Online CryoEM Study Group

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June 10, 2022

Contents

1 Dates and Topics

Meetings are generally on Thursdays (morning Pacific time, afternoon Eastern time, evening Europe)

Date	Time	Topic
Fri 21 Jan 2022	9 AM PST	Mathy cryoem paper: VESPER (Han et al., 2021)
Fri 28 Jan 2022	9:15 AM PST	Guest: Qinwen (Wendy) Huang - Weakly Supervised Learning for Joint Image Denoising and Protein Localization in Cryo-EM
Fri 4 Feb 2022	9 AM PST	Rotations - encoding of S0(3)
Fri 11 Feb 2022	9 AM PST	Mathy cryoem paper: Transformations in SPARX and EMAN2 (Baldwin & Penczek, 2007)
Fri 18 Feb 2022	9 AM PST	no meeting
Fri 25 Feb 2022	9 AM PST	Rotating Averaging (Hartley et al., 2013)
Fri 4 Mar 2022	9 AM PST	Mathy cryoem paper: End-to-end orientation estimation from 2D cryo-EM images (Lian et al., 2022)
Fri 11 Mar 2022	9 AM PST	Guest: Lys Sanz Moreta - Deep Probabilistic Programming and 3D Protein Structure (Moreta et al, 2019; Moreta et al 2020)
Fri 18 Mar 2022	9 AM PST	CryoAI - Part I, overview
Fri 25 Mar 2022	9 AM PST	CryoAI - Part II, supplementary material
Fri 1 April 2022	9 AM PST	Guest: Andrew Hanson - Discovering Quaternion Applications
Fri 8 April 2022	9 AM PST	Mathy cryoem paper: Cryofold (Zhong et al., 2021)
Fri 29 April 2022	9 AM PST	Mathy cryoem paper: Molecular Prior - Wilson Statistics (Gilles & Singer, 2022)
Fri 13 May 2022	9 AM PST	Mathy cryoem paper: Molecular Prior - Wilson Statistics (Gilles & Singer, 2022) II
Fri 27 May 2022	9 AM PST	Guests: Ngan Nguyen and Feng Liang, Differentiable Electron Microscopy Simulation (Ngan et al. 2022)
Fri 3 Jun 2022	9 AM PST	Stochastic gradients (Schulman et al., 2015)
Fri 10 Jun 2022	9 AM PST	Mathy cryoem papers: ManifoldEM

2 General Information

2.1 Archived material

Meetings from 2020-2021 are archived here. The audience was a mix of beginners and advanced practitioners, and computational methods developers.

2.2 Audience and Streams

Feel free to share this document and direct people to sign up at `https://forms.gle/BUeUW14vV4pyQbDDA` so I have the emails in one place. Online meeting links are emailed to those on this list. **Please join the Slack group** `https://cryoemstudygroup.slack.com/` **and ask questions there, rather than emailing me.** Here is a link to join `https://join.slack.com/t/cryoemstudygroup/shared_invite/zt-171mmkqdd-KORjSqcss~A6HNUbOtCpbQ`. If the link is expired, please remind me to update it.

2.3 Audience

In 2022, I am catering to a computational methods development audience. I see this group as a way for computational methods developers to get together in a "pre-competitive" learning environment.

Practitioners → computational methods developers: You are a structural biologist, or biochemist, and perhaps an advanced cryo-EM practitioner. You would like to train in computational methods development, either to do very advanced data processing, or develop your own methods.

Pure computational discipline → cryo-EM computational methods developers: You have a background in computer science (computer vision, deep learning, statistics, electrical/computer engineering) and would like to develop methods for the "killer application" of cryo-EM.

2.4 Pre-requisites

The bar is quite high, and this group is not for all. There are very good resources out there for self-study; see this annotated a bibliography. If you have done an undergraduate degree in an advanced computational program (physics, chemistry, computer science, statistics, applied math) or are a PhD student in a computational field, then you are in good company in this group.

2.5 Scope

2.5.1 Math / Computer science

1. Amortized inference, model learning

2. Physics aware/inspired/infused deep learning
3. Deep learning of the image formation model (rotation, etc)
4. Computationally modelling uncertainty in the image formation model
5. Geometric deep learning and invariance/equivariance in cryo-EM
6. Computational optimal transport
7. Computational differential geometry
8. Optimization
9. Custom GPU kernels, including gradient for backprop/autodiff

2.5.2 Physics

1. Electron optics
2. Higher order CTF aberrations
3. Multi-slice
4. Sample damage
5. Detector physics
6. Solvation
7. Poisson-Boltzmann equation
8. Modelling choices to encode electrostatic / Coulombic density

2.6 Meeting Format

The meetings are meant to be more informal that is typical in research talks. The point is to learn and discuss with other learners, experienced practitioners, and experts. They are also more comprehensive than typical journal clubs. We may stick with a papers or series of papers for multiple weeks to sufficiently learn the material.

2.7 Slack

We will use the Slack channel 'cryoem_study_group' for asynchronous chat. Please join the Slack group and ask questions there, rather than emailing me. You can request a link to join by emailing me.

2.8 Testimonials

- *Suvrajit Maji, Associate Research Scientist at Columbia University Irving Medical Center & Joachim Frank, Chemistry Nobel Laureate (cryo-EM for biomolecules 2017), Professor of Biochemistry and Molecular Biophysics and Professor of Biological Sciences at Columbia University.* Fortunately, scripts of a tutorial for math underlying single-particle cryo-EM processing are already available in a github repository (15) [`https://github.com/geoffwoollard/learn_cryoem_math`, Geoffrey Woollard & Ricardo Righetto]. These include notebooks on numerous subtopics on the physics of the electron microscope and image formation (electron optics, electron diffraction patterns, contrast-transfer function), on mathematical concepts (Fourier transforms, convolution, coordinate systems and rotations, interpolations, Fourier slice theorem, 3D reconstruction), on Bayesian inference and estimation theory (maximal likelihood, 2D and 3D image classification, covariance), and on some important image processing tools (alignment via cross-correlation, 3D reconstruction via weighted back-projection, resolution estimation via Fourier shell correlation, detector noise modeling). There are simple object models to experiment with and learn about the basics of phase contrast image formation and data processing.

- *Shayan Shekarforoush, PhD student with Marcus Brubaker and David Fleet, Jan 2021.* I joined this reading group in mid October and I wish I would have done so much earlier. Although I joined when the group was in the middle of reading a fascinating, recently published book in Single-Particle cryoEM, everyone was so welcome that I did not feel I am way behind others. My background is in CS and I do research as a method developer in this field. With that said, I learned a lot from discussions of people with expertise in experimental side of this area. I believe that this group helped me to build a better intuition and now I feel more comfortable with the underlying math and physics of this topic. This group also provided the opportunity to attend talks of prominent researchers in cryoEM where anyone could openly ask their questions and have clear discussions. Looking forward to having more collaborations with the members of this group.

3 Learning Resources

1. I have made an annotated bibliography organized thematically here.

2. Coding notebooks to play around with are here. If there is incompatibility between the notebook and the code base in the repo, that is because the code base has been updated. Older version of the code are available via past commits.

4 Upcoming Meetings

4.1 10 June 2022 - ManifoldEM

–Pre-reading

1. Seitz, E., Acosta-Reyes, F., Maji, S., Schwander, P., & Frank, J. (2022). Recovery of Conformational Continuum from Single-particle Cryo-EM Images: Optimization of ManifoldEM Informed by Ground Truth. IEEE Transactions on Computational Imaging, PP, 1–1. http://doi.org/10.1109/tci.2022.3174801

2. Dashti, Ali and Schwander, Peter and Langlois, Robert and Fung, Russell and Li, Wen and Hosseinizadeh, Ahmad and Liao, Hstau Y. and Pallesen, Jesper and Sharma, Gyanesh and Stupina, Vera A. and Simon, Anne E. and Dinman, Jonathan D. and Frank, Joachim and Ourmazd, Abbas (2014). Trajectories of the ribosome as a Brownian nanomachine. Proceedings of the National Academy of Sciences of the United States of America, 111(49), 17492–17497. http://doi.org/10.1073/pnas.1419276111

3. Oliver Oswald. Laplace-Beltrami Operator Intuition, YouTube, 2021. https://youtu.be/ZSh5d03YsEM

4. de la Porte, J., Herbst, B. M., Hereman, W., & van der Walt, S. J. An Introduction to Diffusion Maps. https://inside.mines.edu/~whereman/talks/delaPorte-Herbst-Hereman-vander pdf

–Questions

1. How do diffusion maps work (cf. SI section "Manifold Embedding by Diffusion Map" of Dashti et al, PNAS, 2014)?

2. How does ManifoldEM compare and contrast against other methods to study continuous heterogeneity?

3. What synthetic data sets were considered in Seitz et al, 2022?

4. Consider the references in the SI of Dashti et al, PNAS, 2014. What mathematical perspectives / subject areas are they from? Besides cryo-EM, what other applications are there for this type of mathematics?

5. How should you (biologically) interpret the movies in Dashti et al, PNAS, 2014? *These movies consist of a sequence of NLSA reconstructed snapshots, ordered along the minimum-energy path in the energy landscape (Movies S1–S5).* (p. 2, SI). What is the biological meaning of a circular manifold (cf. Figure 3, and discussion in SI section "Patching together information from different orientations").

6. *Reconstruction of the energy landscape for all 320 projection directions requires ?7 d on the same cluster.*, (SI section "Computational expense and scaling" of Dashti et al, PNAS, 2014). How long would practitioners be typically willing to wait for computational results that are highly informative and interpretable?

7. How is the uncertainly propagated or otherwise accounted for in the ManifoldEM computational pipeline (see SI section "Number of Conformational Classes" of Dashti et al, PNAS, 2014)?

8. Overall, how interpretable is ManifoldEM to you? What makes it more/less interpretable? How much of its interpretability depends on your understanding of the technique, and how much is intrinsic to the technique itself?

4.2 May/June 2022 - Guest: Nina Miolane.

–Pre-reading

1. Donnat, C., Levy, A., Poitevin, F., & Miolane, N. (2022). Deep Generative Modeling for Volume Reconstruction in Cryo-Electron Microscopy, 1-26.

4.3 March/April 2022 - Guest: Roy Lederman.

–Pre-reading

1. Lederman, R. R. (2017). Numerical Algorithms for the Computation of Generalized Prolate Spheroidal Functions. ArXiv, (1), 1-23.

2. Lederman, R. R., & Singer, A. (2017). Continuously heterogeneous hyper-objects in cryo-EM and 3-D movies of many temporal dimensions, 1-33.

4.4 Summer 2022? - Guest: David Silva. MD simulations and 2D cryo-EM measurements

4.5 Summer 2022? - Guest: Ellen Zhong. CryoDRGN2

–Pre-reading

1. Zhong, E. D., Lerer, A., Davis, J. H., & Berger, B. (2021). CryoDRGN2 : Ab initio neural reconstruction of 3D protein structures from real cryo-EM images. Iccv, 4066-4075.

–Questions

1. Write out a schematic (computational graph) of CryoDRGN2. What goes into and out of the encoder and decoder?

2. How could cross validation be encorporated into CryoDRGN? What would count as "out of distribution" for various computational workflows, and how would CryoDRGN perform?

5 Reading list

5.1 Advanced treatment of rigid-body rotations (SO(3))

–Pre-reading

1. (2019). On the continuity of rotation representations in neural networks. http://doi.org/10.1109/CVPR.2019.00589
2. (2022). Bingham Policy Parameterization for 3D Rotations in Reinforcement Learning. https://arxiv.org/pdf/2202.03957.pdf
    - SO(3) distribution
3. Orthogonal Procrustes problem https://en.wikipedia.org/wiki/Orthogonal_Procrustes_problem
4. (2021). Brofos, J. A., Brubaker, M. A., & Lederman, R. R. Manifold Density Estimation via Generalized Dequantization. https://arxiv.org/pdf/2102.07143.pdf
5. (2017). State Estimation for Robotics. State Estimation for Robotics. Cambridge: Cambridge University Press. http://doi.org/10.1017/9781316671528
6. (2021). Implicit-PDF: Non-Parametric Representation of Probability Distributions on the Rotation Manifold https://implicit-pdf.github.io/
7. (2020). A Smooth Representation of Belief over SO(3) for Deep Rotation Learning with Uncertainty. https://arxiv.org/pdf/2006.01031.pdf
8. (2021). Eliminating Topological Errors in Neural Network Rotation Estimation Using Self-selecting Ensembles. https://dl.acm.org/doi/pdf/10.1145/3450626.3459882
9. (2021). On the Continuity of Rotation Representations in Neural Networks. https://arxiv.org/pdf/1812.07035.pdf
10. (2021). Learning Rotation Invariant Features for Cryogenic Electron Microscopy Image Reconstruction. https://arxiv.org/pdf/2101.03549.pdf
11. (2020). SE(3)-Transformers: 3D Roto-Translation Equivariant Attention Networks. https://arxiv.org/pdf/2006.10503.pdf
12. (2020). Falorsi, L., de Haan, P., Davidson, T. R., & Forr, P. Reparameterizing distributions on Lie groups. AISTATS 2019 - 22nd International Conference on Artificial Intelligence and Statistics, 89. https://arxiv.org/pdf/1903.02958.pdf

13. (2020). Dmitry Kostyaev. Hands on Tutorials: Better rotation representations for accurate pose estimation. Towards Data Science. https://towardsdatascience.com/better-rotation-representations-for-accurate-pose-estimation-e890a7e1317f

    - Optimization
    - SO(3) encoding

5.2 Assorted mathy cryoem papers

–Pre-reading

1. Masoumzadeh, A., & Brubaker, M. (2020). HydraPicker: Fully automated particle picking in cryo-em by utilizing dataset bias in single shot detection. 30th British Machine Vision Conference 2019, BMVC 2019, (September).

2. Zehni, M., & Zhao, Z. (2021). UVTOMO-GAN: An adversarial learning based approach for unknown view x-ray tomographic reconstruction. Proceedings - International Symposium on Biomedical Imaging, 2021-April, 1812–1816. http://doi.org/10.1109/ISBI48211.2021.9433970

3. Tagare, H. D., Kucukelbir, A., Sigworth, F. J., Wang, H., & Rao, M. (2015). Directly reconstructing principal components of heterogeneous particles from cryo-EM images. Journal of Structural Biology, 191(2), 245?262. http://doi.org/10.1016/j.jsb.2015.05.007

4. Zivanov, J., Nakane, T., & Scheres, S. H. W. (2019). A Bayesian approach to beam-induced motion correction in cryo-EM single-particle analysis. IUCrJ, 6(1), 5?17. http://doi.org/10.1107/S205225251801463X

5. Katsevich, E., Katsevich, A., & Singer, A. (2015). Covariance matrix estimation for the cryo-em heterogeneity problem. SIAM Journal on Imaging Sciences, 8(1), 126-185. http://doi.org/10.1137/130935434

6. Penczek, P. A. (2010). Resolution Measures in Molecular Electron Microscopy. In Methods in Enzymology (1st ed., Vol. 482, pp. 73?100). Elsevier Inc. http://doi.org/10.1016/S0076-6879(10)82003-8

7. Ede, J. M. (2020). Review: Deep learning in electron microscopy. ArXiv. http://doi.org/10.1088/2632-2153/abd614

8. Zhu, D., Wang, X., Fang, Q., Etten, J. L. Van, Rossmann, M. G., Rao, Z., & Zhang, X. (n.d.). Pushing the resolution limit by correcting the Ewald reconstructions. Nature Communications, (2018), 1-7. http://doi.org/10.1038/s41467-018-04051-9

9. Maji, S., Liao, H., Dashti, A., Mashayekhi, G., Ourmazd, A., & Frank, J. (2020). Propagation of Conformational Coordinates across Angular Space in Mapping the Continuum of States from Cryo-EM Data by Manifold Embedding. Journal of Chemical Information and Modeling, 60(5), 2484?2491. http://doi.org/10.1021/acs.jcim.9b01115

10. Zhong, E. D., Lerer, A., Davis, J. H., & Berger, B. (2021). CryoDRGN2 : Ab initio neural reconstruction of 3D protein structures from real cryo-EM images. Iccv, 4066-4075.

5.3 Advanced microscopy

–Pre-reading

1. Nguyen, N., Liang, F., Engel, D., Bohak, C., Wonka, P., Ropinski, T., & Viola, I. (2022). Differentiable Electron Microscopy Simulation: Methods and Applications for Visualization, (1), 1–22.

2. Glaeser, R. M., Hagen, W. J. H., Han, B. G., Henderson, R., McMullan, G., & Russo, C. J. (2021). Defocus-dependent Thon-ring fading. Ultramicroscopy, 222(October 2020), 113213. http://doi.org/10.1016/j.ultramic.2021.113213

3. Russo, C. J., & Egerton, R. F. (2019). Damage in electron cryomicroscopy: Lessons from biology for materials science. MRS Bulletin, 44(12), 935?941. http://doi.org/10.1557/mrs.2019.284

4. Russo, C. J., & Henderson, R. (2018). Ewald sphere correction using a single side-band image processing algorithm. Ultramicroscopy, 187, 26-33. http://doi.org/10.1016/j.ultramic.2017.11.001

5. Fanelli, D., & Öktem, O. (2008). Electron tomography: A short overview with an emphasis on the absorption potential model for the forward problem. Inverse Problems, 24(1). http://doi.org/10.1088/0266-5611/24/1/013001

6. Electron optics textbook chapters (Hawkes and Kasper; Spence; Reimer and Kohl)

6 Past Meetings

6.1 21 Jan 2022 - VESPER: global and local cryo-EM map alignment using local density vectors.

–Pre-reading

1. Han, X., Terashi, G., Christoffer, C., Chen, S., & Kihara, D. (2021). VESPER: global and local cryo-EM map alignment using local density vectors. Nature Communications, 12(1). `http://doi.org/10.1038/s41467-021-22401-y`

–Questions

1. Consider Figure 1 and the Z-score results. When aligning two different maps, and getting DOT scores, how would one re-scale to a Z-score? And to what end?

2. Have you developed any algorithms that start from aligned maps? where did the initial alignment arise from?

3. How do you compare aligning ?by eye? with the algorithms mentioned in the paper, such as the popular fitmap in Chimera. what do you think you are doing in your mind, and how would you break it down into algorithmic steps?

4. Figure 2i (and text on last paragraph of p. 4) shows a case where VESPR did not perform well. two different proteins were predicted to be close in shape ?because they have an overall similar shape and also because these maps are largely hollow inside, and thus inconsistency inside the maps were not much penalized?. Relate this to the DOT score. What other approaches would not suffer from this?

5. In Table 2 there are some cases where the RMSD goes up (worse alignment) with finer grained sampling (rotation angle and translation). Why might this be happening?

6. In the Discussion, the authors explain intuitively how cross correlation (CC) and their DOT score result in different alignments: *In CC, positions with large absolute density values, such as those in a high-density region in a map, influence more to the overall CC value. On the other hand, for the DOT score the contribution of each aligned position pair is essentially the same because the vectors are normalized to the same length. But this also means that the DOT score can be affected by changes in local gradient caused by small structure variations.*

   Consider the following cases and think through how the result might be different.

   (a) Continuous conformational heterogeneity: Blurred out flexible region with partial density.

   (b) Discrete conformational heterogeneity: Mixed discrete states in one map vs one of the partners.

(c) Hinge like displacement (local rigid-body transformation of a domain).

(d) Two maps +/- domain.

7. What do you think of the opinion in the Discussion: *Note that, in general, the optimal parameter setting for a method differs for each map and the purpose of the computation. Thus, a perfectly fair comparison is not possible, and the comparison shown in this work is to characterize the performance of VESPER but not to rank the methods.* Hint: see **Responses to Comments by Reviewer #2**, p. 10.

8. The authors chose a $7\mathring{A}$ grid spacing. Why? How do you suspect the performance of VESPER to change with other grid spacings? Does the method require the same grid spacing when comparing maps? Hint: read the section **Exploration of parameter combinations.**

9. Reviewer #1 asked the authors to justify what map-to-map alignment is useful. Why would a researcher want to align maps (vs model-to-model and model-to-map)?

6.2 28 Jan 2022 - Guest: Qinwen (Wendy) Huang - Weakly Supervised Learning for Joint Image Denoising and Protein Localization in Cryo-EM

–Pre-reading

1. Qinwen Huang, Ye Zhou, Hsuan-Fu Liu, & Alberto Bartesaghi (2021). Weakly Supervised Learning for Joint Image Denoising and Protein Localization in Cryo-EM `https://www.mlsb.io/papers_2021/MLSB2021_Weakly_Supervised_Learning_for.pdf`

–Questions

1. This paper exploits analytical likelihood-prior-posterior conjugacy between gaussian distributions. How could we extend this to Poisson noise with Poisson-Gamma conjugacy? What would be Poisson, and what would be Gamma?

6.3 4 Feb 2022 - SO(3) - encoding of S0(3)

–Pre-reading

1. `https://en.wikipedia.org/wiki/Euler_angles#Rotation_matrix`

2. Gregory G. Slabaugh. Computing Euler angles from a rotation matrix

3. Axis/Angle Representations for Rotations

4. Quine, JR. 4. Orthogonal Transformations and Rotations in Mathematical Techniques in Structural Biology, pp. 25-31

5. Visualizing quaternions (4d numbers) with stereographic projection

6. Quaternions and 3d rotation, explained interactively

7. Visualizing quaternions An explorable video series

8. Wiki: Quaternions and spatial rotation

9. Maxime Tournier's Research Notes. Quaternions

–Questions

1. Problems (e.g. problem 7) from Quine, JR. 4.5 Problems in *Mathematical Techniques in Structural Biology*, pp. 30-31

2. Implement the pseudo-code in Figure 1 of Gregory G. Slabaugh. Computing Euler angles from a rotation matrix, and use it to compute the Euler angles from a rotation matrix.

3. Go to the doublecover interactive, and select the 4D tab on the bottom. Find the quaternion that corresponds to a rotation of 90 deg in the cw direction about the z axis.

4. Quaterions can be used to describe the rotation of the point $r = \langle r_x, r_y, r_x \rangle \in \mathbb{R}^3$ about angle θ in the direction of vector $\langle q_x, q_y, q_z \rangle$ as follows. $p_{rotated} = q * p * q^{-1}$, where $q = \langle \cos(\theta/2), q_x \sin(\theta/2), q_y \sin(\theta/2), q_j \sin(\theta/2) \rangle$ and $q^{-1} = \langle \cos(-\theta/2), q_x \sin(-\theta/2), q_y \sin(-\theta/2), q_z \sin(-\theta/2) \rangle$ encode the rotation; $\vec{r}$ is represented as the quaternion $p = (0, r_x, r_y, r_z)$. Question: using pencil and paper, compute the rotation of the point $r = \langle 1, 0, 0 \rangle$ about the z-axis by $\theta = 180^\circ$ (ccw or cw, it's the same for this angle). Do you get $r = \langle -1, 0, 0 \rangle$? You will have to use the quaternion multiplication table ($ij = k$, etc.), and know how to interconvert quaternions to cartesian 3D vectors.

5. Study the sections Conversion to and from the matrix representation and Performance comparisons on the wiki page for Quaternions and spatial rotation. If we want to rotate many xyz points in space with a 3D rotation, what is the efficient way to do this? Taking into account interconversions between different rotation conventions (Euler angles, rotation matrices, axis-angle, and quaternions) how should we efficiently store rotations versus do rotations?

6. Besides storing and doing rotations, we have to search over rotations. Wikipedia has a section on Differentiation with respect to the rotation quaternion. Unpack the

equation for $\frac{\partial \mathbf{p}'}{\partial \mathbf{q}}$. Would this equation ever be useful in cryoEM? When would we want to estimate the rotation by optimization, and how would we do so with this equation?

6.4 11 Feb 2022 - Transformations in SPARX and EMAN2

–Pre-reading

1. (2007). Baldwin, P. R., & Penczek, P. A. The Transform Class in SPARX and EMAN2. Journal of Structural Biology, 157(1), 250-261. `http://doi.org/10.1016/j.jsb.2006.06.002`
   (a) 2.1. Eulerian angles in different conventions
   (b) 2.1.1. Internal representations and usage
   (c) 2.2. Composition of rotations, distance between rotations, volume element for rotation parameters
   (d) 2.2.1. Composition of rotations
   (e) 2.2.2. The similarity between rotations; metric
   (f) 2.2.3. Volume element
   (g) 2.3. Quasi-uniform sampling of rotation parameters

–Questions

1. 2.1: Compare eq. 7 to the treatment in `https://en.wikipedia.org/wiki/Rotation_matrix#Rotation_matrix_from_axis_and_angle`. What is $\hat{n}\hat{n}$?
2. 2.1: Show that eq. 8 follows from eq. 7.
3. 2.1.1: Compute the RHS of eq 17. Multiply two 4x4 matrices of the form eq. 16 and show they are still of the form in eq. 16. What is the new translation of $\bar{M}$?
4. 2.2: Derive eqs. 18,19,20,21
5. 2.2: Derive eq. 23 and the accompanying equation for $(d\hat{n})^2$ in the paragraph that follows.
6. 2.3: Unpack eq. 27. $\delta\theta = \arctan(1/R)$.
7. 2.3: Connect Figure 1 to eqs. 28,29.
8. What other ways are there to do uniform sampling on the sphere?

6.5 25 Feb 2022 - SO(3) - encoding of S0(3)

–Pre-reading

- (2013). Hartley et al., Rotation Averaging. `https://link.springer.com/content/pdf/10.1007/s11263-012-0601-0.pdf`
    1. Introduction
    2. Previous Work on Rotation Averaging
    3. Alternative Pictures of Rotation Space
    4. Distance Measures on SO(3)
    5. Single Rotation Averaging

–Questions

1. **See accompanying notebook I made** `https://github.com/geoffwoollard/learn_cryoem_math/blob/master/nb/rotation_averaging.ipynb`
2. Does the rotation averaging problem arise in cryo-EM? If so, where? What about the conjugate and multiple rotating averaging problems?
3. 4.1.5: Table 2 Relationship between the different metrics on SO(3): Code up the various metrics (d_{chord}, d_{quat}) and numerically verify these relationships on some (arbitrary) rotations
4. A ball in SO(3) is defined in the appendix. To build some intuition, compute the angular distance between R_1 and R_2 that are (1) about the same axis and differ by angle θ; (2) rotate the same angle (e.g. 5 degrees), but about axes that sweep from the Z-axis, down to the X-axis, through the ZX plane. Relate (1) and (2) to the ball.
5. Draw a picture illustrating **Proposition 2**.
6. 5.3: Code up Algorithm 1 for several rotations. How do you do the log and exp in step 3 and 7? Does your mean seem "in the middle"?
7. We can average together quaternions to get a new quaterion. This is a solution to the rotation averaging problem using what distance?

6.6 4 March 2022 - End-to-end orientation estimation from 2D cryo-EM images

–Pre-reading

- Lian, R., Huang, B., Wang, L., Liu, Q., Lin, Y., & Ling, H. (2022). End-to-end orientation estimation from 2D cryo-EM images. Acta Crystallographica Section D Structural Biology, 78(2), 174?186. http://doi.org/10.1107/s2059798321011761

–Questions

1. 1. Introduction: Discuss the problem setup in Figure 1. What are the inputs and output of their method? What sort of specimens (flexibility, symmetry, etc) would it be suitable for?

2. 2.4 Network architecture: Do you understand the network architecture? Draw a schematic (in more details than Figure 2) of the architecture and point out the "256 feature maps with spatial dimensions 16 x 16" and "2048 feature maps with spatial dimensions 4x4".

3. 2.5. Loss functions: engage with this claim: "A straightforward loss function is the L1/L2 distance between the predicted normalized quaternion and the ground truth". Consider the distances for rotations defined in Hartley et al (see past meeting on 24 Feb 2021, 6.5)

4. 2.5: What is the connection between Eqs 11 and 12? Consider the Taylor expansions of cos and arccos

5. 2.5: The authors cite *Manhardt et al., 2019* in support of the claim "Naively regressing the annotated orientations often ends up with predicting an orientation that is closest to all results in the symmetry group". Look up this reference, and learn how it supports their claim.

6. 2.6. Data sets: Given the datasets they used to demonstrate the feasibility of their method, in what scenarios could the method be applied?

7. 2.7: How does the differentiable projection process enter into their method?

8. 2.8: How is the RMSE in Eq 16 different from the reprojection loss in Eq. 13 and 14?

9. 3.1 Orientation estimation from synthetic cryo-EM images: Consider Figures 3 and 4. What are the main take-homes?

10. 3.1: How does the surrounding area (nearby particles) in Figure 4d (real experimental data) affect the optimization, given the formulation of the RMSE and loss?

11. 3.3. Cross-validation tests for w: Study Figures 6 and 7. What story do they tell?

12. 3.4. How is SNR defined here (cf. method section on cryoSPARC)?

13. : 3.4: What is the influence of different noise levels? How much is too much noise? Is the noise in Figures 8 and 9 realistic to empirical data? How close is the sim-to-real gap here?

14. 3.5. Comparison of different representations of 3D orientations: why do you think quaternions performed better than axis-angle?

15. 3.6. 3D reconstruction using network estimation: What is your impression of the visual results shown in Figure 11a-d?

16. 4.3. Evaluation metric and reconstruction quality: How could we "modify the training objective by adding another loss term based on FSC"?

17. 4.4. Handling real cryo-EM images: how could we "efficiently process larger network inputs without significantly increasing the computational cost"?

6.7 11 March 2022 - Guest: Lys Sanz Moreta

–Pre-reading

1. Moreta, L. S., Al-Sibahi, A. S., Theobald, D., Bullock, W., Rommes, B. N., Manoukian, A., & Hamelryck, T. (2019). A Probabilistic Programming Approach to Protein Structure Superposition. 2019 IEEE Conference on Computational Intelligence in Bioinformatics and Computational Biology, CIBCB 2019. `http://doi.org/10.1109/CIBCB.2019.8791469`

2. Moreta, L. S., Al-Sibahi, A. S., & Hamelryck, T. (2020). Bayesian protein superposition using Hamiltonian Monte Carlo. Proceedings - IEEE 20th International Conference on Bioinformatics and Bioengineering, BIBE 2020, 1, 1?11. `http://doi.org/10.1109/BIBE50027.2020.00009`

–Questions

- Moreta et al, 2019

    1. II. METHODS. A.: Overall model: Consider Eq. 1. What types of conformational heterogenity does this model capture?
    2. II.A.: What is the form of the matrix-normal distribution, and how do the covariance matrices $\mathbf{U}, \mathbf{V}$ govern this distribution?
    3. II. B. Bayesian posterior: Where did the covariance matrix $\mathbf{V}$ go?
    4. II. C. Prior for the mean structure: What do you think of the prior of $\mathbf{M}$? Would it make sense to put in more knowledge about how proteins are folded polymer-chains of covalently bonded atoms?

5. II. D. Prior over the rotation: Why do Eqs. 7-9 result in a uniform rotation over the sphere? How can we define a measure of uniformity on SO(3)?
6. II. E. Prior over the translation: Consider Eq. 11. Some alignment algorithms assume that structures share a centre of mass, and first translate to a shared centre of mass before optimizing over rotation. In what sort of scenarios would having a probabilistic model for translation help alignment? What do you think of the magnitude of covariance in Eq. 11?
7. II. F. Prior over U: How exactly is $\mathbf{U}$ sampled? Which elements are dependent and which independent?
8. II. G. Likelihood: Why does "the matrix-normal likelihood of THESEUS [reduce] to a product of univariate Student?s t-distributions."
9. II.G.: Unpack the right hand side of Eq. 13, so that you understand each term and could in principle code it up.
10. II. I. Initialization: The authors choose to initialize $\mathbf{u} = (0.9, 0.1, 0.9)$. Why is this near the identity matrix (ie., no rotation)? Would other choices be suitable?
11. II. J. Maximum a-posteriori optimization: How exactly does Pyro perform MAP? Look up the documentation to Pyro's AutoDelta guide.
12. III. MATERIALS. Proteins: What do you think of the choice of an NMR ensemble? Does it match their modelling choices?
13. IV. RESULTS. Consider Table I. The "Length (Amino Acids)" are variable. How does the algorithm handle this?
14. IV. Consider Figure 2. Are you satisfied with the results?

- Moreta et al, 2020

1. What does The No-U-Turn Sampler (NUTS) add to Hamiltonian Monte Carlo (HMC)?

–Meeting Recording

https://ubc.zoom.us/rec/share/IhUJnNpL-n-XCt8OMOB5a1b3XiNgJUBEv7oQAnVqEVXWa79yubR_DG7c3rAHCnNk.1Xul52lwhvisRzw0

Access Passcode: `ujd&kq5%`

6.8 18 March 2022 - CryoAI - Part I, overview

–Pre-reading

1. Levy, A., Poitevin, F., Martel, J., Nashed, Y., Peck, A., Miolane, N., Ratner, D., Dunne, M., & Wetzstein, G. (2022). CryoAI: Amortized Inference of Poses for Ab Initio Reconstruction of 3D Molecular Volumes from Real Cryo-EM Images. `http://doi.org/10.48550/arXiv.2203.08138`

6.9 25 March 2022 - CryoAI - Part II, supplementary material

–Pre-reading

1. Levy, A., Poitevin, F., Martel, J., Nashed, Y., Peck, A., Miolane, N., Ratner, D., Dunne, M., & Wetzstein, G. (2022). CryoAI: Amortized Inference of Poses for Ab Initio Reconstruction of 3D Molecular Volumes from Real Cryo-EM Images. `http://doi.org/10.48550/arXiv.2203.08138`

–Questions

1. A. Experimental Details. How are the simulated datasets be different from empirical data? How could simulated datasets be more challenging and narrow the sim to real gap?

2. C.1 Electrostatic Potentials in Fourier Space: Why does the radial average of $|\hat{V}(\mathbf{k}))|$ differ over only 2 orders of magnitude, while the central slice differs over 5, that is, 1000 fold more?

3. D.1 Handedness Ambiguity in cryo-EM: Prove $FR_i = \tilde{R}_i F$. Note that left multiplying by a diagonal matrix (here F) is a row multiplication, and right multiplying is a column multiplication. Also note that $\cos(\theta+\pi) = -\cos(\theta)$ and $\sin(\theta+\pi) = -\sin(\theta)$.

4. D.1: Follow the derivation, line by line.

5. D.2 Spurious Planar Symmetries and Symmetrized Loss: What would the loss / energy landscape look like for higher symmetries, e.g. $C_n, D_n;\ n \in \{2, 3, ..., 8, ...\}$?

6. F.1 Full Evaluation of Poses: Consider Figure S8 and discuss the use cases of cryoAI. How do you think practitioners will use amortized inference in practice? Consider the use cases of *ab initio* versus iterative refinement.

6.10 1 April 2022 - Guest: Andrew Hanson. Discovering Quaternion Applications

–Reference Material

1. Hanson, A. J. (2020). The quaternion-based spatial-coordinate and orientation-frame alignment problems. Acta Crystallographica Section A: Foundations and Advances, 76, 432?457. `http://doi.org/10.1107/S2053273320002648`

2. Hanson, Andrew, J. (2006). Visualizing Quaternions. `http://doi.org/0-12-088400-3`

–Questions

1. Figure 2: What parameters are learned and what are fixed during training?

2. Eq 3: Unpack the equation – what do all the terms refer to?

3. 4.2 Heterogeneous reconstruction: compare and contrast Cryofold with other methods for heterogeneous reconstruction.

4. 4.3 Local minima: "A major shortcoming of this atomic reconstruction approach is the existence of many local minima of the loss function that do not approximate the true atomic coordinates." What might a local minima look like and why exactly do they exist?

5. 5. Results: Architecture and training: What might you want to change/investigate about training?

6. Are you surprised at the results presented in "5.1 Homogeneous reconstruction", "5.2 Heterogeneous reconstruction", Figures 3-5? Why or why not? What is the main story?

7. 6 Discussion: What prior beliefs do you have of you sample, and how strongly are these held?

8. 6 Discussion: How do you validate these methods?

6.11 8 April 2022 - Cryofold

–Pre-reading

1. Zhong, E. D., Lerer, A., Davis, J. H., & Berger, B. (2021). Exploring generative atomic models in cryo-EM reconstruction, 1-13.

–Questions

1. Figure 2: What parameters are learned and what are fixed during training?

2. Eq 3: Unpack the equation – what do all the terms refer to?

3. 4.2 Heterogeneous reconstruction: compare and contrast Cryofold with other methods for heterogeneous reconstruction.

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6.12 29 April 2022 - Molecular Prior - Wilson Statistics

–Pre-reading

1. Gilles, M. A., & Singer, A. (2022). A Molecular Prior Distribution for Bayesian Inference Based on Wilson Statistics, 1?11.

6.13 13 May 2022 - Molecular Prior - Wilson Statistics II

–Pre-reading

1. Gilles, M. A., & Singer, A. (2022). A Molecular Prior Distribution for Bayesian Inference Based on Wilson Statistics, 1?11.

2. `https://github.com/ma-gilles/wilson_prior`

6.14 27 May 2022 - Guests: Ngan Nguyen and Feng Liang, Differentiable Electron Microscopy Simulation

–Pre-reading

1. Nguyen, N., Liang, F., Engel, D., Bohak, C., Wonka, P., Ropinski, T., & Viola, I. (2022). Differentiable Electron Microscopy Simulation: Methods and Applications for Visualization, (1), 1–22. `http://arxiv.org/abs/2205.04464`

–Questions

1. In the focal frequency loss (FFL) in Eq. 2, what is the mean over? What is the "normalize" over in w (see paragraph of text below Eq. 2)? Does it have a probabilistic interpretation (i.e. is it the log loss of some distribution)?

2. In what dose regimes is the parametric form they use of the MTF appropriate? What happens at high dose or low dose? When would the MTF not be circularly symmetric?

3. In Algorithm 1, what happens in the step $I_{p_n} \leftarrow\leftarrow I_{p_n}$?

4. In Algorithm 2, how do they compute line 12: $loss \leftarrow SL(I_n, P, ll)$?

5. What do you think are the bottle necks in the performance in Table 2? What could in principle be sped up through parallelization, and what is intrinsically sequential in an advanced simulator?

6. How exactly does the TEM simulator use the multi-slice method?

–Meeting Recording

https://ubc.zoom.us/rec/share/Nxqm3qRFrowV_bQIjKp9y4qESTcnVziP2OhYHYxNBPvQ1zudFCAA7thofsZqsuBi.4ZUgIN3GQEyXf6oh
Access Passcode: `jNv5P+qv`

6.15 3 June 2022 - Stochastic Gradients (Schulman et al., 2015)

–Pre-reading

1. Schulman, J., Heess, N., Weber, T., & Abbeel, P. (2015). Gradient estimation using stochastic computation graphs. Advances in Neural Information Processing Systems, 2015-Janua, 3528?3536. `https://arxiv.org/abs/1506.05254`

–Questions

1. What distributions do not have continuous parameters? If, in a simulator, the parameters come out of a discrete distribution, and then are used as parameters of a continuous distribution, does this violate the assumptions in Schulman et al.?

2. Consider a simple example of one pixel, going through Poisson and then Gaussian (noise) distributions, where the sampled Poisson random variable is the mean for the Gaussian. What does the estimated gradient look like?