UNIVERSITÀ DEGLI STUDI
DI TRENTO

Dipartimento di Fisica

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Friday 22nd of March 2019 at 14.30
Polo Ferrari 1 - Room A211

"WAVEGUIDE-PAINT: AN OPEN PLATFORM FOR LARGE FIELD-OF-VIEW SUPER-RESOLUTION IMAGING"

Abstract:

Single molecule localization microscopies have been widely adopted due to their demonstrated performance, which made it possible to routinely probe specific protein structures with nanometric localization precision. Yet, common stochastic photoswitching super-resolution methods must find a balance between the labeling density and the fluorophore photo-switching kinetics, limiting their actual potential. Indeed, the mean distance between neighboring localized fluorophores must satisfy the Nyquist-Shannon sampling theorem in order to achieve the desired resolution, and the temporal separation of fluorophores in the same diffraction limited area must be ensured as well to allow distinguishing the molecule signals. This limitation is circumvented by methods that exploit binding and dissociation of fluorescent probes, such as 'points accumulation in nanoscale topography' (PAINT) and extensions thereof which include complementation between target and imager DNA strands in DNA-PAINT [1].

To allow binding and dissociation to occur, PAINT requires a reservoir of fluorescent labelled DNA oligos in solution surrounding the sample, which brings its own limitations to the method. Specifically, it requires axial optical sectioning to reject the background signal from fluorophores in solution and an integration time per localization more than 10x longer than for stochastic photoswitching.

In this work, we extend the waveguide TIRF approach [2] to enable increased throughput and data quality for the PAINT technique, by generating a highly uniform ~100×2000 µm2 area evanescent field for TIRF illumination [3]. To achieve this, we designed and fabricated waveguides optimized for efficient light coupling and propagation, incorporating a carefully engineered input facet and taper. We also developed a stable, low-cost microscope and 3D-printable waveguide chip holder for easy alignment and imaging. We demonstrate the capabilities of our open platform by using DNA-PAINT to image multiple whole cells or hundreds of origami structures in a single field of view.

Bio: I got a Bachelor degree in Physics at Padova University with a thesis on SiPM detectors characterization, under the guidance of Prof. Stroili and Prof. Dal Corso. I did my Master Thesis in Physics at Trento University, where I participated to a collaborative project between the Department of Physics and the Fondazione Bruno Kessler (FBK) Research Centre to develop a FLIM (Fluorescence Lifetime Imaging Microscopy) system based on a novel fast time-gated array of SPAD (Single Photon Avalanche Diode) under the supervision of Prof. Haase and Prof. Pancheri.

I then moved to EPFL for an internship as graduate student in Prof. Manley laboratory where I focused on other fluorescence microscopy methods that belong to the single molecule localization microscopy (SMLM) family. In this context, I participated to the development of a large field of view microscope, developing the analysis techniques and algorithms necessary to characterize the focal plane CMOS camera, and benchmarking different localization algorithms through CMOS-noise based simulations.

During my PhD I have been involved as committee member, to the Single Molecule Localization Challenge 2016 for evaluating existing 3D localization algorithms and for providing robust simulated datasets.

I am now completing my PhD in Prof. Manley's Laboratory of Experimental Biophysics, where I participated in numerous image processing and data analysis activities for cell shape dynamic studies, and personally ideated and realized a waveguide platform for DNA-PAINT microscopy (Points Accumulation for Imaging in Nanoscale Topography) in collaboration with Prof. Radenovic laboratory of Nanoscale Biology at EPFL.

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