

## Localization of High-Density Fluorophores using Wedged Template Matching

Shigeo Watanabe, Teruo Takahashi, Tomochika Takeshima  
System Division, Hamamatsu Photonics K.K.

812 Joko-cho, Higashi-ku, Hamamatsu City, 431-3196, Japan

E-mail : shigeo-w@sys.hpj.co.jp

**KEY WORDS:** super-resolution microscopy, single molecule, precision localization, overlapping molecule, algorithm, camera simulation,

The core principle of precision localization microscopy (PLM) is selectively exciting single non-adjacent molecules over time, allowing the sub-diffraction location of each molecule to be mathematically determined. This elegant approach has limits including the development and implementation of switchable fluorophores and the need for many raw image frames to produce meaningful reconstructions. Solving the issues associated with overlapping fluorophore point spread functions will open the door to larger application of PLM techniques including live cell microscopy [1-3].

To address these problems we have developed a PLM image reconstruction algorithm with two key features: 1) implementation of dynamic background subtraction and 2) wedged template matching (WTM). This new algorithm has the capability to localize molecules with simultaneously overlapping emitters. We tested our algorithm using simulated [4] and experimental data sets. Our results (Fig. 1) show super resolution structures can be seen in reconstructed images from a single frame.

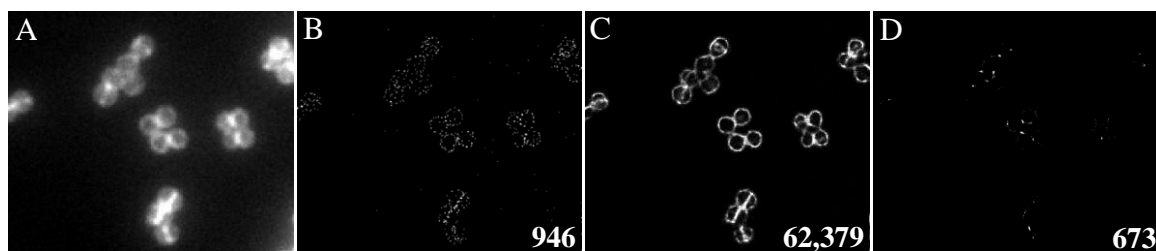


Fig 1. Comparison of MaLiang reconstruction algorithm to wedged template matching (WTM). A) Single raw STORM image. B) 1 frame WTM. C) 100 frame WTM. D) 100 frame MaLiang reconstruction. Numbers in bottom right indicate the molecules localized. WTM localizes nearly two orders of magnitude more molecules. Raw data courtesy of Dr. Ashley Cadby, University of Sheffield, UK.

[1] T. W. Quan, H. Y. Zhu, X. M. Liu, Y. F. Liu, J. P. Ding, S. Q. Zeng and Z. L. Huang, “High-density localization of active molecules using Structured Sparse Model and Bayesian Information Criterion” *Optics Express*, **19**, 16963-16974 (2011)

[2] S. Cox, E. Rosten, J. Monypenny, T. Jovanovic-Taliman, D. T. Burnette, J. Lippincott-Schwartz, G. E. Jones and R. Heintzmann, “Bayesian localization microscopy reveals nanoscale podosome dynamics” *Nature Methods* (2011), doi:10.1038/nmeth.1812

[3] F. Huang, S.L. Schwartz, J. M. Byars and K.A. Lidke, “Simultaneous multiple-emitter fitting for single molecule super-resolution imaging” *Biomedical Optics Express*, **2**, 1377 – 1393 (2011)

[4] S.M. Fullerton, K. Bennett, E. Toda and T. Takahashi, “Camera Simulation Engine Enables Efficient System Optimization for Super-Resolution Imaging.” *Proc. SPIE*, **8228**, In press.