A new high-resolution microscopy technique

Victor Moro[1], Joan Canals[1], Nil Franch[1], Sergio Moreno[1], Albert Romano-Rodríguez[1], Anna Vilà[1], Jan Gülink[2], Daria Bezshlyakh[2], Andreas Waag[2], Joan Daniel Prades[1], Angel Diéguez[1]

[1]Departament of Electronics and Biomedical Engineering, Universitat de Barcelona

[2]Institute for Semiconductor Technology, University of Technology Braunschweig

Abstract

Superresolution microscopy is a new and advanced technique that allows to resolve objects smaller than traditional optical microscopes. Several studies have been done in this subject and there exist various types of microscopy techniques. These techniques can be grouped into two groups, non-fluorescence-based techniques and fluorescence-based techniques. Non fluorescence based techniques are Near Field Random Mapping (NORM) [1] and Near Field Scanning Optical Microscopy (SNOM) [2]. Fluorescence based techniques are Stimulated Emission Depletion (STED) [3], Reversible Saturable Optical Linear Fluorescence Transmissions (RESOLFT) [4], Photo-Activated Localization Microscopy (PALM) [5] and Stochastic Optical Reconstruction Microscopy (STORM) [6].

In this work we present the first prototype for a new optical microscopy technique: nano-illumination microscopy. This microscope prototype bases its resolution in spatially resolved illumination. With this microscope the importance of the sensor pixel size and pitch disappears, and the light source size and pitch are what determines the resolution of the microscope. This microscope prototype consists on an array of 64 micro-LED which is distributed in 8 rows and 8 columns. Custom discrete driving electronics were designed to turn on the micro-LED array. The detector used is a SPAD camera designed in AMS 350nm technology with 10 µm diameter pixels. The micro-LEDs size is 5 µm with a pitch of 10 µm. The smallest object observed with this microscope prototype is a 5 µm width line, but by decreasing the pixels size and pitch, which in the next microscope version would be 400nm size with 800nm pitch, there would be an upgrade of the microscope resolution. The final prototype using this technique aims to achieve a resolution below Abbe diffraction limit.

Images

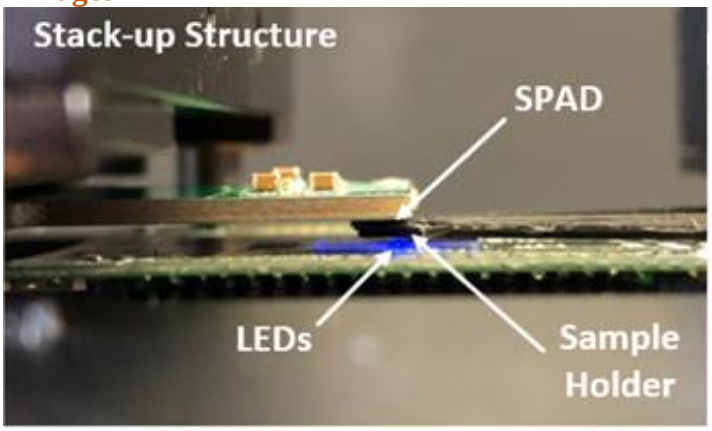

Figure 1. Microscope setup showing LEDs, the Sample Holder with an EBL sample in it and the SPAD detector.

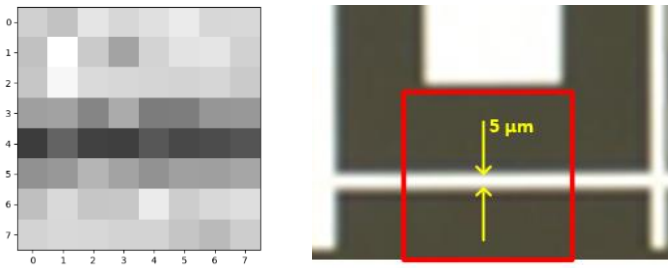

Fig. 2. An image taken with our microscope (left) of a line pattern of 5 µm width. In the right it is shown an image taken with a conventional microscope.

Biography

Victor Moro has his expertise in electronics engineering and biomedical engineering. He is performing his PhD in microelectronics and performing as Adjunct Professor in University of Barcelona in the Faculty of Physics. The PhD he is performing is based on a Horizon 2020 FET-Open project, Chipscope (Grant agreement 737089).

Email: vmoro@el.ub.edu

References:

[1] Y. V. Miklyaev, S. A. Asselborn, K. A. Zaytsev, and M. Y. Darscht, "Superresolution microscopy in far-field by near-field optical random mapping nanoscopy," *Appl. Phys. Lett.*, vol. 105, no. 11, 2014.

[2] Y. Oshikane, T. Kataoka, M. Okuda, S. Hara, H. Inoue, and M. Nakano, "Observation of nanostructure by scanning near-field optical microscope with small sphere probe," *Sci. Technol. Adv. Mater.*, vol. 8, no. 3, pp. 181–185, 2007.

[3] K. I. Willig, J. Keller, M. Bossi, and S. W. Hell, "STED microscopy resolves nanoparticle assemblies," *New J. Phys.*, vol. 8, 2006.

[4] B. Huang, M. Bates, and X. Zhuang, “Super-Resolution Fluorescence Microscopy,” *Annu. Rev. Biochem.*, pp. 993–1016, 2009.
[5] B. O. Leung and K. C. Chou, “Review of super - Resolution fluorescence microscopy for biology,” *Appl. Spectrosc.*, vol. 65, no. 9, pp. 967–980, 2011.
[6] M. J. Rust1, M. Bates, and X. Zhuang, “Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution,” *Nat. Methods*, vol. 3, no. 10, pp. 793–795, 2009.