**Supplementary File 1**

**TITLE:**

# Cryo-Electron Tomography Remote Data Collection and Subtomogram Averaging

**AUTHORS AND AFFILIATIONS:**

Yuewen Sheng1, Kyle Morris1, Julika Radecke1\*, Peijun Zhang1,2,3\*

1Electron Bio-Imaging Centre, Diamond Light Source Ltd, Harwell Science & Innovation Campus, Didcot OX11 0DE, UK

2Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK

3Chinese Academy of Medical Sciences Oxford Institute, University of Oxford, Oxford, OX3 7BN, UK

\*Email addresses of the corresponding authors:

Julika Radecke ([julika.radecke@diamond.ac.uk](mailto:julika.radecke@diamond.ac.uk))

Peijun Zhang ([peijun.zhang@diamond.ac.uk](mailto:peijun.zhang@diamond.ac.uk))

**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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% 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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

tomoCprDefocusRefine=0

tomoCprDefocusRange=500e-9;

tomoCprDefocusStep=20e-9;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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]

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

Tmp\_samplingRate=6

Tmp\_threshold=800

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%%%%%%%%%% 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)]

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% 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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% 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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

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

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%%%%%%%%%% 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