A COMBINED PALM-SOFI APPROACH FOR LIVE CELL IMAGING OF FOCAL ADHESIONS

Hendrik Deschout[1], Tomas Lukes[1,2], Azat Sharipov[1], Daniel Szlag[1], Lely Feletti[1], Wim Vandenberg[3], Peter Dedecker[3], Johan Hofkens[3], Marcel Leutenegger[4], Theo Lasser[1], Aleksandra Radenovic[1]

[1]Lab. of Nanoscale Biology & Lab. d'Optique Biomédicale, EPFL, Switzerland
[2]Dep. of Radioelectronics, Czech Technical University in Prague, Czech Republic
[3]Dep. of Chemistry, University of Leuven, Belgium
[4]Dep. of NanoBioPhotonics, MPI for Biophysical Chemistry, Germany
E-mail : aleksandra.radenovic@epfl.ch

KEY WORDS: PALM, SOFI, focal adhesion, live cell imaging

1. INTRODUCTION

Focal adhesions are complicated assemblies of hundreds of proteins that allow cells to sense and to adhere to the extracellular matrix [1]. Although most focal adhesion proteins have been identified, the spatial organization and dynamics of focal adhesions remains challenging to observe. In order to address this problem, we merged photo-activated localization microscopy (PALM) with super-resolution optical fluctuation imaging (SOFI) by applying both techniques to the same data [2].

2. RESULTS

We investigated the complementarity between PALM and SOFI, using simulations and experimental data of focal adhesions in fixed cells, see Figure 1. The image quality of both techniques was assessed with a methodology that integrates a resolution and a signal-to-noise metric. We also applied our PALM–SOFI concept as a quantitative imaging framework, allowing exploration of focal adhesions through the estimation of molecular parameters such as the fluorophore density. We demonstrated our PALM–SOFI framework by imaging focal adhesions in a living cell with a temporal resolution of 10 s. This allowed us to visualize focal adhesion dynamics, revealing a mean velocity of 190 nm/min.

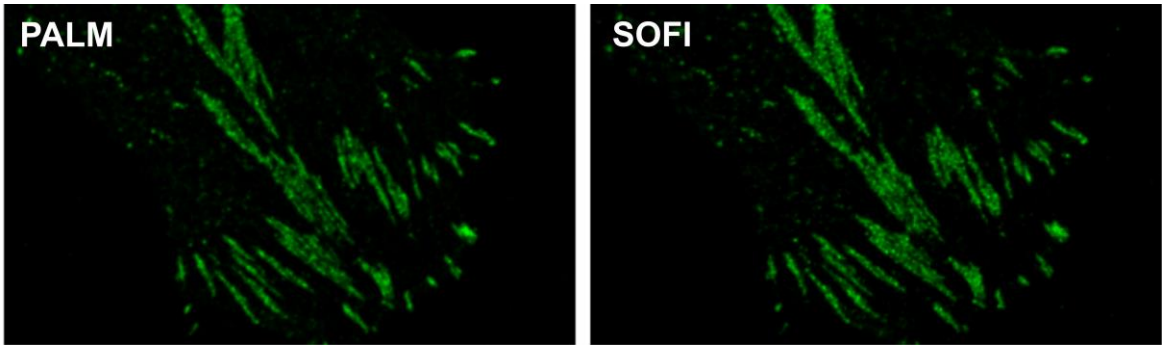

Figure 1 : PALM and SOFI image of a cell expressing paxillin labelled with psCFP2.

[1] B. Geiger, J.P. Spatz, and A.D. Bershadsky, "Environmental sensing through focal adhesions," *Nat. Rev. Mol. Cell Bio.*, **10**, 21-33 (2009).
[2] H. Deschout, T. Lukes, A. Sharipov, D. Szlag, L. Feletti, W. Vandenberg, P. Dedecker, J. Hofkens, M. Leutenegger, T. Lasser, and A. Radenovic, "Complementarity of PALM and SOFI for super-resolution live-cell imaging of focal adhesions," *Nat. Commun.*, **7**, 13693 (2016).