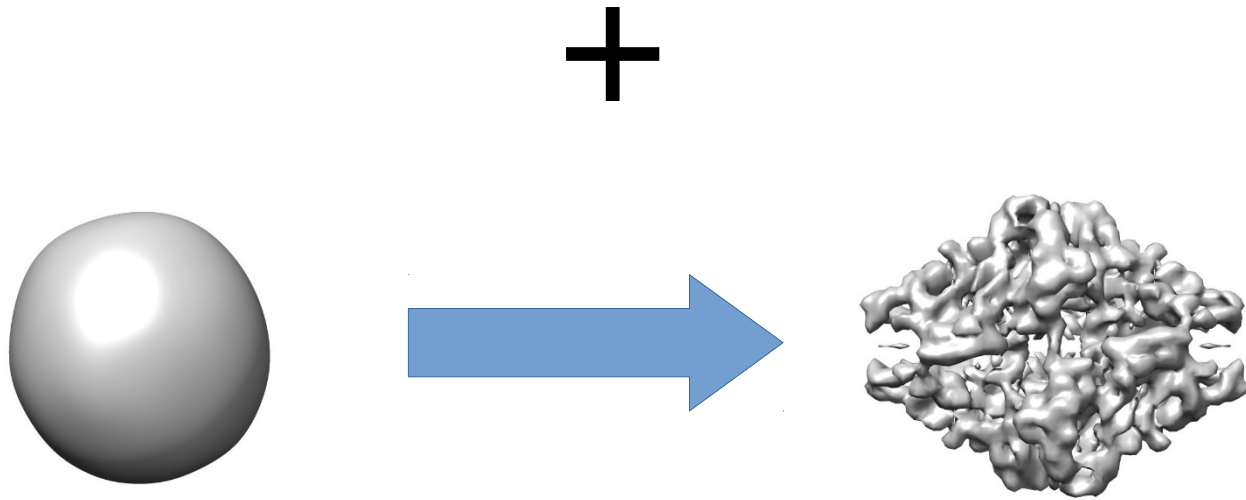


Computational Imaging System for Transmission Electron Microscopy





Computational Imaging System for Transmission Electron Microscopy



Tim Grant



Alexis Rohou



Nikolaus Grigorieff

Tools for Single Particle Cryo-EM



Process

Mag. distortion correction

CTF determination

Movie processing

Exposure filtering

3D refine. & reconstr.

B-factor sharpening

Tool

mag_distortion

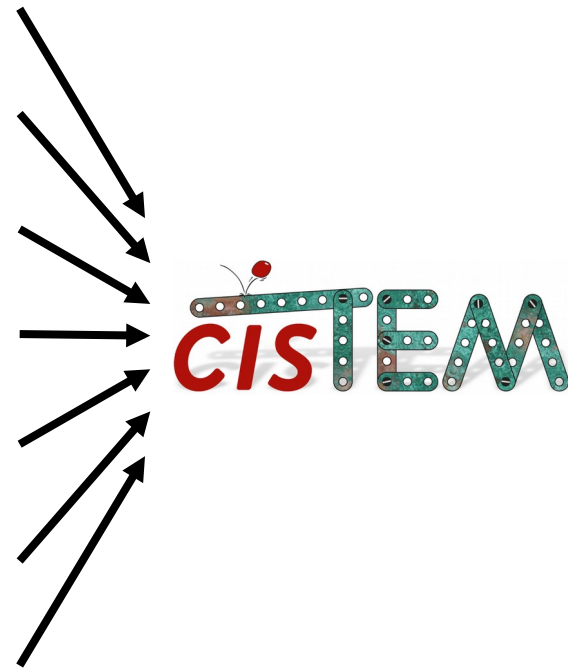
CTFFind4

Unblur

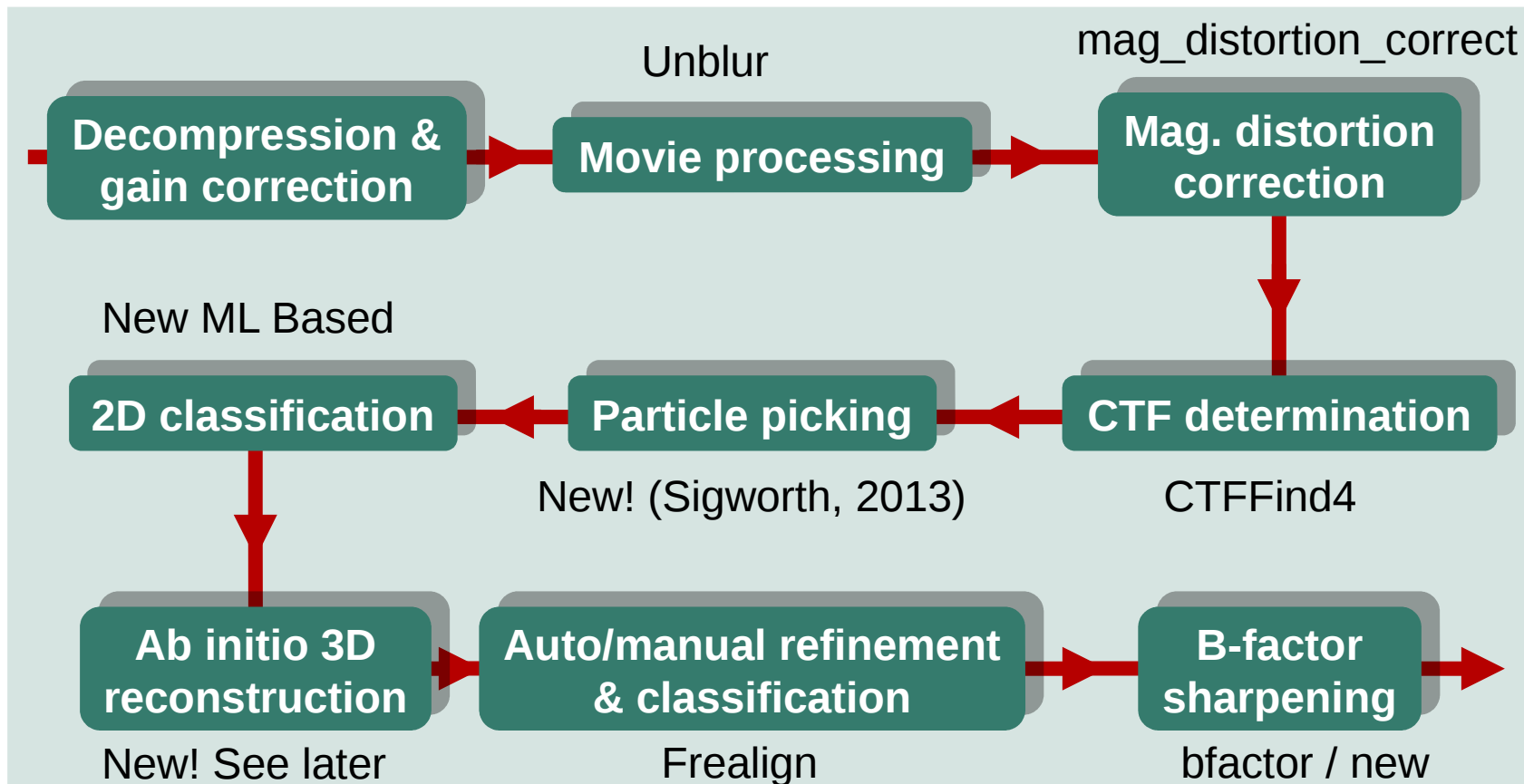
Unblur

Frealign

bfactor

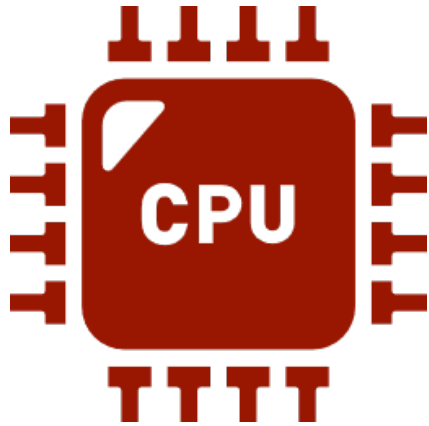


Complete Pipeline





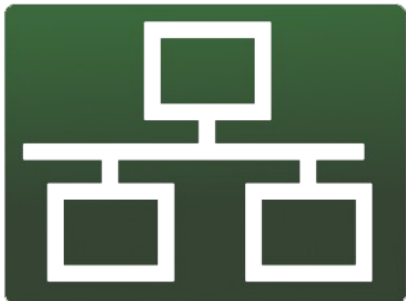
Robust Architecture



- Parallelized for CPUs, no GPUs
- Written entirely in C++ (Open-Source)

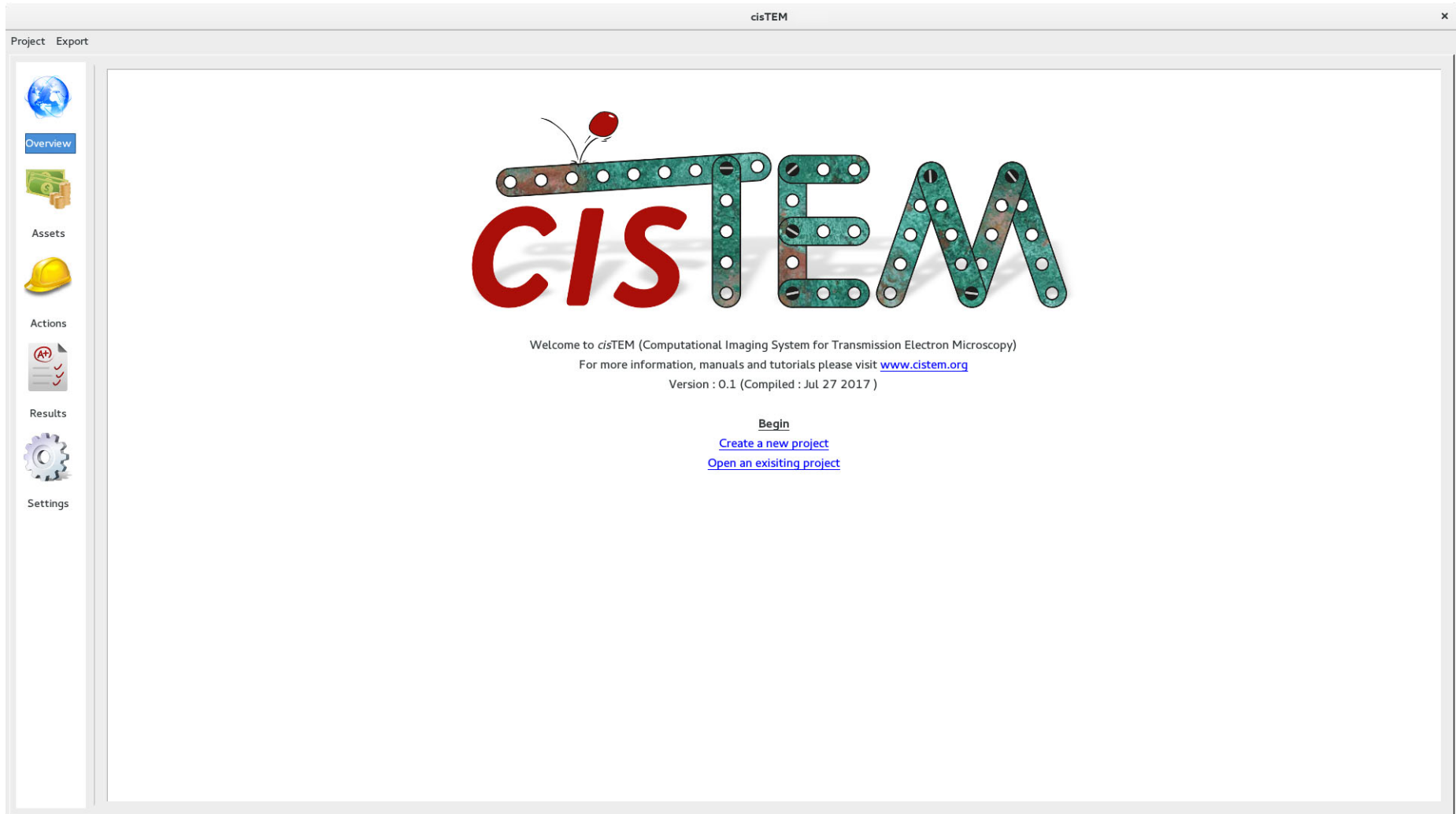
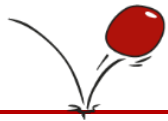


- SQLite database stores all meta data



- Custom socket-based parallelization

cisTEM GUI

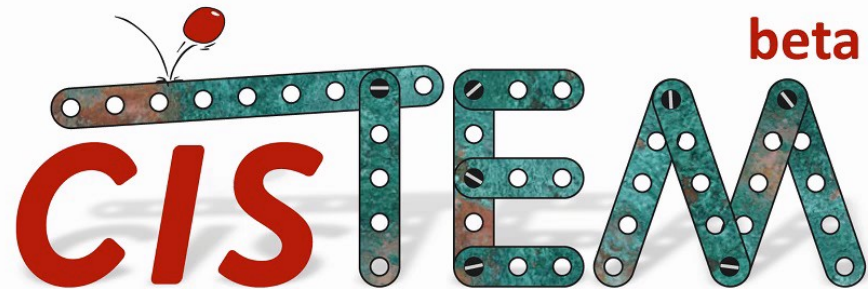




GUI Design

- No command line / editing of text files (and command line only)
- Single window
- Easy presentation / sorting of results
- Easy selection of good results
- Live feedback while jobs are running
- Only enter information once
- Easy Import / Export to other packages

GUI Movie



Project summary

Project name: ApoNew2
Project directory: /home/grantt/ApoNew2
Total job runtime : 37.68 hours (1.57 days)
Total number of jobs run: 1476



erview



ssets



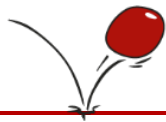
tions



esults

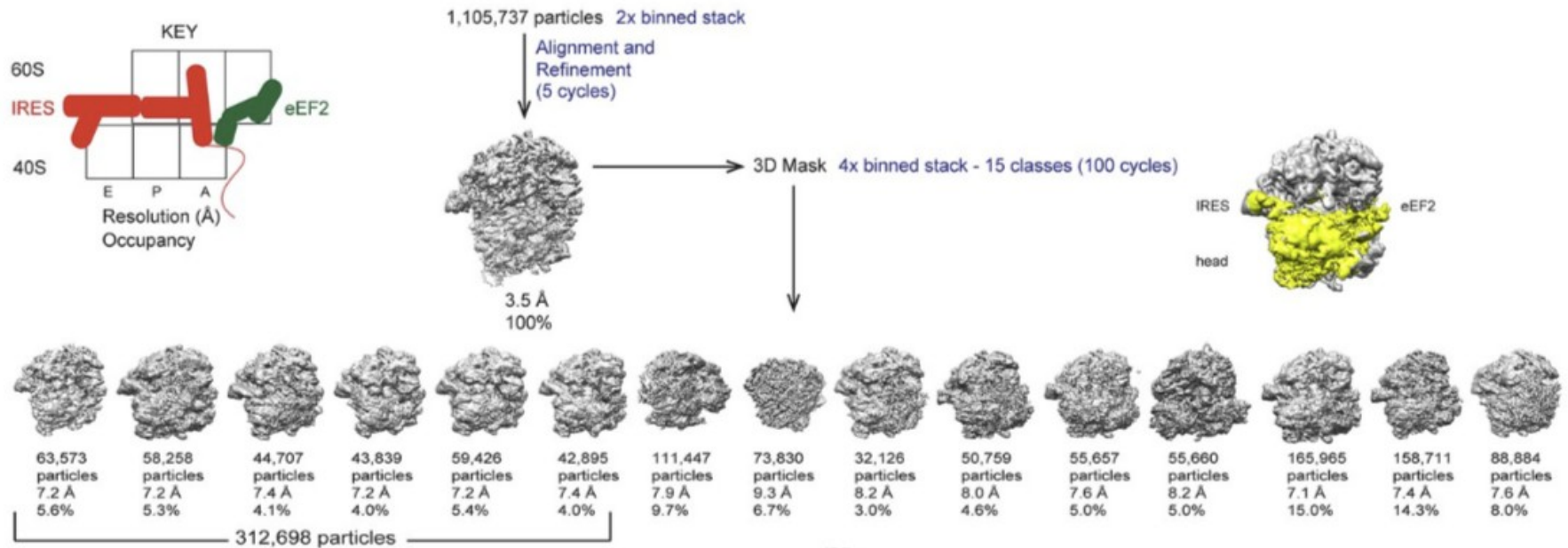


ettings



*cis*TEM is fast!

- Optimized code (especially MKL FFT)
- On the fly binning
- On the fly adjustment of particle no. / resolution
- Only 3D classification is ML, not refinement





Benchmark: β -galactosidase

2.2 Å resolution cryo-EM structure of β -galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,^{1*} Alan Merk,^{1*} Soojay Banerjee,¹ Doreen Matthies,¹ Xiongwu Wu,² Jacqueline L. S. Milne,¹ Sriram Subramaniam^{1†}

Processing on single workstation

2 x Xeon (44 cpu cores)

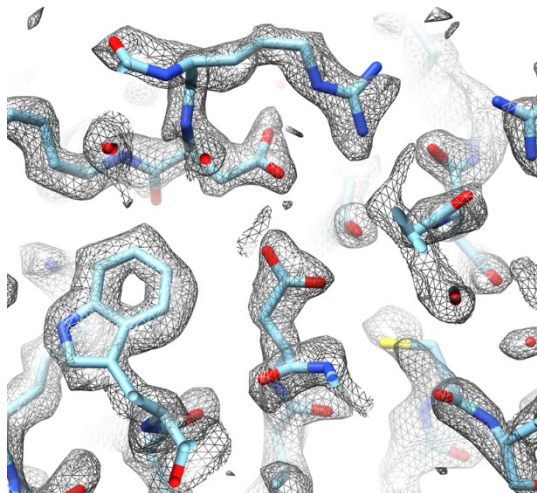
512 GB Memory

16TB SSD Scratch

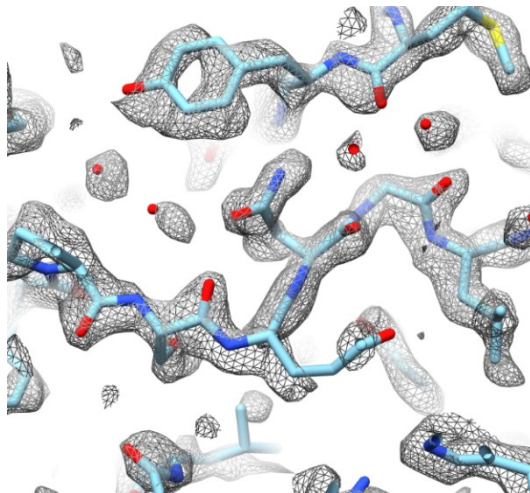
Sits under my desk

Processing Step	Details	Time (hours)
Movie Processing	1539 movies, 38 frames, super-resolution	1.1
CTF Determination	On Images	0.1
Particle Picking	131,298 particles	0.1
2D Classification	50 classes, 28 selected with 119,523 particles	0.8
Ab-initio 3D	40 iterations	0.8
Auto refinement	8 iterations, final resolution 2.2 Å	1.4
Manual refinement	1 iteration (incl. defocus), final resolution 2.2 Å	0.4
Total		4.7

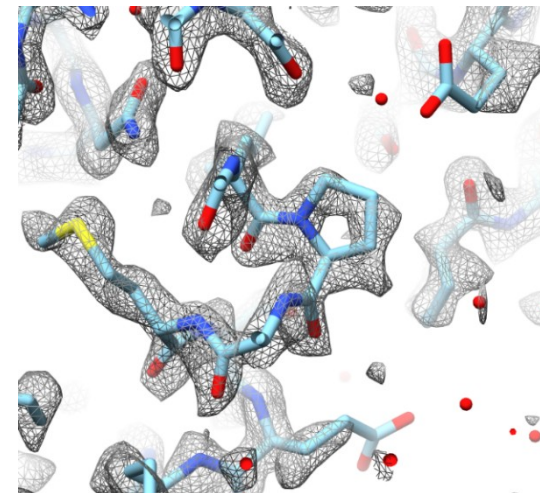
Benchmark: β -galactosidase



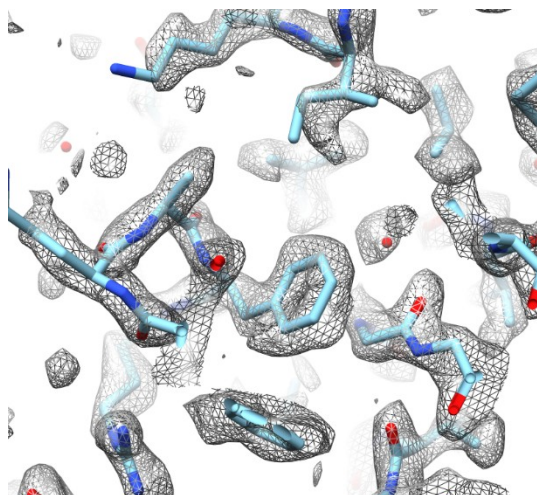
Trp, Asp...



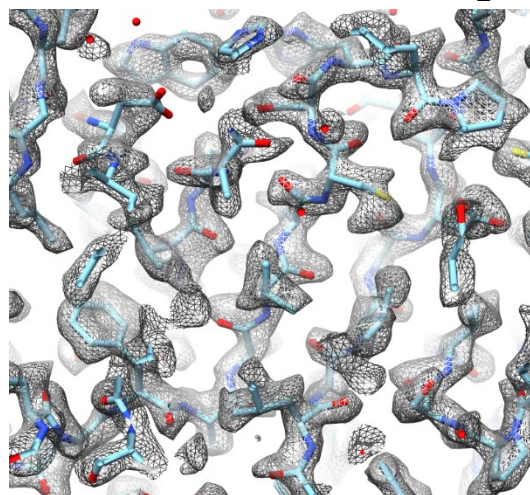
Tyr, Asn, carbonyl, H₂O



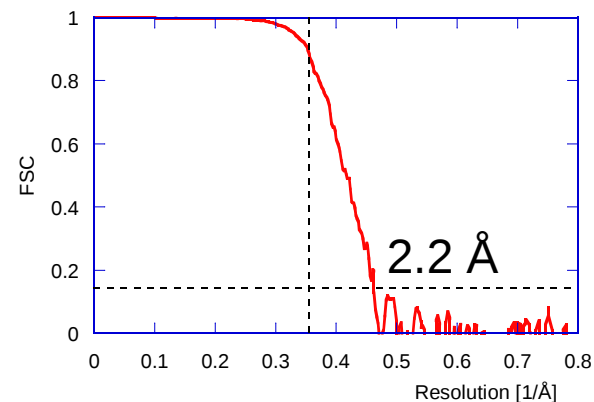
Pro, Met...



Phe, Ile, α -helix



Trp, carbonyl, β -sheet



(no masking)



Web Page: cistem.org



Computational Imaging System for Transmission Electron Microscopy

[Software](#) [Documentation](#) [FAQs](#) [Forums](#) [My account](#) [My Watched Posts](#) [Log out](#)

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[View](#) [Edit](#) [Manage display](#)



cisTEM is user-friendly software to process cryo-EM images of macromolecular complexes and obtain high-resolution 3D reconstructions from them. It was developed by Tim Grant, Alexis Rohou and Nikolaus Grigorieff and comprises a number of tools to process image data including movies, micrographs and stacks of single-particle images, implementing a complete "pipeline" of processing steps to obtain high-resolution single-particle reconstructions. cisTEM is distributed under the Janelia Research Campus Software License and can be downloaded here. We recommend downloading and using the pre-compiled binaries, rather than compiling the source code, for best performance. New users are encouraged to follow the tutorial, which provides a quick way to become familiar with the most important functions of cisTEM.

Support

Please visit the documentation pages for help. If you encounter any problems using cisTEM, or have questions, please use the forums.

Current Release

- [cistem-1.0.0-beta-intel-linux.tar.gz \(recommended\)](#) displayed 1701 times
- [cistem-1.0.0-beta-source-code.tar.gz](#) displayed 266 times

You are watching this page and will be emailed about updates, click to stop watching.

Topics from the cisTEM Forum

- Exporting or accessing Particle Positions (2 replies)
- corelation between stack and particle (2 replies)
- Cannot find Align Movies output files (3 replies)
- Error while viewing result (3 replies)
- replacing binned stack with unbinned stack (9 replies)
- Merge 200kV and 300kV data (1 replies)
- generate mask for manual refine (1 replies)
- cisTEM gets hung when trying to do a global search on only Psi and Y (1 replies)
- Particle picking and recentering (1 replies)
- Time estimate for movie alignment? (3 replies)

Visit the cisTEM Forum to read up on more topics.

Previous Releases

cisTEM, user-friendly software for single-particle image processing

Timothy Grant*, Alexis Rohou*, Nikolaus Grigorieff*

Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, United States

Abstract We have developed new open-source software called cisTEM (computational imaging system for transmission electron microscopy) for the processing of data for high-resolution electron cryo-microscopy and single-particle averaging. cisTEM features a graphical user interface that is used to submit jobs, monitor their progress, and display results. It implements a full processing pipeline including movie processing, image defocus determination, automatic particle picking, 2D classification, ab-initio 3D map generation from random parameters, 3D classification, and high-resolution refinement and reconstruction. Some of these steps implement newly-developed algorithms; others were adapted from previously published algorithms. The software is optimized to enable processing of typical datasets (2000 micrographs, 200 k – 300 k particles) on a high-end, CPU-based workstation in half a day or less, comparable to GPU-accelerated processing. Jobs can also be scheduled on large computer clusters using flexible run profiles that can be adapted for most computing environments. cisTEM is available for download from cistem.org.

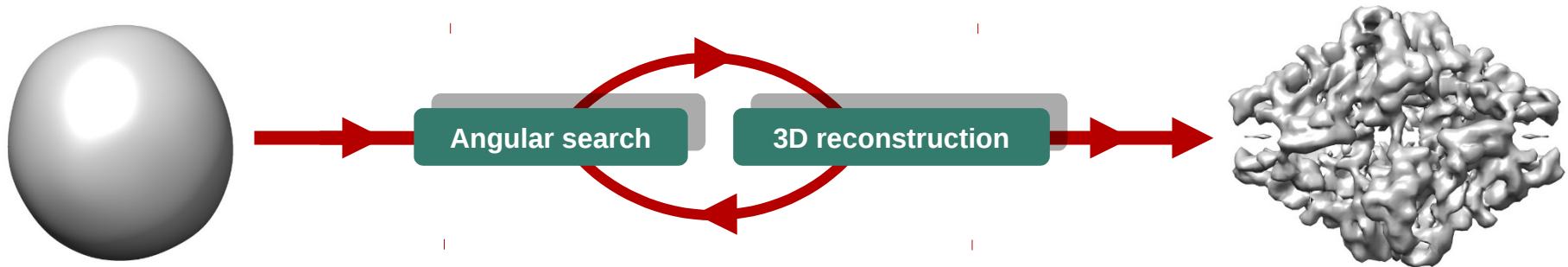
DOI: <https://doi.org/10.7554/eLife.35383.001>





*cis*TEM Ab-initio

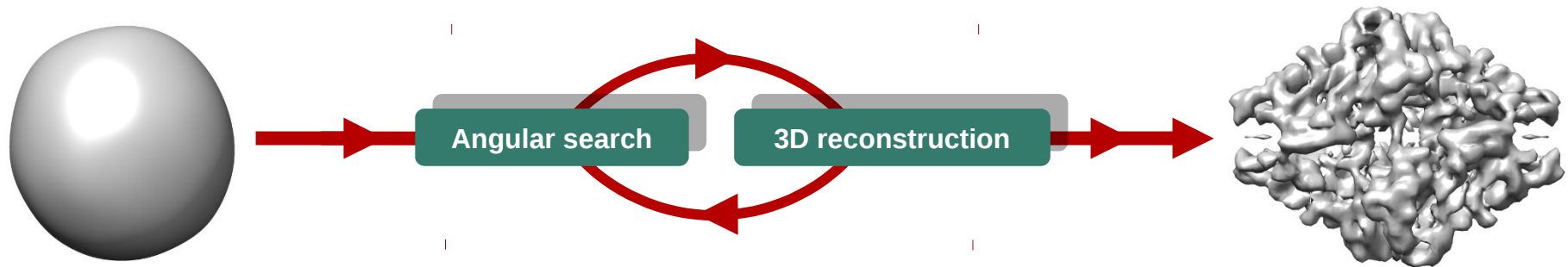
Ideal case, ab-initio is just a refinement



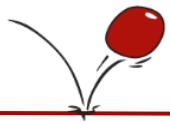


*cis*TEM Ab-initio

Ideal case, ab-initio is just an iterative refinement

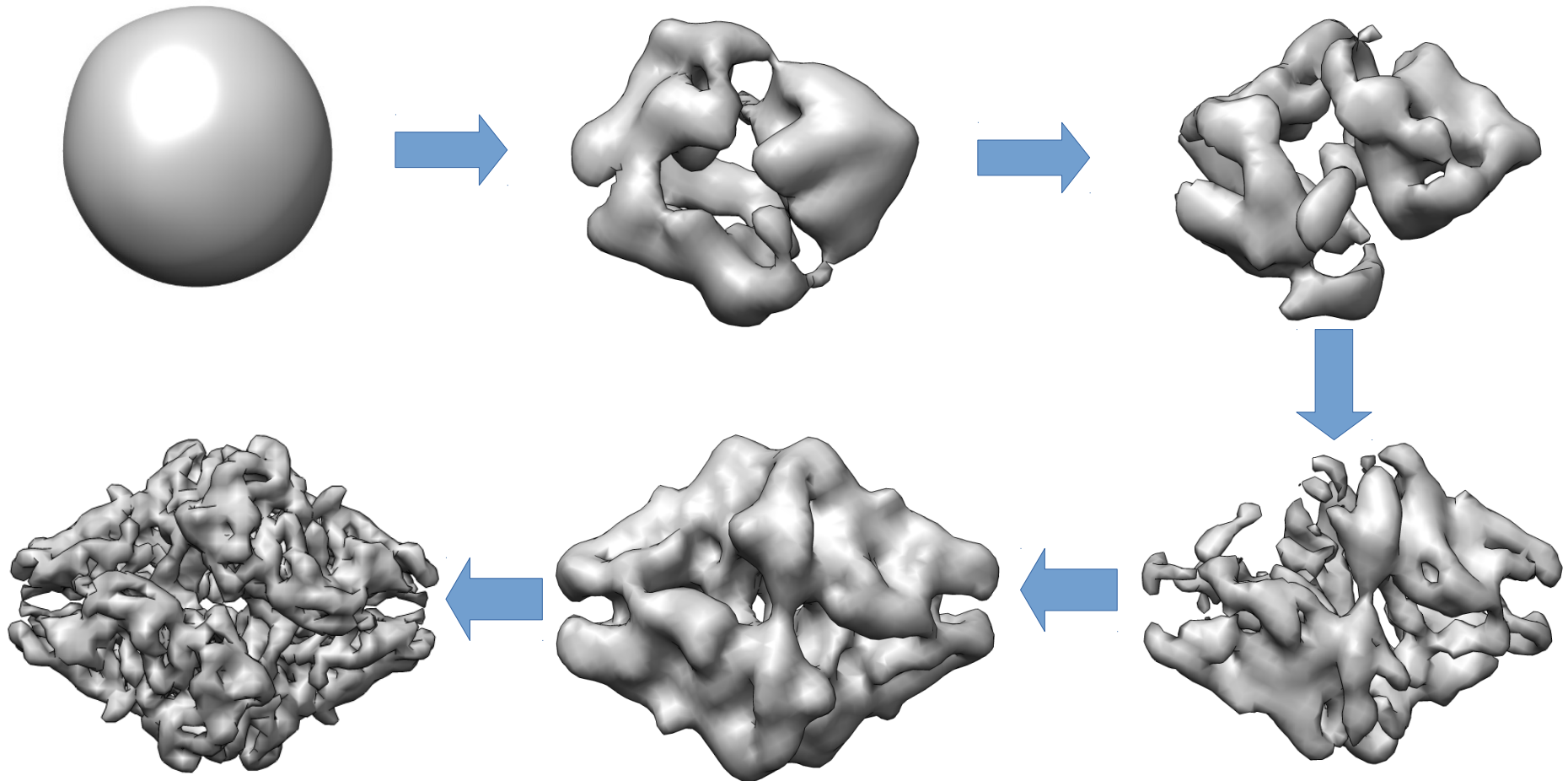


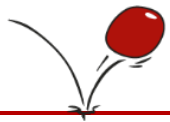
How to prevent getting stuck in local minima?



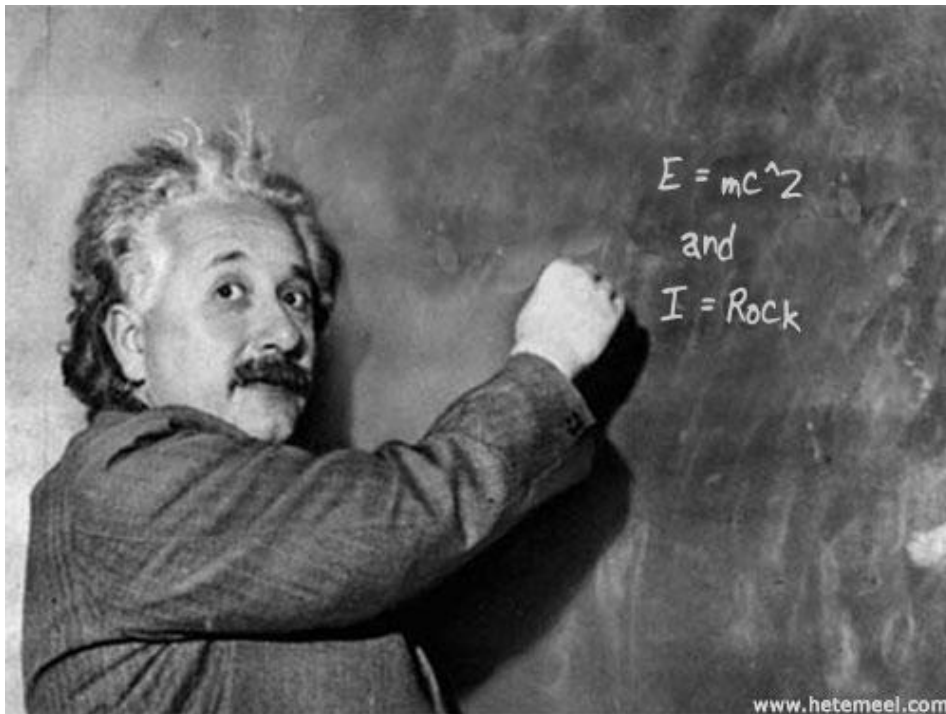
*cis*TEM Ab-initio

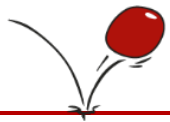
*cis*TEM does 40 rounds of global refinement



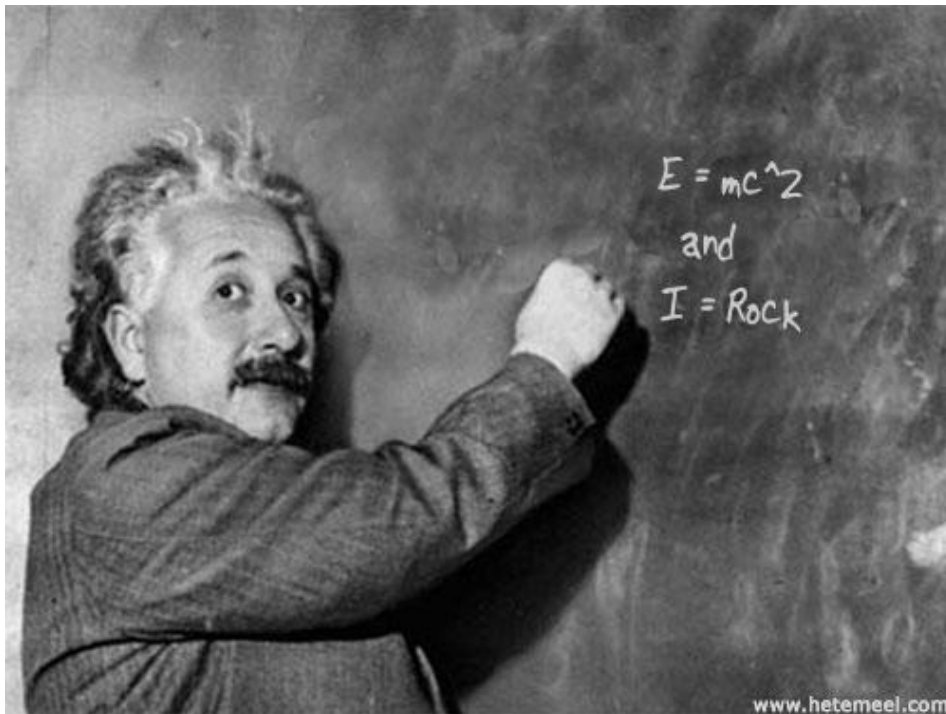


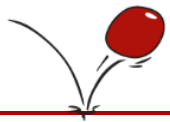
*cis*TEM Ab-initio



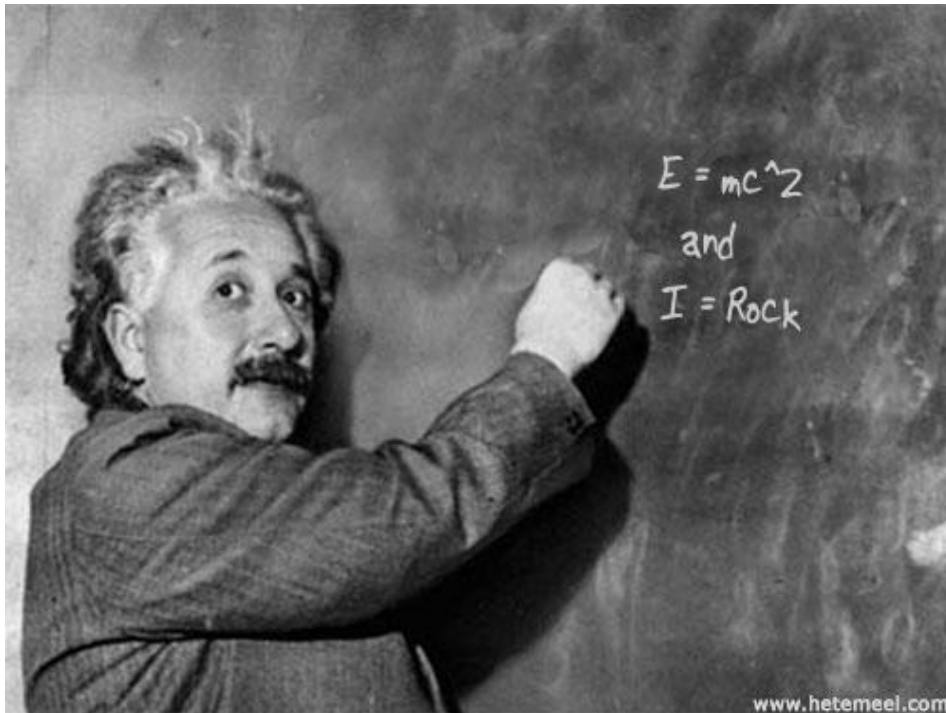


*cis*TEM Ab-initio





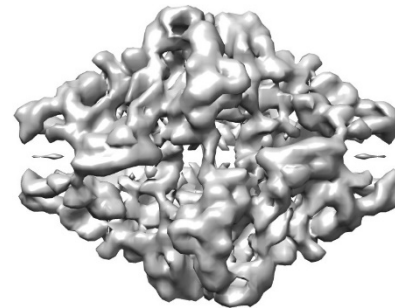
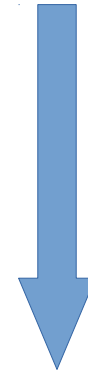
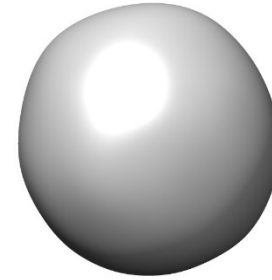
*cis*TEM Ab-initio



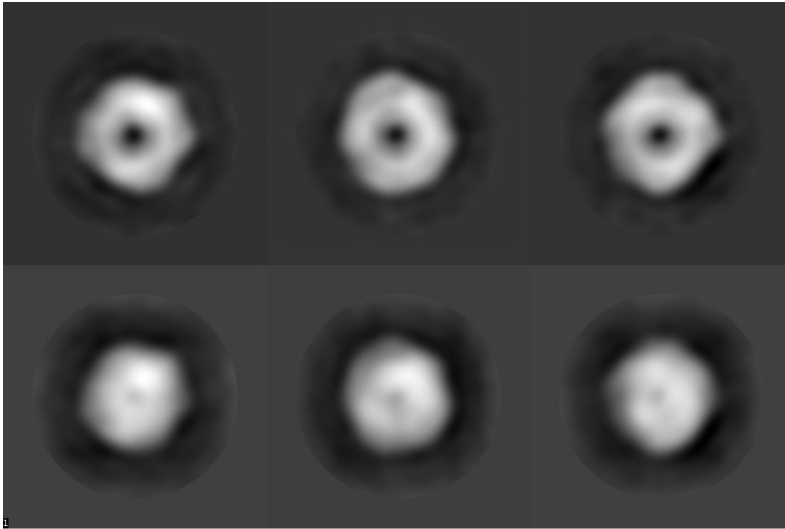
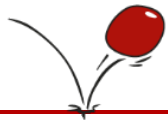
“Heuristic” approach?

CisTEM Ab-initio

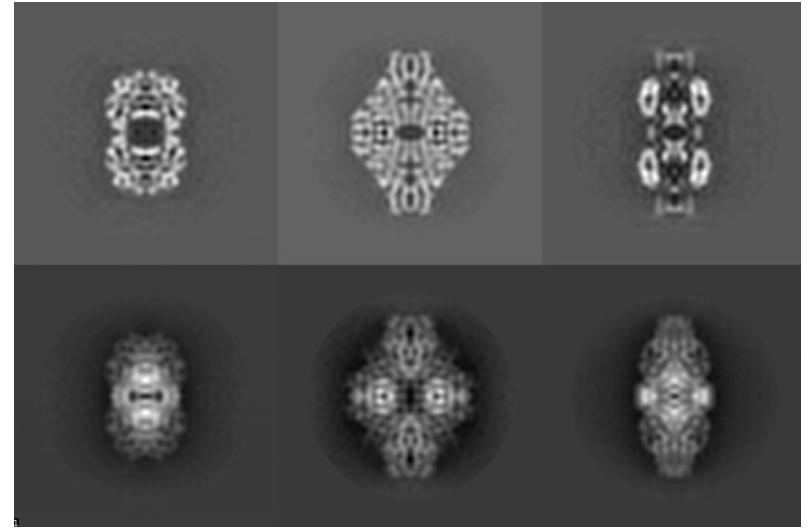
1. Start at low resolution
2. Random subsets of particles
3. Sloppy alignment
4. Use best 1/3rd particles
5. Good automasking
6. C1 with symmetry alignment



Low Resolution to High

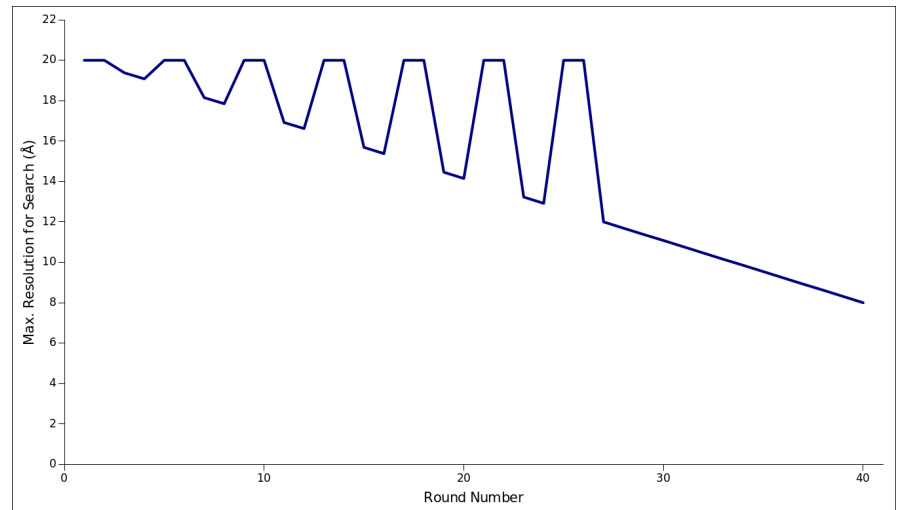


Round 1 (20 Å)

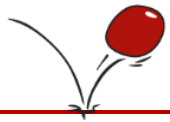


Round 40 (8 Å)

- Smoother search space at low resolution
- Some high resolution probably required for distinguishing right / wrong (protein dependent)
- Jumping back eliminates high-res bias.

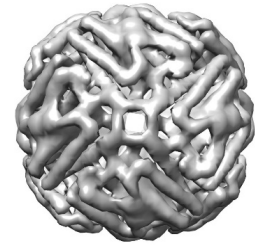
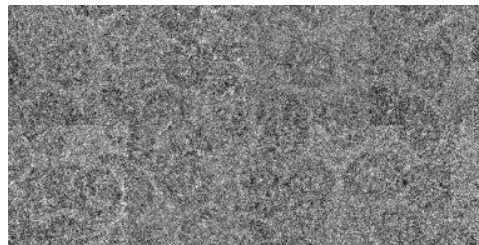
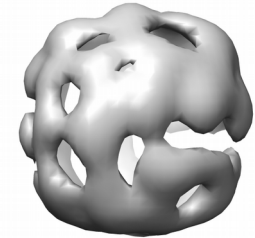
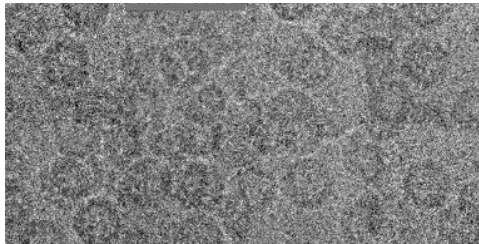
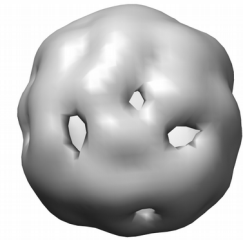
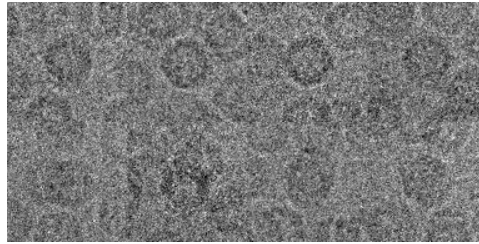


Random Subsets of Particles

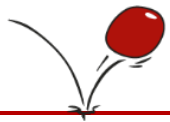


-Faster!

-Also improves
convergence / reduces
overfitting.

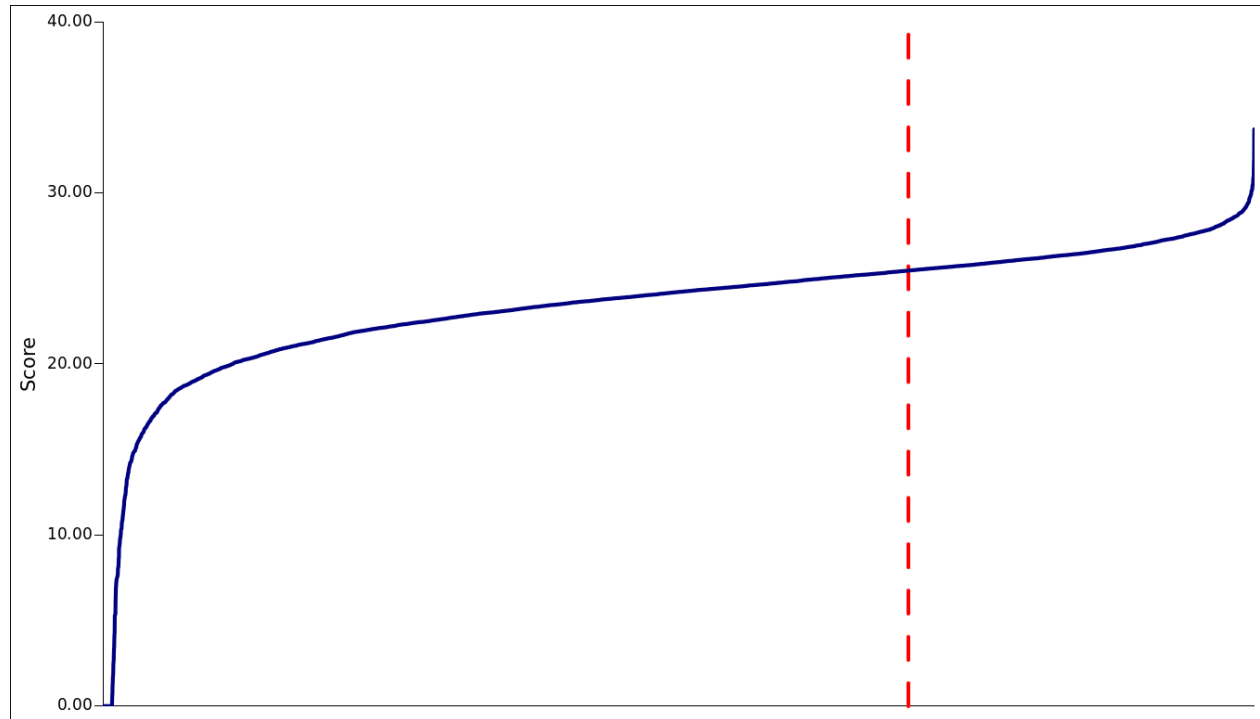


$$N_1 = 2500, N_{40} = 10000$$



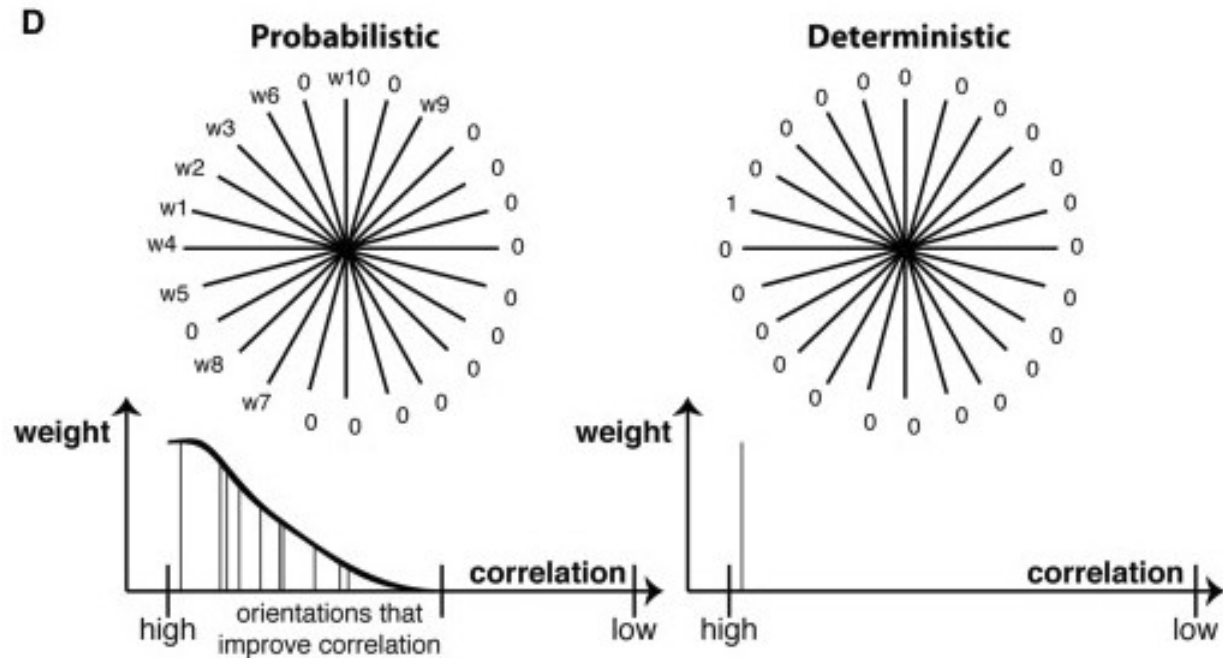
Best 3rd of particles

Taking the best 3rd of scores appears to improve convergence.



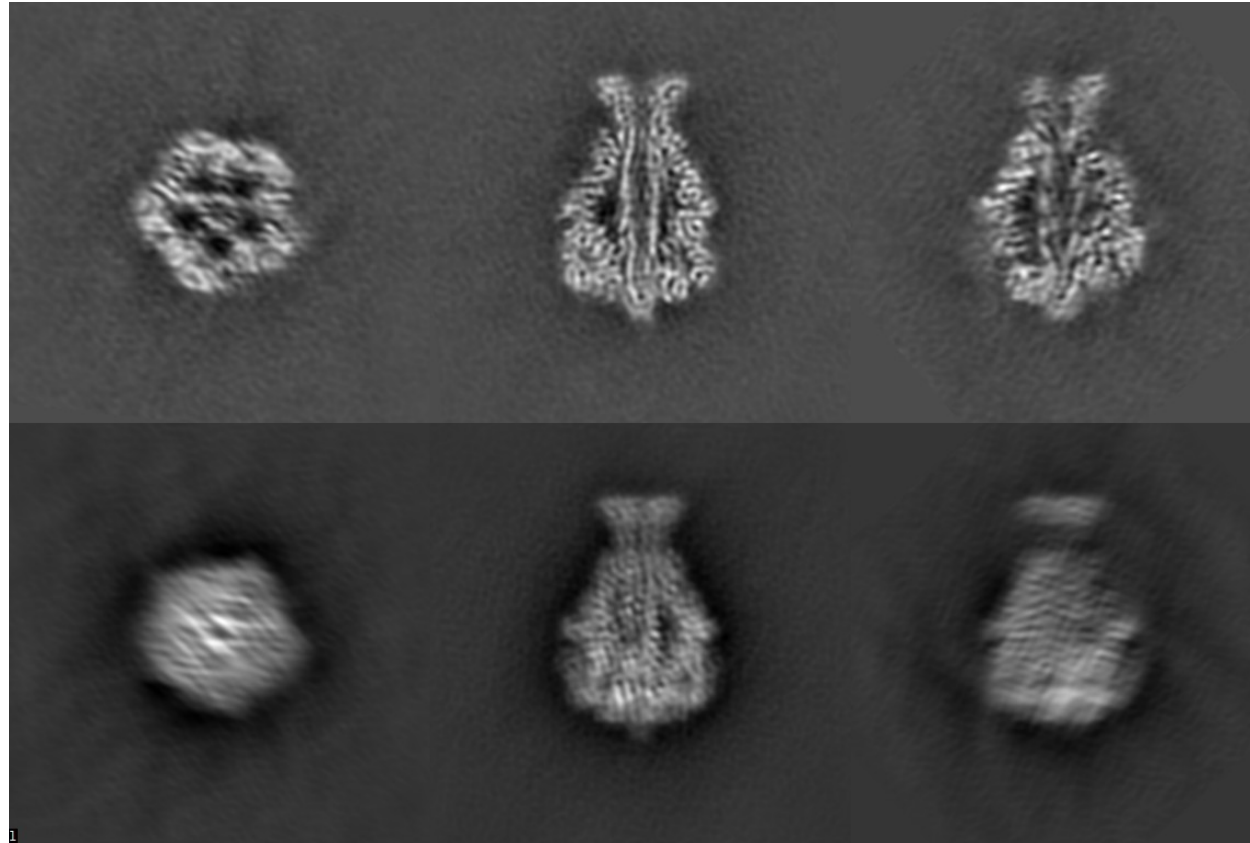
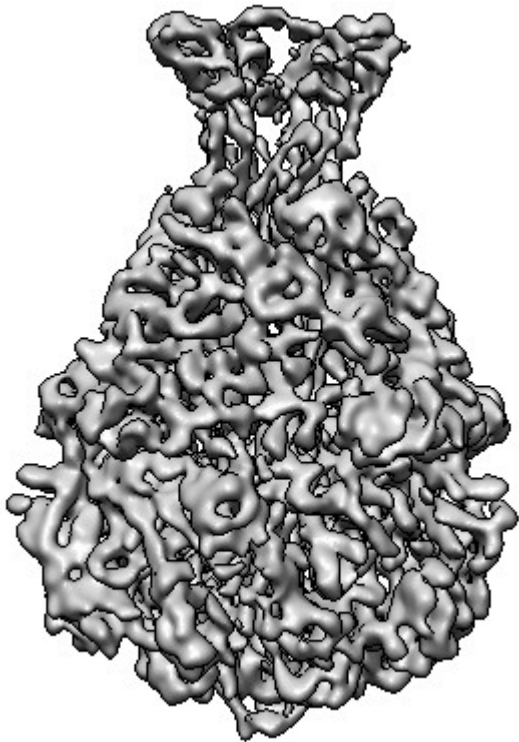
“Sloppy” alignment

cisTEM alignment is “deterministic”



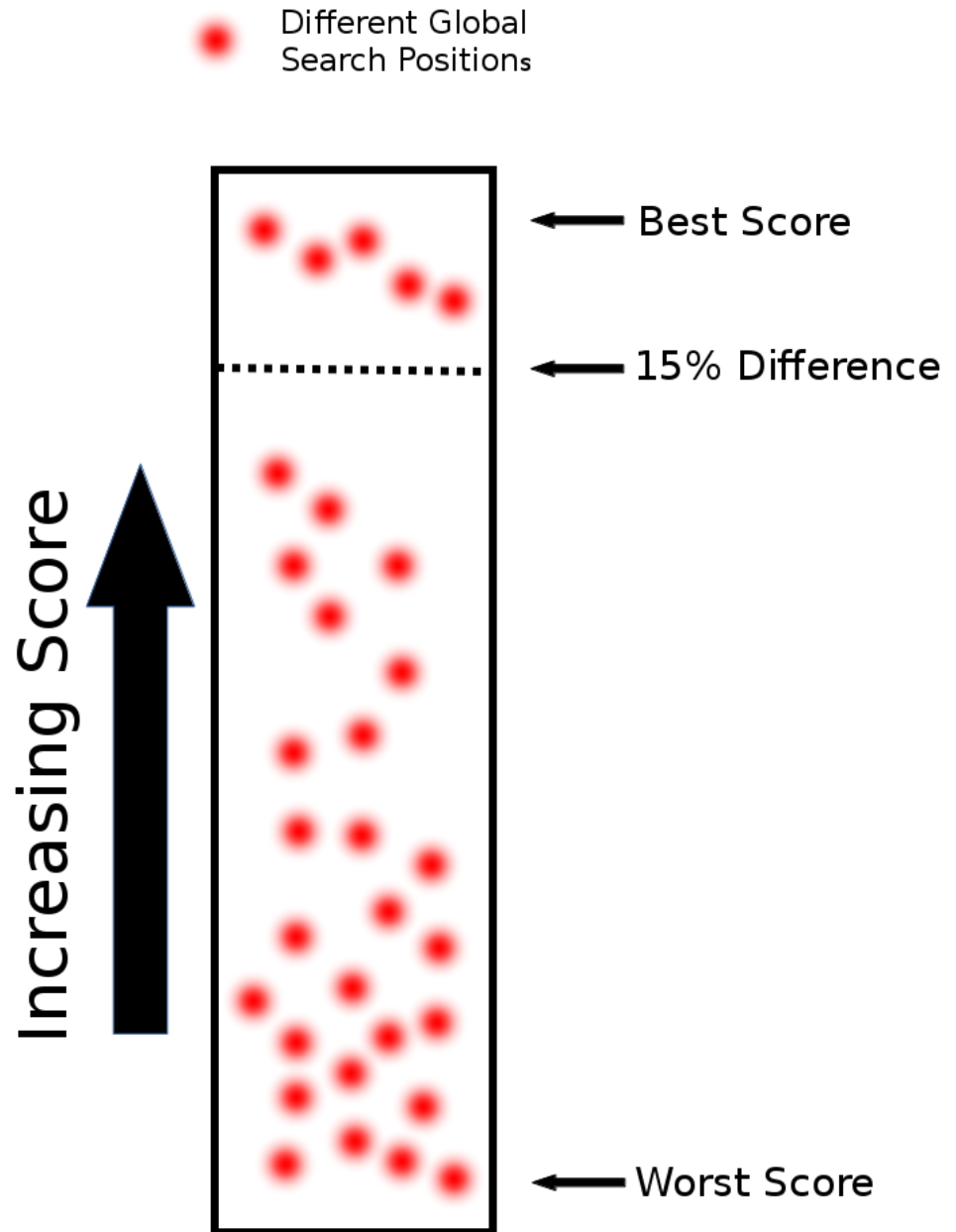
Elmlund et al, 2013

“Sloppy” alignment

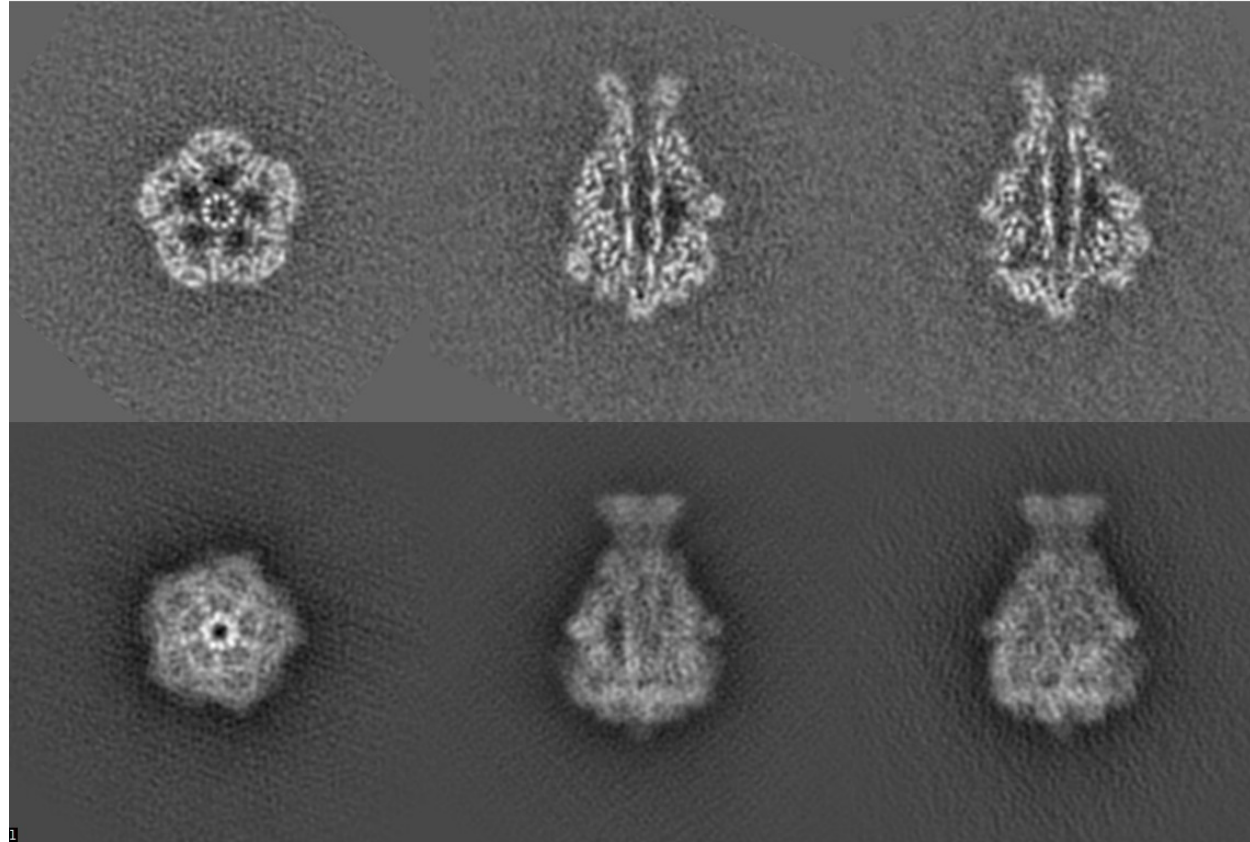
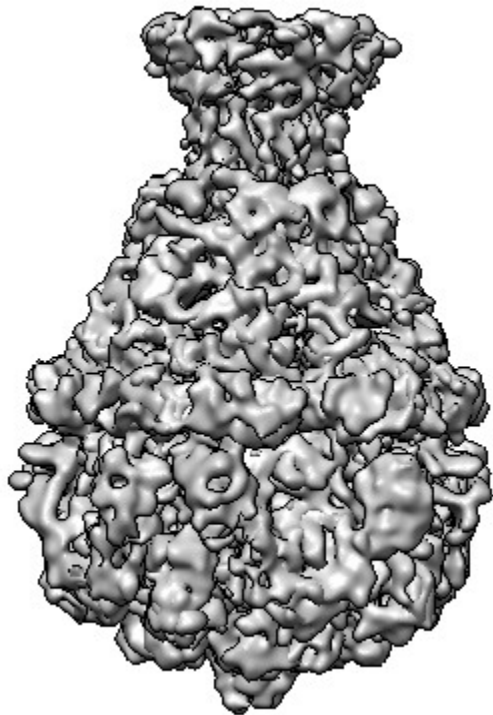


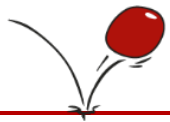
Randomly select from all global search results whose score is in the top 15% of the difference between the best and worst score.

Actually *Faster* than normal cisTEM algorithm.



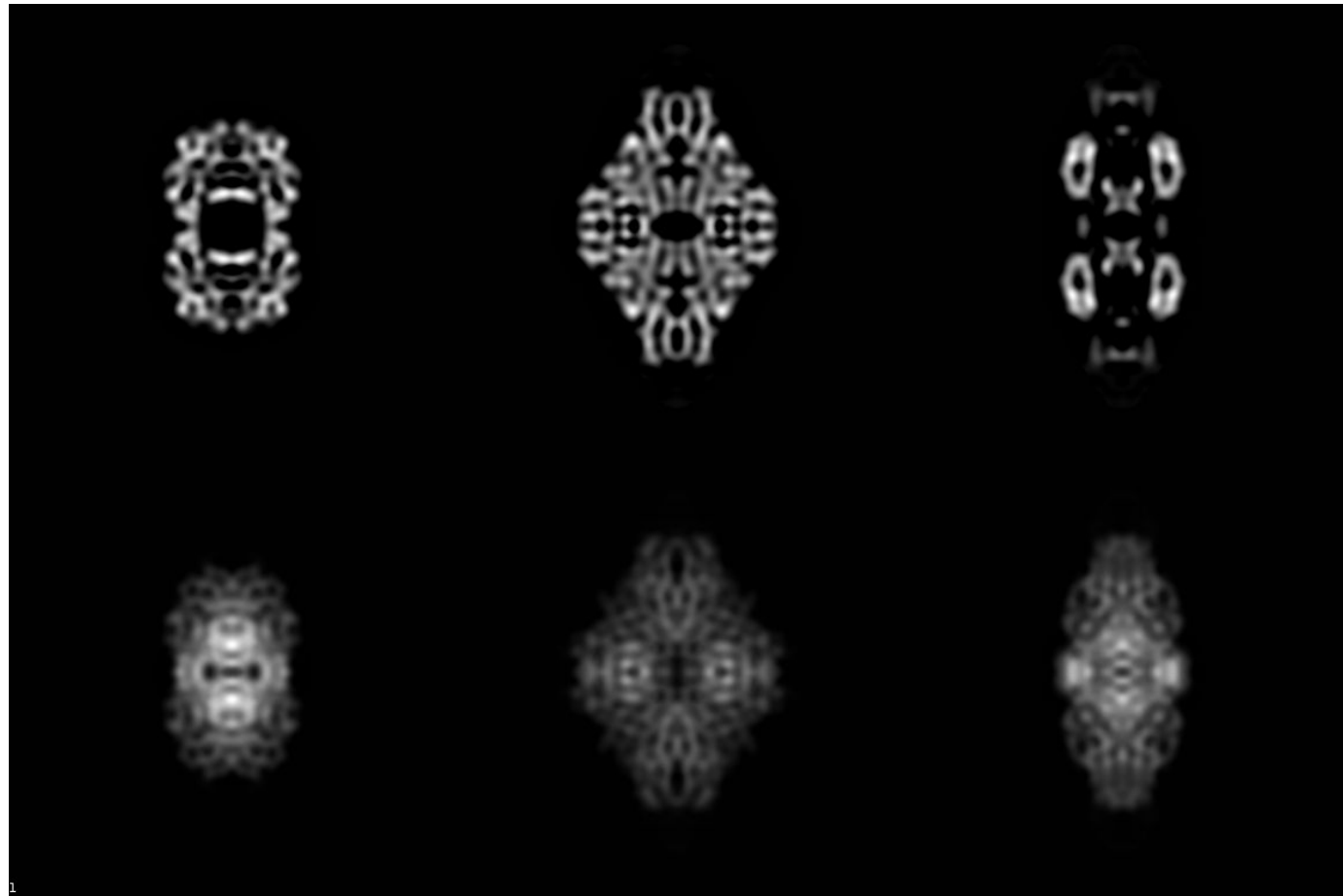
“Sloppy” alignment





Auto masking

Apply, then
threshold



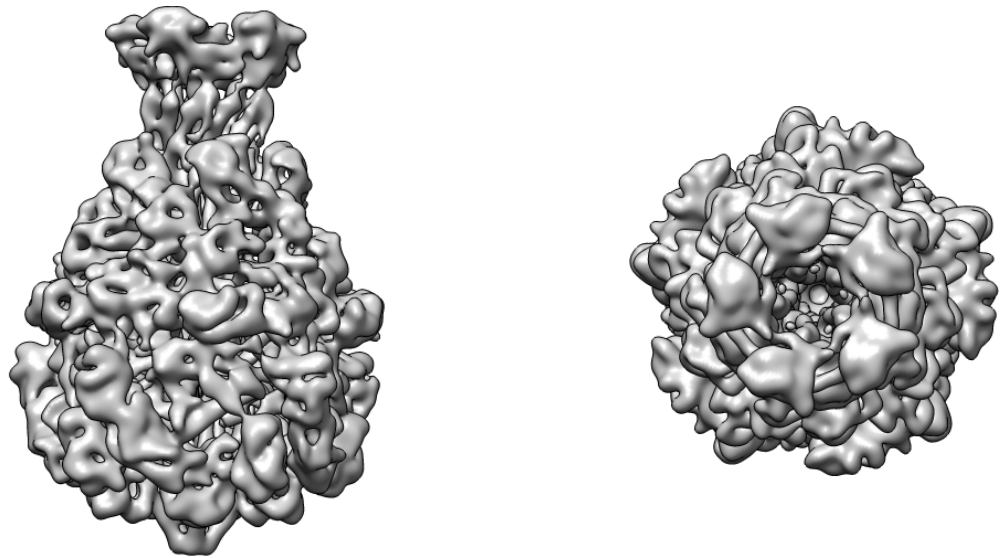


Symmetry Alignment

Best result from starting in C1 then finishing with symmetry.

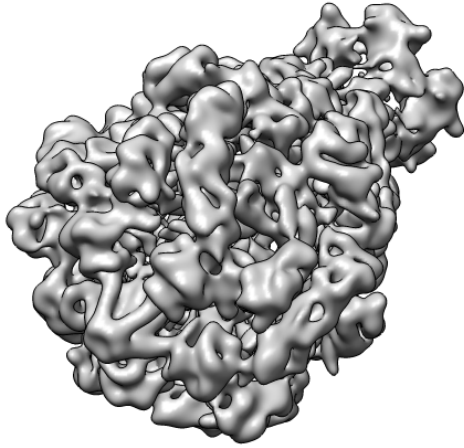
Need to align to symmetry axes.

Needs to be fast.

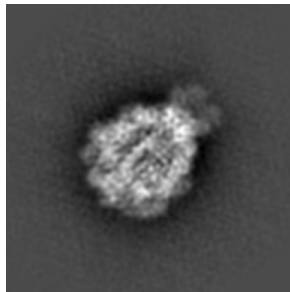
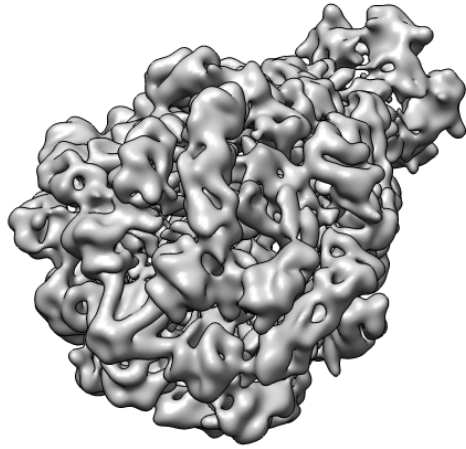


Gatsogiannis et al, EMPIAR-10089

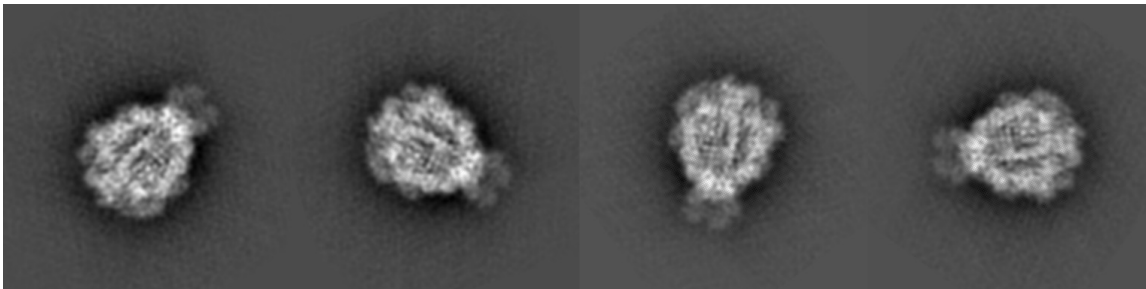
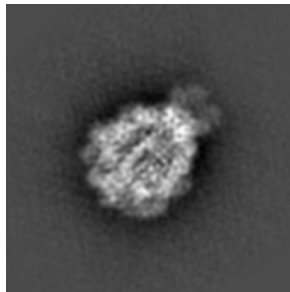
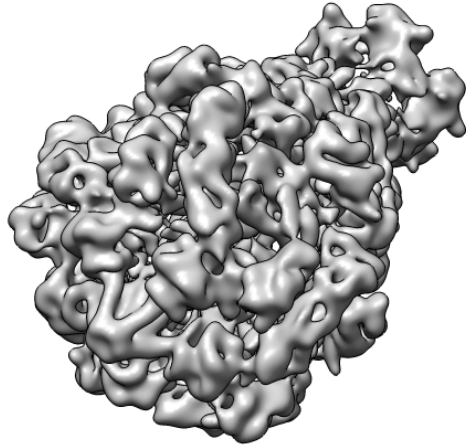
Symmetry Alignment



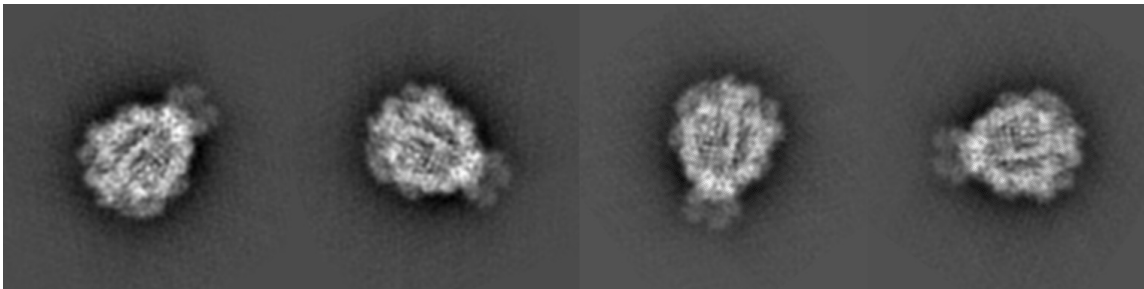
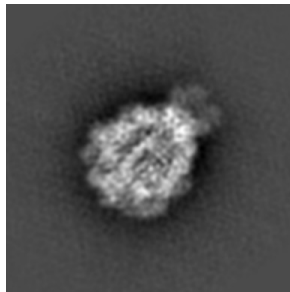
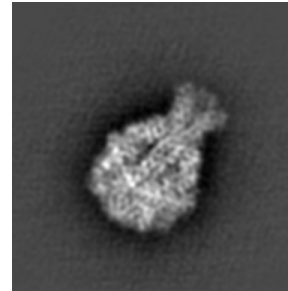
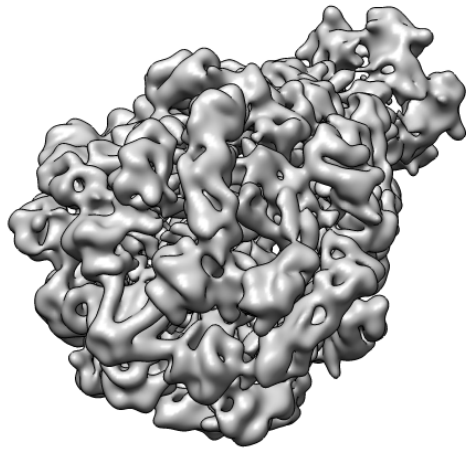
Symmetry Alignment

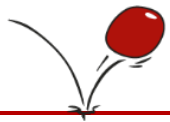


Symmetry Alignment

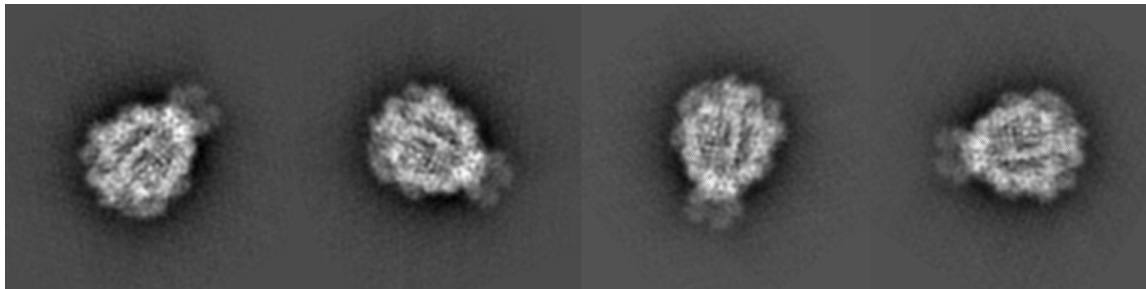
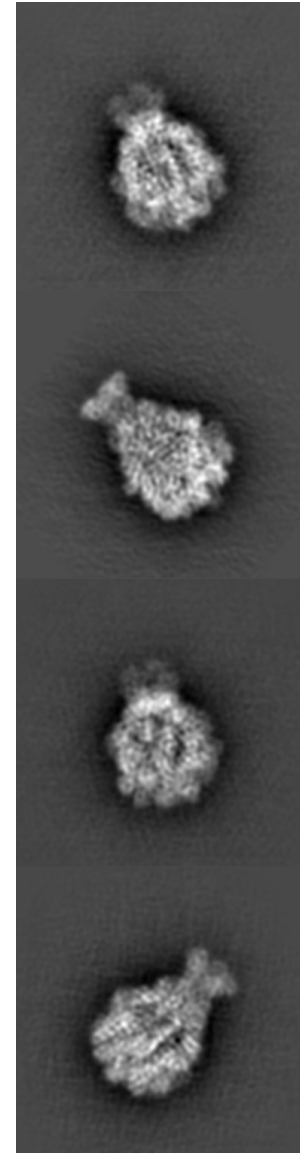
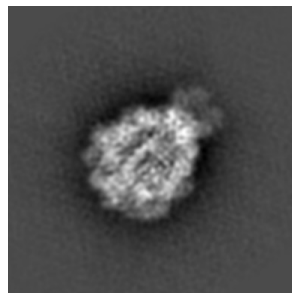
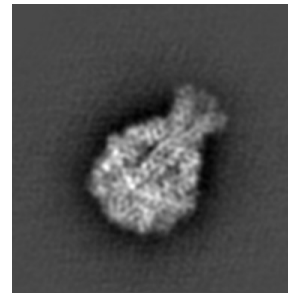
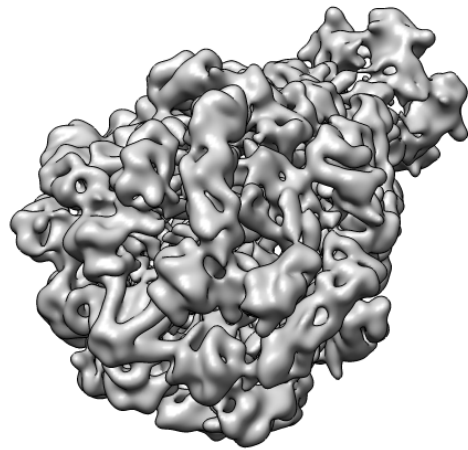


Symmetry Alignment





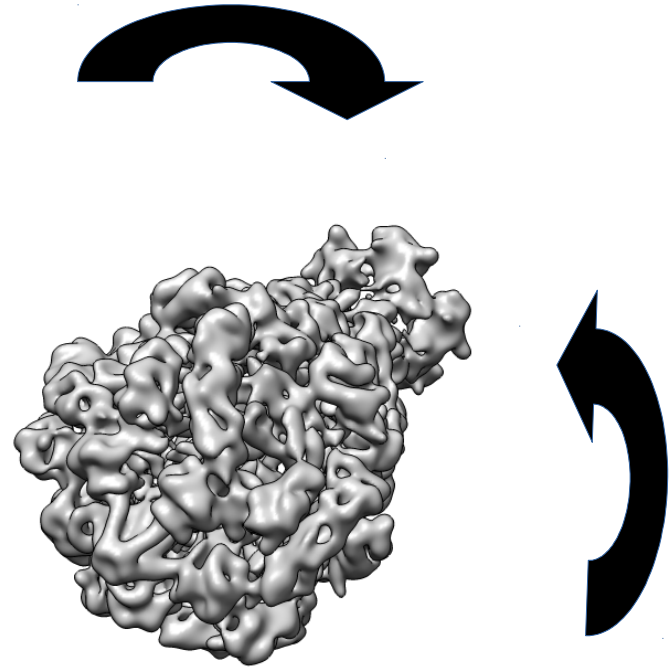
Symmetry Alignment

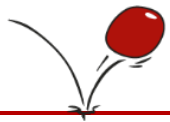


Symmetry Alignment

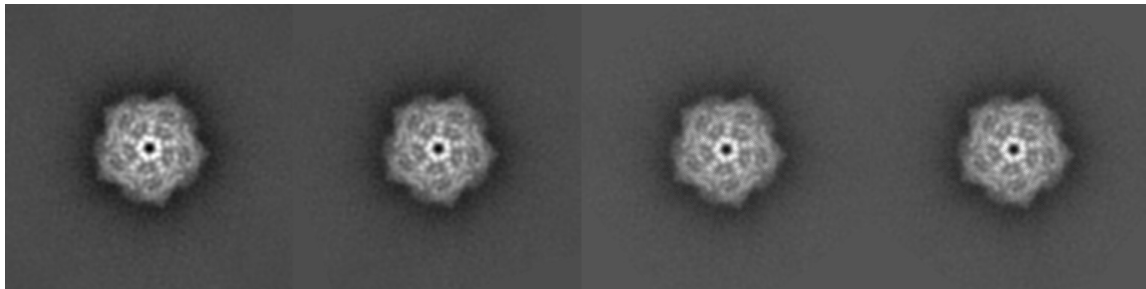
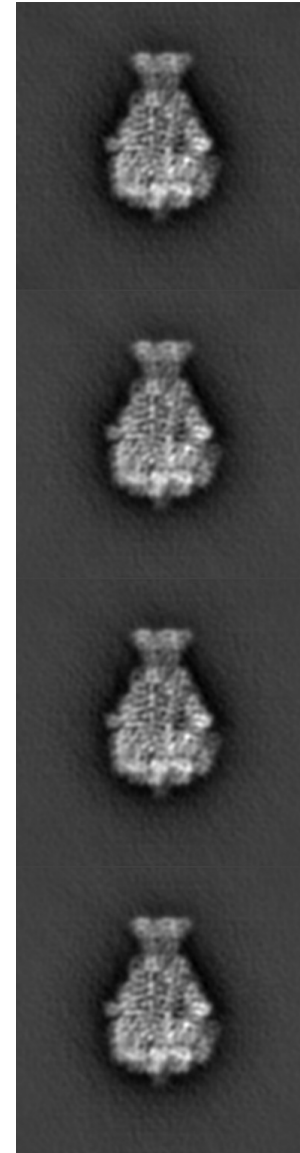
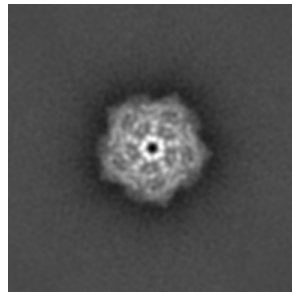
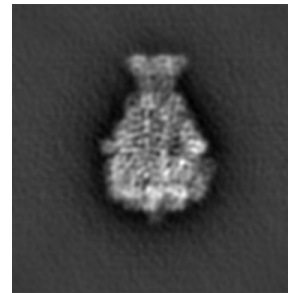
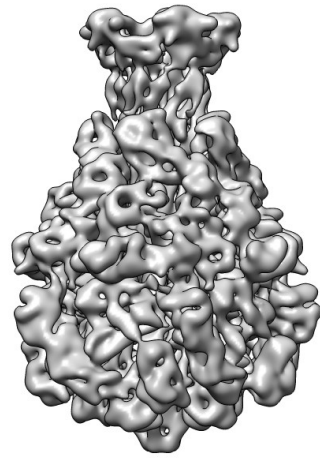


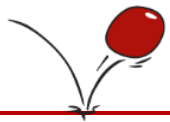
Brute Force search over angles





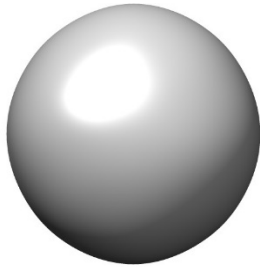
Symmetry Alignment



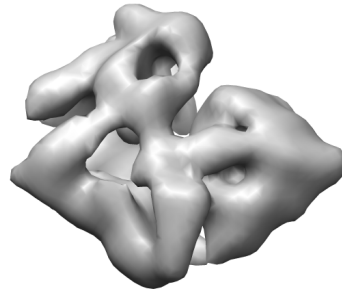


Seems robust...

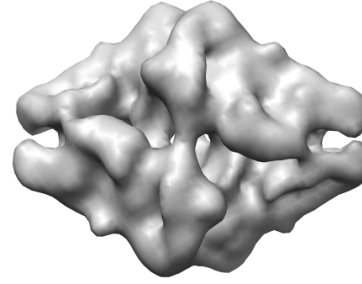
D2
460 kDa



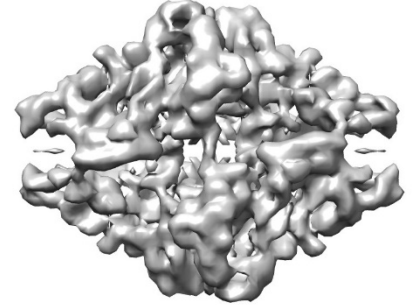
Start



Cycle 9

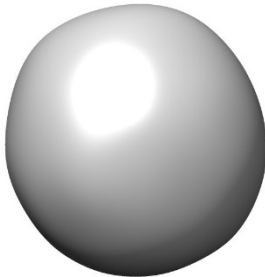


Cycle 27

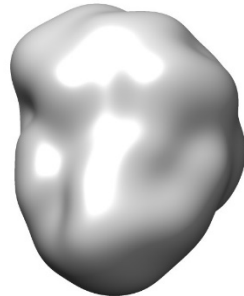


Cycle 40 **1.0 h**

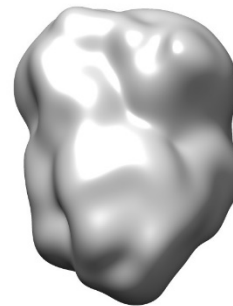
C1
240 kDa



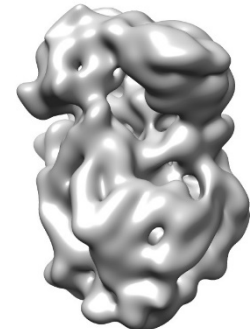
Start



Cycle 9

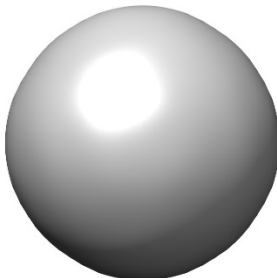


Cycle 27

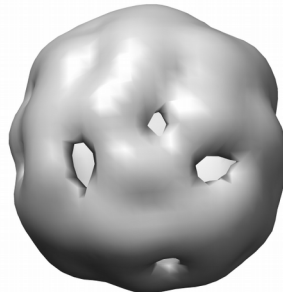


Cycle 40 **4.2 h**

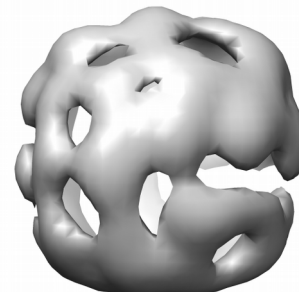
O
440 kDa



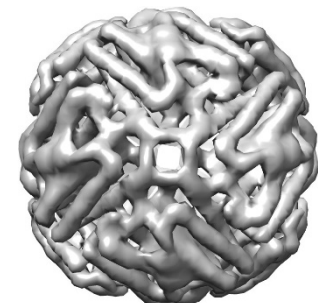
Start



Cycle 9



Cycle 27



Cycle 40 **0.3 h**

Thanks!



Web Page: cistem.org



Computational Imaging System for Transmission Electron Microscopy

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cisTEM is user-friendly software to process cryo-EM images of macromolecular complexes and obtain high-resolution 3D reconstructions from them. It was developed by Tim Grant, Alexis Rohou and Nikolaus Grigorieff and comprises a number of tools to process image data including movies, micrographs and stacks of single-particle images, implementing a complete "pipeline" of processing steps to obtain high-resolution single-particle reconstructions. cisTEM is distributed under the Janelia Research Campus Software License and can be downloaded here. We recommend downloading and using the pre-compiled binaries, rather than compiling the source code, for best performance. New users are encouraged to follow the tutorial, which provides a quick way to become familiar with the most important functions of cisTEM.

Support

Please visit the documentation pages for help. If you encounter any problems using cisTEM, or have questions, please use the forums.

Current Release

[cistem-1.0.0-beta-intel-linux.tar.gz](#) (recommended) displayed 1701 times

[cistem-1.0.0-beta-source-code.tar.gz](#) displayed 266 times

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Exporting or accessing Particle Positions (2 replies)
correlation between stack and particle (2 replies)
Cannot find Align Movies output files (3 replies)
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replacing binned stack with unbinned stack (9 replies)
Merge 200kV and 300kV data (1 replies)
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Previous Releases

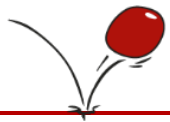
cisTEM, user-friendly software for single-particle image processing

Timothy Grant*, Alexis Rohou*, Nikolaus Grigorieff*

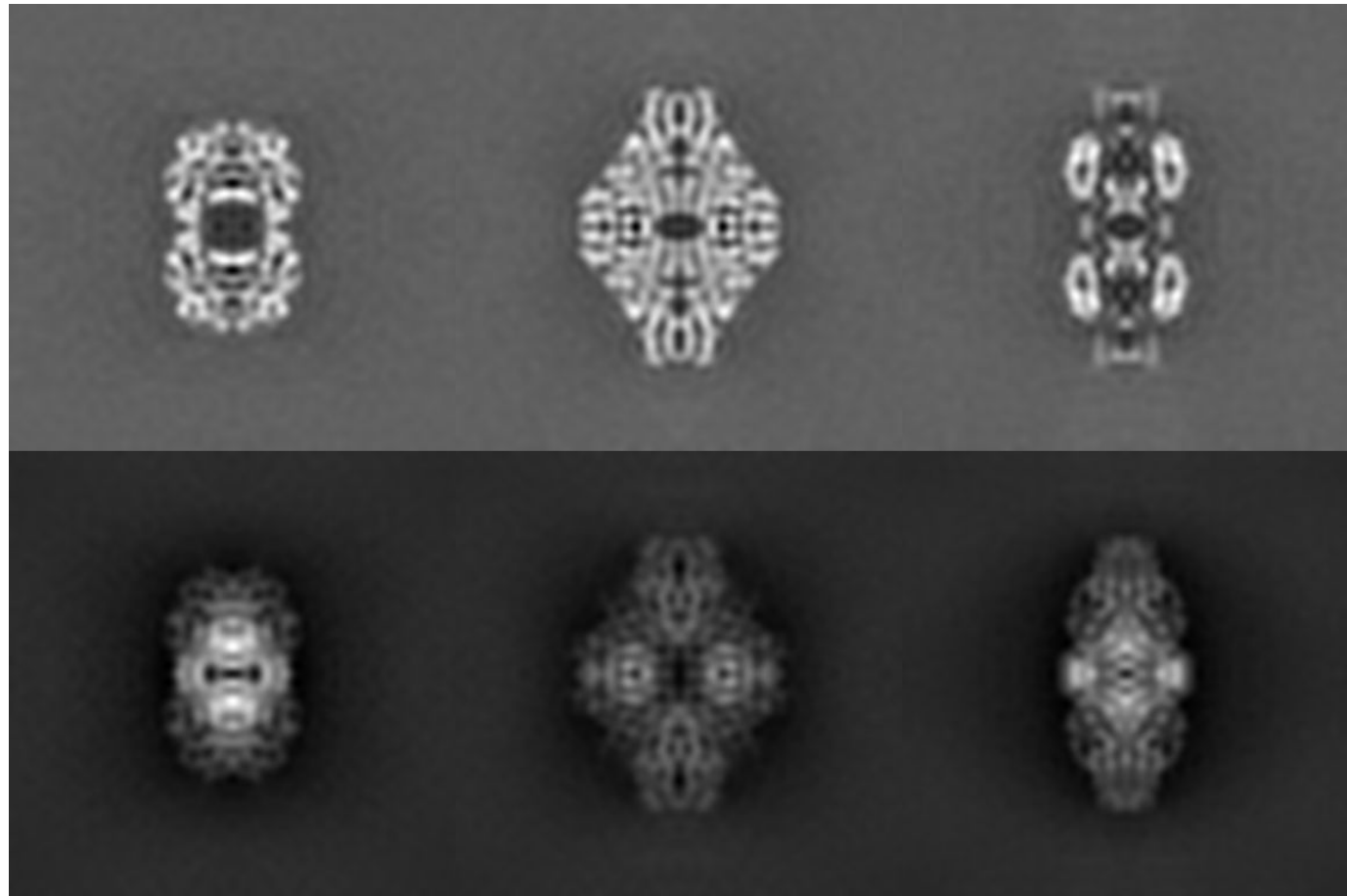
Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, United States

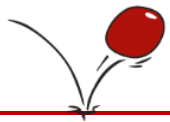
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DOI: <https://doi.org/10.7554/eLife.35383.001>



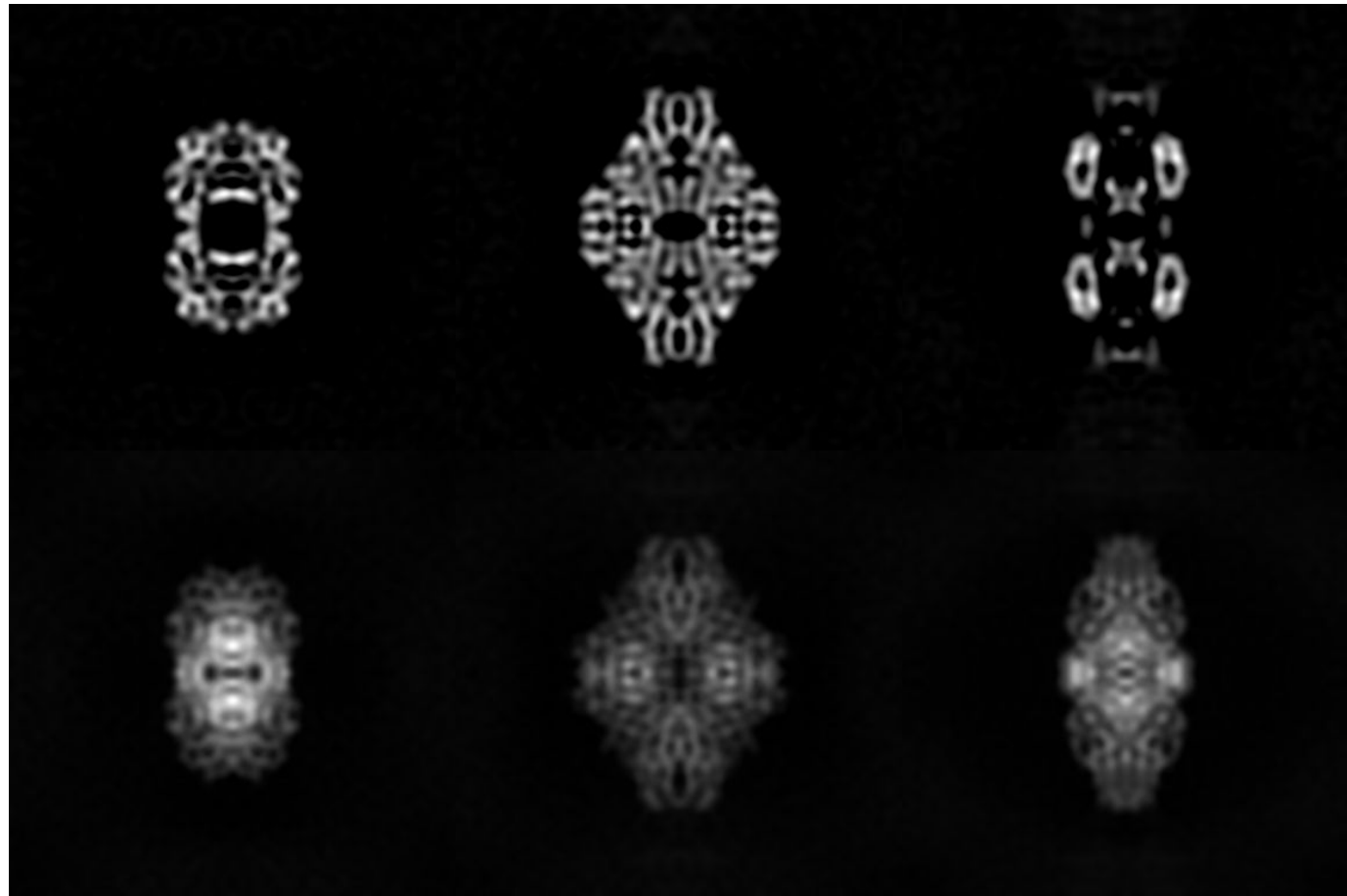
Auto masking





Auto masking

Threshold to
average value
at edges.



Auto masking



Filter to 50 Å.





Web Page: cistem.org



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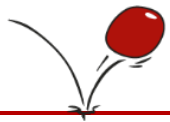
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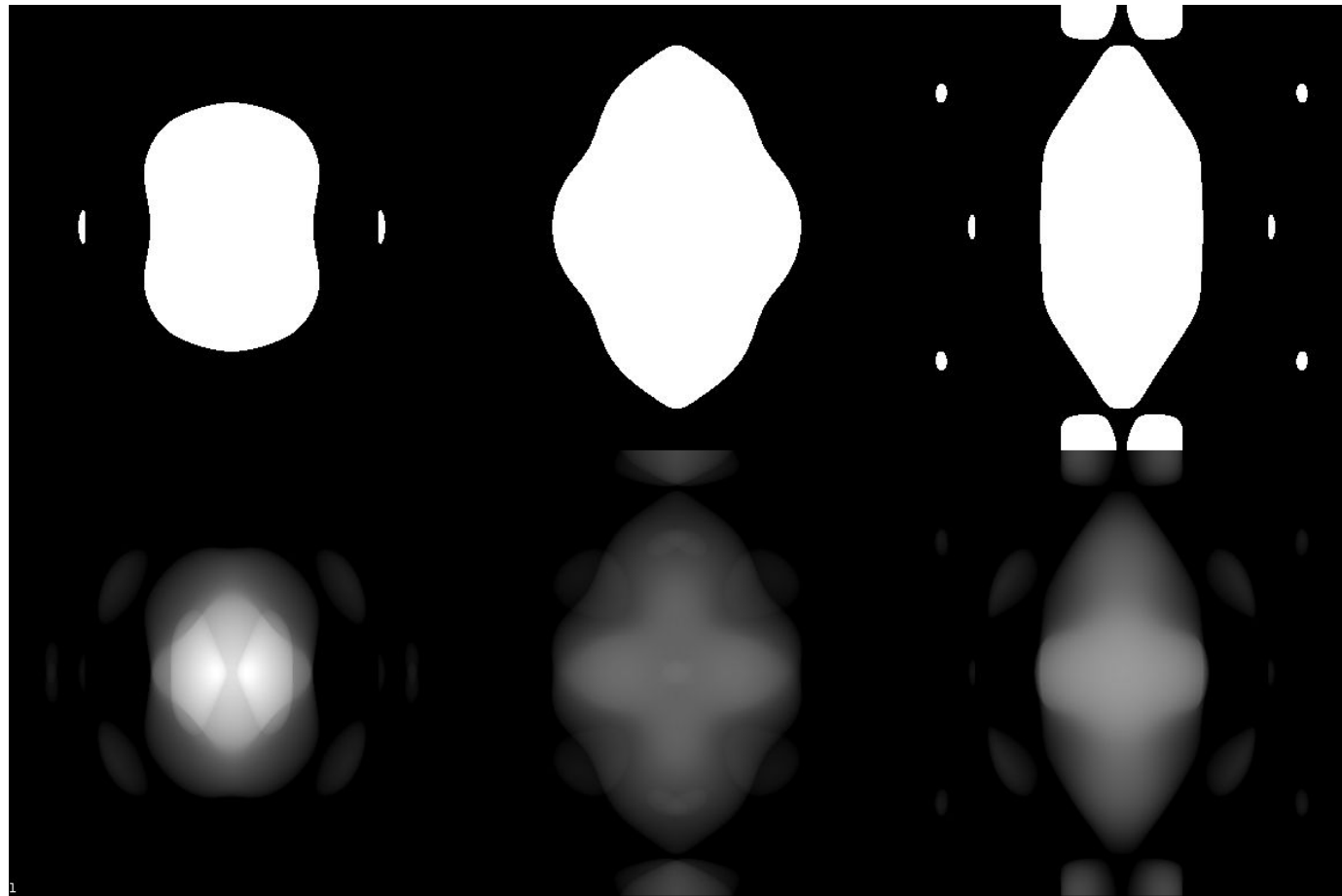
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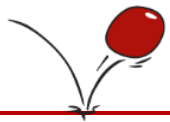




Auto masking

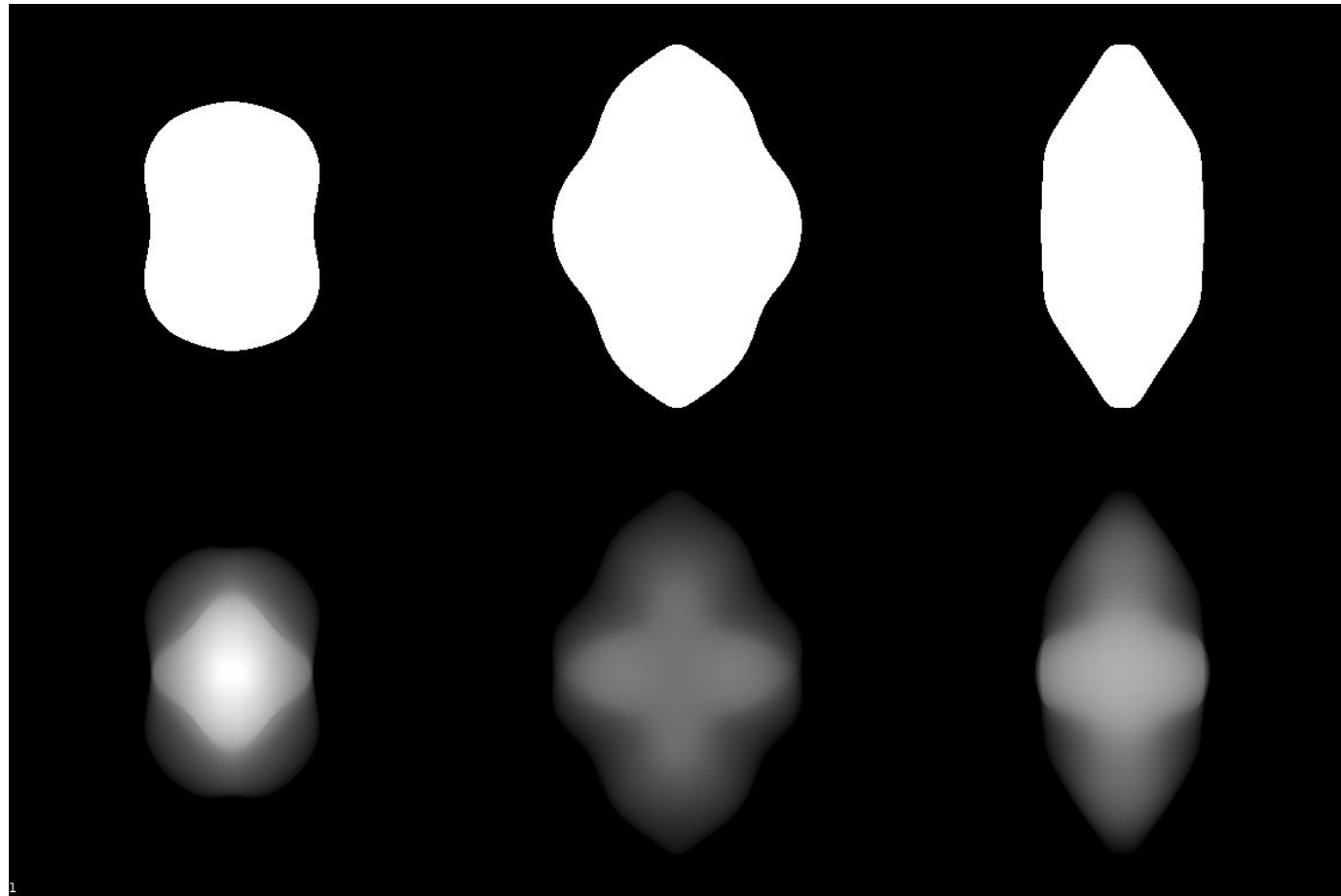
Binarise.



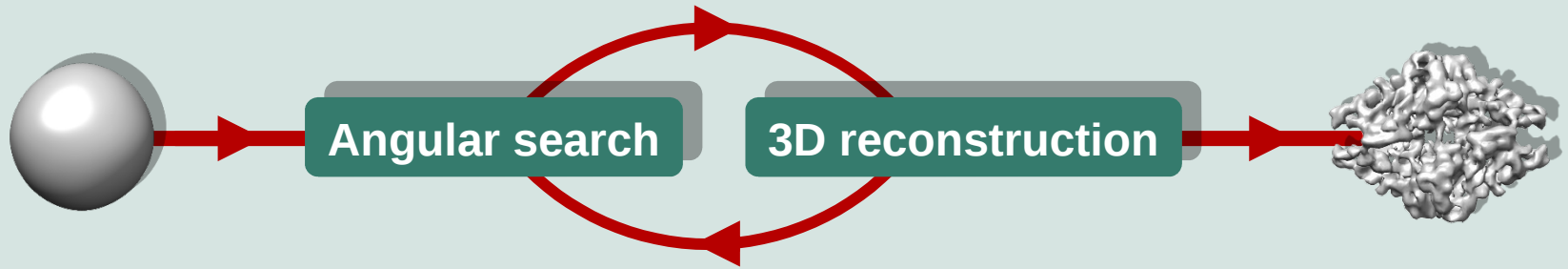


Auto masking

Remove
disconnected
density.



Ab-Initio 3D Procedure



40 cycles

Resolution incremented from 20 Å to 8 Å

3N particles

$N_1 = 2500, N_{40} = 10000$

**N particles with
highest scores**

Repeat if needed

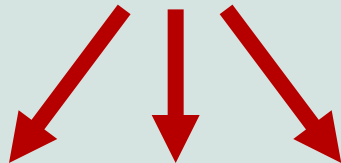
Custom Parallelization

Workstation

CISTEM GUI



Job controller



Slave jobs

Workstation

CISTEM GUI



Cluster Head

Job controller



Cluster Nodes

Slave jobs



Run Profiles

Project Help

Run Profiles

Overview

Assets

Actions

Results

Settings

Default Local

Local

Cluster

Add

Rename

Remove

Duplicate

Import

Export

Total Number of Processes : 33

Manager Command :-

ssh -f login1 "nohup \$command"

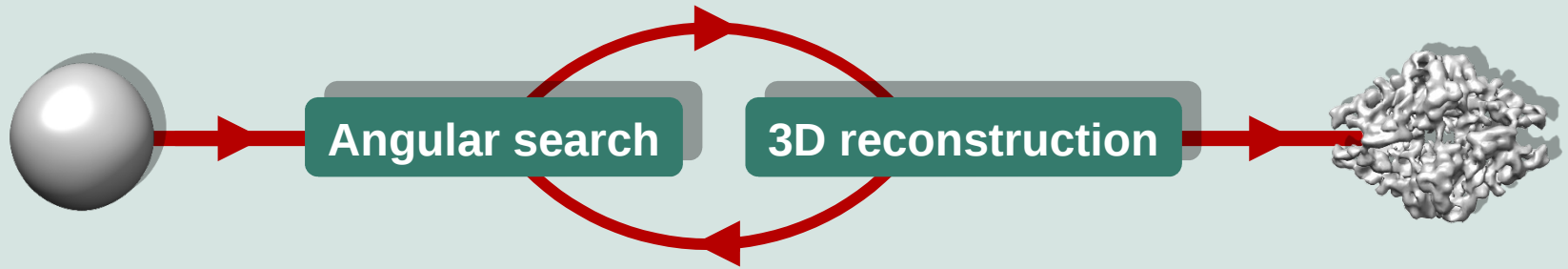
Gui Address : Automatic

Controller Address : Automatic

Command	No. Copies	Launch Delay (ms)
qsub -N cisTEM -j y -o /dev/null -b y -cwd -V \$command	33	70

Add Edit Remove Save

Ab-Initio 3D Procedure



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**N particles with
highest scores**

Repeat if needed