Online CryoEM Study Group

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1 General Information

In just a few days since my message on 3DEM there were over 100 responses to the survey. Below is the plan, which will kick off this upcoming week on **Thursday 19 Nov at 9 AM PST. Dr. Frederick Sigworth will be our special guest for this inaugural meeting.** Now is the time to extend invitations far and wide. 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 link to follow (Zoom). Forgive typographical errors for this document, which I wanted to get out as soon as possible. **Please join the Slack group and ask questions there, rather than emailing me, as there are too many of us and I will be overwhelmed!**

1.1 Meeting Frequency and Length

The most popular meeting frequency was every 1-2 weeks (59) followed by once a month (32). We'll hit the ground running and start off every 1-2 weeks. Depending on how things go we way move to once a month. For length the popular times were 60-90 min (47), followed closely by 30-45 min (41). Thus the initial format will be:

- Every 1-2 weeks
- 60-90 min

1.2 Audience and Streams

Over 2/3 of the audience are grad students. There rest of the audience is a mix of long term staff (13, facility manager, seniour scientist, research associate, unspecified industry position, etc.), principal investigators (4, industry or academic), postdocs (9), and undergraduate (1). After carefully reading the responses, I think it would be best to have two streams each meeting. I have code named them the **blur** and **sharpen** stream, referring to map sharpening. I am counting on your sense of humour and humility for this playful naming schema! Perhaps its a bit more gentle than low and high resolution? Remember

that both blurred and sharpened maps are important for being able to see what is going on and build a model in to the map. The goal of this study group is to have both intuition and be able to connect that back to the math, never missing the forest for the trees.

Blur stream: Beginners and intermediates with may have years of expertise doing sample prep, collecting data on the scope, making maps, and building models. However, many people with such expertise expressed a desire to go deeper into the fundamentals, and understand things better under the hood, and requested help to become more confident in the math. One person put it bluntly, "I am interested in being more than a button pusher."

Sharpen stream: This stream can be for people with a high degree of expertise that are looking to sharpen their skills, and interact with other experts. For example: methods developers with perhaps not that much experience processing datasets from start to finish, university instructors, facility managers who have a strong working knowledge but want to go deeper into the foundations and refresh, people with strong math and physics backgrounds that are fairly new but will catch on quickly, experts in crystallography that are switching over to cryoEM but that have a strong theoretical basis in the underlying math already.

After reading through the responses a few times, I would estimate the ratio of the streams to be about 3/4 blur to 1/4 sharpen. In the first meeting you can choose your own stream, and go to breakout rooms with this in mind. I will make multiple breakout rooms so that there can be small group discussion of 4-8 people. If you are an expert feel free to go to the blur stream and help out. If you judge yourself an intermediate-beginner, you can test the waters in the sharpen stream. The two stream format may evolve over time (e.g. three streams, a lecture with everyone together), but let's try out two streams and see how it goes.

1.3 Meeting Format

We will start with the "flipped classroom" and "breakout room discussions" to get things off the ground with minimal overhead. After some initial meetings we will try out a mix of the following formats listed below, with their surveyed popularity listed next to them. **If you would like to volunteer to give a lecture or be available for office hours then please send me an email to volunteer yourself.**

- Lecture with Q&A (72)
- Flipped classroom: i.e. we have a syllabus with pre-reading (textbook chapter, review paper) that we go through before and then discuss online (57)
- Breakout room discussion (small groups organized thematically) (35)
- Office hours with an expert available to answer questions (24)

2 Dates and Topics

Date	Time	Stream	Topic
Th 19 Nov 2020	9 AM PST / 12 PM EST	blur/sharpen	defocus phase contrast
~~Th 26 Nov 2020~~			(cancelled for US Thanksgiving)
Th 3 Dec 2020	9 AM PST / 12 PM EST	blur	Fourier transform
Th 10 Dec 2020	9 AM PST / 12 PM EST	sharpen	Fourier transform
Th 17 Dec 2020	9 AM PST / 12 PM EST	blur	convolution, sampling, Nyquist
Th 7 Jan 2021	9 AM PST / 12 PM EST	sharpen	convolution, sampling, Nyquist
Th 17 Dec 2020	9 AM PST / 12 PM EST	blur	phase-contrast in the EM
Th 7 Jan 2021	9 AM PST / 12 PM EST	sharpen	phase-contrast in the EM
Th 21 Jan 2021	9 AM PST / 12 PM EST	blur	2D Expectation-maximization
Th 28 Jan 2021	9 AM PST / 12 PM EST	sharpen	2D Expectation-maximization
Th 4 Feb 2021	9 AM PST / 12 PM EST	blur	2D Expectation-maximization
Th 11 Jan 2021	9 AM PST / 12 PM EST	sharpen	2D Expectation-maximization
Th 18 Feb 2021	9 AM PST / 12 PM EST	blur	image formation (forward model)
Th 25 Jan 2021	9 AM PST / 12 PM EST	sharpen	omage formation (forward model), multislice
Th 21 Jan 2021	9 AM PST / 12 PM EST	...	Wah Chiu et al, lectures, ...
... 2021 ...	...	...	...

3 Meeting Time for a Global Audience

About 94% of those interested in North America and Europe (mainly UK and Germany) (85), with the rest in Israel, China, India, Bangladesh, Australia (5). For now we will have the time at 9 AM PST, thus 7 PM in Israel. I am open to proposals to occasionally have a different time to accommodate a global audience - especially if the presenter is from a less represented time zone. I tried this online time zone tool and it didn't suggest anything that worked. Suggestions are welcome for this aspect.

For the first meeting we will meet at 9 AM PST, which is 12 PM EST, 5 PM GMT, 7 PM GMT+2, 1 AM next day GMT+8, 3 AM next day GMT+10.

4 Organizational Team

To the question 'Would you like to be part of the organizational team?' 39 people answered maybe and 4 people answered yes.

I would appreciate help

- **Giving lectures. You can send a proposal to me including topic, format, time, date.**
- Developing the syllabus, and suggesting (or making) learning content.

- Formulating challenge problems to test comprehension, build intuition, and conceptual understanding.
- **Being the leader of a breakout room to answer questions. You could list your areas of expertise, so people would know to come to you for those things. A sort of 'office hours'.**
- Zoom co-host.
- Suggesting improvements to me. You could ask around and see how people are finding things.

5 Slack

We will use the Slack channel 'cryoem_study_group' for asynchronous chat. The link to join is https://join.slack.com/t/cryoemstudygroup/shared_invite/zt-j66wuws3-~UcfsdmtQow~7qYC~iJu_g. Please join the Slack group and ask questions there, rather than emailing me, as there are too many of us and I will be overwhelmed!

6 Syllabus

We will start off drawing heavily from the content developed by Dr Frederick Sigworth, `https://cryoemprinciples.yale.edu/video-lectures`.

If you haven't already gone through Grant Jensen's popular online course 'Getting Started in Cryo-EM' , now would be a good time to do so. I think enough people have gone through this on their own that we will mainly draw from other material.

6.1 19 Nov 2020

6.1.1 Blur

–Pre-reading

- Complex numbers and the complex exponential (10 min)
- Review of complex numbers (3 pages)
- Defocus phase contrast (35 min)

–Questions

- What is physically happening to the sample when the electron is detected at small diffraction angles vs large diffraction angles? What else can happen to the electron?

6.1.2 Sharpen

–Pre-reading

- Defocus phase contrast (35 min)
- 6.2 The Wave Equation for Fast Electrons in 'Advanced Computing in Electron Microscopy', Kirkland (2000), pp. 156-159.

–Questions

- In Sigworth's derivation of $|\Psi|^2$ in the 'Defocus phase contrast' video, he made various assumptions such as small theta, small epsilon. Under what extreme conditions would they break down. Do these occur in cryoEM? In other experimental regimes besides what is typical in cryoEM?
- Around the 23-25 min mark of the 'Defocus phase contrast' video the CTF seems to oscillate to zeros. Is this observed in practice? Why or why not?
- Work through the derivation with pencil and eraser, justifying each step as best you can. come with your questions to the group study.
- Biological samples are made of atoms that give faint contrast, when compared to samples with higher atomic numbers. Where does the atomic number of the sample come into the equations presented here?

6.2 26 Nov 2020

6.2.1 Blur

–Pre-reading

- The Fourier transform in one dimension (35 min)
- The 1D Fourier Transform (7 pages)
- Interactive coding notebook 'Practical 1 - Fundamentals of Image Processing' in the High-resolution imaging course at TUDelft. Note there are multiple notebooks: Introduction, Fourier Series, Frequency Spectrum, 2-D Fourier Analysis. See the links at the bottom.

–Questions

- Assume you have a 128 Åbox size, with pixel size of 1 Å. If the spacing between bins in Fourier space is *, and there are 64 bins in the negative direction and 65 bins in the positive direction, what length ranges (in units of Å) do the first few and last few frequency bins correspond to? How many frequency bins are between 10 and 5 Å, versus 5 and 2.5 Å?
  Hint: $\langle [..., [0, 1/128), [1/128, 2/128), ..., [\frac{128/2-2}{128}, \frac{128/2-1}{128}), [\frac{128/2-1}{128}, \frac{128/2}{128})\rangle$.

6.2.2 Sharpen

–Pre-reading

- The Fourier transform in two and three dimensions (43 min)
- 2D and 3D Fourier transforms (9 pages)
- Interactive coding notebook 'Practical 1 - Fundamentals of Image Processing' in the High-resolution imaging course at TUDelft. Note there are multiple notebooks: Frequency Spectrum, 2-D Fourier Analysis. See the links at the bottom.

–Questions

- Sample a simple function (e.g. exp, sin, gaussian) in 1D or 2D. Then use some library to compute the FFT. Then compute the DFT in your own implementation. How close is the error? Work our the solution analytically for the continuous case. What should the answer be at some discrete points according to the continuous case, and how is it different from what the FFT gave? What is the typical floating point error? What was the speedup of the FFT?

6.2.3 3 Dec 2020

6.2.4 Blur

–Pre-reading

- Fourier transform: convolution, sampling and Nyquist (37 min)
- Interactive coding notebook 'Practical 1 - Fundamentals of Image Processing' in the High-resolution imaging course at TUDelft. Note there are multiple notebooks: 2-D Fourier Analysis, Convolutions. See the links at the bottom.

6.2.5 Sharpen

–Pre-reading

- Fourier transform: convolution, sampling and Nyquist (37 min)
- Interactive coding notebook 'Practical 1 - Fundamentals of Image Processing' in the High-resolution imaging course at TUDelft. Note there are multiple notebooks: 2-D Fourier Analysis, Convolutions. See the links at the bottom.

6.3 10 Dec 2020

6.3.1 Blur

–Pre-reading

- Phase-contrast imaging in the EM' (10 pages)
- Interactive coding notebook 'Practical 1 - Fundamentals of Image Processing' in the High-resolution imaging course at TUDelft. Note there are multiple notebooks: CTF. See the links at the bottom.

6.3.2 Sharpen

–Pre-reading

- Phase-contrast imaging in the EM' (10 pages)
- Interactive coding notebook 'Practical 1 - Fundamentals of Image Processing' in the High-resolution imaging course at TUDelft. Note there are multiple notebooks: CTF. See the links at the bottom.

6.4 17 Dec 2020

6.4.1 Blur

–Pre-reading

- Nelson, P. C. (2019). Chapter 12 : Single Particle Reconstruction in Cryo-electron Microscopy. In Physical Models of Living Systems (pp. 305?325).
- Interactive coding notebook 'Practical 2 - 2D/3D reconstruction' in High-resolution imaging course at UTDelft.

6.4.2 Sharpen

–Pre-reading

- Nelson, P. C. (2019). Chapter 12 : Single Particle Reconstruction in Cryo-electron Microscopy. In Physical Models of Living Systems (pp. 305?325).
- Interactive coding notebook 'Practical 2 - 2D/3D reconstruction' in the High-resolution imaging course at TUDelft.

6.5 7 Jan 2020 2020

6.5.1 Blur

–Pre-reading

- NCCAT SPA short course 2020, Lecture 4: Algorithms and foundational math Part I & 2, Fred Sigworth (1:18:10 - 1:28:46, 10 min)

6.5.2 Sharpen

–Pre-reading

- Section '2. Theory' in Vulović, M., Ravelli, R. B. G., van Vliet, L. J., Koster, A. J., Lazić, I., Löcken, U., ? Rieger, B. (2013). Image formation modeling in cryo-electron microscopy. Journal of Structural Biology, 183(1), 19?32. `http://doi.org/10.1016/j.jsb.2013.05.008`
- Supplementary material associated with the article 'Image Formation Modeling in Cryo-Electron Microscopy' (18 pages)
- 6.4 The Multislice Solution in 'Advanced Computing in Electron Microscopy', Kirkland (2000), pp. 162-165

6.6 21 Jan 2020

By this time the anticipated textbook by Wah Chiu, Robert Glaeser, and Eva Nogales will hopefully be out, and we can see how it looks. We could also plan some lectures.