13. A Bayesian approach to cryo-EM structure determination

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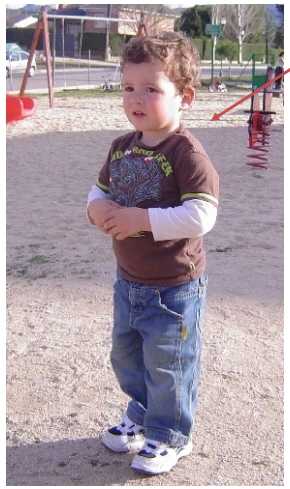
Birkbeck College, London

Agenda

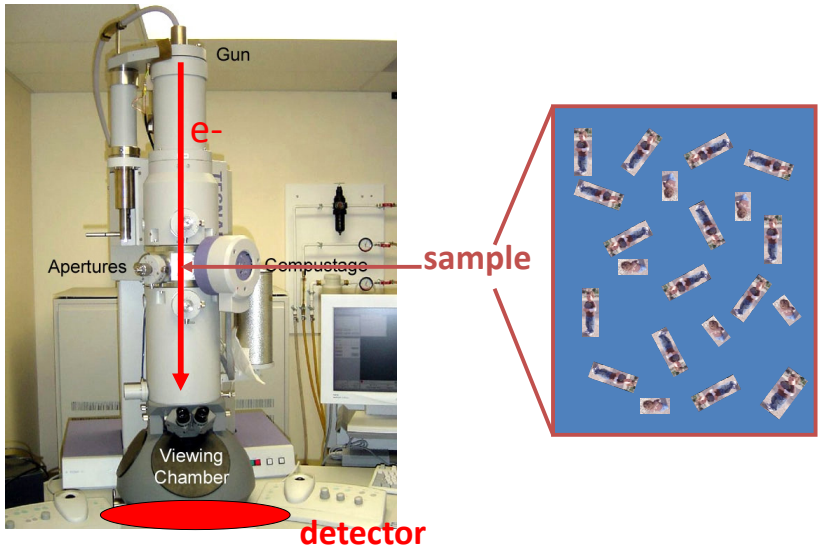
- An intuitive introduction
- Alignment
  - Dealing with the incomplete problem
  - maxCC vs ML (real-space)
- Classification
  - Multi-reference alignment in 2D
  - and in 3D
- Fourier-space formulation
  - Regularised likelihood optimisation (Bayesian approach)

An intuitive introduction

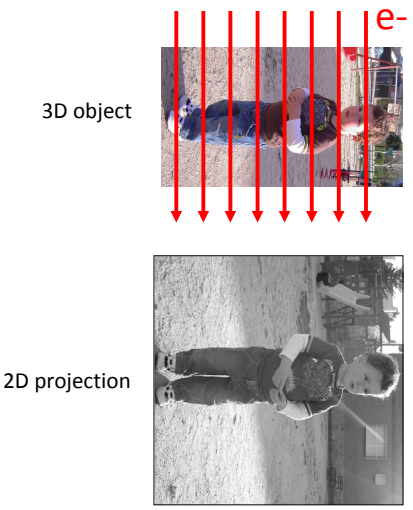
An example "protein"



Experimental setup



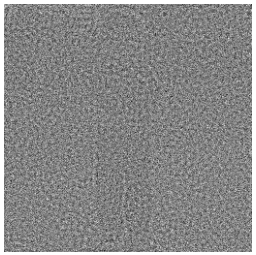
Electron microscopy imaging



We collect data in 2D, but we want 3D info!

Further inconveniences

- Microscope imperfections introduce artefacts
  - Contrast Transfer Function (CTF)
- Large amounts of noise

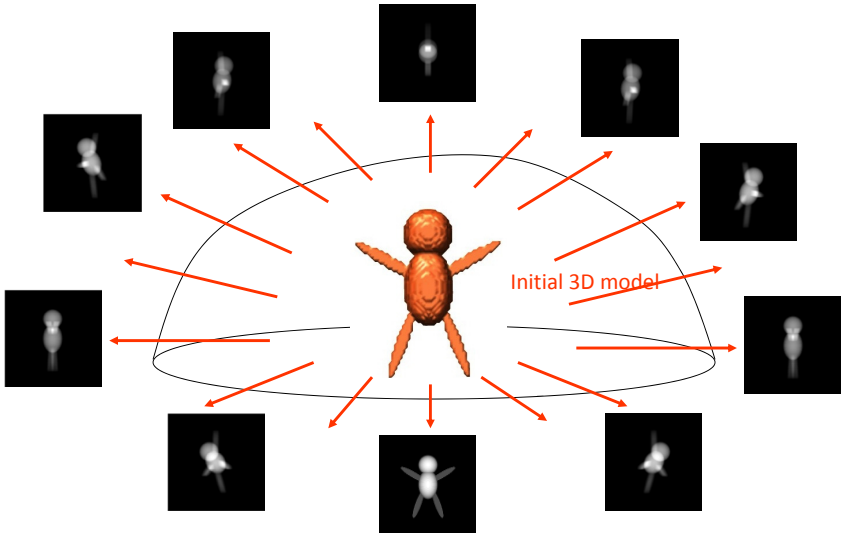


Single particle analysis

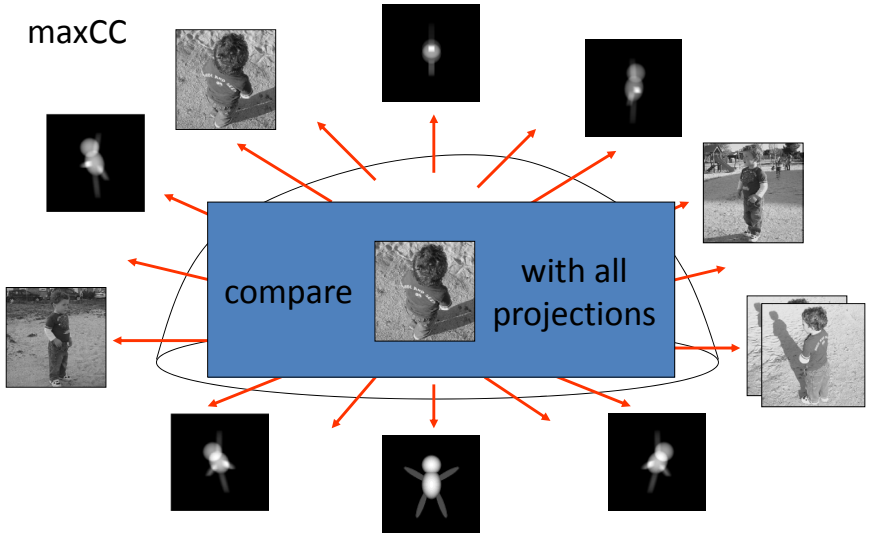
- Embedded in ice: many unknown orientations

- Combine all 2D projections into a 3D reconstruction

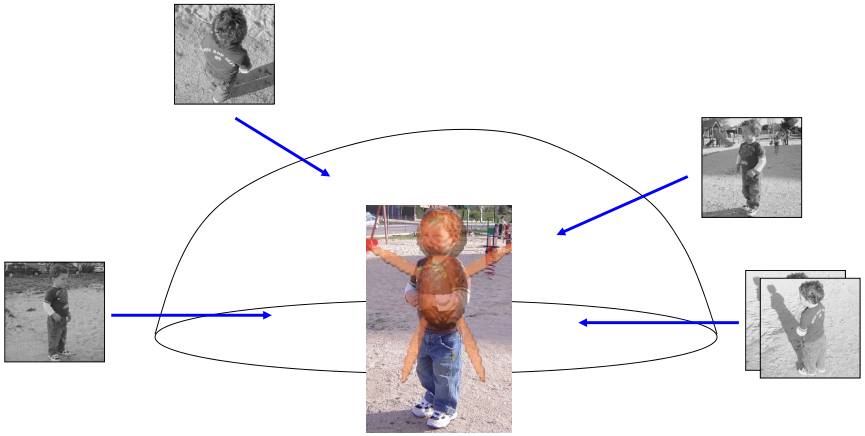
Projection matching



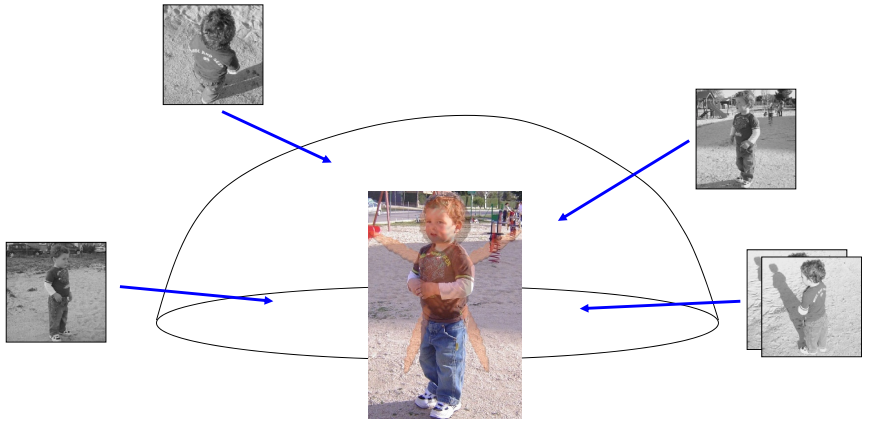
Projection matching



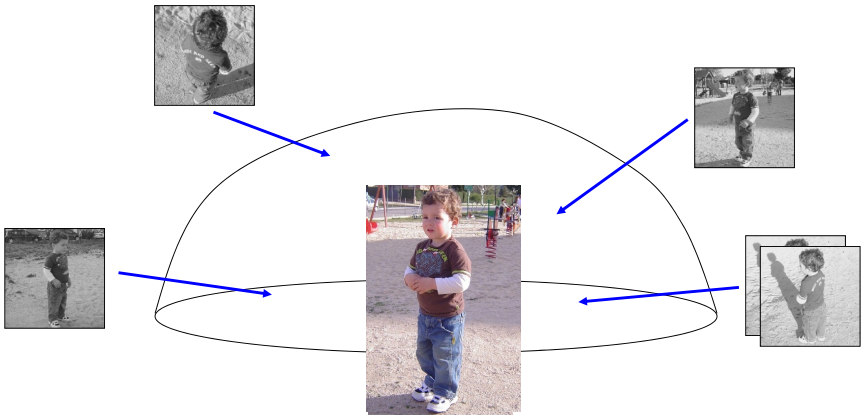
3D reconstruction



Iterative refinement



Iterative refinement



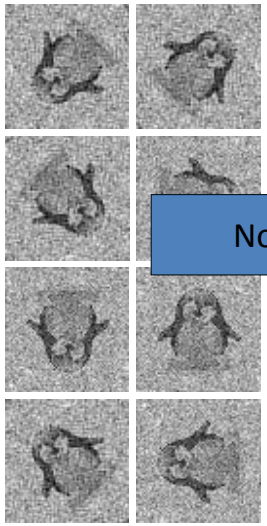
Alignment

Or how to ‘match’ projections

Incomplete data problems

- Part of the data was not observed experimentally
  - Orientations
  - Class assignments
- Difficult to solve!
  - Iterative methods?
- Complete data problem would be very easy to solve
- (Another famous one: the phase problem in XRD)

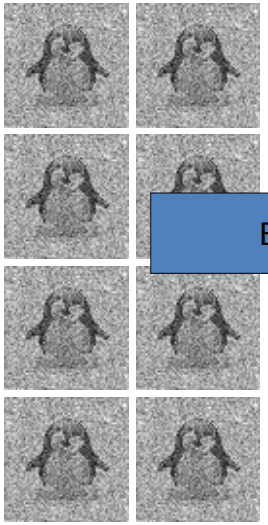
Incomplete data problems



Observed data (*X*): images

Missing data (Y): orientations

Complete data problems



white Gaussian noise

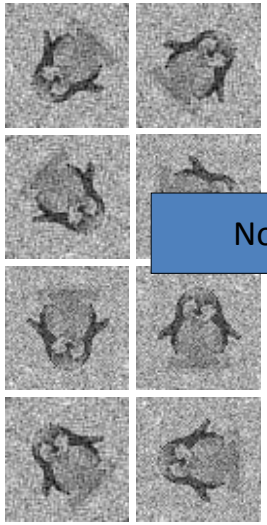
$$L(\Theta) = P(X \mid \Theta)$$

Easy!

$$A^{MLE} = \frac{1}{N}\sum_{i=1}^{N} X_i$$

Observed data (*X*): images

Incomplete data problems



Not easy

Observed data (*X*): images

Missing data (Y): orientations

Incomplete data problems

- Option 1: add *Y* to the model → Maximum cross-correlation / least-squares

$$L(Y,\Theta) = P(X \mid Y,\Theta)$$

- Option 2: marginalize over *Y* → Maximum Likelihood

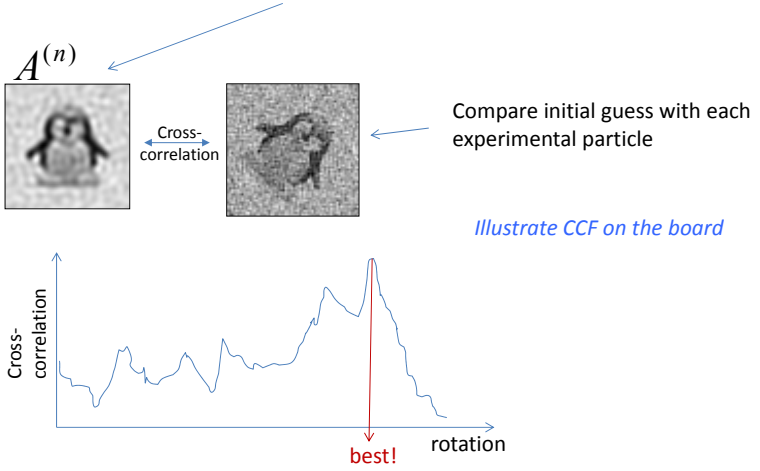
$$L(\Theta) = P(X \mid \Theta) = \int_Y P(X \mid Y,\Theta)P(Y \mid \Theta)d\phi$$

Probability of X, regardless Y

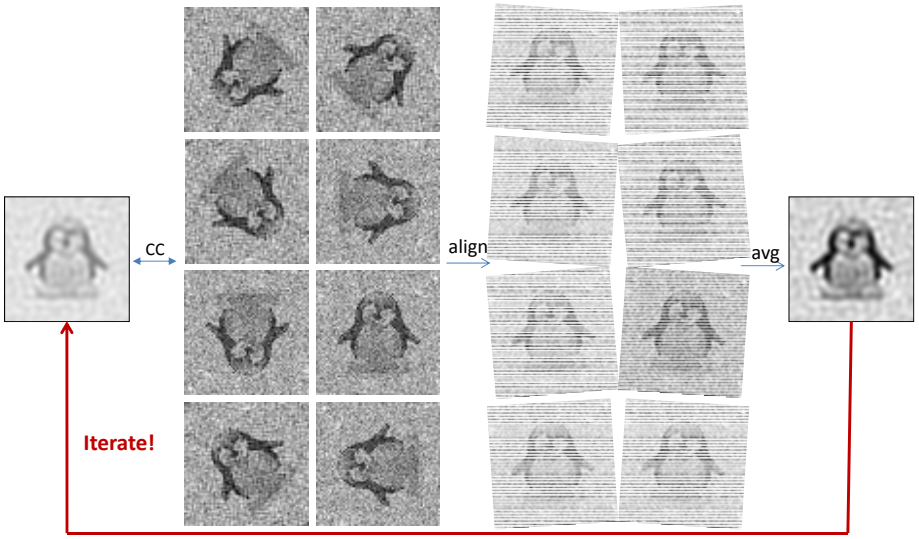
The maxCC approach

Reference-based alignment

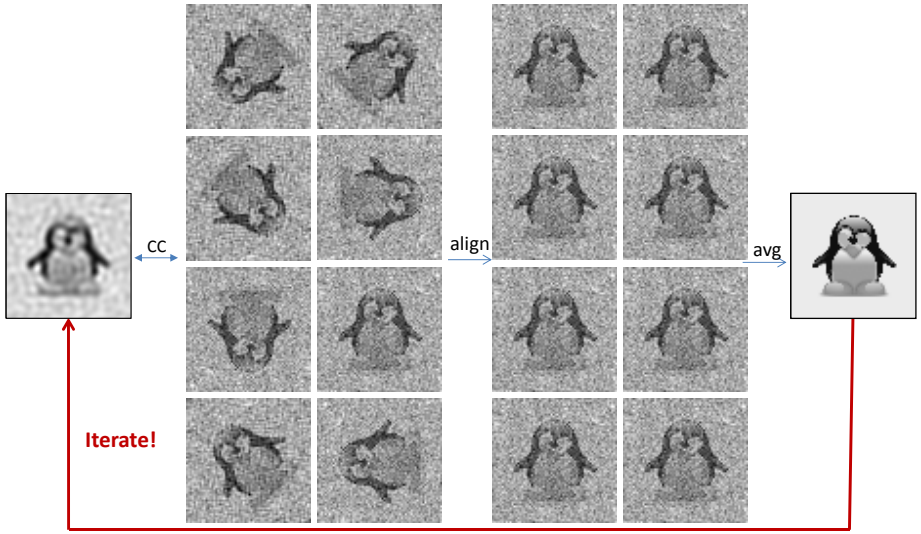
- Starts from some initial guess about the structure



Align and average

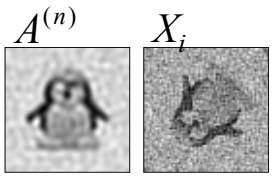


Align and average



The ML approach

Maximum likelihood



Statistical model

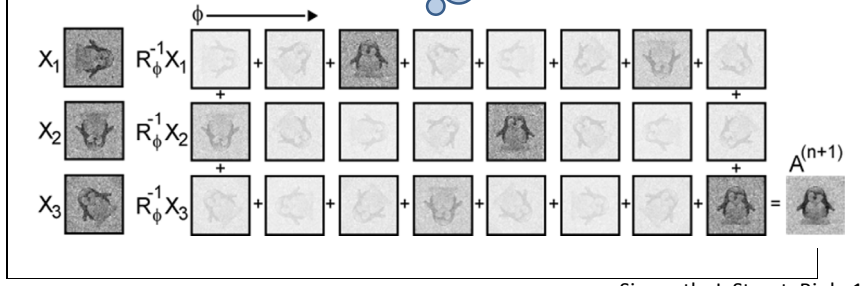
$$P(X_i \mid \phi, \Theta)$$

Based on Gaussian error model

$$P(X_i \mid \phi, \Theta) = \prod_{j=1}^{J} \frac{1}{2\pi\sigma^2} \exp\left(\frac{\left(\left[\mathbf{P}_\phi V \right]_j - X_{ij} \right)^2}{-2\sigma^2} \right)$$

Maximum li

Do not assign discrete orientations if the noise in the data does not allow this...



Sigworth, J. Struct. Biol., 1998

Incomplete data problems

- Option 1: add *Y* to the model

$$L(Y, \Theta) = P(X \mid Y, \Theta)$$

- Option 2: marginalize over *Y*

$$L(\Theta) = P(X \mid \Theta) = \int_Y P(X \mid Y, \Theta) P(Y \mid \Theta) d\phi$$

Probability of X, regardless Y

Maximum cross-correlation

Maximum Likelihood

Incomplete data problems

In the limit of **noiseless data** the Two techniques are equivalent!

Maximum cross-correlation

Maximum Likelihood

Many software packages now use ML: cryoSPARC, SPARX/SPHIRE, FREALIGN, XMIPP, RELION

Read more? See *Methods in Enzymology*, **482** (2010)

Classification

The 2D multi-reference algorithm

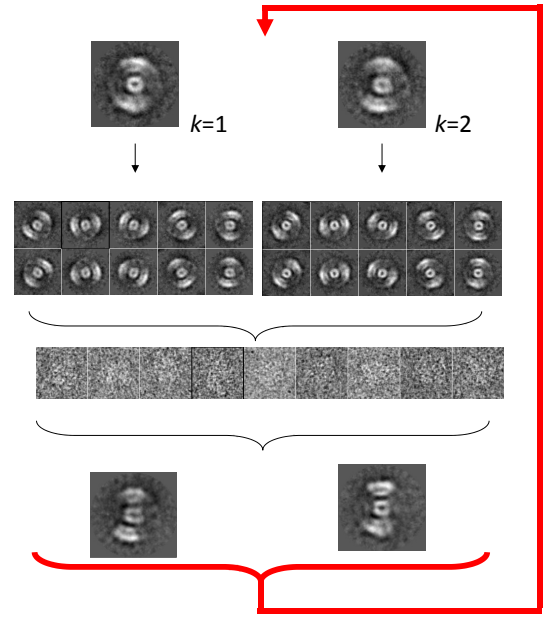
estimates for *K* 2D objects

sampled rotations 360°

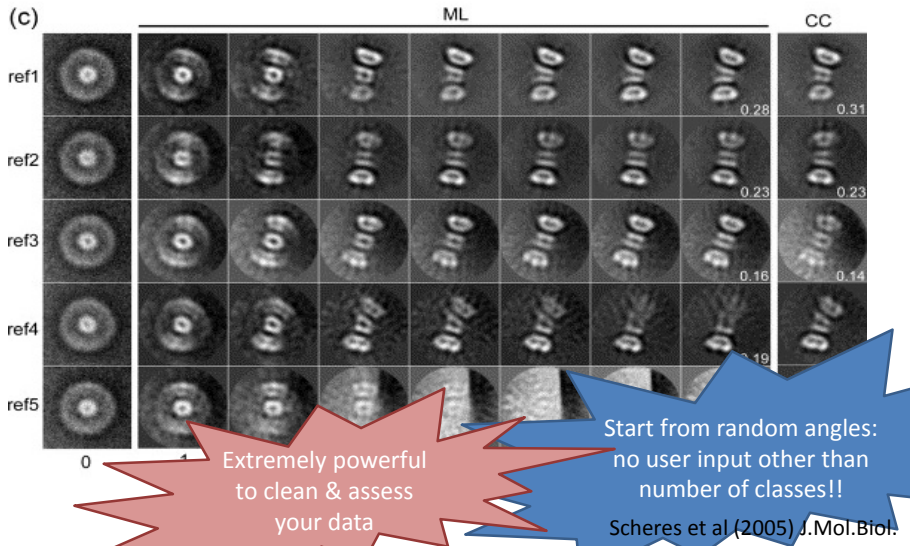
for each image, calculate all

$$P(\text{image}_i \mid k, \text{rot})$$

calculate new 2D average as *probability weighted averages*



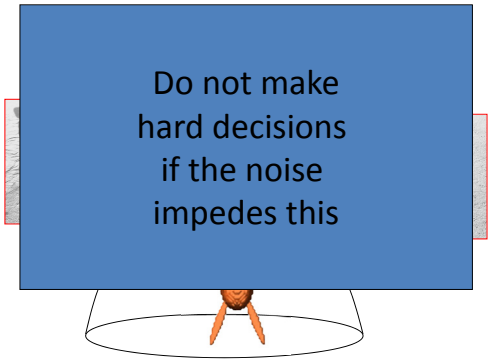
Reference-free 2D class averaging



Scheres et al (2005) J.Mol.Biol.

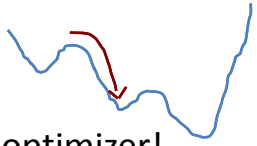
3D alignment & classification

3D ML refinement



"Probability-weighted angular assignment"

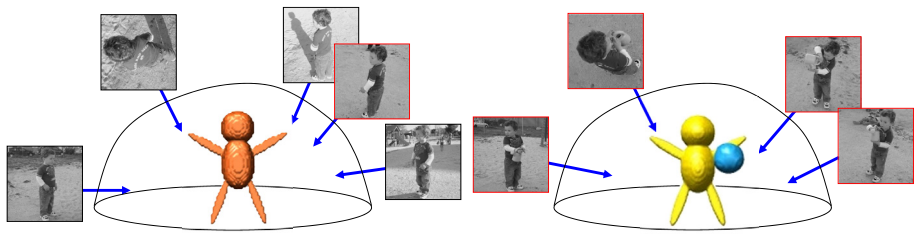
Initial model



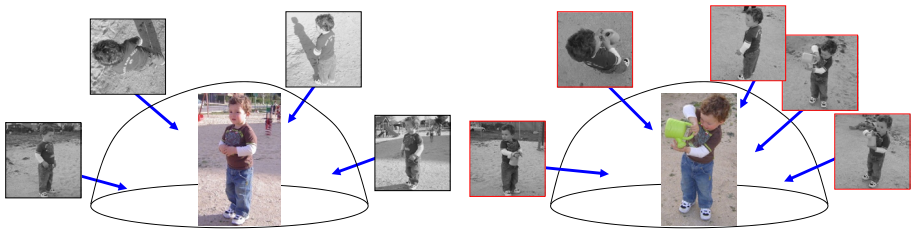
- Expectation-Maximisation is a local optimizer!
  - Gets stuck in nearest (local) minimum
- Bad model in -> bad model out!!!
  - Much less of a problem with high-resolution data
- Stochastic methods may reach global minimum
  - Stochastic Hill Climbing (SIMPLE)
  - Stochastic Gradient Descent (cryoSPARC & RELION)

Structural heterogeneity

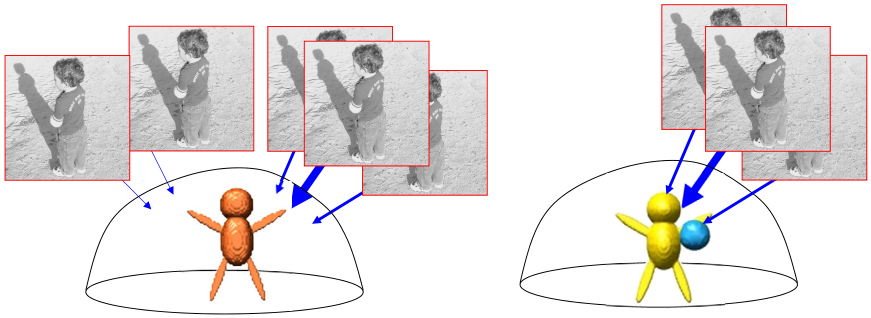
Multi-reference refinement



Multi-reference refinement



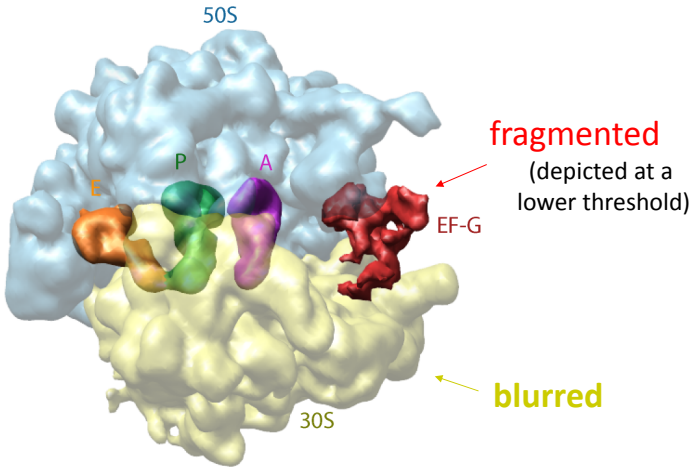
ML3D classification



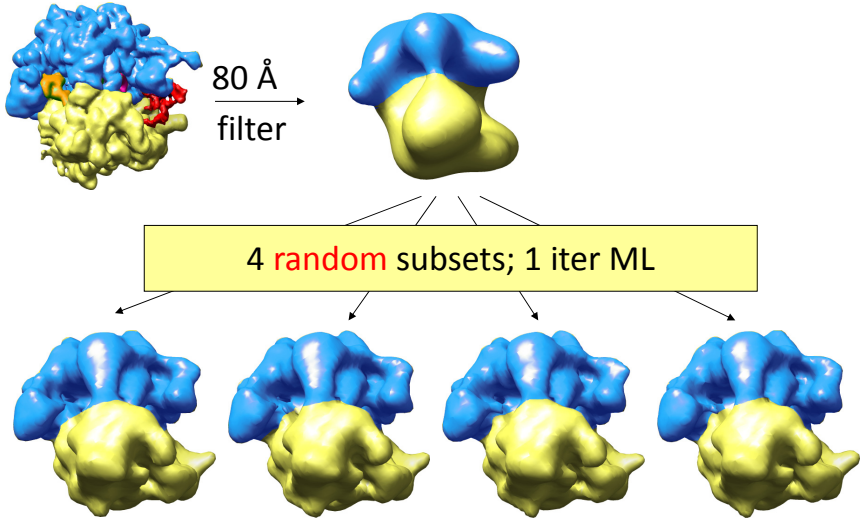
"Probability-weighted angular assignment"

Prelim. ribosome reconstruction

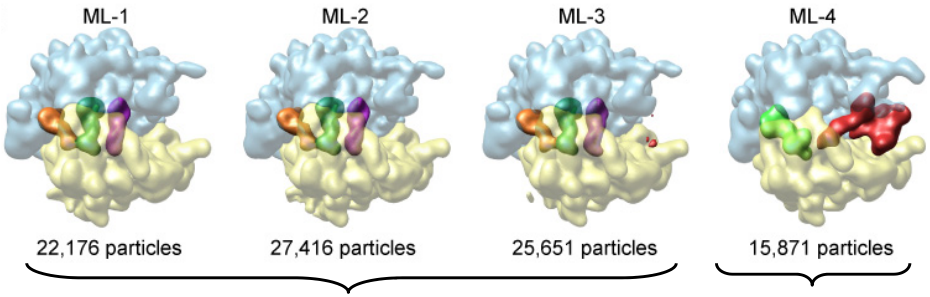
91,114 particles; 9.9 Å resolution



Seed generation



ML-derived classes



no ratcheting; no EF-G; 3 tRNAs
differences: overall rotations

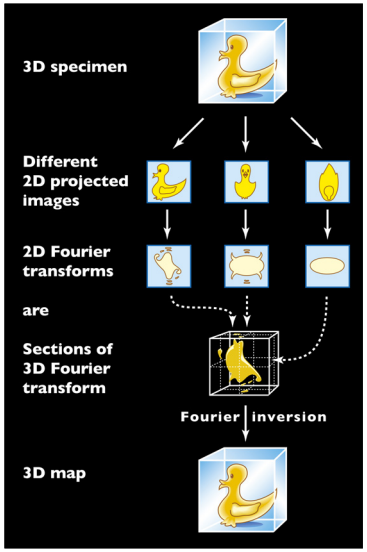
ratcheting,
EF-G, 1 tRNA

(Results coincided with a supervised classification)

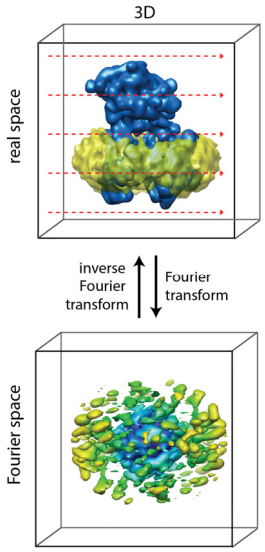
Scheres et al (2007) Nat. Meth.

Fourier-space formulation

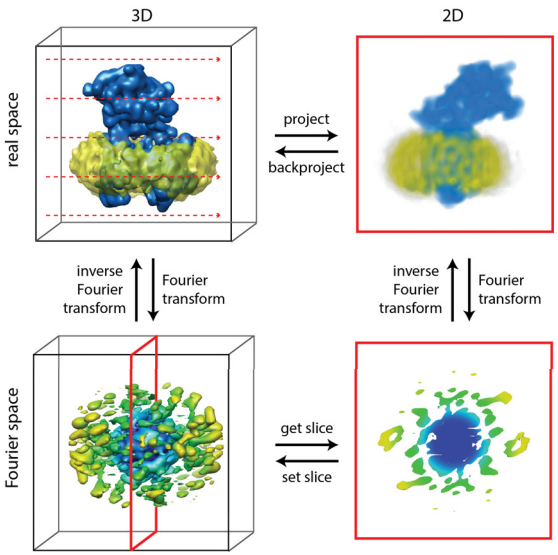
Projection-slice theorem



Projection-slice theorem



Projection slice theorem



Data model

- Real-space

$$X_i = \mathrm{CTF}_i \otimes \mathbf{P}_\varphi V_k + N_i$$

- Convolute w/ CTF
- $\mathbf{P}_\phi$ implements integrals
- N_i describes white noise

- Fourier space

$$X_i = \mathrm{CTF}_i \mathbf{P}_\varphi V_k + N_i$$

- Multiply w/ CTF
- $\mathbf{P}_\phi$ takes a slice
- N_i describes coloured noise

Regularised Likelihood

Maximum-likelihood estimators

- The best one can do…
- …in the limit of *infinitely large data sets*

- But my data set is limited in size, right?!
  - Even with Krios, K3 & EPU!

The bad news

- The experimental data alone is not enough to determine a unique solution!
- There are many noisy reconstructions that describe the data equally well…
- Danger of incorrect interpretation…

The good news

- By incorporating external information, a different problem may be solved for which a unique solution does exist!
- Regularisation
- Conventional regularisation approaches
  - Wiener filtering
  - Low-pass filtering

A Bayesian view on regularization

$$P(\Theta \mid X) = \frac{P(X \mid \Theta)P(\Theta)}{P(X)}$$

$$\text{Posterior} = \frac{\text{Likelihood} * \text{Prior}}{\text{Evidence}}$$

Regularised likelihood optimisation

Likelihood

- Assume noise is Gaussian and independent
  - in Fourier space
  - with spectral power $\sigma^2(\upsilon)$: *coloured noise*

$$P(X_i \mid k, \phi, \Theta) = \prod_{j=1}^{J} \frac{1}{2\pi\sigma_{ij}} \exp\left(\frac{\left\| X_{ij} - \mathrm{CTF}_{ij}(\mathbf{P}_\phi V_k)_j \right\|^2}{-2\sigma_{ij}^2} \right)$$

Prior

- Assume signal is Gaussian and independent
  - in Fourier space
  - Limited power $\tau^2(\upsilon)$: *smoothness in real space!*

$$P(\Theta) = \prod_l \frac{1}{2\pi\tau_{kl}} \exp\left\{ \frac{\left\| V_{kl} \right\|^2}{-2\tau_{kl}^2} \right\}$$

Expectation maximization

$$V^{(n+1)} = \frac{\sum_{i=1}^{N} \int_{\phi} \Gamma_{i\phi}^{(n)} \mathbf{P}_{\phi}^{\mathrm{T}} \frac{\mathrm{CTF}_i}{\sigma_i^{2(n)}} X_i d\phi}{\sum_{i=1}^{N} \int_{\phi} \Gamma_{i\phi}^{(n)} \mathbf{P}_{\phi}^{\mathrm{T}} \frac{\mathrm{CTF}_i^2}{\sigma_i^{2(n)}} d\phi + \frac{1}{\tau^{2(n)}}}$$

→ Wiener (optimal) filter for CTF-corrected 3D reconstruction / 2D class averages

$$\sigma_i^{2(n+1)} = \frac{1}{2} \int_{\phi} \Gamma_{i\phi}^{(n)} \left\| X_i - \mathrm{CTF}_i \mathbf{P}_{\phi} V^{(n)} \right\|^2 d\phi$$

→ Estimate resolution-dependent power of noise from the data

$$\tau^{2(n+1)} = \frac{1}{2} \left\| V^{(n)} \right\|^2$$

→ Estimate resolution-dependent power of signal from the data

$$\Gamma_{i\phi}^{(n)} = \frac{P(X_i \mid \phi, \Theta^{(n)}) P(\phi \mid \Theta^{(n)})}{\int_{\phi'} P(X_i \mid \phi', \Theta^{(n)}) P(\phi' \mid \Theta^{(n)}) d\phi'}$$

3D Wiener filter

$$V^{(n+1)} = \frac{\sum_{i=1}^{N} \int_{\phi} \Gamma_{i\phi}^{(n)} \mathbf{P}_{\phi}^{\mathrm{T}} \frac{\mathrm{CTF}_i}{\sigma_i^{2(n)}} X_i d\phi}{\sum_{i=1}^{N} \int_{\phi} \Gamma_{i\phi}^{(n)} \mathbf{P}_{\phi}^{\mathrm{T}} \frac{\mathrm{CTF}_i^2}{\sigma_i^{2(n)}} d\phi + \frac{1}{\tau^{2(n)}}}$$

- Calculates SSNR(υ) (as a 3D function)
- Handles uneven orientational distribution
- Handles astigmatic CTFs & CTF en
- Corrects CTF & low-pas
- *Optimal linear filter*

WITHOUT ARBITRARINESS!

Recapitulating

- Alignment & classification are incomplete problems
  - Best dealt with by marginalisation (ML)
- 2D and 3D problems are very similar
- Fourier-space is most convenient
  - CTF multiplication
  - Slices instead of line integral projections
  - Coloured noise-model
  - Regularised Likelihood function -> ‘optimal’ filters

Further Reading

- Penczek, Fundamentals of Three-Dimensional Reconstruction from Projections, *Methods in Enzymology*, , **482** (2010) p 1
- Penczek, Image restoration in cryo-electron microscopy, *Methods in Enzymology*, , **482** (2010) p 35
- Sigworth, Doerschuk, Carazo & Scheres, An Introduction to Maximum-Likelihood Methods in Cryo-EM, *Methods in Enzymology*, **482** (2010) p 263
- Scheres, Classification of Structural Heterogeneity by Maximum-Likelihood Methods, *Methods in Enzymology*, **482** (2010) p 295
- Scheres, Processing of Structurally Heterogeneous Cryo-EM Data in RELION, *Methods in Enzymology*, **579** (2016) p 125
- www2.mrc-lmb.cam.ac.uk/relion (tutorial & Wiki pages)