λ/100 RESOLUTION BY TEMPLATE-FREE 2D PARTICLE FUSION IN LOCALIZATION MICROSCOPY

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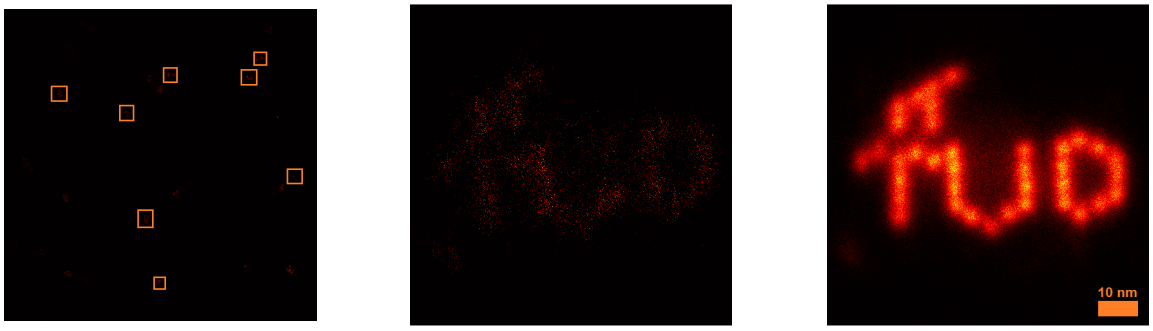
Low density labeling and limited photon count of emitters are among the factors that limit the achievable resolution in localization microscopy. This problem can be mitigated if instances of the same structure are fused (combined) properly. This technique is similar to Single Particle Analysis from cryo-EM. Typically, these particles have arbitrary pose, are degraded by low photon count, false localizations and missing labels. If the structure to be imaged is known a priori, one approach is to register all particles on a template [1]. However, it introduces the so-called template bias which can occur if too strong prior knowledge is imposed on the data. To address this issue, a novel template-free data fusion method is proposed which assumes no prior knowledge about the sample.

Pairs of particles are registered in a tree structure from leaves to root. For each pair, a cost function which uses the uncertainties is optimized for rigid transform parameters. To speed up the optimization, a Gaussian mixture registration method is used [2]. The test samples consist of logos shaped by DNA-origami and imaged with DNA-PAINT. The average number of localizations per particle is ~2660 and the mean lateral uncertainty is 0.83 nm. With 456 particles, a logo of ~1.2*10^6 localizations is reconstructed. We evaluate the resolution by Fourier Ring Correlation [3]. While the initial particles have FRC values in the range of 10-30 nm, the top reconstructed particle is ~4.0 nm which is close to the best possible resolution one can get from this data as the distance of the binding sites is ~5 nm.



Superresolution image of TUD logo. **Left**: Regions of interest containing raw TUD logos. **Middle**: A zoom-in on one of the ROIs. **Right**: The fusion of 456 identical TUD logos.

[1] A. Löschberger, et al.: "Super-resolution imaging reveals eightfold symmetry of gp210 proteins around the nuclear pore complex and resolves the central channel with nanometer resolution", *Journal of Cell Science*, 125:570-575, 2012.
[2] B. Jian and B. Vemuri, "Robust point set registration using Gaussian mixture models", *IEEE Trans. Pattern Anal. Mach. Intell.* , vol. 33, no.8, pp. 1633–1645, Aug. 2011.
[3] Nieuwenhuizen, R.P.J. et al. "Measuring image resolution in optical nanoscopy", *Nat. Methods 10*, 557–62, 2013.