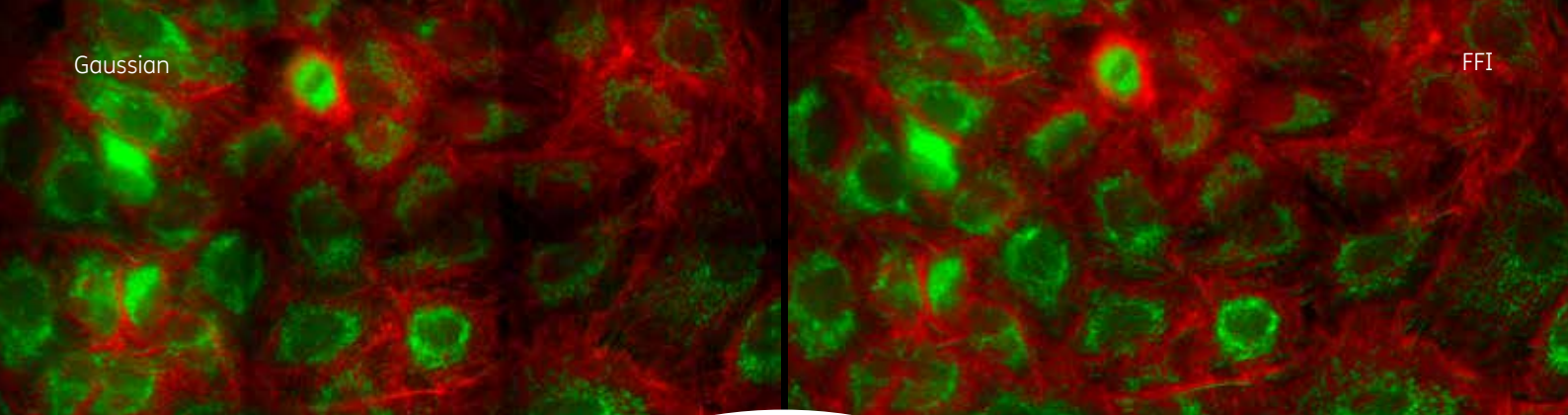


asphericon

Flat-field illumination for quantitative fluorescence imaging

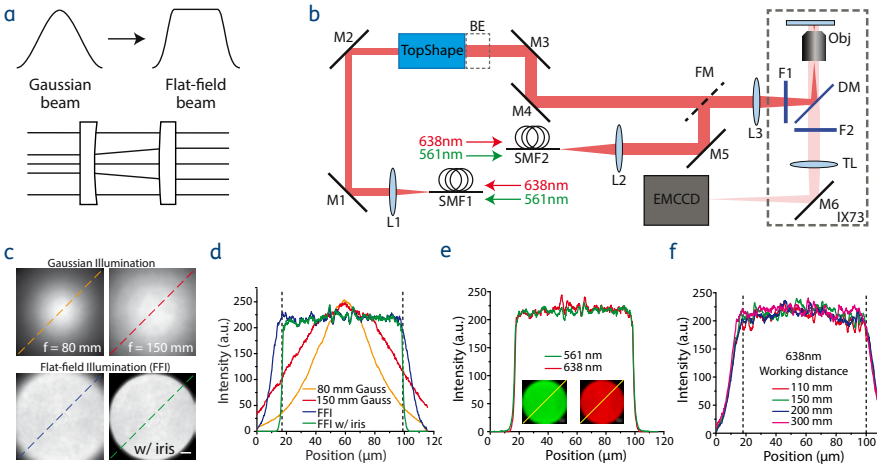
Project description:

The uneven illumination of Gaussian profiles makes quantitative analysis in laser-based wide-field fluorescence microscopy highly challenging. Many factors including light sources and illumination optics contribute to the uniformity. These characteristics are particularly difficult where a large FOV on the scale of several hundreds of microns or millimeters is desired. A grid of images is acquired such that the borders overlap and following the images are stitched together in post-processing. If the illumination is not uniform, the final stitched image has dimmed borders around each individual image. Therefore, measurements in cell and tissue samples are unreliable. Another disadvantage of non-uniform illumination is the uneven activation of molecules. Those being closest to the center of the beam fluoresced more strongly than those near the periphery.

Project realisation:

A research team from the College of Optics and Photonics, University of Central Florida in Orlando, FL/USA, could overcome these problems while using asphericons TopShape and BeamExpander in their microscopy setup (b), both very compact and high-precision refractive optical components consisting of aspheres. Thus, they were able to present flat-field illumination (FFI), where the Gaussian beam was shaped into a uniform flat-top profile (a). The beam shaping device is extremely tolerant to variations in size of the incoming laser beam by accepting ± 10%, while being achromatic (e) as well. The long working distance (f) and high spatial coherence of FFI allowed to accomplish uniform epi[1] and TIRF[2] illumination for multi-color single-molecule imaging. The unbeatable optical performance of the used optical devices, with a homogeneity > 95%, allows uniform illumination (c & d) and thus an even activation of molecules. In addition, FFI enabled borderless stitched imaging with minimal image overlap (5%).

Experimental characterization of FFI



Figures:

(a) Beam shaping schematic.

(b) Experimental setup. FFI was expanded 1.5x by using the BeamExpander behind the TopShape to provide a full FOV illumination to the sample.

(c) Beam profiles of Gaussian beams collimated by a lens with 80 mm or 150 mm focal length and FFI beams without and with an iris.

(d) Cross-sections taken from beam profiles in (c) along dashed lines. Vertical dashedlines in (d) indicate detection region of camera.

(e) Excitation wavelength dependence of FFI. Cross-sections taken from multicolor images (inserts) with an iris.

(f) Working distance dependence of FFI with 638 nm laser.

Explanations: [1] Epi-illumination mode means illumination and detection from one side of the sample; [2] TIRF is the abbr. for total internal reflection fluorescence

Image and text reference: I. Khaw, B. Croop, J. Tang, A. Moehl, U. Fuchs, K. Y. Han: „Flat-field illumination for quantitative fluorescence imaging“, In: OPTICS EXPRESS, Vol. 26, No. 12, 11 Jun 2018, pp. 15276-15288

asphericon GmbH | Stockholmer Straße 9 | 07747 Jena | Germany | Phone: +49(0)3641-3100 500 | Fax: +49(0)3641-3100 501 | Email: sales@asphericon.com
www.asphericon.com