A FLUORESCENT CALIBRATION SPECIMEN FOR LIVE SAMPLES

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MICROSCOPE CALIBRATION: Fluorescence microscopy has yet to identify a universal standard that can provide an accurate measure of microscope performance. This is particularly true for water-immersed live samples. Fluorescent beads in agarose take a long time to prepare, tend to aggregate, are mobile in the gel and must be disposed of after a few days.

DIRECT LASER WRITING: By tightly focusing a pulsed infra-red laser source into a suitable polymer substrate, it is possible to write bright fluorescent patterns [1,2]. These patterns can be used to provide several measures of system performance: illumination uniformity, image distortion, detector uniformity, multiple channel alignment (Figure 1(A)). Moreover, by fabricating just above the material threshold it is possible to exploit the non-linear nature of the fabrication process to write sub-diffraction fluorescent features. These can be used instead of 100 nm beads to obtain a point spread function of the system. The points can be arranged in a grid to map changes in aberration over an image volume (Figure 1(B)) and has been demonstrated for a high index substrate (n=1.58).

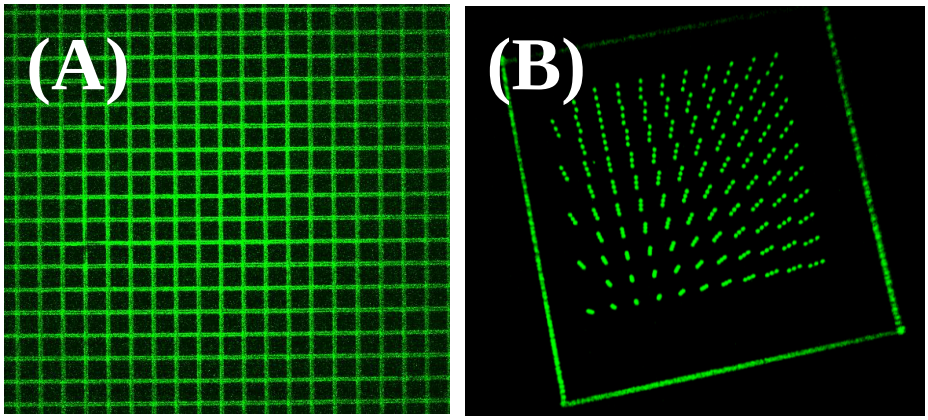

Figure 1: Fluorescence images of a grid pattern (A) and 3D point array (B) in a polymer substrate with refractive index of 1.34

AN INDEX-MATCHED STANDARD: In this talk we will demonstrate how direct laser writing has been extended to the writing of calibration patterns in a polymer with an index of 1.34. This will enable the accurate calibration of microscopes that use water dipping lenses when imaging live samples and allow calibration errors to be corrected in post-processing.

REFERENCES:

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