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Scientific Computing

Cross-Validation for Measuring and Preventing Overfitting in Cryo-EM

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Ada Lovelace Centre

Agenda

- **Introduction to Cryo-EM**
- Project Motivation
- Measuring Overfitting
- The Cross-Validation Workflow
- Project Extensions

What is Cryo-EM?

- Biological macromolecules imaged in electron microscope
- Molecules rapidly frozen in a thin layer of vitreous ice – captures molecules in their native state.
- Randomly oriented and positioned in ice layer.

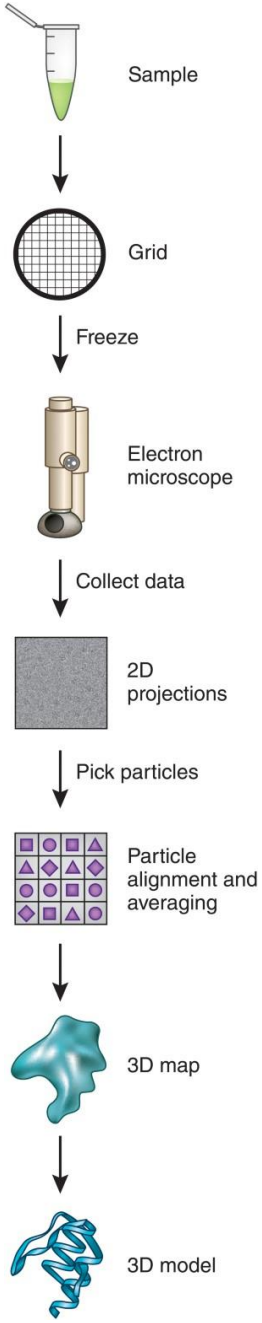
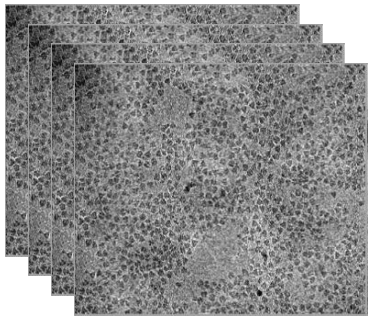


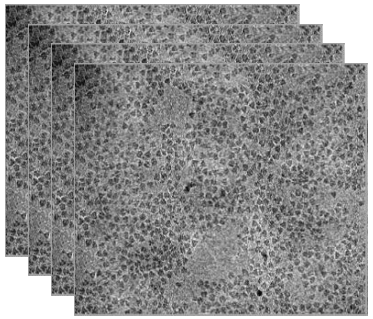
Figure: Doerr, A. Single-particle cryo-electron microscopy. Nat Methods 13, 23 (2016). https://doi.org/10.1038/nmeth.3700

Cryo-EM Image Processing



Movie Frames

Cryo-EM Image Processing



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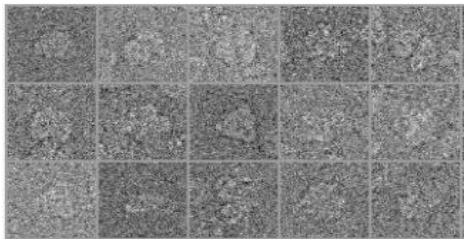

Particle picking
and extraction

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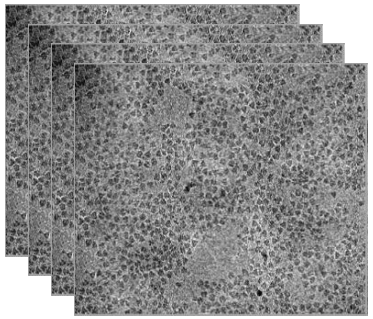
Cryo-EM Image Processing



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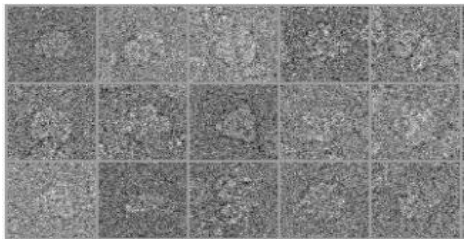

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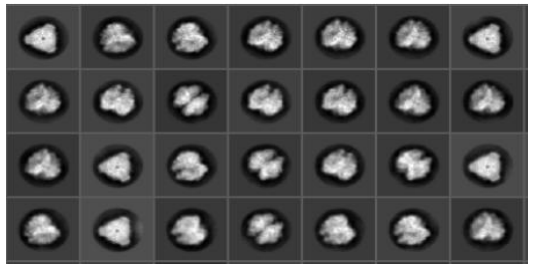

Particle alignment
and classification

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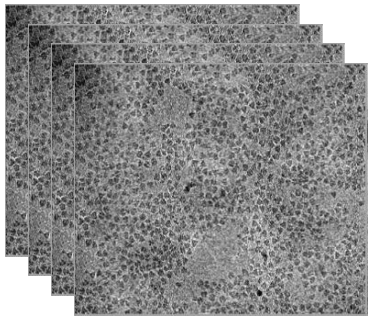
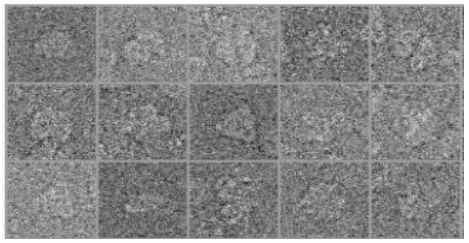
Cryo-EM Image Processing



Movie Frames



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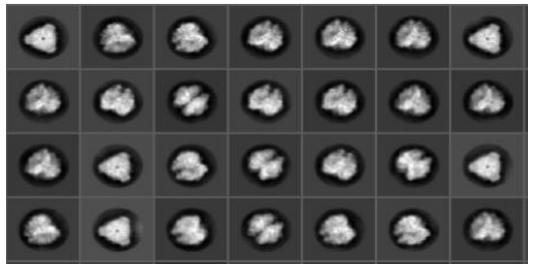

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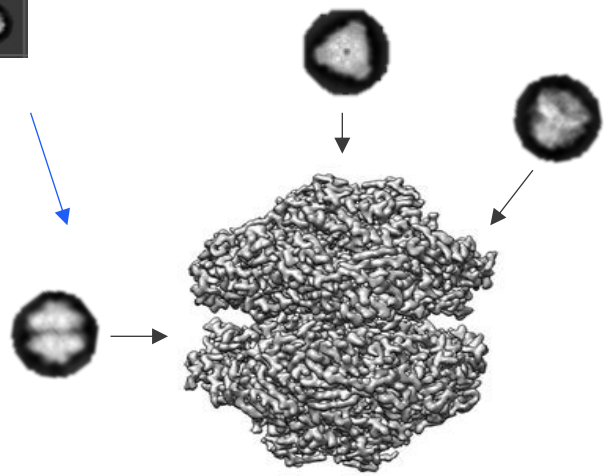

3D Reconstruction

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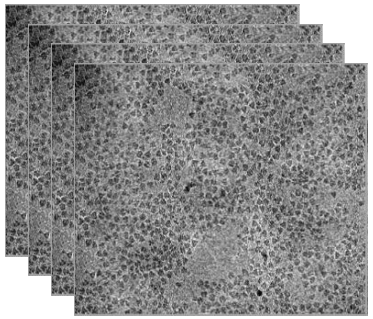
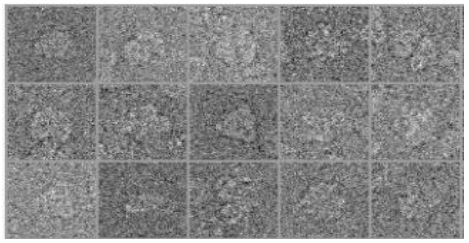
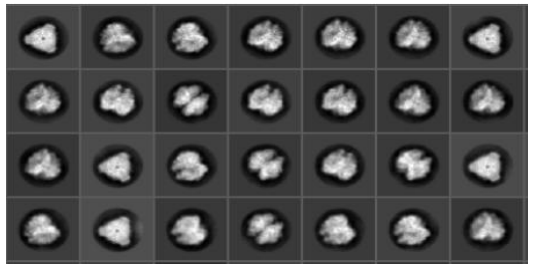
Cryo-EM Image Processing



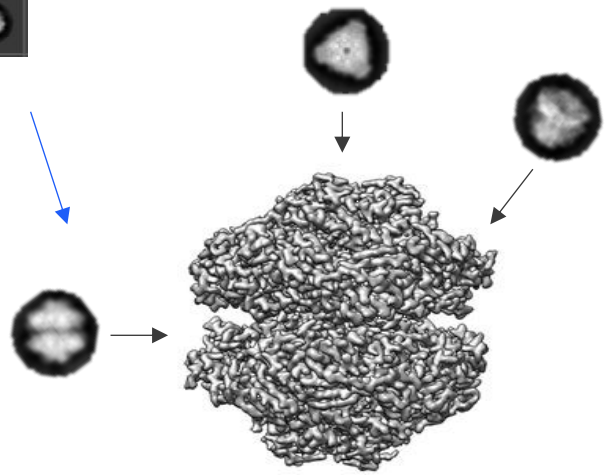
Movie Frames



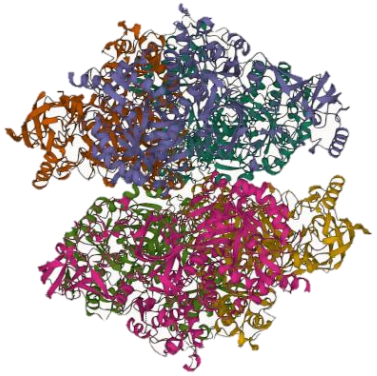
Particle picking and extraction



Particle alignment and classification



3D Reconstruction



Model Building

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- **Project Motivation**
- Measuring Overfitting
- The Cross-Validation Workflow
- Project Extensions

Project Motivation

- Images are noisy!

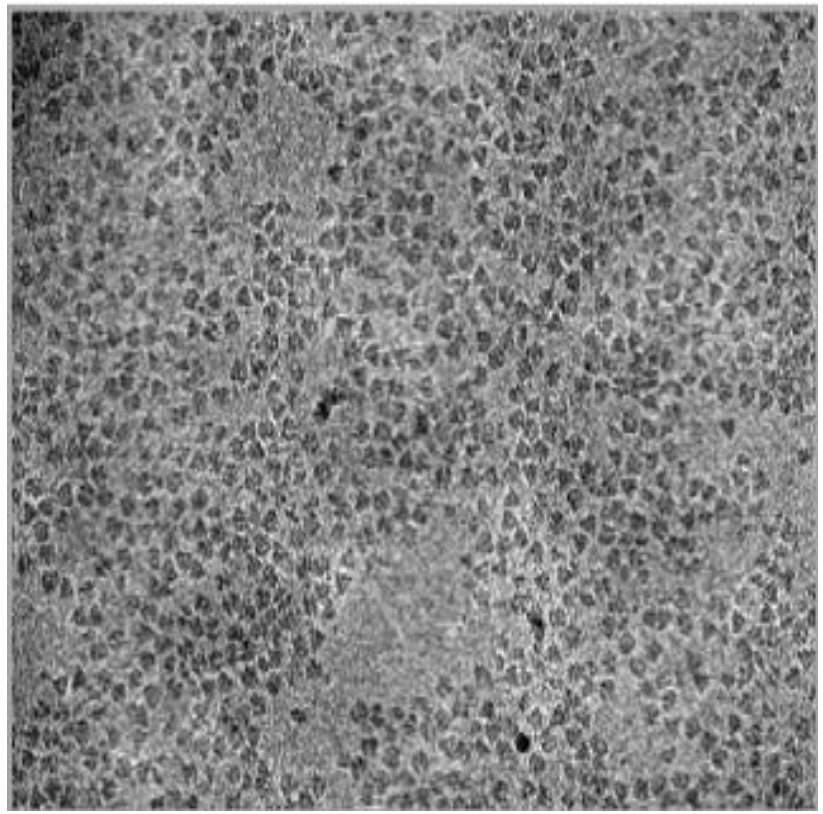


Image Formation Model

Usually, all movie frames are summed together to calculate a 3D reconstruction. Instead, consider a “single-frame reconstruction”.

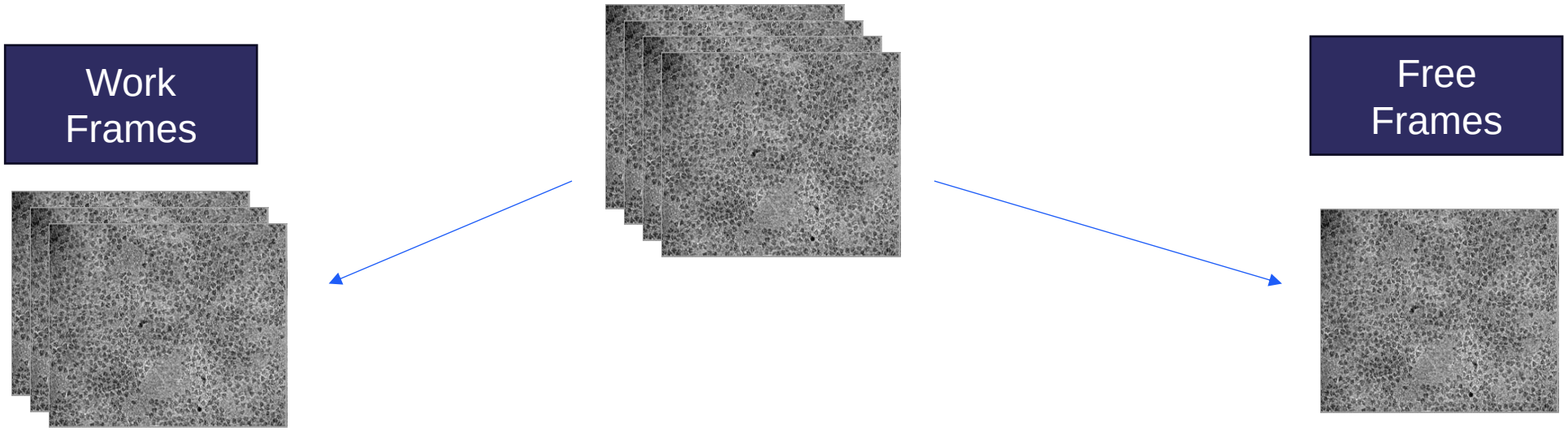
In reciprocal space, each structure factor F_i can be written as

$$F_i = F_S + N_i$$

for (randomly-chosen) frame with index i

F_S is “true” signal shared by all frames, and N_i is the noise contribution from that particular frame

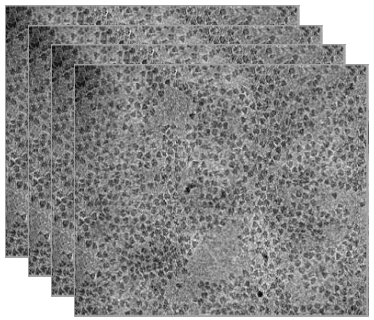
Work, Free, and Test Sets



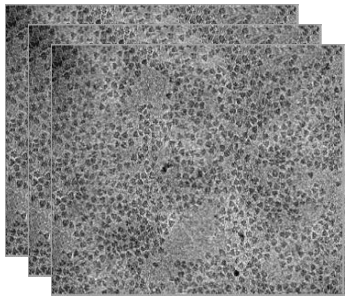
$$F_w = n_w F_s + N_w = n_w F_s + \sum_i^{n_w} N_i$$

$$F_f = n_f F_s + N_f = n_f F_s + \sum_{n_w+1}^{n_w+n_f} N_i$$

Work, Free, and Test Sets

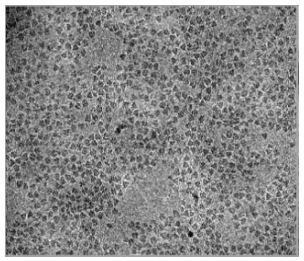


Work Frames



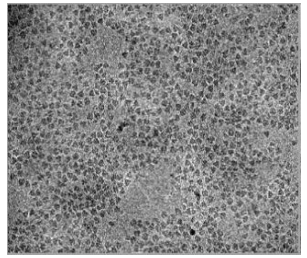
$$F_w = n_w F_s + N_w = n_w F_s + \sum_i^{n_w} N_i$$

Test Frames



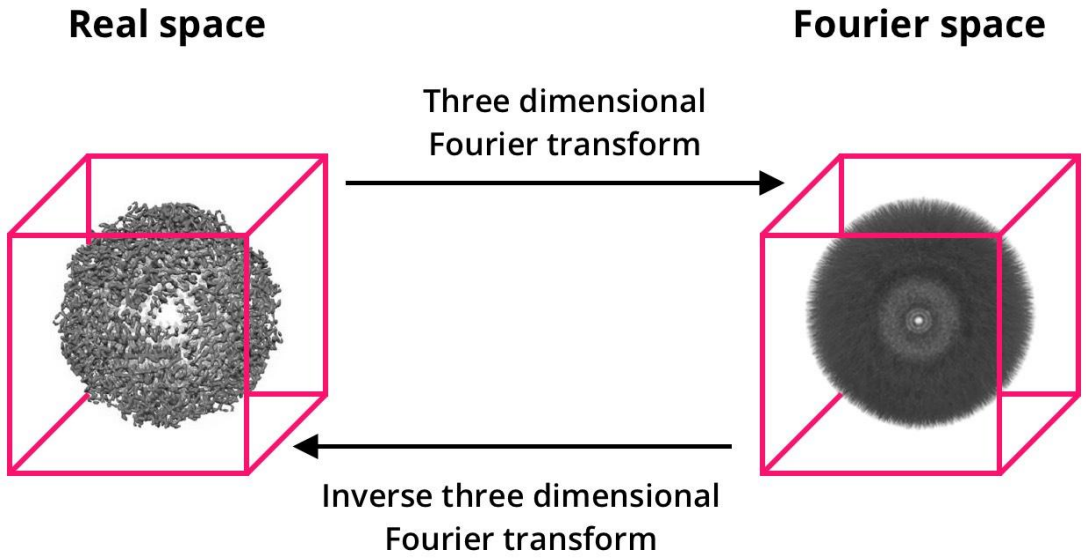
$$F_t = n_f F_s + N_t = n_f F_s + \sum_i^{n_f} N_i$$

Free Frames



$$F_f = n_f F_s + N_f = n_f F_s + \sum_{n_w+1}^{n_w+n_f} N_i$$

Fourier Shell Correlation

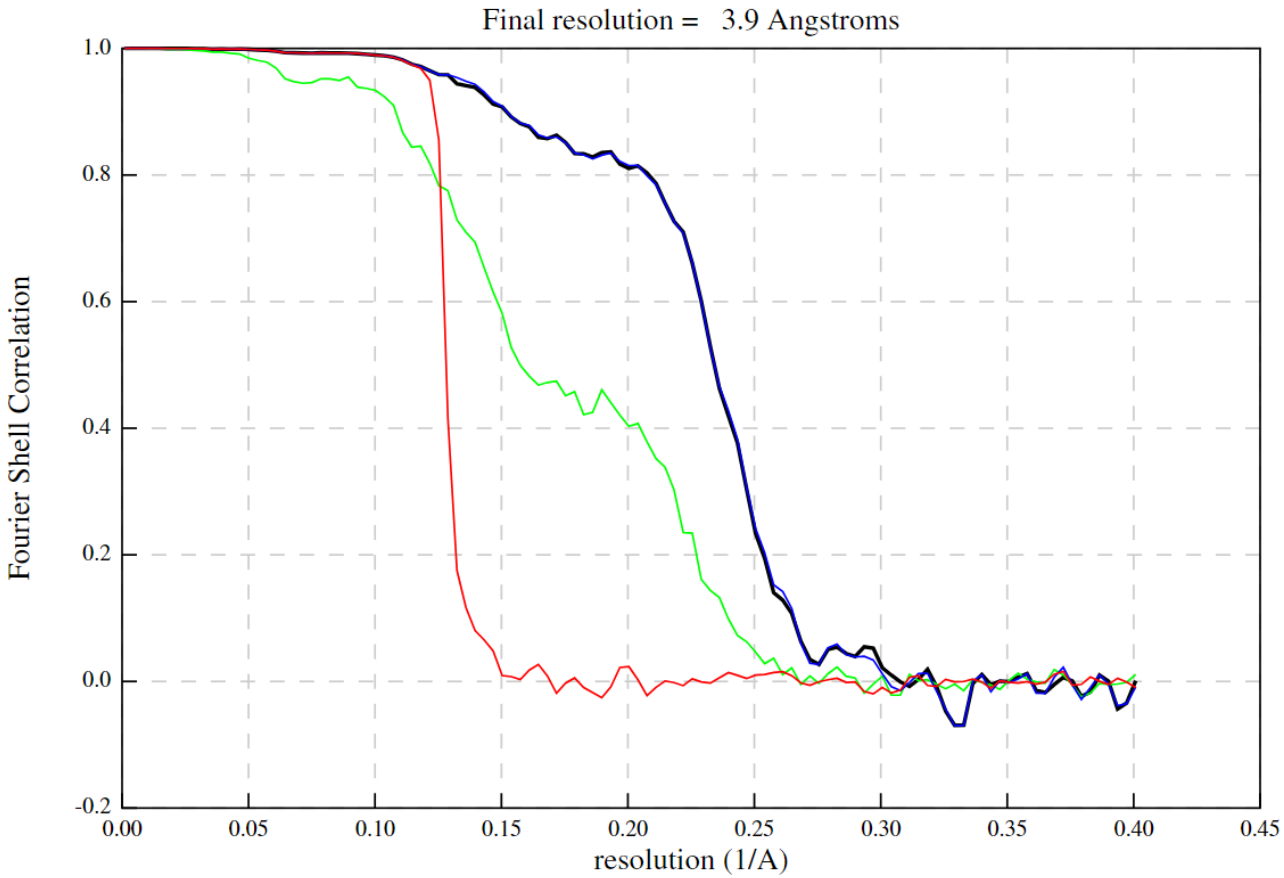


- Widely used method to assess agreement between 3D volumes in cryo-EM
- Defined as the Pearson Correlation Coefficient between two complex variables:

$$FSC(r) = Corr(F_A, F_B)$$
$$= \frac{Cov(F_A, F_B)}{\sqrt{Var(F_A)Var(F_B)}}$$

Figure: https://myscope.training/CRYO_Why_do_Fourier_transforms

Fourier Shell Correlation



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Estimating FSC curves

- There are several FSC curves that can be directly calculated between structure factors from an atomic model (F_c) and the work, free, and test maps.
- Can use these to estimate what we want to know:
  - How closely does the fitted model match the real structure observed in the data, i.e. FSC_{cs}
  - How much has the model been over-fitted to noise, i.e. FSC_{cn}

Assumptions

- All frames are equally well fitted to the final reconstruction, so all common shared signal is included in F_s and noise N_w is uncorrelated with the signal.
- Assume that the power of the noise in each single frame reconstruction is the same and noise variables N_i are uncorrelated to each other

Estimating FSC curves

Starting from Fisher Z-transform, $z = \frac{1}{2}\ln(\frac{1+r}{1-r})$

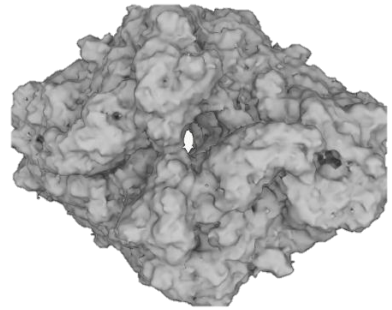
$$FSC_{cs} = \tanh(\frac{1}{2}\ln(\frac{\sqrt{FSC_{tf}}+FSC_{cf}}{\sqrt{FSC_{tf}}-FSC_{cf}}))$$

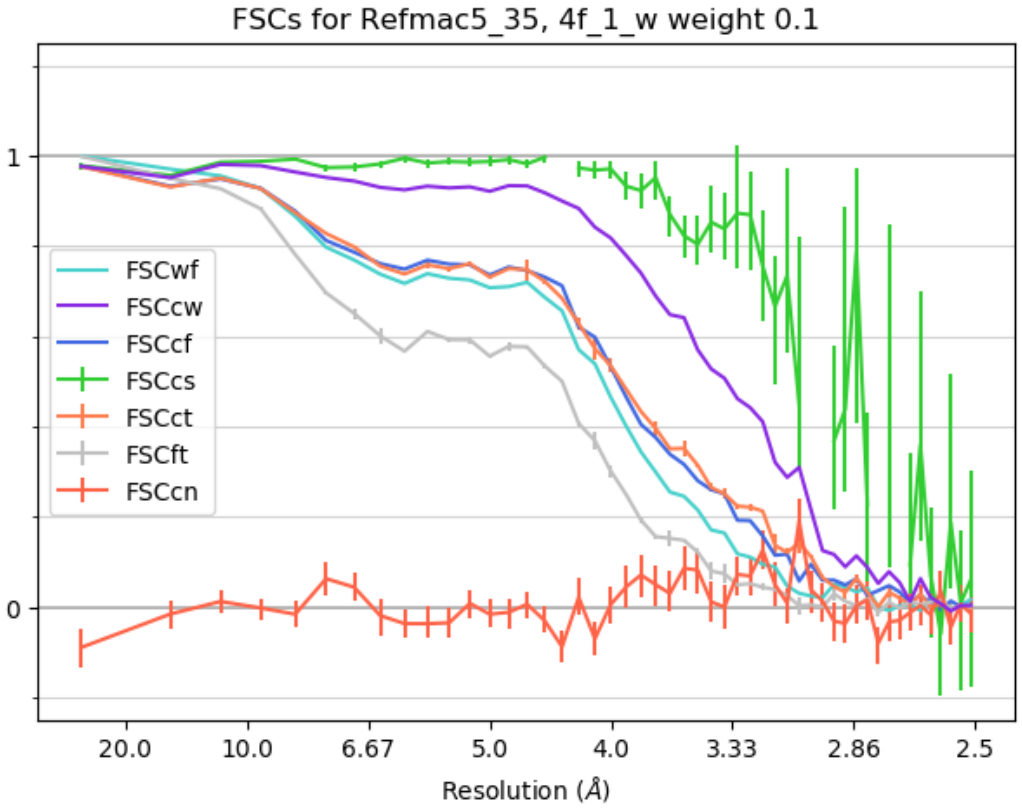
$$FSC_{cn} = \tanh(\frac{1}{2}\ln(\frac{\sqrt{1-FSC_{tf}}+\sqrt{\frac{n_w}{n_f}}(FSC_{ct}-FSC_{cf})}{\sqrt{1-FSC_{tf}}-\sqrt{\frac{n_w}{n_f}}(FSC_{ct}-FSC_{cf})}))$$

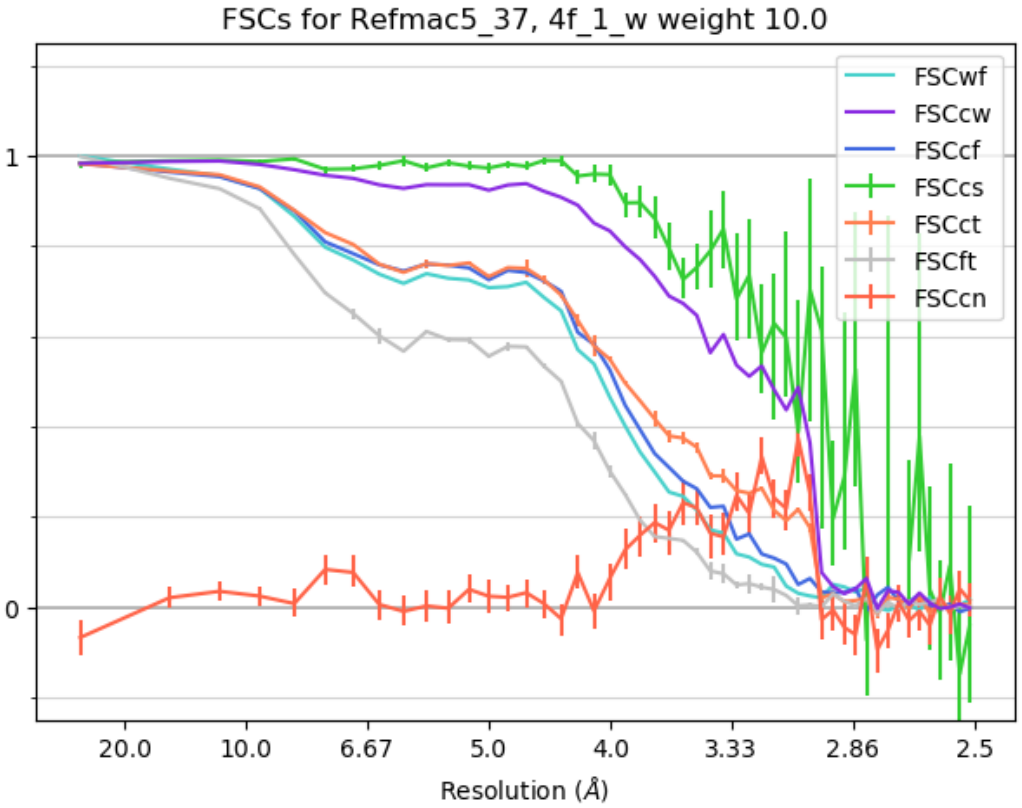
Uncertainty Quantification

- Bootstrapping used estimate 95% confidence intervals
  - Repeatedly sample Fourier components for each Fourier shell **with replacement**
- Need many bootstrap iterations (~1000) for a good estimate of underlying distribution
  - Slow to compute but embarrassingly parallel

Beta-Galactosidase Dataset







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- **Software Implementation**
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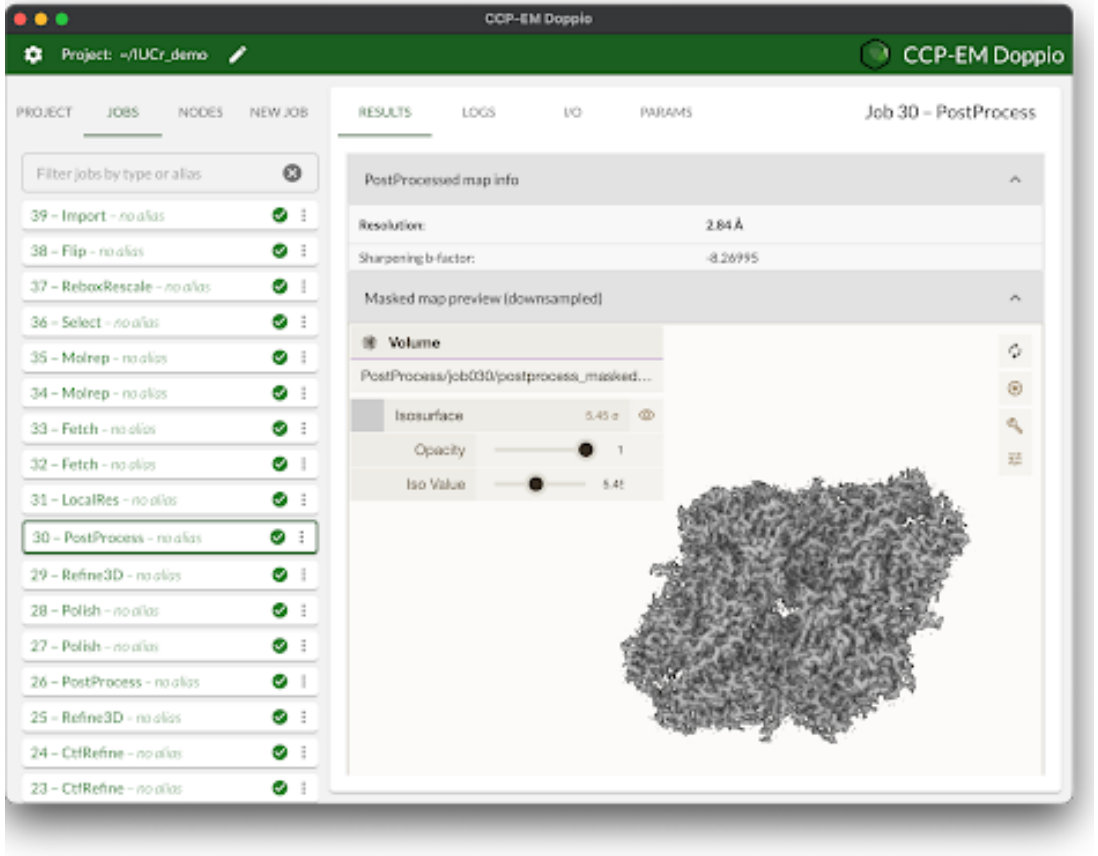
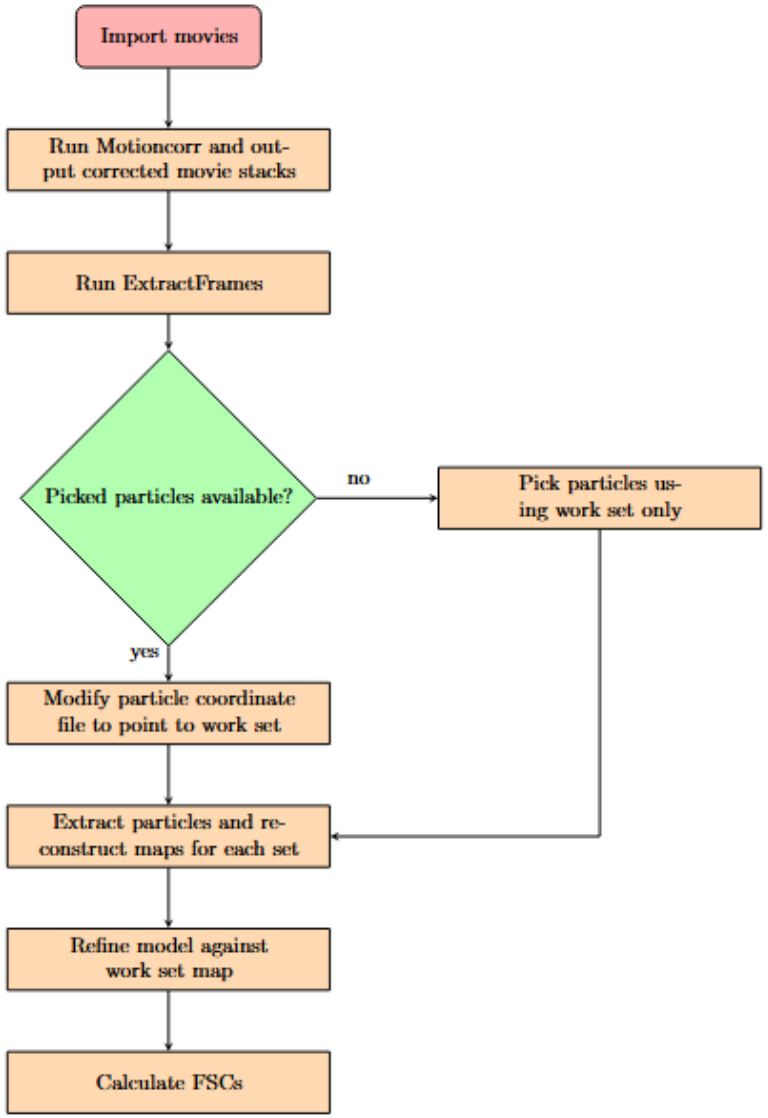
The Cross-Validation Workflow





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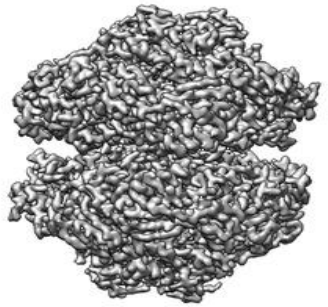
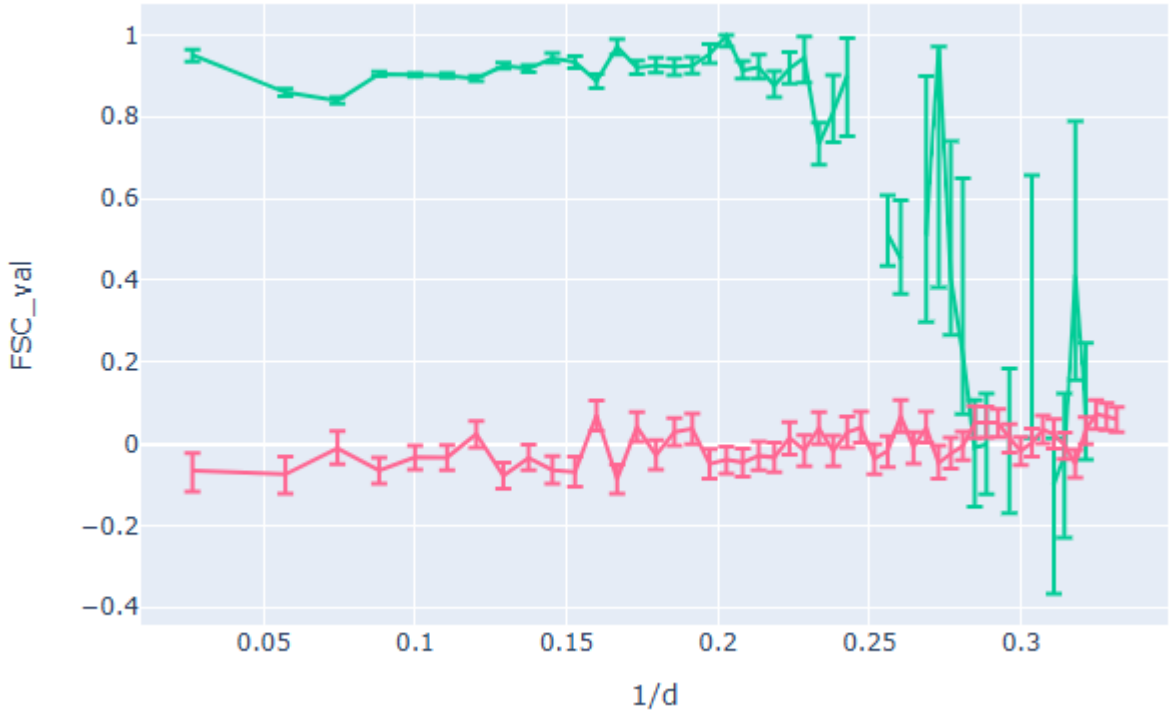
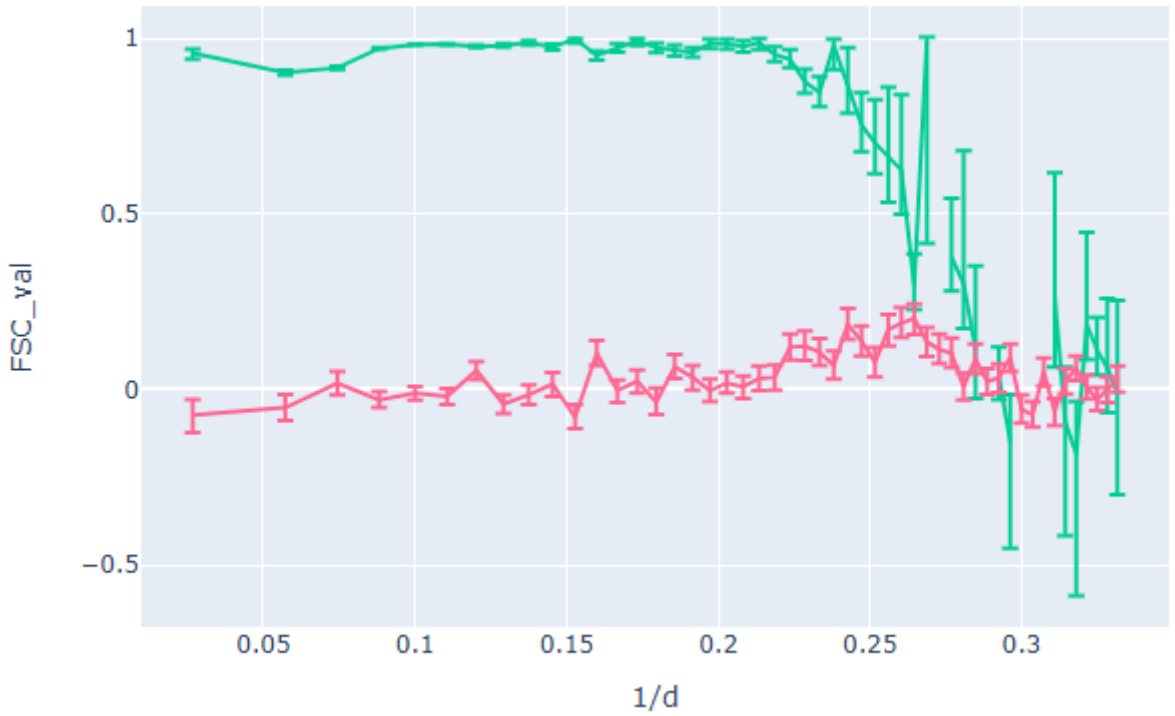
Jack Bean Urease Dataset







FSCcs FSCcn

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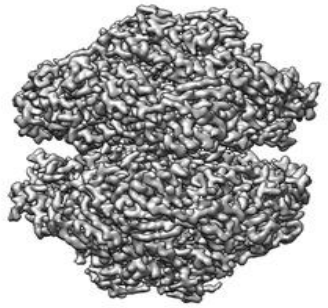
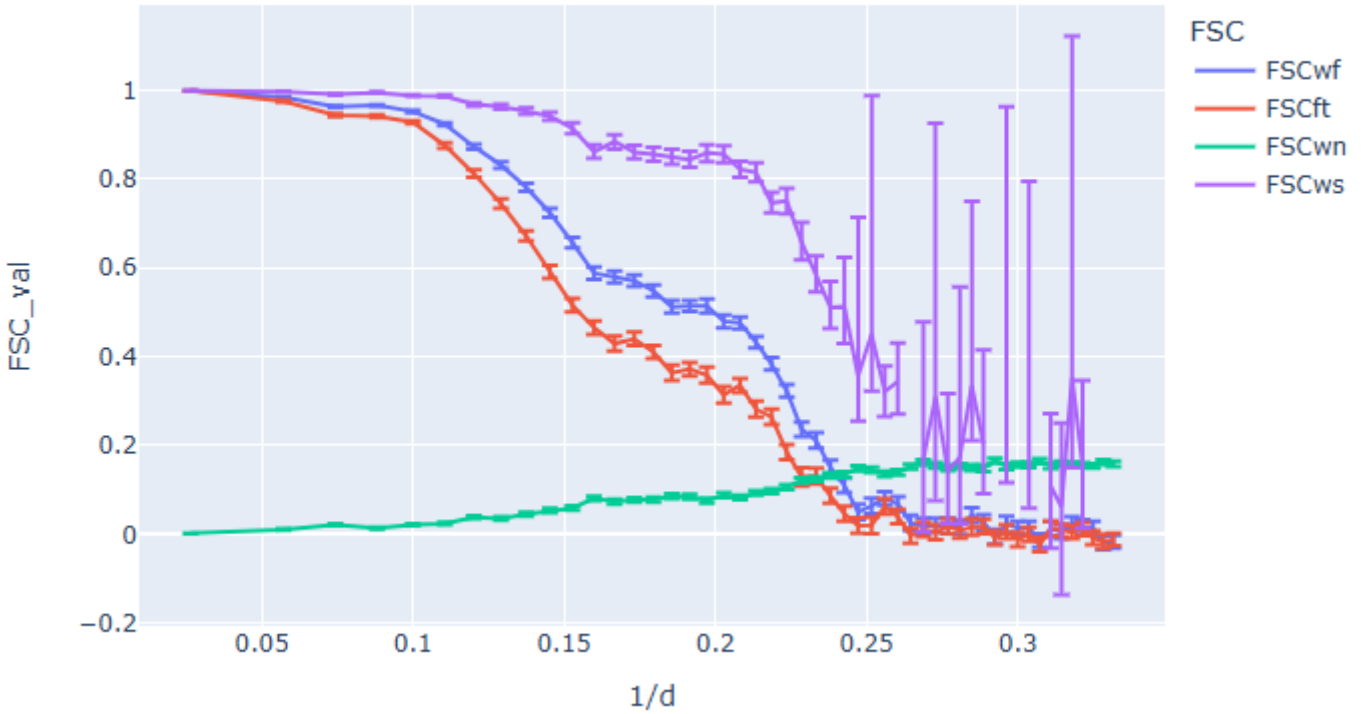
Jack Bean Urease Dataset





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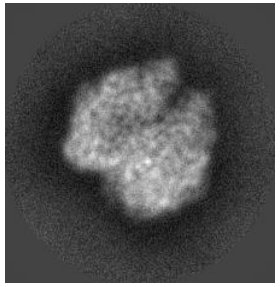
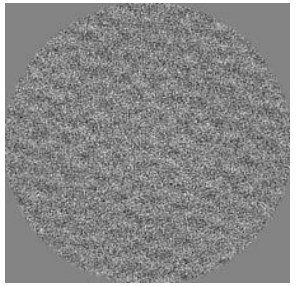
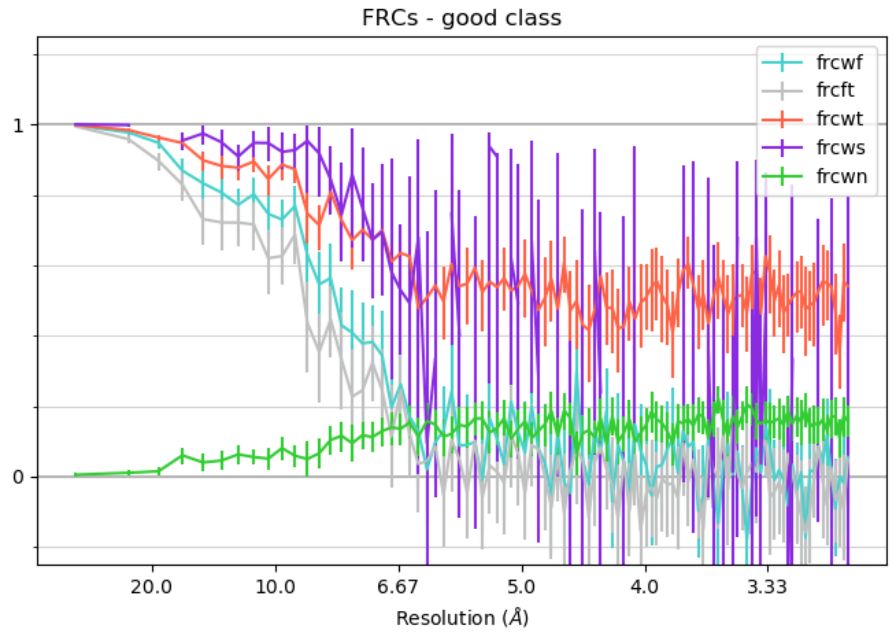
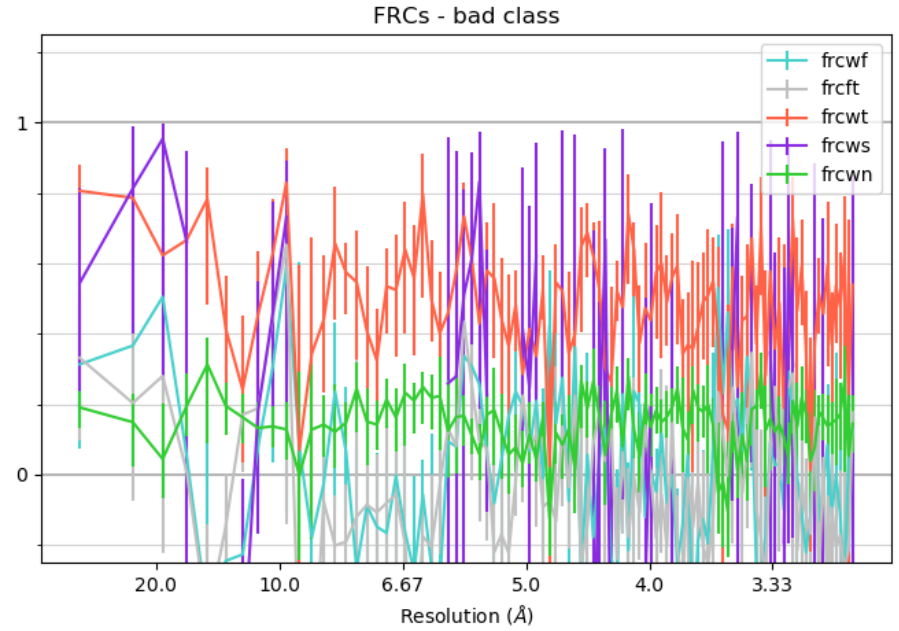
Validating 2D Class Averages









What's Next?

- More testing!
  - Involvement of electron Bio-Imaging Centre (eBIC) scientists
  - How to effectively summarise 2D class average FRCs?
  - Can we account for electron damage across movie frames?

- Method Refinements
  - Properly accounting for degrees of freedom for bootstrapping
  - How to account for variability of free set sampling?
  - How to handle noisy data points?

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Thank you

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