**Supplementary File 1**

**TITLE:**

# Cryo-Electron Tomography Remote Data Collection and Subtomogram Averaging

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**Parameter file (param0.m):**

% This is a comment

% Inline comments will break the parser.

% String to name the structure that contains all of the metadata, projectName

subTomoMeta=ApoF\_Krios2

fastScratchDisk=

% Number of GPUS

nGPUs=4

nCpuCores=20

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%%%%%%%%%%%%%%% Mask parameters %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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% The particle radius in x,y,z Angstrom, smallest value to contain particle.

% For particles in a lattice, neighboring particles can be used in alignment

% by specifying a larger mask size, but this paramter must correspond to your

% target, a cetral hexamer of capsid proteins for example.

particleRadius=[65,65,65]

% Estimated particle mass in megaDa

particleMass=0.5

Ali\_mType=sphere

Cls\_mType=sphere

% For special cases where repeated motifs are present which might cause one

% subtomo to drift to a neighbor. This allows a larger alignment mask to be used

% for the rotational search (Ali\_m...) but limits the translational peak search.

%Peak\_mType=sphere

%Peak\_mRadius=[20,20,20]

% mask radius and center - and center in Angstrom. Mask size is determined

% large enough to contain delocalized signal, proper apodization, and to

% avoid wraparound error in cross-correlation.

Ali\_mRadius=[70,70,70]

Ali\_mCenter=[0,0,0]

Cls\_mRadius=[70,70,70]

Cls\_mCenter=[0,0,0]

phakePhasePlate=1

% Sampling rate

Ali\_samplingRate=4

Cls\_samplingRate=4

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%%%%%%%%%% Tomo-constrained projection refinement parameters %%%%%%%%%%%%%%%%

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% I advise to avoid using this experimental feature for now.

tomoCprDefocusRefine=0

tomoCprDefocusRange=500e-9;

tomoCprDefocusStep=20e-9;

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%%%%%%%%%%%%%%%%%%% subTomogram alignment %%%%%%%%%%%%%%%%%%%%%%%%%

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Raw\_className=0

% Second row specifies C1 symmetry

Raw\_classes\_odd=[0;4.\*ones(2,1)]

Raw\_classes\_eve=[0;4.\*ones(2,1)]

symmetry=O

Raw\_angleSearch=[0,0,45,5]

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%%%%%%%%%%%%%%%%%%% Template matching parameters %%%%%%%%%%%%%%%%%%%%%%%%%

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Tmp\_samplingRate=6

Tmp\_threshold=800

Tmp\_angleSearch=[180,15,180,15,0]

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%%%%%%%%%%%%%%%%%% Class reference %%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%%%%%%%%param0.m%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

Cls\_className=9

Cls\_classes\_odd=[1:9;1.\*ones(1,9)]

Cls\_classes\_eve=[1:9;1.\*ones(1,9)]

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%%%%%%%%%%%%%%%%%%%%%% FSC Paramters %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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% On/Off anisotropic SSNR calc

flgCones=1

% B-factor applied to weighted averages and refs. Should be < 20. Can be a vector

% where the 2:end positions generate independent maps at that sharpening

% when "avg paramN.m N FinalAlignment is run.

Fsc\_bfactor=10

% For very tightly packed subTomos set to 1 to avoid mixing halfsets

% form overlaping peripheral density.

fscGoldSplitOnTomos=1

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%%%%%%%%%%%%%%%%%%% Classification Paramters %%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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% On/Off classification. This must be on when "avg paramN.m N RawAlignment"

% is run at the begining of a cycle where classification is to be run.

flgClassify=0

% List of different cluster sizes to try, eg [3;4]

Pca\_clusters=[2,3]

% Maximum number of eigenvalues/vectors to save

Pca\_maxEigs=36

% Different resolution bands to run PCA on. Not all need to be used for subsequent

% clustering. (Angstrom)

pcaScaleSpace=[8,14,21];

% Random subset of particles used to reduce the burden of PCA

% This is ignored if flgPcaFull is true in the call to "pca"

Pca\_randSubset=0

% Different ranges of coefficients to use in the clustering. At times, the

% missing wedge can be a strong feature, such that ignoring the first few

% eigen values can be usefule. [2:40 ; 6;40 ; 10:40]

% Each row must have the same number of entries, and there must be a row

% for each scale space, even if it is all zeros.

Pca\_coeffs=[7:11;7:11;7:11]

% The number of subtomos to process at once before pulling tempDataMatrix off

% the gpu and into main memory.

PcaGpuPull=1200

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%%%%%%%%%%%%%%%%%%%%%%%%% Parameters for CTF all si (meters, volts)%%%%%%%%%%%%%

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%%%%%%%%%% Microscope parameters %%%%%%%%%%

% Of the data saved in fixed stacks - MUST match header

PIXEL\_SIZE=1.34e-10

% Currently any super-resolution data is cropped in Fourier Space after alignment

% allowing for finer sampling when interpolating the stacks, while then

% filtering out noise due to aliasing.

SuperResolution=0

% Spherical abberation

Cs=2.7e-3

% Accelerating voltage

VOLTAGE=300e3

% Percent amplitude contrast

AMPCONT=0.10

% search range - generally safe to test a wide range

defEstimate=3.5e-6

defWindow=2e-6

% The PS is considered from the lower resolution inflection point

% past the first zero to this cutoff resolution

defCutOff=7e-10

% Total dose in electron/A^2, assumed constant rate

CUM\_e\_DOSE=102

% Gold fiducial diameter

beadDiameter=10e-9

use\_v2\_SF3D=1

% Boolean: If a graduated dose scheme is used, where applied dose is ~ Thickness (1/cos(tiltAngle))

oneOverCosineDose=1

% Degrees: first tilt collected

startingAngle=0

% String: Direction after first tilt (pos or neg)

startingDirection=pos

% Boolean: Group size on either side of zero, e.g. 2 (with pos) would give, 0, 3, 6, -3, -6, 9, ...

doseSymmetricIncrement=2

% Float: Dose applied per tilt (at zero degrees)

doseAtMinTilt=2.5