sizes by DFM is envisaged very useful for the screening of bead-based assays. For such assays, microspheres are used as support profiles for biomolecules, such as DNA or peptides, and are screened in high throughput instruments such as flow cytometers. The precise characterisation of the spheres will enable the quantification of fluorescence and scattering signals measured by flow cytometry [3].

Fig 1: (a) Optical scatter profile of 2 µm sphere (b) Radial profile of optical scatter pattern and sample image as inset (c) Size histogram

References