NANOSCALE TOPOGRAPHY OF CELLS IN ADHESION REVEALED BY VARIABLE-ANGLE TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

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Total Internal Reflection Fluorescence (TIRF) microscopy is a widespread technique used to study cellular process occurring near the contact region with the glass substrate. In this field, determination of the accurate distance from the surface to the plasma membrane is crucial to investigate the physical basis of cellular adhesion process. However, quantitative interpretation of TIRF pictures regarding the distance z between a labeled membrane and the substrate is not trivial. Indeed, the contrast of TIRF images depends on several parameters more and less well known (local concentration of dyes, absorption cross section, angular emission pattern…). To get around this problem we propose to exploit a series of TIRF pictures recorded at different incident angles in evanescent regime. This technique called variable-angle TIRF microscopy (vaTIRF), allows mapping of the membrane-substrate separation distance with a nanometric axial resolution (≈10 nm) as shown in Figure 1 [1, 2]. vaTIRF is an old technique introduced in the mid-80s and quickly forgotten due to the high complexity of the first experimental setup. We propose an improved straightforward version of vaTIRF adapted to modern TIRF setup (no azimuthal scanning needed). In Comparison with usual single-molecule based super-resolution techniques (PALM, STORM...), cells do not need to be fixed in vaTIRFM. Thus, our technique can be employed for real-time observations, which is important for any relevant investigations about adhesion. Therefore, we demonstrate that vaTIRFM is useful not only to quantify the adhesion of living cells on various substrates, but also to probe membrane-surface interactions.

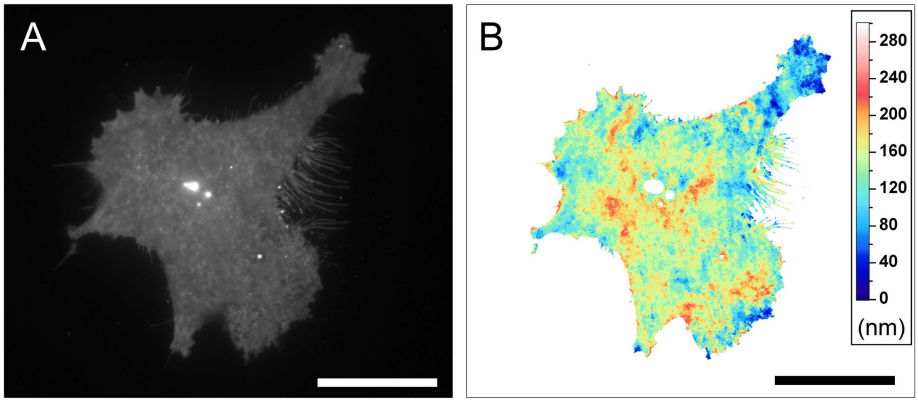

Figure 1: MDA-MB-231 cell in adhesion on fibronectin. The plasma membrane was labeled with DiO. (A): TIRF image, (B): corresponding cell topography (bar = 20 μm).

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[2] M. Cardoso Dos Santos, R. Déturche, C. Vézy and R. Jaffiol, "Topography of Cells Revealed by Variable-Angle TIRF Microscopy,"*Biophysical Journal*, **111**, 1316-1327 (2016).