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PSRT: Progressive Stochastic Reconstruction Technique for Cryo Electron Tomography

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Cryo Electron Tomography (cryoET) is one of the essential techniques in Structural Biology, as it allows us to study the structure of macromolecular complexes in their native environment *in situ*. The tomographic reconstruction in cryoET is a particularly challenging task as the input data suffers from very low contrast, high noise, and limited tilt range. Moreover, the scanned specimen is larger than the detector, introducing the interior problem into the reconstruction process, which causes vignetting artifacts on the edges of the reconstructions. To alleviate some of these limitations, high-resolution protocols such as Subtomogram Averaging (SA) are applied to obtain structures of individual macromolecular complexes from a tomogram. Results of these protocols are highly dependent on the quality of the reconstruction. Current state-of-the-art methods such as Weighted Back Projection (WBP) or Simultaneous Algebraic Reconstruction Technique (SART) deliver noisy and low-contrast reconstructions and thus manual intervention is often needed during SA.

We present a novel iterative approach to tomographic reconstruction in cryoET called Progressive Stochastic Reconstruction Technique (PSRT), which is designed to improve the reliability and decrease the need for manual intervention in the high-resolution protocols [1]. The approach is based on a different mathematical framework than the existing techniques. It uses Monte Carlo random walks to reconstruct the volume by gradually placing spherical elements, called samples, of a given size and intensity into the volume (Fig. 1). The position of each sample is generated randomly and the sample is accepted only if the improvement of the current volume estimate is sufficient with respect to a selected error metric. This is guided by a sampling strategy similar to Metropolis-Hastings, where the areas with higher acceptance potential are sampled more densely, thus speeding up the convergence of the method. During the reconstruction process we progressively decrease both the size and the intensity of the samples, performing a coarse-to-fine reconstruction. Furthermore, we can design additional importance sampling that allows us to focus on specific parts of the volume. This is of special importance in SA, where one is interested in individual structures distributed within the volume which, however, represent only a small part of it. The current state-of-the-art methods lack this ability and reconstruct every part of the volume with the same procedure. PSRT also provides a memory efficient solution to specimen-level interior problem and removes all associated artifacts.

We compare the method to the state-of-the-art techniques, validate it on synthetic data and show on experimental data of known biological systems that it is able to reconstruct the correct high-resolution structures. Moreover, PSRT delivers smoother reconstructions with enhanced contrast and fewer artifacts. This improves template-based localization and thereby enables better automation in SA (Fig. 2).

References:

[1] B Turoňová *et al*, J Struct Biol (2015) doi: 10.1016/j.jsb.2015.01.011.
[2] We thank Massimiliano Maletta from NKI AVL and Peter J. Peters from Department of Health Medicine and Life Science at Maastricht University for providing us with the experimental data of the 70S ribosome.

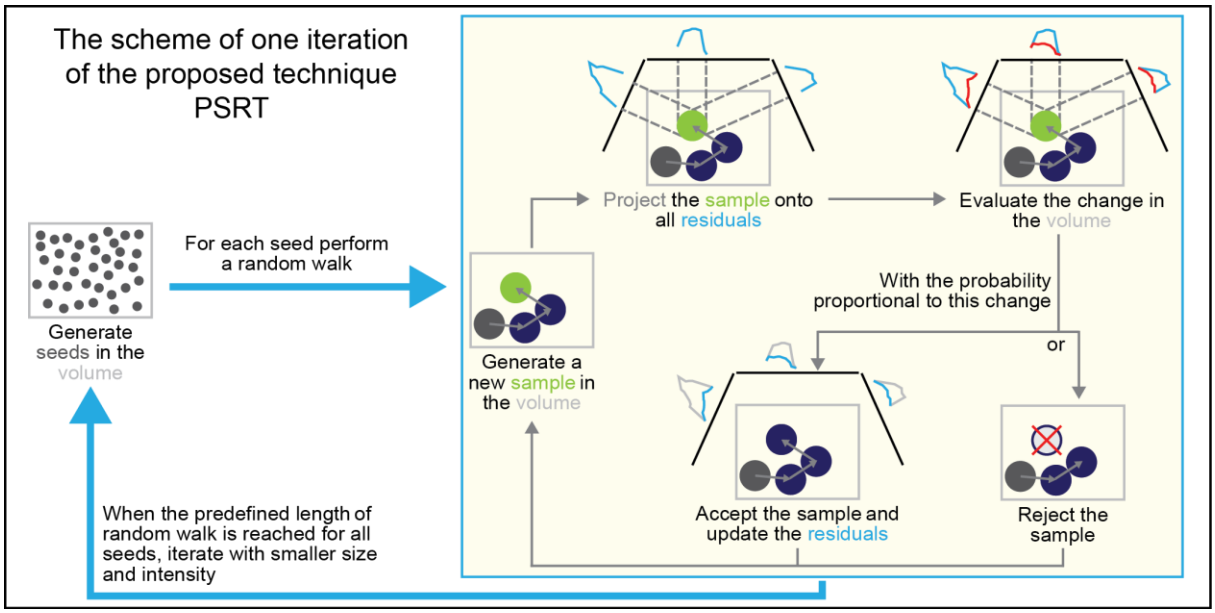

Figure 1. Scheme of one iteration of PSRT. In each iteration we generate starting samples, called seeds, with given size and intensity and for each seed we perform a random walk. The iteration has completed once all seeds and their corresponding random walks were processed. The next iteration starts with seeds of smaller size and intensity.

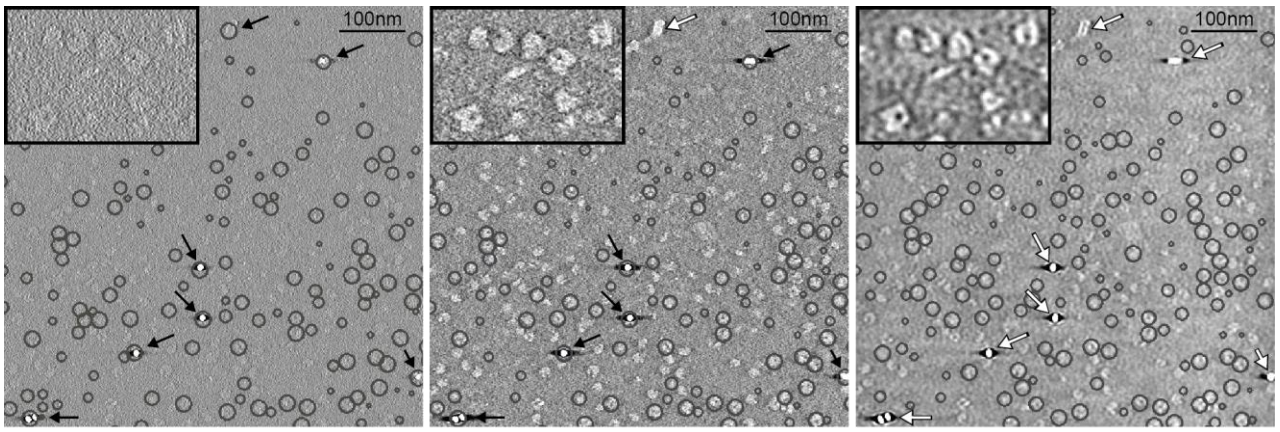

Figure 2. Comparison of template-based localization for (a) WBP, (b) SART (with relaxation parameter 0.1 and one iteration), and (c) PSRT. The enlargements (2x) demonstrate contrast properties of the reconstructions. Black arrows indicate false positives in the localization for WBP and SART. In the PSRT reconstruction, only ribosomes were localized and no manual correction of the localization results was therefore necessary.