LOCALIZING SINGLE MOLECULES WITH INTERFEROMETRY

Lusheng Gu, Yuanyuan Li, Shuwen Zhang, Wei Ji, Tao Xu
Institute of Biophysics, CAS
No. 15, Datun Rd., Chaoyang District, Beijing, P.R.China
E-mail: gulusheng@ibp.ac.cn

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Here we proposed a novel fluorescent imaging strategy named repetitive optical selective exposure (ROSE). This method provides the ability to measure fluorescent signals under multiple illumination conditions simultaneously. We demonstrated this method with single molecule interferometric localization along lateral directions. The result showed that the localization precision could be doubled, compared with centroid fitting with the same photon budget. As locating single molecules is the basis of single molecule localization microscopy (SMLM), the localization precision will directly impact the resolving ability. Compared with widely used centroid fitting methods such as 2D Gaussian fitting, interferometry shows higher precision but only applied in the axial direction before, such as iPALM and W-4PiSMSN. Another technique called MINFLUX provides ultra-high precision, but the field of view (FOV) is restricted to a small area. Our method provides higher precision along lateral directions, with large FOV which will be limited only by the imaging sensor and laser power. DNA origami structures as well as cellular structures were used to test this method, showing a doubled resolution over a large FOV of 25 x 25 micrometers. Also this method could be extended to 3D and multi-color imaging, making this method a useful tool for fluorescent imaging and super-resolution imaging.

[1] L.S. Gu, Y.Y. Li, S.W. Zhang, Y.H. Xue, W.X. Li, D. Li, T. Xu W. Ji, “Molecular resolution imaging by repetitive optical selective exposure”, *Mature Methods*, 1114-1118 (2019)