ASTROTIRF: total internal reflection fluorescence microscopy away from the coverslip

James D. Manton, Christopher J. Rowlands, Eric J. Rees, Clemens F. Kaminski
Department of Chemical Engineering & Biotechnology
University of Cambridge
Pembroke Street, Cambridge, CB2 3RA, United Kingdom
E-mail: ajdm2@cam.ac.uk

KEY WORDS: fluorescence microscopy, total internal reflection fluorescence, optical sectioning, three-dimensional imaging, super-resolution

High resolution is of critical importance in biological fluorescence microscopy, but fundamental optical principles limit confocal or multiphoton imaging to an axial resolution of ~600 nm. Total internal reflection fluorescence microscopy (TIRF) can be used to confine the excitation of fluorescence to a thinner region, typically around 100 nm, through the use of evanescent waves [1]. However, this thin excitation region is confined to the surface of the coverslip, preventing the observation of structure and dynamics deeper within the cell.

We propose a new method, Axial Section TRanslation Of TIRF (ASTROTIRF), to circumvent this limitation, allowing TIRF-like illumination of arbitrary sections without compromising axial resolution. By interfering multiple evanescent waves, we create excitation planes at depth, with minimal intensity at the coverslip surface. Manipulating the relative phases and amplitudes of illumination beams permits control of the axial location and extent of the illuminated plane, with ~50 nm thick (FWHM) sections being possible within a few hundred nanometres of the interface, and 200 nm-thick sections possible up to 1 micron away from the coverslip.

In contrast to other axially-resolved TIRF methods [2, 3], we do not need to illuminate the entire sample up to the desired depth, reducing phototoxicity and photobleaching. In addition, images are produced in real time without costly computational reconstruction, and there is no requirement to bleach successive sections. We will discuss the design parameters of ASTROTIRF, its implementation, and progress made towards imaging biologically-relevant samples.

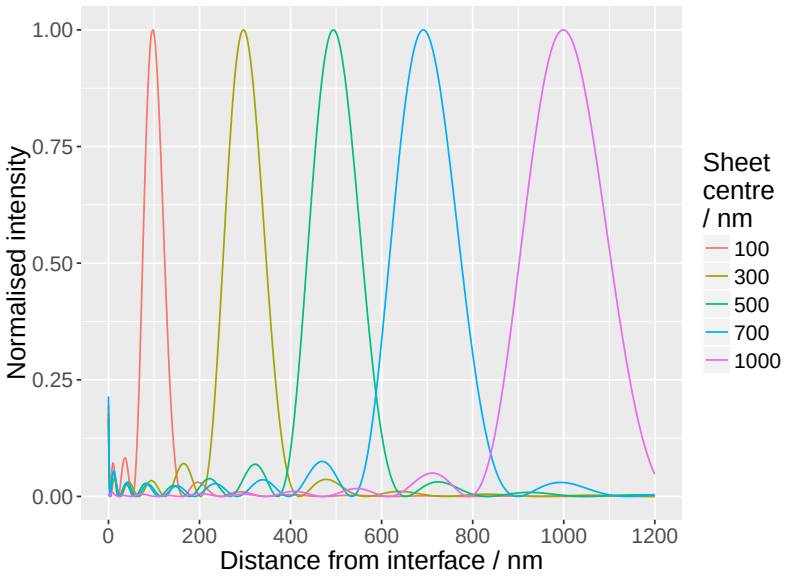

Figure 1: Gaussian-like axial sections of increasing depth and width generated through ASTROTIRF using 15 interfering evanescent waves of 488 nm wavelength.

[1] Axelrod, D, "Cell-substrate contacts illuminated by total internal reflection fluorescence", *J. Cell Biol*, **89**, 141 (1981).

[2] Boulanger, J, et al., "Fast high-resolution 3D total internal reflection fluorescence microscopy by incidence angle scanning and azimuthal averaging", *Proc Natl Acad Sci USA*, **111**, 17164–17169 (2014).

[3] Fu, Y, et al., "Axial superresolution via multiangle TIRF microscopy with sequential imaging and photobleaching", *Proc Natl Acad Sci USA*, **113**, 4368–4373 (2016).