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Deep orientation estimation of macromolecules in cryo-electron tomography

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Background incl. aims

The standard method for detecting macromolecules in cryo-electron tomography (cryo-ET) images is template matching (TM), which suffers from high computational complexity and difficulties in identifying particles with similar structures. In TM, one uses template density maps of a specific macromolecular particle and computes the cross-correlation score at every voxel across the whole tomogram. The highest-ranked cross-correlation scores correspond to possible particle locations. In addition to the particle location, one also obtains an estimation of the particle orientation. As a final step, sub-volumes are extracted at those locations which can be used in turn for other tasks like classification and segmentation.

Recently, the investigation of crowded cell environments using cryo-ET has been attempted with deep learning (DL) methods. Models like DeepFinder [1] improve particle picking by being much faster while at the same time providing a reasonable accuracy. Prediction of the particle orientation using DL methods, however, has, to the best of our knowledge, not yet been achieved. This, we believe, is mainly because learning based on representations like Euler angles or quaternions fails due to discontinuities of the representation space [2].

Here, we investigate DL-based particle orientation estimation using a continuous representation with six degrees of freedom (6DoF) that empowers neural networks for the optimal estimation. Input to the neural network is a 3D image patch containing the particle. Since for experimental data, usually no ground truth is available, we generate test data using the PolNet software that was recently published [3]. We evaluate the accuracy of the orientation estimation using an end-to-end model on this test data. Our promising results suggest that particle orientation using DL methods is indeed feasible.

Methods

Different rotation representations may be used to train a machine learning model for orientation estimation. Inspired by the work of Zhou et al. [2], we address the problem of orientation estimation as a regression problem. We use M =[a1, …, an] and SO(n) to denote a rotation matrix and the space of n-dimensional rotations, respectively, and ai represents a column vector. In consequence, having a set of 3D rotations, one can define the original space $X=SO(3)$. Zhou et al. [2] showed that representations for 3D rotations are discontinuous in four or lower dimensions; hence, representation spaces for rotations based on Euler angles and quaternions are discontinuous.

Let R and X be the representation space and the original space of rotations; then the neural network should predict an intermediary representation in R that can be mapped into the original space X. In other words, we are looking for a representation (f, g) such that $f:R \rightarrow X$ maps from the representation space to the original space and $g:X \rightarrow R$ maps back to R, preserving the continuity. Figure 1 illustrates these definitions. However, the problem is that the limit of g is undefined for zero rotation, i.e. limit of g in one direction gives 0 and in the other 2π.

One possible solution is to employ identity mapping. Although this guarantees that the network output is back in SO(3), it results in matrices of size 3 × 3 which can be computationally exhaustive due to orthogonalization. As a result, we perform the orthogonalization in the representation space. Having the original space $X=SO(3)$, a representation space $R=R^{3\times 2}\backslash D$, and a rotation matrix M, the mapping g is simply defined as dropping the last column of the rotation matrix, resulting in
[a1, a2], as suggested by Zhou et al. Here, the set D represents that part of the space that f cannot map to SO(3) [2].

In addition to these challenges, designing a network structure that can reduce the computational cost while accurately estimating the orientation is a profound task. One such structure is a multi-layer perceptron (MLP). We use a four-layer fully connected network with 32 nodes per layer and a regression layer with tanh activation function. The number of output nodes in the regression layer equals the dimension of the orientation representation, i.e. 3, 4, and 6 for Euler angles, quaternions, and 6DoF, respectively.

Results

We performed experiments on a synthetic dataset generated by PolNet [3] that for uniform random sampling of the rotations uses the Algorithm S2 in [4]. We generated 150 tomograms of resolution 10A° containing the ribosomal complex (4v4r) and Thermoplasma acidophilum 20S proteasome (3j9i). We extracted centred patches of size 40^3 for all 4v4r particles, leading to 26703 samples. 130 tomograms (23128 samples) were used for training and validation, and 20 tomograms (3575 samples) for testing. Note that the synthesized tomograms contain the missing-wedge artifact and noise.

We trained our network for 50 epochs using Adam optimization, batch size 32, learning rate 0.0001, and patch size of 40^3. We used Huber loss to calculate differences between ground truth and predicted orientation representation. Our experimental results suggest that the continuous representation performs much better in practice. Figure 2, left, shows the ground truth and predicted orientations on a test tomogram for the 6DoF representation. Green and red colors represent ground truth and prediction, respectively. Our model predicted 81% of the test samples correctly using the Crowther criterion [5] with an angle difference threshold of 20 degrees. Visual analysis shows that most incorrect predictions occur in regions where the particles form a cluster. While training time was about 1 hour, inference time was only approximately 25 seconds on a single GPU. We achieved an R2 score of 0.96 on training data and 0.87 on test data for the 6DoF representation. These values downgrade to 0.76, -0.07 on training and -0.47, -0.07 on test data for the quaternion and Euler representations, respectively. Figure 2, right, shows a histogram chart of the angle differences for all three representations.

Conclusion

We studied the use of MLP to estimate macromolecule orientation using various representation spaces, namely Euler angles, quaternions, and a 6DoF-continuous. The continuous representation space shows a huge advantage over the others. Our future work includes developing more complex models to perform multiple downstream tasks along rotation estimation.

Graphic:

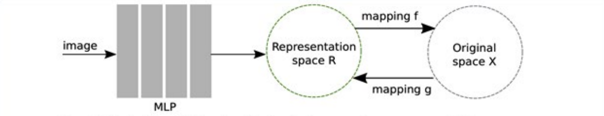

Figure1: Illustration of MLP network trained using a continuous representation space

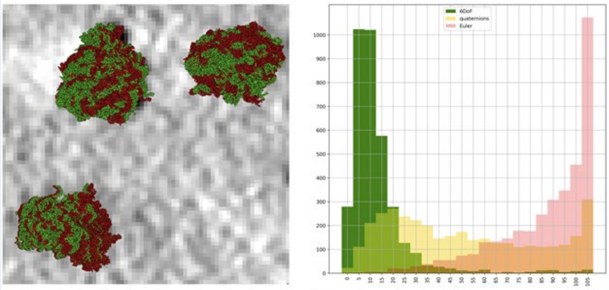

Figure 2: Visualization of ground truth and predicted orientations using 6DoF for a test tomogram (left) histogram chart of the angle differences with a 20° bin width and a 10 step Crowther criterion (right)

Keywords:

machine learning, cryo-electron tomography, orientation

Reference:

[1] E. Moebel, A. Martinez-Sanchez, L. Lamm, R. D. Righetto, W. Wietrzynski, S. Albert and D. Larivière, "Deep learning improves macromolecule identification in 3D cellular cryo-electron tomograms," Nature methods, pp. 1386-1394, 2021.

[2] Y. Zhou, C. Barnes, J. Lu and J. Yang, "On the Continuity of Rotation Representations in Neural Networks," Long Beach, CA, USA, 2019.

[3] A. Martinez-Sanchez, M. Jasnin, H. Phelippeau and L. Lamm, "Simulating the cellular context in synthetic datasets for cryo-electron tomography," bioRxiv, 2023.

[4] A. Martinez-Sanchez, W. Baumeister and a. V. Lučić, "Statistical spatial analysis for cryo-electron tomography," Computer methods and programs in biomedicine, 2022.

[5] M. L. Chaillet, G. v. d. Schot, I. Gubins, S. Roet, R. C. Veltkamp and F. Förster, "Extensive angular sampling enables the sensitive localization of macromolecules in electron tomograms," International Journal of Molecular Sciences, 2023.