A platform for 3D tracking of single molecules in living cells

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KEY WORDS: 3D tracking, living cells, HILO microscopy, out-of-focus imaging.

In the last years, a wide range of microscopy techniques able to localize fluorescent dyes with a few nanometers accuracy have been developed, opening new avenues for super-resolution techniques such as STORM and PALM. Despite their power in pushing for higher spatial resolution all these techniques show some limitations when applied to the study of proteins inside a living cell. Some are limited by the small penetration depth of the fluorescence excitation (TIRF configuration), some other by the low temporal resolution, while looking at both diffusion and active transportation processes inside a cell requires three-dimensional localization over a few microns range, high SNR images and high temporal resolution (ms order of magnitude). We developed an apparatus which combines different microscopy techniques in order to satisfy all the technical requirements for a **nanometer accuracy 3D tracking of fluorescent single molecules inside living cells**. To account for the optical sectioning of thick samples we built up a HILO (Highly Inclined and Laminated Optical sheet) microscopy system [1] through which we can excite the sample in a widefield (WF) configuration by a thin sheet of light that is able to follow the molecule up and down along the z axis spanning the entire thickness of the cell with a SNR much higher than traditional WF microscopy. Since protein dynamics inside a cell involve all three dimensions we included a method to measure the x, y, and z coordinates with nanometre accuracy, exploiting the properties of the point-spread-function of out-of-focus quantum dots bound to the protein of interest[2],[3],[4],[5]. Finally, a feedback system stabilizes the microscope from thermal drifts [6], assuring accurate localization during the entire duration of the experiment.

REFRENCES:

[1] M. Tokunaga, N Imamoto & K. Sakata-Sogawa," Highly inclined thin illumination enables clear single-molecule imaging in cells", *Nature Methods* 5, 159 - 161 (2008)

[2] Ahmet Yildiz, Paul R. Selvin, "Fluorescence Imaging with One Nanometer Accuracy: Application to Molecular Motors", *Acc. Chem. Res.*, 2005, *38* (7), pp 574–582

[3] M. Speidel, A. Jonáš and E.L. Florin, "Three-dimensional tracking of fluorescent nanoparticles with subnanometer precision by use of off-focus imaging", *Optics Letters, Vol. 28, Issue 2*, pp. 69-71 (2003)

[4] E. Toprak , H. Balci , B. H. Blehm and P. R. Selvin, "Three-Dimensional Particle Tracking via Bifocal Imaging", *Nano Lett.*, 2007, *7* (7), pp 2043–2045

[5] M. Capitanio, D. Maggi, F. Vanzi and F S Pavone, "FIONA in the trap: the advantages of combining optical tweezers and fluorescence", 2007 *J. Opt. A: Pure Appl. Opt.* 9 S157

[6] Vanzi F.' MECHANICAL STUDIES OF SINGLE RIBOSOME/mRNA COMPLEXES' PhD Thesis 2002