DESIGN, DEVELOPMENT AND DEPLOYMENT OF REAL-TIME HOLOGRAPHIC PHOTO-MANIPULATION CAPABLE HIGH-SPEED CONFOCAL MICROSCOPY

Ahsan M. Memon[1,2], Henry De Belly[3], Ruby Peters[2], Sohaib Abdul Rehman[4], Martin Lenz[1,2], Leila Muresan[1,2], Philip H. Jones[5], Guillaume Salbreux[6], Ewa K. Paluch[2], Kevin O'Holleran[1,2]
[1]Cambridge Advanced Imaging Centre, University of Cambridge, Cambridge, UK
[2]Department of Physiology, Development & Neuroscience, University of Cambridge, UK
[3]Cardiovascular Research Institute, University of California San Francisco, US
[4]Department of Molecular and Cellular Biology, Harvard University, US
[5]Department of Physics & Astronomy, University College London, UK
[6]The Theoretical Physics of Biology Laboratory, The Francis Crick Institute, UK
Downing Site, Cambridge, CB2 3DY, United Kingdom
Email: amm277@cam.ac.uk (A.M.M.)

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ABSTRACT

Microscopic imaging of rapid biophysical processes often relies on high contrast, high resolution, and high-speed acquisition. However, confocal microscopes capable of such imaging lack the capacity to manipulate the sample or its surrounding environment it in real time [1]. As a result, the possibility to non-invasively initiate, alter or halt a biochemical process during imaging is restricted. To overcome this limitation, we have established a versatile optical system equipped with multiple photo-perturbation techniques including photo-manipulation, photo-activation, photo-ablation, optical-trapping and optogenetics, combined with confocal imaging and the capacity for structured illumination super-resolution microscopy. This hybrid system is comprised of a spinning disk confocal unit, a spatial light modulator and a digital micromirror device, and is able to accurately elucidate the dynamics of molecules, precisely measure local forces, and re-localise or switch molecular behaviour with high specificity. Here, we present the first application of this hybrid system to the study of cell shape regulation and the role of effective membrane tension in resisting external deformational forces [2]. We demonstrate that by simultaneously trapping and unfolding the cell membrane, quantitatively imaging actin network dynamics and measuring cellular forces, allows for a multi-level understanding of how the interplay of membrane tension and actin dynamics governs cell shape. More generally, our findings have the potential to expand our understanding of how mechanical properties of the cell surface are locally and globally responsible in driving cell shape changes in physiological and disease conditions.

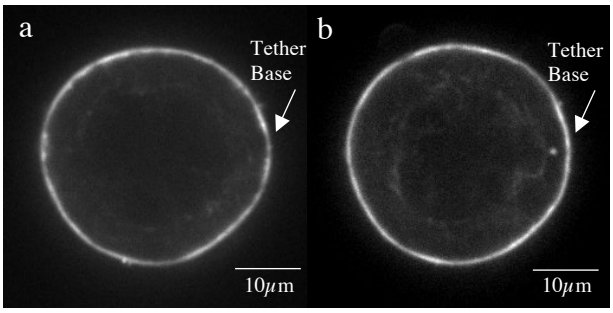

Figure 1: Live-cell confocal images of the actin cortex in mitotic HeLa cells before (a) and after (b) optical trapping.

REFERENCE

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