

# VARIABLE-ANGLE TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY: DELVING INTO SINGLE CELL ADHESION

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Understanding how cells interact with surfaces has been considerably facilitated by innovative super-resolution strategies. Achieving a nanometric axial resolution is the keystone to decipher adhesion and migration processes. For this purpose, we propose a straightforward version of variable-angle Total Internal Reflection Fluorescence Microscopy (va-TIRFM) [1,2]. This prismless technique involves the recording of several TIRF images at different incident angles. A simple image processing can then be used to restore the cell topography with a nanometric axial resolution (10-20 nm), i.e. a map of the distance between the stained membrane and the substrate, as illustrated in Figure 1. Moreover, we can extract the effective refractive index distribution, which includes the combined influence of the water gap thickness and cytoplasmic refractive index. By combining the cell membrane topography with effective refractive index distribution, we have demonstrated that we are able to observe focal adhesion distribution without any specific protein labeling, as illustrated in Figure 1.

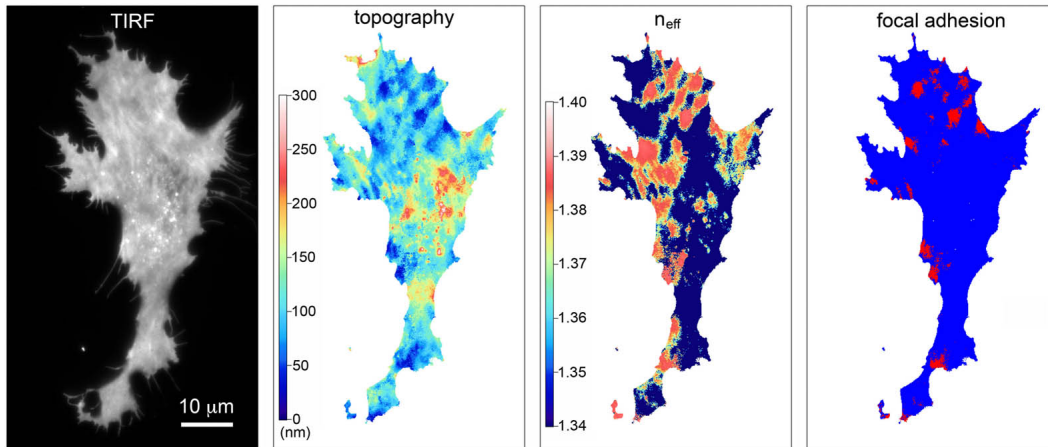


Figure 1: U87 MG cell in adhesion on fibronectin. The plasma membrane was labeled with DiO. From left to right: TIRF image, and corresponding cell topography, effective refractive index distribution, focal adhesion distribution.

[1] M. Cardoso Dos Santos, R. D eturche, C. V zy and R. Jaffiol, "Topography of Cells Revealed by Variable-Angle TIRF Microscopy," *Biophysical Journal*, **111**, 1316-1327 (2016).

[2] M. Cardoso Dos Santos, C. V zy, H. Morjani and R. Jaffiol, "Single cell adhesion strength assessed with variable-angle total internal reflection fluorescence microscopy," *AIMS Biophysics*, **4**, 438-450 (2017).