Pop Microscopy and Optical Nanoscopy: the "marvelous real" at the nanoscale

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For more than 350 years Optical microscopy has been unique in allowing imaging and understanding of biological systems in the spatial and temporal dimensions. The picture (Credits: Agnese Abrusci, IIT) brings together the first microscope realized by Antoni van Leeuwenhoek (1650 circa) and the latest implementation (in the background) of a light sheet fluorescence microscope endowed of spatial super resolution abilities (1). In both cases, the main target is related to the chance of following biological systems during their activities at details sharper than the ones provided by the human eye. Three-dimensional (3D) fluorescence optical microscopy has had a tremendous development in the last thirty years, related to the different converging approaches and technologies (2) and to the fact that it fits perfectly with the need for a detailed knowledge of living systems which are 3D by nature. Living systems that – as clearly defined by their biological condition – allow and demand the exploration of the 4th temporal dimension. So far, confocal and multi-photon microscopy pushed the optical sectioning ability for getting 3D information (3). The temporal dimension is naturally added towards 4D (x-y-z-t) bio-imaging also thanks to the possibility of performing long term experiments, like the ones in developmental biology. We can state that today microscopy is popular and we can say that the related images are definitely "Pop". Our eyes and mind became skilled at processing images at the microscale, what about the nanoscale? Incredibly beautiful images of cells, molecules, organs and tissues attracted the interest of ordinary people. The "marvellous real", using the Carpentier's oxymoron, emerged from optical microscopes allowing scientists to decipher secrets of life, also facilitating the bringing of science to the people. Within this amazing scenario, thanks to developments encouraged late in the forties (4) and experimented since the early nineties (5), an incredible advance came when unlimited resolution in space was demonstrated (6). Terms like super resolution microscopy, super or ultra microscope and optical nanoscopy refer to the possibility of producing images at an unlimited spatial resolution. The fact that spatial resolution using an optical microscope is unlimited does not scarify any physical law. The way to get spatially super resolved images is strongly related to the ability to control, precisely, at least two molecular states of our targets (7), namely: fluorescent or notfluorescent, absorbing or nonabsorbing, scattering or noscattering, spin up or spin down, and more. Moreover such an optical ability can be merged with other high resolution methods like scanning probe microscopy (8) (9) (10). So, optical microscopes can be pushed to unlimited spatial resolution providing new marvelous insights at the nanoscale in a Pop Microscopy context.



References

- Cella Zanacchi, F., Z. Lavagnino, M. Perrone Donnorso, A. Del Bue, L. Furia, M. Faretta, and A. Diaspro. 2011. Live-cell 3D super-resolution imaging in thick biological samples. Nat Methods. 8: 1047–1049.
- 2. Zanacchi, F.C., P. Bianchini, and G. Vicidomini. 2014. Fluorescence microscopy in the spotlight. Microsc Res Tech. 77: 479–482.
- 3. Diaspro, A., others. 2002. Confocal and two-photon microscopy: foundations, applications, and advances. Wiley-Liss New York.
- 4. Toraldo Di Francia, G. 1952. Super-gain antennas and optical resolving power. Nuovo Cim. 9: 426–438.
- 5. Hell, S.W. 2009. Microscopy and its focal switch. Nat Methods. 6: 24–32.
- 6. Diaspro, A. 2014. Circumventing the diffraction limit. Il Nuovo Saggiatore 30: 45–51.
- Hell, S.W., S.J. Sahl, M. Bates, X. Zhuang, R. Heintzmann, M.J. Booth, J. Bewersdorf, G. Shtengel, H. Hess, P. Tinnefeld, A. Honigmann, S. Jakobs, I. Testa, L. Cognet, B. LOUNIS, H. Ewers, S.J. Davis, C. Eggeling, D. Klenerman, K.I. Willig, G. Vicidomini, M. Castello, A. Diaspro, and T. Cordes. 2015. The 2015 super-resolution microscopy roadmap. Journal of Physics D: Applied Physics. 48: 443001.
- 8. Chacko, J.V., F.C. Zanacchi, and A. Diaspro. 2013. Probing cytoskeletal structures by coupling optical superresolution and AFM techniques for a correlative approach. Cytoskeleton (Hoboken). 70: 729–740.
- 9. Monserrate, A., S. Casado, and C. Flors. 2013. Correlative Atomic Force Microscopy and Localization-Based Super-Resolution Microscopy: Revealing Labelling and Image Reconstruction Artefacts. ChemPhysChem. 15: 647–650.
- 10. Harke, B., J.V. Chacko, H. Haschke, and C. Canale. 2012. A novel nanoscopic tool by combining AFM with STED microscopy. Optical Nanoscopy.