A FLUORESCENT CALIBRATION SPECIMEN FOR THE CHARACTERISATION OF OPTICAL MICROSCOPES

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BACKGROUND: Fluorescence microscopy is an essential tool in nearly all fields of scientific discovery. It is therefore all the more surprising to find that there are no widely adopted standards for the calibration of fluorescent microscopes. Calibration provides a wide range of information relating to microscope performance. Without calibration, images taken on two separate microscopes cannot be directly compared as they may have differing magnifications, illumination intensities or detector sensitivities. As the range of microscopy techniques capturing 3D information continues to increase [1–3], the need for standardization becomes ever greater.

THE PROBLEM: Widely used methods for determining microscope performance are currently limited to basic techniques such as fluorescent beads, which don't form a regularly spaced pattern and reflective etched gratings, which are limited to being two-dimensional and require changes to the microscope filter sets.

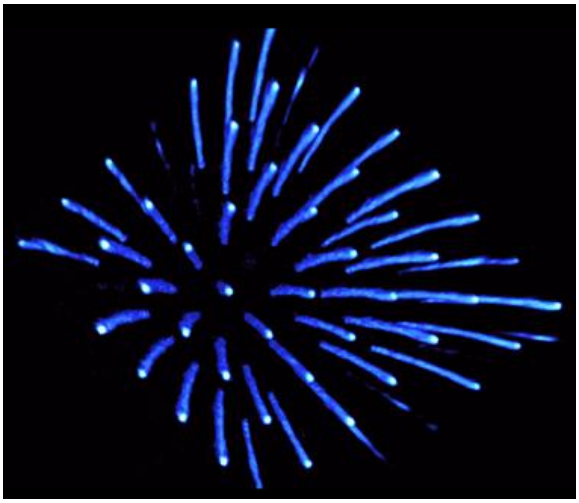

Figure 1: Rendering of raw data taken from a fluorescent calibration slide.

THE SOLUTION: Laser processing of plastic substrates has shown that it is possible to generate bright fluorescent patterns which meet many of the criteria needed to develop a fluorescent calibration standard in optical microscopy. The new standard will be able to quantify a range of parameters that determine microscope performance. For example, spatial distortions within the field of view can be quantified by a regular array of bright fluorescent points (Figure 1). Other patterns can determine factors such as detector linearity, field flatness and changes in the point spread function across the field of view and over depth.

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