TEMPLATE-FREE 3D PARTICLE FUSION IN LOCALIZATION MICROSCOPY

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The achievable resolution in localization microscopy is limited by the localization uncertainty and labelling density. When many identical copies of the imaged structure are available ("particles"), the limiting density factor can be eliminated if we can fill in missing information by fusing information from acquisition of similar structures. Previously, we developed a novel template-free particle fusion method which is capable of aligning 2D particles with very low degree of labelling (DOL)[1]. As an ongoing work, we have extended our approach to 3D SMLM particles.

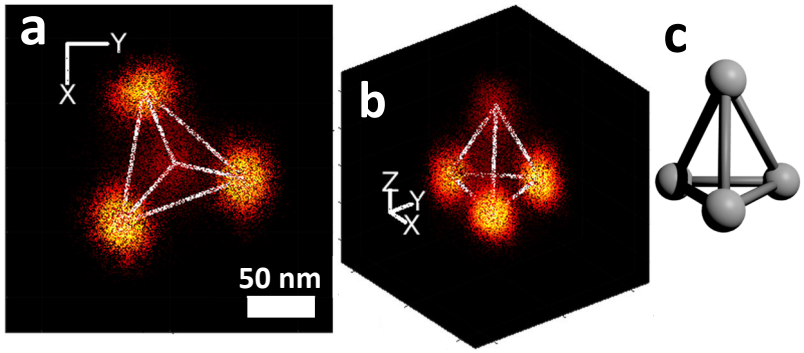

Figure 1 | 3D particle fusion. The result of fusing 50 tetrahedrons using the proposed method (**a**) Top x-y view (**b**) Side view (c) 3D origami design.

The existing methods for 3D particle fusion of SMLM data are either limited to those that use a template[2] or the ones that copy techniques from cryo-EM[3] and fuse 2D projections, instead of actual 3D localization data. While the former is prone to template bias, the latter are ignoring the fundamental differences in image formation of SMLM and EM images, and most importantly cannot handle 3D data acquired typically in SMLM. We developed a method which is template-free and directly works on 3D localization data. It is based on the maximum information that can in principle be extracted from aligning N particles. Firstly, all segmented particles are aligned to each other using a pair registration method. This will provide us with $N(N-1)/2$ (redundant) relative motion parameters (3D rotation and x, y, z translation). Then, the Lie-algebraic representation of motion parameters are averaged in the L_1 sense which is subsequently followed by removal of outliers and a bootstrapping procedure.

We have evaluated our developed method on an experimental dataset containing tetrahedron-shaped DNA-origami nanostructures imaged with 3D DNA-PAINT[4]. Figure 1a-b show the result of fusing 50 manually segmented tetrahedrons containing ~34k localizations. The reconstruction is fully compatible with the designed 3D patterns of vertices (figure 1c).

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